MEMORANDUM OF MEETING

Date: January 29, 2001

Time: 1:00 - 3:00 PM

Location: 12th floor Conference Room
1110 Vermont Ave., NW

Participants:

Visitors

Robert Hill	Hill Research Associates, Inc.
Charles Morin	Morin & Krasny, LLP
Bertjan Ziere	Pharming B.V.
Paul Leufkens	Pharming B.V.

FDA

Kathleen McAveney Jones	HFS-206
Rudaina Alrefai	HFS-206
Rebecca Edelstein	HFS-246
Susan Carberry	HFS-246
George Pauli	HFS-205
Linda Kahl	HFS-206
Paulette Gaynor	HFS-215
Michael DiNovi	HFS-246
Sue Anderson	HFS-831
Mel Dong	HFS-225
Antonia Mattia	HFS-207

Subject: Product Under Development

The visitors requested the meeting to consult with FDA regarding Pharming's recombinant human lactoferrin (rHLF) derived from transgenic cows. The visitors had met with FDA to discuss this product on two earlier occasions. Prior to the meeting, the visitors provided a package of background information. A copy of that material is attached to this memorandum.

administration, as well as an ingredient for non-infant formula food uses.

The visitors discussed a proposed 90-day toxicity study with add-ons intended to address reproductive and developmental issues. This prompted discussion from FDA about appropriate safety testing for Pharming's product. Dr. Sue Anderson emphasized the importance of using the food-grade rather than pharmaceutical-grade product for food-related safety studies. Dr. Linda Kahl discussed safety assessment studies comparing rHLF with HLF found in breast milk. Dr. Kahl informed the visitors that the "Red Book" provided guidance, not requirements for safety testing. She stated that the basic approach to assessing the safety of rHLF would be the same for evaluating the safety of any protein. She recommended determining the real safety issues presented by this substance, and performing studies relevant to these issues.

Dr. Toni Mattia informed the visitors that she could not chart a path for them because the safety of a food ingredient was dependent upon many things. Dr. Mattia recommended examining the differences between rHLF and HLF derived from breast milk, and evaluating animal and human studies on digestion, nutrition and allergenicity which were currently available. She discussed standard metabolic studies and endpoints. Dr. Mattia stressed the importance of the diet used in studies because of its impact on results. She also discussed the grade of the material used for safety studies as compared with the grade of material intended for use in the food product, and recommended characterizing multiple lots of material for toxicology testing. Dr. Sue Carberry emphasized the importance of providing exposure levels. Drs. Pauli and Mattia questioned the purpose of the reproduction add-on to the 90-day study. Dr. Mattia stated that anticipating a particular reproductive event would beg for a separate reproduction study.

Dr. Sue Anderson stressed the importance of considering infant physiology and development when assessing the safety of rHLF for use in infant formula, and inquired about the feasibility of performing toxicological studies at earlier time points. Drs. Kahl and Anderson recommended that the digestibility of rHLF versus breast milk by infants be considered. Dr. Anderson also informed the visitors of the requirements for infant formulas citing the Infant Formula Act of 1980 and subsequent amendments in 1986, and the Proposed Rule for Current Good Manufacturing Practice, Quality Control Procedures, Quality Factors, Notification Requirements, and Records and Reports, for the Production of Infant Formula (61 FR 36153).

should be breast milk LF?

The potential allergenicity of Pharming's product was discussed, due to possible contamination with bovine lactoferrin (BLF), a known food allergen present in cow's milk. Dr. Kahl also discussed issues related to non-infant formula uses of rHLF. Because HLF is immunologically as well as biologically active, it was recommended that Pharming address whether any of these effects might be adverse, rather than beneficial, for some people.

Dr. Kahl presented an overview of the GRAS notification process, comparing it with the food additive petition process. Drs. Kahl and Pauli emphasized that for a food ingredient to be considered GRAS, there must be safety data that is generally available plus consensus among qualified experts. Dr. Kahl made a recommendation that if the visitors were considering convening

a GRAS panel, that the panel should consist of scientists and/or physicians with the appropriate expertise, and should be consulted early in the process in order to provide input as to the appropriate safety testing. Dr. Kahl emphasized that there was no clear path to proving the safety of a food ingredient.

Kathleen McAveney Jones, Ph.D.

cc: HFA-224 HFS-200 HFS-207 HFS-215 HFS-225 HFS-246 HFS-831
FDA participants

R/D: KMcAveneyJones: HFS-206:3/2/01:Pharming.wpd
Edited and initialed: LSKahl: HFS-206:3/2/01
F/T: KMcAveneyJones: HFS-206:3/6/01

MEMORANDUM OF CONFERENCE

Date: July 20, 1998

Time: 1:00 - 2:30 p.m.

Location: 7th Floor Conference Room
Vermont Ave. Bldg.,1110 Vermont Ave., NW

Participants:

Visitors:

Joost van Bree	Pharming Group N.V.
Juha Koivurinta	Pharming Group N.V.
Patrick van Berkel	Pharming Technologies B.V.
Charles Morin	Burditt and Radzius

FDA:

Nega Beru		HFS-206
Mika Alewynse		HFV-228
John Matheson		HFV-200
Bill Price		HFV-200
Isabel Chen		HFS-207
George Pauli		HFS-205
Jeanette Glover-Glew		HFS-207
Zofia Olempska-Beer		HFS-207
J. Eugene LeClerc		HFS-237
Anita Chang		HFS-225
Wendy Dixon		[redacted]
Stephanie McQuilkin		HFS-200
Linda Kahl		HFS-206
Felicia Satchell	(by phone)	HFS-158
Nick Duy	(by phone)	HFS-456
Gillian Robert-Baldo	(by phone)	HFS-456
Sue Anderson	(by phone)	HFS-465
Linda Tonucci	(by phone)	HFS-456

Subject: Recombinant Human Lactoferrin

The visitors requested the meeting in order to consult FDA regarding Pharming's recombinant human lactoferrin (rhLF) derived from transgenic cows. The visitors stated that while they envisaged other uses in the future and that they intended to consult the appropriate FDA centers regarding these uses, rhLF at present is intended for use in infant formulas. Prior to the meeting, the visitors provided a package of information consisting of

a historical perspective of the company and its development and characterization of rhLF. The package also contained draft preclinical study protocols aimed at addressing the safety of rhLF for its intended use.

During the meeting, the visitors presented information regarding the generation of the transgenic animals including the genetic constructs that were used. They also enumerated the tests aimed at comparing the structural and functional characteristics of rhLF to that of native lactoferrin isolated from human milk. The visitors reported that most of the studies have been completed and that the assays conducted to date show that rhLF and natural human lactoferrin appear similar except in the relative concentrations of the different forms of the protein that are normally present due to heterogeneity in N-linked glycosylation.

The preclinical study protocols were discussed both with respect to the product as well the dose that is appropriate to use in the studies. We stated that we were in the midst of developing guidance for macro food additive testing and that we would be willing to provide further guidance. We suggested that they provide us with a list and purpose of preclinical and clinical studies they intend to conduct for our review. We also noted that the July 9, 1996, issue of the *Federal Register* contains a proposed rule amending the infant formula regulations and that this document includes guidance on clinical studies for infant formulas.

The visitors inquired regarding the possibility of use of the milk after the removal of rhLF in the production of other food products such as cheese; they noted that not all of the rhLF can be removed from the milk quantitatively. We stated that assuming that there were no safety concerns they would need to discuss with FDA regarding appropriate labeling and regarding how the product might be used in standardized foods.

We also discussed the proposed premarket notification for generally recognized as safe (GRAS) substances including the basis for making GRAS determinations, the information that should be contained in a notification, and possible agency responses. We indicated that FDA is accepting notifications pending finalization of the proposed rule. Finally, we noted that GRAS notification cannot be used in lieu of, and does not replace the mandatory premarket notification requirement for infant formulas.

Nega Beru, Ph.D.

cc: HFS-200 HFS-205 HFS-206 HFS-207 HFS-225 HFS-235 HFS-246 HFS-456
HFS-158 HFS-247 HFS-13 HFV-228 HFV-200
R/D:HFS-206:NBeru:418-3097: 7/22/98:Saved as meet0720.98
F/t: HFS-206:NBeru:srd:8/20/98

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#73988

January 5, 2001

George H. Pauli, PhD (Room 1250)
Director (HFS-205)
Division of Product Policy
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition
Food and Drug Administration
1110 Vermont Avenue, N.W.
Washington, D. C. 20201

CONFIDENTIAL

Re: Request for meeting

Dear Dr. Pauli:

Thanks very much for returning my phone call and for taking the time to discuss aspects of GRASing human lactoferrin for use in infant formulas and supplementing other foods. This will memorialize the substance of our conversation and provide you with additional information.

First, please find attached a copy of my July 2, 1998 **confidential** letter to you which provided information pertinent to the meeting subsequently held on July 20, 1998. It covers all of the information we discussed.

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Second, please find attached a copy of my July 22, 1998 **confidential** memorandum summarizing the substance of the July 20th meeting with FDA. Please note that it includes a list of those who were in attendance.

Third, we discussed having a meeting towards the end of January (our prioritized list of dates for such meeting was as follows:

1. 1/26 (first choice);
2. 1/29 (second choice); or
3. 1/22 (third choice))

at from approximately 1:30 – 3:00 p.m. in the afternoon.

Fourth, the purpose of the meeting is to discuss exactly what preclinical tox testing should be done in order to satisfy the needs of **both** the OPA group **and** the Infant Formula group. As of this date, two studies had been agreed to, i.e.:

1. the first, a 90-day study in rats; and
2. the second, a 90-day study in dogs.

Please note that both studies are currently drafted to start exposure to the pups earlier than is usual **and** both have a reproductive evaluation added at the end. Are these the battery of tests that are still required?

Finally, please feel free to invite any FDA employee to the meeting that you feel may play an important role in any future GRAS Notification that my client may file pertinent to the above-referenced uses.

As you can appreciate, this entire matter is very **confidential**; thus, we trust that the enclosed information will not be discussed or released, except as necessary to prepare FDA personnel for the end of January meeting.

Please call me if you have any questions.

Thanks again for your help.

Sincerely,

Charles Morin

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FILE

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July 2, 1998

George H. Pauli, PhD (HFS-205)
Director (Room 1250)
Division of Product Policy
Office of Premarket Approval
Office of Programs
Center for Food Safety & Applied Nutrition
Food and Drug Administration
1110 Vermont Avenue, N.W.
Washington, D.C. 20201

CONFIDENTIAL

Re: Pharming Health Care Products
Meeting (7/20/98) concerning
use of recombinant lactoferrin

Dear Dr. Pauli:

Pursuant to Nega Beru's instructions and in preparation for Pharming's meeting with CFSAN on July 20th, I am forwarding to you information concerning Pharming Health Care Products ("Pharming") and its recombinant lactoferrin ("rhLF") product which should serve to background you and your colleagues concerning the need for the meeting. Unless I hear differently from you, we will arrive at 1110 Vermont Avenue on Monday, July 20th at approximately 12:45 p.m. in preparation for the meeting to be held in your seventh floor conference room between 1 p.m. and 2:30 p.m.

The company

Pharming is a biotech company whose corporate offices are located in Leiden (a large, university city), The Netherlands. It was incorporated in 1988. Pharming focuses on the research, development, and commercialization of human

health care products derived from milk, primarily from transgenic animals. To this end, Pharming has developed, and will continue to develop, a proprietary production technology using transgenic animals, particularly transgenic dairy cattle. Such transgenic animals are generated from a one-cell animal embryo whose genetic make-up has been modified in the laboratory via the insertion of specifically designed sequences of DNA, so-called gene constructs or transgenes. Pharming has developed proprietary transgenes so as to produce transgenic animals which, in turn, produce proteins in their milk for use in human health care applications.

In June, 1995, Pharming acquired the Finnish Company, Oy FinnGene Ltd., which was subsequently renamed Pharming Oy. As a wholly owned subsidiary, Pharming Oy conducts certain research and development activities focused on the generation of transgenic cattle. The acquisition served to increase Pharming's commercial flexibility by expanding its scientific and operating base.

In June, 1996, Pharming established a subsidiary in Belgium, which is named Pharming N.V. This subsidiary will focus exclusively on production and commercialization of recombinant proteins produced in milk of transgenic rabbits.

Pharming is currently the leader in the field of production technology using transgenic dairy cattle, including having produced the world's first scientifically documented transgenic dairy calf, i.e., the well-known "Herman" the bull. This technology creates product opportunities which are otherwise difficult or even impossible to address. Transgenic cattle are the production route of choice for complex biomedical proteins which either have to be produced in very large quantities at low cost, or which, while representing a small volume, are very difficult to produce. In both cases, manageable numbers of transgenic cows suffice to produce sufficient product to satisfy market demands.

Pharming is also pursuing production technology using other transgenic animals, such as mice and rabbits. In some instances, manageable numbers of

these transgenic animals will also suffice to produce sufficient product to satisfy market demands.

Pharming is dedicated to achieving technological excellence and, particularly, a leading intellectual property position. Cutting-edge technology and adequate patent protection are extremely important in the biotechnology industry To this end, since 1989, the Company has filed, on a worldwide basis, a number of basic patent applications covering a wide range of methods, products and product applications in the area of transgenic animal technology. Pharming's first patent was issued in August, 1993. Since then, various other patents have been issued, including a basic U.S. patent in April, 1994. This latter event made Pharming the first transgenic farm animal company to receive patent protection in a major market. With regard to the other patents, currently Pharming owns or controls such patents in the USA, Canada, Europe, Australia and New Zealand. In addition, patent applications are also pending in these and many other countries.

The product (hLF)

Lactoferrin is the major iron-binding protein in the milk of many mammalian species, including humans. Its concentration in mature human milk ranges from 1-2 grams/liter. This makes it one of the most abundant proteins in human milk. In contrast, the concentration of lactoferrin in mature bovine milk is less than 0.1 grams/liter.

Several biological functions have been ascribed to lactoferrin. The function that is probably most relevant and important to infants consuming human milk is lactoferrin's ability to regulate bacterial growth. It has been demonstrated that lactoferrin promotes growth of Bifidobacterium spp. which are the predominant organisms of the intestinal flora of healthy infants that are breast-fed. In addition, it has been shown that lactoferrin has a strong antibacterial effect on many organisms that are potentially pathogenic. In accordance with these observations, it is well-known that the intestinal flora of children being breast-fed is dramatically

different from children consuming infant formula. Although the exact composition of the intestinal flora is probably regulated by several factors, lactoferrin is probably one of the more important regulators.

Since most infant formulas are derived from bovine milk, they contain very little lactoferrin. Accordingly, addition of human lactoferrin to an infant formula would make the formula more closely resemble human milk. However, since infants fed with breast-milk consume more than 1 gram of human lactoferrin per day, the amounts of lactoferrin needed to supplement infant formula have, to date, been prohibitively large and unavailable. Classical recombinant-DNA methods are not very suitable on a very large scale for producing proteins, such as lactoferrin. In addition, isolation of the protein from other sources, such as pooled human milk, is not desirable or practical.

Transgenic mice were used to demonstrate the feasibility of producing recombinant human lactoferrin in the milk of a different mammal. Human lactoferrin gene sequences were cloned from DNA libraries prepared from healthy human individuals. These lactoferrin sequences were subsequently fused to regulatory sequences derived from regions of the bovine αS1-casein gene. These regions direct mammary gland-specific expression of the casein gene. The casein/lactoferrin gene construct was injected into the pronucleus of fertilized mouse oocytes which were subsequently transferred into recipient animals. After birth, animals were analyzed for integration of the transgene and, if positive, were bred to non-transgenic mice to obtain F1-offspring. Milk was collected from transgenic females and analyzed for the presence of recombinant human lactoferrin. In all mice analyzed, such human lactoferrin was detectable in the milk. In the majority of the cases, expression was higher than the levels observed in human milk. No adverse effect on the physiology and health of the lactating mother as well as of the pups was demonstrable.

The protein was subjected to a large number of assays (such as those pertinent to N-terminal protein sequencing, determining immunological

characteristics, the ability of the protein to bind to a large variety of ligands, purification properties, migration pattern on SDS-PAGE, N-terminal glycosylation, and iron-binding) to compare its structural and functional characteristics with those of native lactoferrin isolated form human milk. It was concluded that the recombinant protein was very similar to the human protein. The primary difference that was observed between the recombinant and native lactoferrin appears to relate to a difference in the relative concentrations of the different forms of the protein that are normally present due to heterogeneity in glycosylation.

Expression of the transgene in mice is primarily restricted to the mammary gland of lactating females. It was also demonstrated that the size of the transcript corresponded precisely with the expected size. The transgene was transmitted to the offspring in Mendelian fashion. In the limited number of lines that were analyzed, the structure of the transgene appeared to be stable throughout several generations.

Given the foregoing mouse results, Pharming then developed methods to produce recombinant human lactoferrin in milk of transgenic cows at high levels. Transgenic cows are animals that contain, in their genome, one or more copies of a gene that is derived from another species. In this case, a gene construct was used that directs expression of human lactoferrin in the milk of the animal. Since dairy cows can produce up to 12,000 liters of milk per year, a single animal is expected to produce at least 10 kilograms of lactoferrin per year, depending on the expression level occurring in each cow. Therefore, a cow herd of manageable size could produce enough lactoferrin to supplement infant formula with human lactoferrin.

Casein/lactoferrin gene constructs selected for their ability to function efficiently in transgenic mice and to direct high levels of lactoferrin expression were also used to generate transgenic cattle. Oocytes were derived from ovaries of slaughtered dairy cows or via OPU technique (ovum pickup) and fertilized in vitro with sperm of elite bulls. DNA was injected into one of the pronuclei after which

embryos were allowed to develop in vitro for another five days. After that period, two cells were removed from the embryo and analyzed for the presence of the transgene. Positive embryos were transferred non-surgically into the uterus of recipient cows (see attached article for additional information).

Pharming first produced recombinant human lactoferrin from transgenic cow's milk in 1996. Such rhLF is of excellent quality and has been demonstrated – in terms of biological activity – to be very similar to natural hLF. Pharming now has a similar, but growing, production herd of transgenic cows capable of producing the necessary quantities of rhLF for testing and commercial use.

Use of rhLF

As indicated above, Pharming desires to commercialize rhLF for use in infant formulas intended to more closely simulate human mother's milk. Given hLF's natural and strong anti-microbial activity, Pharming may, in the future, also decide to commercialize rhLF for uses deemed by FDA to be foods associated with health claims, medical foods, or drugs/biologics. To the extent use concerns health claims or medical foods, Pharming desires to have CFSAN's input about such use. (Drug/biologic use will be discussed at another time with CBER).

The regulatory interest

Pharming is now ready to initiate preclinical testing of rhLF. Before doing so, Pharming thought it would be productive for both FDA and itself to thoroughly inform FDA as to what has transpired thus far, to discuss its commercial intentions, and to discuss the regulatory implications of such intentions, especially the best regulatory approach (e.g., GRAS affirmation, GRAS notification, a FA petition, or other (?)).

Preclinical studies

Pharming intends to initiate three studies to evaluate the safety of its rhLF. These include an Ames test, a standard 90-day oral study in dogs, and a non-standard 90-day (plus) oral study in rats.

Draft protocols for these studies are attached for your review.

Pharming would appreciate having CFSAN's input, if any, concerning the adequacy of these protocols before they are initiated.

The attendees

Pharming intends to have the following representatives present at the meeting:

1. Joost B.M.M. van Bree, PhD
   Vice President, Clinical Development & Regulatory Affairs;

2. Juha Koivurinta
   Vice President, Pharming Holding N.V.;

3. Patrick van Berkel, PhD
   Senior Scientist; and

4. the undersigned

all of whom will be prepared to present and discuss in detail the information referenced above and outlined on the attached agenda.

We encourage CFSAN to have present any and all FDA personnel that may play a significant role in any future hLF regulatory submission sent to CSFAN. Such persons might include there with responsibility for infant formulas,

toxicology, chemistry, microbiology, environmental, regulatory, review and supervising.

Any future submission (pertinent to Pharming's rhLF product) will include information which demonstrates, among other things, that the rhLF is sufficiently comparable to human derived hLF. Such information will also indicate that the rhLF ingredient is produced by transgenic cows that incorporate no pathgenic or toxicogenic capabilities. It also will indicate that all production methods and substances used are appropriate for food use. Finally, the information will indicate that the finished hLF ingredient has been thoroughly tested and found to be safe for use in human food.

As you can appreciate, this entire matter is very **confidential**; thus, we trust that the enclosed information will not be discussed or released, except as necessary to prepare FDA personnel for the July 20th meeting.

Thank you very much for your continuing assistance. If you should have questions or need additional information, please let me know.

Sincerely,

Charles L. Morin

CLM: jkm

cc: Joost van Bree

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To: Pharming rhLF File

From: Charles L. Morin

CONFIDENTIAL

Re: Meeting (7/20/98) with FDA (CFSAN)

Date: July 22, 1998

On Monday, July 20, 1998, the following representatives of Pharming

1. Joost B.M.M. van Bree, PhD
   Vice President, Clinical Development & Regulatory Affairs;

2. Juha Koivurinta
   Vice President, Pharming Holding N.V.;

3. Patrick van Berkel, PhD
   Senior Scientist; and

4. Charles L. Morin
   Burditt & Radzius

met with the following representatives of FDA

1. Felicia B. Satchell (HFS-158)
   Branch Chief
   Food Standards Branch
   CFSAN/OFL/DPEPOFL;

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2. Stephanie McQuilkin (HFS-200)
Special Assistant
Office of Premarket Approval
CFSAN/OPA;

3. George H. Pauli (HFS-205)
Branch Chief
Division of Product Policy
CFSAN/OPA;

4. Nega Beru, PhD (HFS-206)
Team Leader
Regulatory Policy Branch
CFSAN/OPA/DPP;

5. Wendy J. Dixon (HFS-206)
CSO
Regulatory Policy Branch
CFSAN/OPA/DPP;

6. Linda S. Kahl (HFS-206)
Guidelines and Regulations Branch
CFSAN/OFL/DPEPOFL;

7. Isabel S. Chen (HFS-207)
Scientific Support
CFSAN/OPA/DPP;

8. Jeanette Glover Glew (HFS-207)
Environmental Scientist
Scientific Support
CFSAN/OPA/DPP;

9. Zofia S. Olempska-Beer (HFS-207)
Science/technology
CFSAN/OFL/DSATOFL;

000001.017

10. Anita H.C. Chang (HFS-225)
Scientific Support
CFSAN/OPA/DPP;

11. J. Eugene LeClerc, PhD (HFS-237)
Toxicologist
Molecular Toxicology Branch
CFSAN/OPA/DMBRE;

12. Nick Duy (HFS-456)
Regulatory Branch
CFSAN/OSN/DPEPOSN;

13. Gillian L. Robert-Baldo (HFS-456)
Regulatory Branch
CFSAN/OSN/DPEPOSN;

14. Linda H. Tonucci (HFS-456)
Regulatory Branch
CFSAN/OSN/DPEPOSN;

15. Sue A. Anderson (HFS-465)
Scientific Support
CFSAN/OSN/DSATOSN;

16. John C. Matheson (HFV-200)
Senior Environmental Scientist
CVM/OSC/OSCOD;

17. William D. Price, PhD (HFV-200)
Special Assistant
Office of Surveillance and Compliance
CVM/OSC/OSCOD; and

18. Mika G. Alewynse (HFV-228)
Food Safety
Animal Feeds
CVM/OSC/DAF

for the purpose of conveying certain information concerning use of Pharming's rhLF in human foods and discussing the implications of such use. The meeting was held in the 7th floor conference room at 1110 Vermont Avenue, N.W.; it lasted from 1 p.m. until approximately 2:40 p.m.

After brief introductory remarks (by CLM) and self introductions by all attendees, the presentations evolved as indicated on the attached agenda. Other than the information set forth below, the information conveyed to FDA was that indicated on the attached copies of overheads.

The questions asked and/or the points discussed were as follows:

A. Introductions (C.M.)

No questions

B. Overview (J.K.)

No questions

C. Characterization efforts (P.B.)

1. **Question:** Is the genetic effect seen a result of dominance?

   Answer: Yes. The result duplicates a typical Mendelian expectation; thus, 50 percent of the offspring should have the transgene for rhLF.

2. **Question:** Is the glycosylation that occurs an all or none phenomenon?

   Answer: No. The result varies; sometimes it's all, sometimes it differs, and sometimes it's as little as 5 percent.

D. Production process and intended use (J.K.)

No questions

E. Pre-clinical testing (JvB)

Suggestions and Comments:

1. FDA will consider rhLF to be like a macroingredient, given its expected consumption level.

2. You may not be able to feed the dogs and rats the amounts you have indicated in the draft protocols. Feed at the highest level technically feasible.

3. Take in to account human experience, and adjust the protocols accordingly.

4. Suggest you do a dose range study (over 2 weeks) from which you establish the lowest dose that causes an effect.

5. It is critical that the characterized, especially as to foreign substances.

6. It is essential that you be able to explain the impact, if any, of the different glycosylation patterns.

7. Be able to identify qualitatively and quantitatively the nature of any impurities, including processing aids.

8. It is essential that you demonstrate that digestability is not adversely impacted on.

9. Purity needs to be adequately identified.

10. Safety here should focus on infants, not adults.

11. Keep in mind the special place infant formulas occupy in our culture. (Hint! Hint! Hint!)

12. If food additives are used in preparation of your product, for example as processing aids, be sure that they are used only as approved.

13. You should indicate whether infant exposure will be any different than that for adults.

14. We assume that you intend to use the same exposure level (of rhLF) in infant formulas as infants would be exposed to (i.e., hLF) in mother's milk.

15. As to what product should be pre-clinically tested, try and use as downstream a product as reasonable; such testing should cover any upstream product.

16. FDA suggests that we forward to them a list of the pre-clinical tests we intend to conduct for their review. Please also include chemistry information as it relates to safety.

17. Pharming needs to be able to demonstrate equivalence between hLF and rhLF.

18. Normal dairy practice procedures may be enough; Pharming will need to show that they are.

19. Pharming will need to demonstrate that it can control all critical aspects of the production of its rhLF product.

20. For the proper handling and disposition of animals once culled from the production herd see the CDER/CBER PTC document.

21. Pharming will need to show that its rhLF product is biologically equivalent to hLF after being pasteurized.

22. As to the stability of level of production of rhLF during the lactation period, it was indicated that production approximates 0.3 to 1.0 during the first week and 0.8-0.9 over the rest of the period.

23. As to propagation, it was indicated that Pharming only generates female transgenics via its transgenic bull. No markers are involved.

24. As to use of the milk byproducts, they should be able to be used, but may require use of labeling information and may not be able to be represented as or in standardized foods.

25. To date, FDA has received five GRAS notifications and has responded to three.

26. Use of a blue ribbon panel is not a substitute for publication but may support it. Such a panel should be composed of all assets necessary to derive GRASness.

27. FDA strongly encourages use of the GRAS notification process.

28. Approval of rhLF for use in infant formulas will occur (as expected) in two steps – first via GRAS notification, and then via approval by the infant formula group via a submission from an infant formula manufacturer).

29. The narrative portion of the GRAS notification needs to be thorough and to tell a story – the whole story.

30. If after receiving a GRAS notification CFSAN wants more information, it will ask for it.

31. For the new regulations pertinent to clinical testing of infant formulas see the July 9, 1996 FR document. (A copy of it is attached).

32. As to the need for an environmental assessment, please note the new categories that are now excluded. (A copy of this document is attached). Two of these, i.e., numbers 8 and 12, may be applicable to Pharming's product.

33. Note also the "extraordinary circumstances" exception to the subpart 32 (directly above) exemptions. (See subpart C of the attached pertinent EA document).

34. The GRAS notification should include copies of comparison data.

35. The proposed dog study should include dosing which begins just after birth and then for 90 days.

36. Use at least 4 animals per groups (see Red Book, Appendix II, page 45).

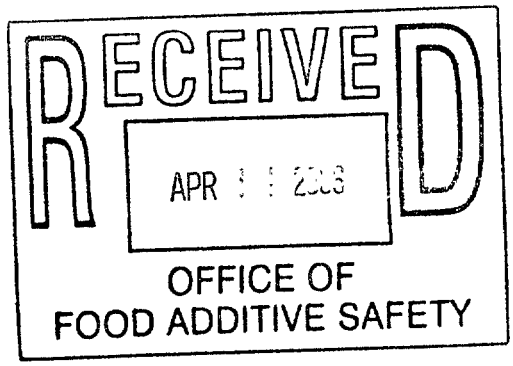
000001.023

Law Offices Of
Morin & Associates

Suite 500
388 Market Street
San Francisco, California 94111
Telephone: (415) 957-0101 e-mail: charleslmorin@earthlink.net Facsimile: (415) 957-5905

April 10, 2005

Antonia Mattia, PhD (HFS-255)
Director
Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied
Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835



Re: Notice of GRAS exemption for human lactoferrin derived from the milk of transgenic cows expressing a human gene encoding human lactoferrin
GRN 000189
CFSAN request for information

Dear Dr. Mattia:

Pursuant to Mr. Fasano's request, please find attached copies of three of the appendices referenced in the above-referenced GRAS Notification, i.e., appendix numbers 15, 16, and 18.

Thank you in advance for your and your colleagues' efforts on behalf of Pharming's notice.

Sincerely,

Charles L. Morin

Cc: Frans de Loos, PhD
Project Director (rhLF)
Pharming Group N.V.

COPY

A-15

TO: Charles L. Morin
Morin & Associates
388 Market Street, Suite 500
San Francisco, CA 94111

FROM: Ian C. Munro, PhD, FRCPath, MSc, (Panel Chair)
Professor
Department of Nutritional Sciences
Faculty of Medicine
University of Toronto
FitzGerald Building
150 College Street
Toronto, Ontario
CANADA M5S 3E2

Jeremy H. Brock, ScD, PhD, MSc
Senior Research Fellow
Department of Immunology
University of Glasgow
Glasgow
SCOTLAND G11 6NT

F. Jay Murray, PhD
President
Murray & Associates
5529 Perugia Circle
San Jose, CA 95138

Jorge A. Piedrahita, PhD, MSc
Professor of Genomics
Department of Molecular Biomedical Sciences
College of Veterinary Medicine
North Carolina State University
4700 Hillsborough Street
Raleigh, NC 27606

DATE: December, 20, 2005

RE: **Expert Panel Opinion Regarding the Generally Recognized as Safe (GRAS) Status of Pharming's hLF Product**

000110

Panel members were provided with a copy of the GRAS Notification and access to all information (including references and appendices) in support of the Notification. The Panel independently and collectively reviewed all information provided and met on December 20, 2005 to consider the information in detail. The Notification contained detailed information on the production of transgenic cattle from which Pharming's hLF is ultimately obtained. In addition, the manufacturing process by which Pharming's hLF is isolated and purified from the milk of transgenic cattle was well-documented in the Notification. The Panel concluded that the process of producing transgenic cattle and the hLF manufacturing process did not raise safety concerns. The Panel also reviewed the proposed specification for Pharming's hLF and confirmed that analytical data on three batches of Pharming's hLF conformed to the specification. The Panel critically evaluated the available data supporting the safety of Pharming's hLF and noted that except for minor differences in glycosylation, hLF derived from transgenic cattle was idential to native hLF. It was the opinion of the Panel that hLF derived from transgenic cattle was substantially equivalent to native hLF. The Panel also was provided with results of a 14-day range-finding toxicity study on Pharming's hLF in rats, a GLP 90-day study in rats, and three genotoxicity assays. The data from the 90-day rat study on Pharming's hLF indicated a NOAEL of 2,000 mg/kg body weight/day.

The Panel also reviewed data in the submission on the potential allergenicity/immunotoxicity of Pharming's hLF. The Panel was informed that since the product was derived from bovine sources, the manufacturer intended to label the product as containing milk ingredients. The Panel was statisfied that hLF derived from transgenic cattle did not present any increased risk of allergenicity or immunotoxicity over conventional milk-derived products. The Panel further noted that the manufacturer intended to use hLF in a variety of sports and functional foods in an amount not to exceed 100 mg of Pharming's hLF per serving of such foods. These uses result in an estimated total population mean and 90th percentile intake of 0.32 and 1.00 Pharming's LF/kg body weight/day, respectively. For users only the mean and 90th percentile intakes are estimated to be 1.91 and 3.95 mg/kg/day, respectively. After reviewing all the available information the Panel concluded that Pharming's hLF derived from transgenic cattle is safe for its intended uses. Thus, the Panel concluded:

Based on our independent collective critical and in-depth evaluation of the available pertinent, scientific (both published and unpublished) and other information, we conclude that Pharming's human lactoferrin – which is derived from the milk of transgenic dairy cattle carrying and expressing a human lactoferrin gene – is manufactured in accordance with good dairy practices and cGMPs, meets the relevant food grade specifications and, based primarily on scientific procedures, is Generally Recognized As Safe (i.e., GRAS) for use in food as described within the GRAS Notification.

Ian C. Munro, PhD, FRCPath, MSc, (Panel Chair) Date
University of Toronto
Toronto, ON CAN

Dec 20 2005

Jeremy H. Brock, ScD, PhD, MSc
University of Glasgow
Glasgow SCOTLAND

20/12/05

F. Jay Murray, PhD
Murray & Associates
San Jose, CA 95138

20 Dec 2005

Jorge A. Piedrahita, PhD, MSc
North Carolina State University
Raleigh, NC 27606

12/20/05

000112

TNO Quality of Life

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek/Netherlands Organisation for Applied Scientific Research

COPY

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Return address Postbus 360, 3700 AJ, Zeist, The Netherlands

Pharming Group N.V.
Attn. Dr F. de Loos
Archimedesweg 4
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TNetherlands

Toxicology and Applied Pharmacology
Location Zeist
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P.O. Box 360
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Date
December 19, 2005

Our reference
TAP-2005

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Direct fax
+31 30 694 49 86

Project number
31657.01 03.01

Subject
Expert Opinion

Statement concerning the bovine glycosylation of Pharming's hLF (chapter 3 "Allergenicity", paragraph "Glycosylation").

Statement:

Next to:
- the various observations addressed in the glycosylation paragraph (chapter 3) that the bovine glycosylation of Pharming's hLF is not likely to be a safety factor in respect to its immunogenicity (sensitizing potential) in comparison to natural hLF,
there is, moreover,
- no indication that the bovine glycosylation of Pharming's hLF will result in clinical symptoms of allergy due to cross reactivity with in particular serum IgE-antibodies against N-glycans (e.g. IgE antibodies to plant N-glycans of pollen allergic individuals).

This statement is supported by the following observations:

- It is clear that the glycosylation of Pharming's hLF is of a mammalian type and that, although Pharming's hLF and native hLF show differences in carbohydrate structures, they do not differ in the number and location of the glycosylation sites (see chapter 5, 6B).
- Concern in respect to the contribution of glycan epitopes to allergy is mainly based on research with plant and invertebrate glycoproteins
- Carbohydrate structures are not generally considered as allergens
- Despite strong in vitro reactivity of IgE antibodies against carbohydrate moieties (Cross-reacting Carbohydrate Determinants, CCD) can occur, it is clear that they have a poor biological activity (Van der Veen and van Ree, 1997; Aalberse et al, 2001)
- From a recombinant human lactoferrin produced in plants (rice) it was shown that, despite 1) two out of the three putative N-glycosylation sites of the natural hLF are glycosylated, 2) serum samples of pollen allergic individuals with IgE-reactivity to plant glycans showed significant binding to the

Nederlandse Organisatie voor
toegepast-natuurwetenschappelijk
onderzoek/Netherlands Organisation
for Applied Scientific Research

recombinant human lactoferrin isolated from rice, but negligible binding to the natural human lactoferrin purified from breast milk, 3) histamine release assays demonstrated that the IgE antibodies against plant N-glycans have a poor biological activity and are of no or limited clinical relevance. Thus even a recombinant lactoferrin produced from rice, which has much stronger differences in glycosylation than Pharming's hLF, is regarded as safe in respect to allergenicity (GRAS notification 162).

In conclusion it is considered very unlikely that the bovine glycosylation of Pharming's hLF will result in clinical symptoms of allergy from the consumption of foods containing Pharming's hLF.

References:

Van der Veen, M., and R. van Ree. Allergens, IgE, Mediators, Inflammatory Mechanisms; Poor Biological activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. J Allergy Clin Immunol, 100, 327-334,1997.

Aalberse, R et al. Cross-reactivity of IgE-antibodies to allergens, Allergy 56, 478-490, 2001.

Yours faithfully,

Dr A.H. Penninks
TNO Toxicology and Applied Pharmacology
Dept. Experimental Immunology

TNO Quality of Life

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek/Netherlands Organisation for Applied Scientific Research

COPY
A-18

TNO Report

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Allergenicity prediction of recombinant human lactoferrine using the database of the Food Allergy Research and Resource Program

pos.nr. 2.5 rhLF archive

name Eric Verhoef

owner FdL

location 6A 130 Dutch Space

Date	21 October, 2005
Authors	J.H.M. van Bilsen, Ph.D.
Sponsor	Pharming Group N.V. Archimidesweg 4 2333 CN Leiden
TNO project no.	31657/01 01.02
Number of pages	8
Number of tables	3

Contents

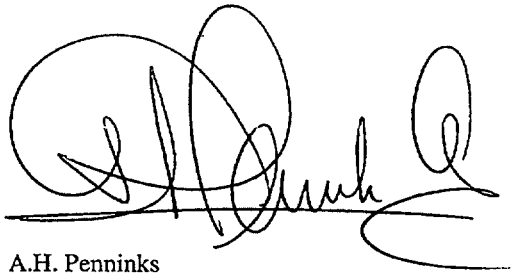
Statement

We, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Quality of Life, and that the study was carried out under our supervision.

J.H.M. van Bilsen, Ph.D

Date: 21 October '05

Project Leader
Business Unit Toxicology and Applied Pharmacology



A.H. Penninks

Date: 21 October 2005

Product manager
Business Unit Toxicology and Applied Pharmacology

1 Introduction

Potential allergenicity of recombinant proteins for consumption must be investigated before their introduction into the food chain. To asses whether the tested recombinant protein is considered to be an allergenic risk, a database search can be performed to reveal a level of homology with known allergens that suggests a potential for cross-reactivity.

To evaluate whether recombinant human lactoferrine (hLF) has the ability to induce an allergy, a BLAST search was performed against the Food Allergy Research and Resource Program (FARRP) Protein Allergen database, using the sequence of hLF.

The FARRP Protein Allergen Database contains a comprehensive list (1191 sequence entries) of unique proteins of known and putative allergens (food, environmental and contact) and gliadins that may cause celiac disease. The 1191 entries were identified by searching publicly available protein databases using the Entrez search and retrieval system, which is a compilation of a variety of databases including SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq. Search terms were the key words "allergen" and "celiac". A few additional entries were identified by searching Medline for allergens that have not been entered in a sequence database. The strength of the evidence regarding the allergenicity of proteins in the database varies greatly. Some entries are from publication of peer reviewed studies demonstrating clear clinical cause and effect for some individuals with a history of allergy to the source material, to those where the authors of an abbreviated note or a sequence database entry claim that protein is an allergen or binds IgE without published proof. However, proteins that are merely similar in sequence to an allergen (homologues) were not included in the database.

2 Materials and methods

In this search we compared the protein sequence of hLF to entries in the FARRP database.

To this end, a complete set of 80-amino acid length sequences (n = 613) derived from hLF, together spanning the entire protein, were prepared and individually compared with all the amino acid sequences of the entries in the FARRP database.

The FARRP database utilizes a sequence comparison routine, FASTA (Pearson and Lipman, 1988). This version of the FASTA search interface utilizes the FASTA3 (Pearson, 2000) algorithm to evaluate whether the hLF protein sequence is identical to, or homologous with known or putative allergens and gliadins in the database. Alignments with high identity scores may indicate a potential for allergenic cross-reactions. However, there is not sufficient scientific data to establish a simple scoring boundary (E-score or percent identity), beyond which cross-reactivity is certain, or below which cross-reactivity is not possible.

Based on historical data, cross-reactivity is not likely for proteins with less than 50% identity over the entire protein sequence, and is fairly common above 75% identity (Aalberse, 2000).

According to the FAO/WHO guidelines for allergenicity evaluation of foods derived from biotechnology, a query protein is potentially allergenic if it either has an identitiy of at least 6 contiguous amino acids or more than 35% sequence similarity over a window of 80 amino acids when compared with a known allergen.

References

- Aalberse, R.C. 2000. Structural biology of allergens. J. Allergy Clin. Immunol. 106:228-238.
- Pearson, W.R. and Lipman, D.J. 1988. Improved tools for biological sequencecomparison. Proc. Natl. Acad. Sci. USA 85:2440-2448.
- Pearson, W.R. 2000. Flexible sequence similarity searching with the FASTA3 program package. Methods Mol. Biol. 132:185-219.

3 Results

The amino acid sequence of recombinant hLF was provided by the sponsor (Table 1)

Table 1. hLF derived protein from cDNA sequence

```
  1  GRRRRSVQWC AVSQPEATKC FQWQRNMRRV RGPPVSCIKR DSPIQCIQAI
 51  AENRADAVTL DGGFIYEAGL APYKLRPVAA EVYGTERQPR THYYAVAVVK
101  KGGSFQLNEL QGLKSCHTGL RRTAGWNVPI GTLRPFLNWT GPPEPIEAAV
151  ARFFSASCVP GADKGQFPNL CRLCAGTGEN KCAFSSQEPY FSYSGAFKCL
201  RDGAGDVAFI RESTVFEDLS DEAERDEYEL LCPDNTRKPV DKFKDCHLAR
251  VPSHAVVARS VNGKEDAIWN LLRQAQEKFG KDKSPKFQLF GSPSGQKDLL
301  FKDSAIGFSR VPPRIDSGLY LGSGYFTAIQ NLRKSEEEVA ARRARVVWCA
351  VGEQELRKCN QWSGLSEGSV TCSSASTTED CIALVLKGEA DAMSLDGGYV
401  YTAGKCGLVP VLAENYKSQQ SSDPDPNCVD RPVEGYLAVA VVRRSDTSLT
451  WNSVKGKKSC HTAVDRTAGW NIPMGLLFNQ TGSCKFDEYF SQSCAPGSDP
501  RSNLCALCIG DEQGENKCVP NSNERYYGYT GAFRCLAENA GDVAFVKDVT
551  VLQNTDGNNN DAWAKDLKLA DFALLCLDGK RKPVTEARSC HLAMAPNHAV
601  VSRMDKVERL KQVLLHQQAK FGRNGSDCPD KFCLFQSETK NLLFNDNTEC
651  LARLHGKTTY EKYLGPQYVA GITNLKKCST SPLLEACEFL RK*
```

The FASTA program was used to compare the complete sequence of hLF to the FARRP Protein Allergen Database. The best scores are depicted in Table 1. The most significant scores are derived from ovotransferrin (chicken) and ovotransferrin precursor)

Table 2 FASTA search with complete sequence of hLF in FARRP database

NCBI link	Name	SW*	z-sc	E-value**
gi|1351295|sp|P02789|TRFE_CHICK	Ovotransferrin precursor chicken	2410	2801.7	3.6e-151
gi|757851|emb|CAA26040.1|	ovotransferrin (chicken)	2402	2792.4	1.2e-150
gi|170743|gb|AAB02788.1|	HMW glutenin subunit Ax2	81	88 8	4.6
gi|21743|emb|CAA43331.1|	high molecular weightgluteni	81	88.7	4.7
gi|18639|emb|CAA33217.1|	glycinin subunit G3	78	88.6	4.7
gi|4102959|gb|AAD01630.1|	ladder protein [Acanthocheil)	71	88.6	4.8
gi|501050|gb|AAA19162 1|	phospholipase A2 inhibitor	71	85 9	6 7
gi|30793446|dbj|BAC76688.1|	27K protein [Triticum aest)	71	85.8	6.8
gi|5381323|gb|AAD42943.1|AF091841_1	2S albumin precursor	69	85.5	7.1
gi|21632054|gb|AAK85129.1|	elongation factor [Juniperu)	68	83.4	9.3
gi|112558|pir||B37330	venom allergen III –red importe)	69	83.2	9.4
gi|118216|sp|P18153|D7_AEDAE	D7 protein precuror	71	83.0	9.7

*Smith-Waterman score

**Expectation value: The number of different alignements with scores equivalent to or better than S that are expected to occur in a database search by chance. The lower the E value, the more significant the score.

The 80-mer sliding window search revealed that hLF shares significant homology with two allergens desribed in the FARRP database: ovotransferrin precursor (chicken) and ovotransferrin (chicken)(Table 2). The best percentage identical amino acids (%ID) in 80-mer sequence in both hits was about 67%. All 613 overlapping 80-mers from hLF showed >35% homology with ovotransferrin (precursor). The percentage identical aminoacids in the full alignment (the whole protein, not just 80-mer sequence), was in both hits 52%.

Table 3 80-mer sliding window search results

Hits	Best %ID	# hits >35%	Full alignment			NCBI links
			E-value	%ID	length	
Ovotransferrin precursor (chicken)	67.55%	613 of 613	3.6e-151	52.2%	693	gi|1351295
Ovotransferrin (chicken)	66.7%	613 of 613	1.2e-150	51.9%	693	gi|757851

4 Conclusion

From this study, it can be concluded that cross-reactivity between recombinant human lactoferrin and ovotransferrin (precursor) has to be considered as an allergenic risk.

Unfortunately, it is currently not possible to define a similarity threshold in allergenicity prediction that can truly discriminate between immunologically cross-reactive and non-crossreactive proteins. In most cases experimental studies will be needed to confirm that two sequence similar proteins may cause allergic cross-reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: February 1, 2006

TIME: 3:00 PM

PHONE NUMBER: 415-957-0101 (initial contact)

PARTICIPANTS:

FDA
Jeremiah Fasano HFS-255

External
Charles Morin Morin & Associates

SUBJECT: Basis of GRAS Claim for GRN 189

Mr. Morin submitted GRN 000189 to FDA on behalf of Pharming, N.V. The notice claims that recombinant human lactoferrin produced in bovine milk is generally recognized as safe for use in a variety of foods, on the basis of both scientific procedures and common use in food prior to 1958.

I contacted Mr. Morin to discuss this dual basis for Pharming's GRAS claim. He returned my call shortly thereafter. I explained that we expected GRAS notices to include a GRAS determination based either on scientific procedures or on common use in food, in accordance with 21 CFR 170.30. I requested that Mr. Morin provide a short written statement clarifying the basis of the notifier's GRAS determination. I noted that, as a practical matter, we would expect to evaluate the notice on the basis of scientific procedures, since we were not aware of any evidence that recombinant human lactoferrin produced in bovine milk had been in common use in food prior to 1958.

Mr. Morin agreed to consider our request.

Jeremiah Fasano

R/D:HFS-255:JMFasano:02/21/06
F/T:HFS-255:JMFasano:05/25/10

FILE COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
College Park, MD 20740

February 14, 2006

Charles L. Morin
Morin & Associates
Suite 500
388 Market Street
San Francisco, CA 94111

Re: GRAS Notice No. GRN 000189

Dear Mr. Morin:

The Food and Drug Administration (FDA) has received the notice, dated December 29, 2005, that you submitted on behalf of Pharming Group N.V., in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)). FDA received this notice on January 3, 2006, filed it on January 12, 2006, and designated it as GRN No. 000189.

The subject of the notice is human lactoferrin derived from the milk of transgenic cows expressing a human gene encoding lactoferrin. On February 8, 2006, you clarified the basis for the GRAS determination. The notice informs FDA of the view of Pharming Group N.V. that their lactoferrin is GRAS, through scientific procedures, for use as an ingredient in sports and functional foods at a level of 100 milligrams per serving.

In accordance with proposed 21 CFR 170.36(f), a copy of the information in the notice that conforms to the information described in proposed 21 CFR 170.36(c)(1) is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html). If you have any questions about the notice, contact me at 301-436-1173 or jeremiah.fasano@fda.hhs.gov.

Sincerely yours,

/s/

Jeremiah Fasano
Division of Biotechnology and
GRAS Notice Review
Center for Food Safety
and Applied Nutrition

Hard copy cc: **GRN 000189** (1 copy)
Filename: Final GRN 189 Acknowledgement Letter
R/D:HFS-255:JMFasano:01/26/06
Init:HFS-255:JGGlew:02/13/06
Comment:HFS-255:PGaynor:02/14/06
F/T:HFS-255:JMFasano:02/14/06

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

Date: April 6, 2006

Time: 3:00 PM

Number: 415-957-0101

Participants:

FDA
Jeremiah Fasano HFS-255

External
Charles L. Morin Morin & Associates

Subject: Request for GRN 189 Appendices, Notice of Questions

Pharming Group, N.V. (Pharming) submitted a notice on December 29 informing FDA that recombinant human lactoferrin produced in the milk of transgenic cows is generally recognized as safe (GRAS) for use in sports and functional foods at a level of 100 milligrams (mg) per serving.

Mr. Morin, Pharming's agent, contacted me for an update on the status of the notice. I explained that our review was in progress, and that we would appreciate the provision of a number of appendices to the notice that were listed but not included with the original submission, namely appendix 15 (Pharming's GRAS panel report), appendix 16 (TNO report concerning allergenicity), and appendix 18 (Expert opinion of Dr. A. H. Penniks concerning potential allergenicity of Pharming's LF). Mr. Morin had previously offered to provide any appendices desired.

I also advised Mr. Morin that we had tentatively identified a number of questions regarding the notice. While we were still developing clear and concise statements of these questions, we considered it appropriate to apprise Pharming of this development.

Mr. Morin asked what our concerns were. I said that the two foremost in our minds at the current time were:

- the potential for adverse effects of increased human lactoferrin exposure in adults, and
- the potential for breakdown of self-tolerance to endogenous human lactoferrin.

FILE COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

I stated that once we had articulated our primary concerns to our own satisfaction, we would be willing to discuss the relevant scientific issues with Pharming if the firm was interested in doing so. Mr. Morin expressed his preference that the request be received in writing, and said that a face-to-face meeting with the company's scientists might be the most effective way of discussing scientific issues associated with the notice.

Mr. Morin agreed to pass on the requested appendices, and I agreed to contact Mr. Morin when we were prepared to communicate our concerns more fully to Pharming.

Jeremiah Fasano

R/D: HFS-255:JMFasano:04/06/2006
F/T: HFS-255:JMFasano:05/26/2010

Fasano, Jeremiah

From: Fasano, Jeremiah
Sent: Wednesday, May 17, 2006 10:18 AM
To: 'charleslmorin@earthlink.net'
Subject: Offer to meet for discussion on GRN 189

Mr. Morin-

We have completed a preliminary evaluation of GRN 000189 for recombinant human lactoferrin expressed in bovine milk. As discussed previously, we are providing some of our concerns in writing for your consideration. This should not be considered an exhaustive list, but does represent what we consider significant questions that we have right now.

Lactoferrin is a known biological response modifier of the immune system. The action of various parts of the immune system can be both beneficial and harmful, depending on the abundance and activation of the effector cell or protein relative to other immune system components, as well as the duration of the specific immune activity. While beneficial effects bear no weight in a GRAS determination, we are concerned about potential adverse effects of lactoferrin consumption. These adverse effects would not necessarily appear in every susceptible individual, and would probably not become apparent in short term human or animal studies.

- Lactoferrin has been shown to enhance Type 1 T helper (Th1) cell activity, as well as the release of specific cytokines in the gut and systemically following oral administration. We are concerned about lactoferrin's ability, through effects on Th1 cells, to potentially exacerbate pro-inflammatory responses by this arm of the adaptive immune system. Chronic pro-inflammatory Th1-mediated immune responses might result in the promotion of autoimmune or other inflammatory disorders, in the gut or elsewhere, in individuals predisposed to such disorders.
- Pharming's lactoferrin is distinct from the endogenous lactoferrin of individual consumers with respect to
    - expected differences between the amino acid sequence of the exogenous lactoferrin and the polymorphic endogenous lactoferrin alleles present in the general population, and
    - the modification of some species of the exogenous lactoferrin with oligomannose glycans not found on endogenous forms.

  Even small structural or biological differences between the native and modified form of a particular protein may have a significant impact on that protein's recognition by the immune system and subsequent response. We are concerned that Pharming's exogenous human lactoferrin may evoke a nonallergic immune response in susceptible individuals that disrupts previous tolerance to endogenous lactoferrin through determinant spreading from alloepitopes, the potential for enhanced pro-inflammatory Th1 responses mentioned above, and increased uptake by antigen-presenting cells via the mannose receptor.

Given these concerns, we have questions about the evidence and information presented in the notice.

- The notice states that lactoferrin is known for its immunomodulatory properties. However, the preclinical studies presented in the notice do not address the immunomodulatory activities of lactoferrin. What preclinical evidence supports the safety of exogenous lactoferrin for its intended use given its activity as a biological response modifier of the immune system?
- The primate and human studies of oral lactoferrin administration cited in the notice are in small populations for relatively short periods of time. Most of the studies with recombinant human lactoferrin focus on efficacy rather than safety, and many of the human studies involve subjects with pre-existing medical conditions. Where safety endpoints are included, they do not appear relevant to the effects of lactoferrin as a biological response modifier of the immune system. Is there clinical evidence that supports the immunological safety of long-term exogenous lactoferrin administration at the proposed use level in the general population?
- The notice provides an acceptable daily intake (ADI) based on the maximal consumption of lactoferrin in human

milk by infants. The infant immune system and gut are different from that of the adult, for example in the infant bias towards Th2 responses relative to Th1. Given this, what evidence supports the use of exposure data derived from infants in setting an ADI for adults that takes into account lactoferrin's activity as a biological response modifier of the immune system?

- The notice provides an assessment of the potential allergenicity of Pharming's lactoferrin and states that there is no evidence to date that anti-lactoferrin antibodies are associated with autoimmune pathology. Other than this statement, the notice does not address the potential for adverse non-allergic responses to Pharming's lactoferrin by the adaptive immune system as described above. To what extent has Pharming evaluated this risk, and what evidence was used in the evaluation?

While we have tried to state the essence of our concerns here, we believe that we could most effectively convey the complexity, significance, and relationships of each concern to the others in a verbal discussion. Such a discussion would provide you with an opportunity to clarify any points that were unclear and obtain as much detail as needed in preparing your response. We would be willing to have a second discussion with you, potentially including members of your GRAS panel, after you have had time to consider the issues we have raised,. In our estimation, these issues are sufficiently complex that we do not expect that you will necessarily be prepared to address them all at our first meeting.

We would be available to meet by phone or in person after May 31st, 2006 to explain our concerns. If this is agreeable, please provide us with a few dates (and time of day) that would be best for you and we will confirm if the appropriate FDA staff are available.

Sincerely,

Jeremiah Fasano

_____

Jeremiah Fasano, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

Note Transition to New Email Address: jeremiah.fasano@fda.hhs.gov

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_____

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Fasano, Jeremiah

From: Charles Morin [charleslmorin@earthlink.net]
Sent: Thursday, June 01, 2006 4:50 PM
To: Fasano, Jeremiah
Cc: Frans de Loos
Subject: Re: FDA-Pharming Discussion - June 29th @ 10 am EST is open

Dear Dr. Fasano,

Thank you for your email concerning reservation of June 29th at 10:00 a.m. EST for our meeting concerning hLF for food use. I understand that the time and date are a go. As of this date, they are also a go for Pharming. However, as indicated to you earlier, we are adding additional expertise (in immunology) to our hLF project team to respond to CFSAN's concerns and hope to be fully prepared so as to be able to proceed on June 29th. If we cannot be fully prepared by that date, then we should know by June 12th and I will let you know.

In any case, I will communicate with you in a week or so as to final details.

Thanks for your help!

Charles L. Morin
Morin & Associates
388 Market Street, Suite 500
San Francisco, CA 94111
US

Phone: (415) 957-0101
Fax: (415) 957-5905

Email: charleslmorin@earthlink.net

> ----- Original Message -----
> **From:** Fasano, Jeremiah
> **To:** 'charleslmorin@earthlink.net'
> **Sent:** Tuesday, May 30, 2006 9:30 AM
> **Subject:** FDA-Pharming Discussion - June 29th @ 10 am EST is open
>
> Mr. Morin-
>
> The 10 am slot on June 29th works for us - I've reserved it for the necessary FDA personnel.
>
> Regards-
>
> -Jeremiah Fasano
>
> _____
> Jeremiah Fasano, Ph.D.
> Consumer Safety Officer
> Division of Biotechnology and GRAS Notice Review
> Office of Food Additive Safety

Center for Food Safety and Applied Nutrition
Food and Drug Administration

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Fasano, Jeremiah

From: Charles Morin [charleslmorin@earthlink.net]
Sent: Monday, June 12, 2006 1:48 PM
To: Fasano, Jeremiah
Subject: CFSAN Meeting with Pharming
Follow Up Flag: Follow up
Flag Status: Completed

Dear Dr. Fasano,

This communication makes two requests. First and with regard to the meeting date (currently set for June 29th at 10:00am), as anticipated (and as mentioned to you in an email on June 1st) it is taking longer to arrange for additional experts and prepare for the meeting than Pharming had hoped. Consequently, so as not to waste CFSAN time and resources, Pharming respectfully requests that the meeting date be changed to July 13th (if possible). Pharming apologizes for any inconvenience this request may cause.

Second, in prior communications with you, you had indicated that (in addition to two major concerns) CFSAN also had some minor concerns/questions. If such questions currently exist, please forward a copy of them to us so that Pharming can proceed to respond (in writing) to all outstanding questions.

Thank you for your continuing efforts.

Best regards.

Charles L. Morin
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San Francisco, CA 94111
US

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Fax: (415) 957-5905

Email: charleslmorin@earthlink.net

Agennix Incorporated

OU

June 27, 2006

Laura M. Tarantino, Ph.D. (HFS-200)
Director, Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Room 3044
University Station
4300 River Road
College Park, Maryland 20740

Re: Safety Concerns Raised by Recombinant Human Lactoferrin from Transgenic Cows (GRN No. 000189 Submitted by Pharming Group N.V.)

Dear Dr. Tarantino:

This letter is to address the claim of Pharming Group N.V. ("Pharming") in GRN No. 000189, submitted to FDA's Center for Food Safety and Applied Nutrition (CFSAN), that recombinant human lactoferrin (rhLF) from transgenic cows is generally recognized as safe (GRAS) for use in sports and functional foods and drinks. As you know, Agennix is a biopharmaceutical company focused on developing protein-based drugs for the treatment of cancer and diabetic ulcers. We have significant experience with rhLF from a fermentation process that conforms to current good manufacturing process (cGMP) requirements for drugs, and have been conducting clinical trials with oral rhLF under Investigational New Drug Applications (INDs) filed with the FDA since 1996. Agennix recently completed blinded, placebo-controlled Phase II clinical trials with rhLF that met their primary efficacy endpoints in indications including non-small cell lung cancer and diabetic foot ulcers.

We have carefully reviewed GRN 000189 and consulted with leading experts qualified by scientific training and experience to assess the safety of transgenic cow-produced rhLF for the proposed uses. As explained more fully below and in the attached scientific assessments, serious concerns and unanswered questions preclude any determination that transgenic cow-produced rhLF is GRAS. Indeed, opinions of qualified experts confirm that rhLF is a potent and complex bioactive molecule for which extensive clinical investigations of appropriate size and duration—far beyond those described in GRN 000189—are warranted to establish safety. Accordingly, we respectfully ask that FDA conclude that this notification does not provide a basis for a GRAS determination. The scientific assessments and other supporting materials on which this request is based are provided in **Appendix Volumes 1** and **2**. 1/

1/ **Appendix Volume 1** provides a detailed assessment of safety concerns raised by the claimed GRAS status of rhLF from transgenic cows. Volume 1 also contains letters from Dr. Simon Roger, Dr. Irma van Die and Dr. Eugene Weinberg commenting on GRN No. 000189, as well as a copy of Pharming's web page that

I. THE GRAS STANDARD

As you are aware, a substance added to food is a "food additive" for which FDA pre-market approval is required unless the substance is GRAS or qualifies for another statutory exemption. The intended use of a substance is GRAS if it is—

> generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use . . . 2/

As the statutory language suggests, a GRAS determination may be based either on "scientific procedures" or common use in food prior to 1958. A GRAS determination based on scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. 3/

Based on the statute, FDA has advised that a GRAS determination requires three elements, all of which must be present:

1. Evidence that a substance is safe for its intended use;
2. A basis for concluding that such evidence of safety is generally available; and
3. A basis for concluding that such evidence of safety is the subject of scientific consensus among qualified scientific experts.

FDA refers to the first element as "technical evidence of safety"; the second and third criteria collectively constitute the "common knowledge" element of the GRAS standard.

Technical evidence of safety requires a showing that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use." 4/ This is frequently paraphrased as demonstrating that there is a "reasonable certainty of no harm." The second element, general availability, requires publication of key data or information in peer-reviewed scientific journals, general reference materials, textbooks, or other appropriate

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suggests pharmaceutically relevant uses for transgenic cow-produced rhLF. **Appendix Volume 2** contains copies of the CV's for the experts contributing to the scientific assessment.

2/ FFDCA § 201(s).

3/ 21 C.F.R. § 170.30(b).

4/ 21 C.F.R. § 170.3(i); 62 Fed. Reg. 18937, 18948 (Apr. 17, 1997).

sources. 5/ The third element, expert consensus, may be demonstrated by the scientific literature, documentation of the opinion of an expert panel, or the pertinent opinion of an authoritative body, such as the National Academy of Sciences (NAS), among other references. 6/ Expert consensus does not require unanimity; however, the existence of a "severe conflict' among experts will preclude a GRAS determination.7/

II. APPLICATION OF THE GRAS STANDARD TO RECOMBINANT HUMAN LACTOFERRIN FROM TRANSGENIC COWS

Pharming fails on all three counts of the GRAS standard. Specifically, (1) GRN 000189 fails to establish that transgenic cow-produced rhLF presents a reasonable certainty of no harm under the intended conditions of use; (2) Pharming fails to cite published studies that credibly support the safety of rhLF from transgenic cows; and (3) a severe conflict exists between scientists consulted by Pharming and numerous highly qualified scientists with specific expertise in lactoferrin, the toxicological significance of glycosylation, immunogenicity of recombinant proteins and other subjects pertinent to an evaluation of GRN 000189.

Technical Evidence of Safety

As the attached scientific assessments state, rhLF is a complex molecule with potent biological activity for which a rigorous safety assessment is warranted. In a drug context, extensive clinical trials and post-market surveillance are needed to adequately assess the safety of a bioactive substance such as rhLF because adverse reactions may not be evident absent extended study. In a food context involving comparable conditions of use, an even greater assurance of safety is essential due to the general availability of the product and absence of direct medical supervision.

In GRN 00189, Pharming asserts that rhLF from transgenic cows is GRAS for use in sports and functional foods and drinks at levels not to exceed 100 mg/serving. The assertion that rhLF from transgenic cows is GRAS is based on (i) claimed substantial equivalence between rhLF from transgenic cows, native human lactoferrin and rhLF from a cGMP fermentation process, and (ii) the opinion of an expert panel that rhLF from transgenic cows presents no immunotoxicity or other safety concerns. As described in the attached assessments, however, Pharming's analysis fails to adequately address numerous important safety issues, including the following:

- Differences of potential toxicological significance between transgenic cow-produced rhLF and other types of lactoferrin, including native human lactoferrin and rhLF from a cGMP fermentation process.

  The glycosylation pattern that is unique to transgenic cow-produced rhLF is of particular concern. Glycosylation can have a significant impact on the function and safety of proteins, including impacts on pharmacokinetics, immunogenicity and

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5/ 21 C.F.R. § 170.30(b); 62 Fed. Reg. at 18942-43.

6/ 62 Fed. Reg. at 18940-43.

7/ *See* 62 Fed. Reg. at 18939.

allergic potential, stability, resistance to thermal or enzymatic degradation and specific activity.

- The absence of relevant studies sufficient to assess the safety of transgenic cow-produced rhLF.

  Only one clinical trial with transgenic cow-produced rhLF is referenced in GRN 000189—an unpublished study with six subjects who consumed two acute 52 mg doses of transgenic cow-produced rhLF over a 24-hour period. Other clinical trials cited by Pharming involved rhLF from a cGMP fermentation process, which differs significantly from transgenic cow-produced rhLF.

- Immunotoxicity concerns.

  The GRAS submission does not adequately address (i) immunotoxicity risks posed by major differences between the composition of transgenic cow-produced rhLF and native lactoferrin, and (ii) the possibility that administration of recombinant *human* lactoferrin with bovine glycosylation, along with up to 10% contaminating foreign proteins and carbohydrates, may induce recognition of rhLF as a foreign protein with resulting cross-reactivity to an individual's native lactoferrin.

- Potential induction or exacerbation of autoimmune disease and generation of anti-lactoferrin antibodies.

  Published literature cited in the GRAS Notice indicates that (i) lactoferrin is a potent immunostimulatory molecule known to induce a systemic immune response in both animals and humans, (ii) anti-lactoferrin antibodies are associated with a host of serious human autoimmune diseases, and (iii) there is animal evidence suggesting that lactoferrin might indeed exacerbate autoimmune disease. These concerns raised by these literature references are not adequately addressed.

- Other risks associated with extended dosing with any rhLF.

  These include risks of iron-overload in susceptible individuals, iron delivery to iron-constrained pathogens, iron delivery to tumors, systemic amyloidosis caused by lactoferrin variants, induced changes to immune function, induction of antibiotic resistance and viral activation.

- An intended daily dose far in excess of exposure to native LF (which is not equivalent to rhLF from transgenic cows in any event).

  The intended daily dose for transgenic-produced rhLF cited by Pharming is up to a hundred times higher than that resulting from the levels of native LF claimed to be present in saliva.

- Concerns relating to the manufacturing of rhLF in transgenic cows.

> Highly controlled systems for production, purification and characterization are required to ensure the integrity and safety of complex recombinant proteins. The production of rhLF in transgenic cows is not sufficiently controlled to allow for a consistent and pure final product that is free from potentially harmful impurities, degradation products, and contaminants. The genetic stability of the host expression system (transgenic cows) has not been established.

Based on these and other concerns, the experts consulted by Agennix found that GRN 000189 raised substantial issues and unanswered questions that preclude a finding of safety for the intended conditions of use. Indeed, these experts believe that rigorous testing, including clinical trials of appropriate size and duration, would be required before transgenic cow-produced rhLF could be considered safe for addition to the food supply.

This last point is particularly important. Although many GRAS determinations have been made, and should continue to be made, based on an established battery of animal toxicology studies and safety factors that establish safe conditions of use, there are some compounds that must necessarily be subject to rigorous clinical testing in order to demonstrate a reasonable certainty of no harm, as required by the statute. This especially includes molecules such as recombinant human proteins with potent biological activities and toxicities that may not be accurately reflected in animal models, and immunomodulatory molecules whose full spectrum of activity can only be observed following extended administration and surveillance in humans. We believe that rhLF, with its potent biological effects demonstrated in Phase II clinical trials in cancer and diabetic ulcers, is one such substance.

Past examples of compounds that also warranted significant clinical testing data include artificial sweeteners and fat substitutes, so FDA has ample precedent to require such significant clinical testing. Even though these other examples were in the context of a food additive petition, the legal standard for showing technical evidence of safety, as noted on page 2 above, is exactly the same for food additives and GRAS substances. We believe, as a matter of scientific evidence, that extensive clinical trials are needed for rhLF in order to adequately investigate the critical question of how humans will react to this compound under widespread conditions of long term use. The scant clinical evidence referenced in GRN 000189 does not even scratch the surface of what is needed to meet the statutory standard and protect public health.

The concerns stated above are serious and should preclude GRAS status for any sports or functional food or drink application of rhLF from transgenic cows. These concerns are even more pressing in light of the perceived therapeutic uses for which transgenic cow-produced rhLF might be consumed. Public statements of Pharming indicate that rhLF from transgenic cows will be expressly or implicitly promoted for therapeutic uses that are functionally indistinguishable from proposed drug uses undergoing critical evaluation by FDA's Center for Drug Evaluation and Research (CDER). Pharming's website states clearly that "human lactoferrin (hLF) is a natural protein that helps to fight and prevent infections and excessive inflammations and strengthens the defense system of the human body . . . and has been shown to fight bacteria that cause infections of the eye and lungs . . . which makes it a good candidate for a number of product applications. Since the protein has the ability to bind iron, is a natural anti-bacterial, anti-fungal and anti-viral, is an antioxidant and also has immunomodulatory properties, large groups of people might benefit from orally administered lactoferrin." These statements suggest

pharmaceutically relevant activities. A copy of Pharming's web page containing these statements is provided in **Appendix Volume 1**.

We recognize that the regulatory classification of a product as a "drug" due to an intent to treat, prevent, cure, or mitigate disease does not typically factor into an assessment of whether the product meets the GRAS standard (i.e., assessment of whether a product is a "drug" under section 201(g) of the Federal Food, Drug, and Cosmetic Act is not usually considered as part of a GRAS assessment conducted under section 201(s)). By law, however, the safety of a substance that will be added to food is to be assessed in light of its intended use, taking into account its "probable consumption." Based on Pharming's apparent intent to market rhLF from transgenic cows for its pharmaceutical or pharmacological benefits, it is entirely appropriate for CFSAN to consider the unique types of harms that may result from individuals consuming rhLF for perceived therapeutic effects in potentially unlimited doses for unlimited periods of time. 8/ Indeed, the law requires consideration of these factors, as GRAS for a food compound must be shown "under the conditions of its intended use." Moreover, a GRAS determination for rhLF should require a safety assessment even more rigorous than that required by CDER, to account for general availability of the substance without prescription or ongoing medical supervision.

The Common Knowledge Element—Publication

Pharming cites no published studies that support the safety of its rhLF from transgenic cows. All of the studies cited by Pharming are either unpublished or are not applicable to rhLF from transgenic cows, including studies conducted with rhLF from a cGMP fermentation process. Further, Pharming's assertions concerning the substantial equivalence of rhLF from transgenic cows to native human lactoferrin or rhLF from a cGMP fermentation process are unfounded in light of published information to the contrary concerning such biologically important features as glycosylation and specific contaminants. Accordingly, GRN 000189 fails to satisfy the second element of the GRAS standard—demonstration that key studies and information supporting the GRAS determination are generally available to qualified experts.

The Common Knowledge Element—Severe Conflict Among Qualified Experts

Finally, Pharming clearly fails to satisfy the third element of the GRAS standard—demonstration that the safety of the proposed use of rhLF from transgenic cows is the subject of expert consensus. Consensus is lacking because more than a dozen experts qualified by scientific training and expertise to evaluate the safety of transgenic cow-produced rhLF do not consider it to be safe or generally recognized as safe for use in food.

As discussed in FDA's GRAS proposal and the pertinent case law, a proponent of a GRAS claim bears the burden of establishing expert consensus (i.e., that experts "generally" consider the ingredient at issue to be safe). The courts and FDA have interpreted this to mean that, although a mere divergence of views will not necessarily preclude GRAS status, as "even properly

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8/ These concerns are exacerbated by the possibility that rhLF may be used disproportionately by susceptible groups including immunocompromised individuals, those with systemic infections and infants.

conducted studies may produce disagreement," 9/ a "severe conflict" of expert opinion will prevent a finding of general recognition. 10/

There is no bright-line test for identifying what constitutes a "severe conflict," but courts have found a "severe conflict" to exist after evaluating the merits of each situation. In one case, even where the proponent of a GRAS claim presented the testimony of seven experts supportive of GRAS status, general recognition was found to be lacking in light of persuasive opposing views offered by "several" government experts. 11/ In another case, "sharply divided testimony" was found to present a severe conflict of opinion. 12/ Expert testimony critical of general recognition in that case suggested that the studies presented did not prove safety or efficacy and that the studies were not "well controlled" within the meaning of FDA's regulations. 13/ Although these and other cases addressing expert consensus involve drug products, the expert consensus standard is the same for both food products and drugs. 14/ For both food products and drugs, determining whether there is a meaningful and substantive dispute is key.

Expert credentials are also important when assessing whether expert consensus exists. In one case evaluating the status of a drug for the treatment of various vaginal infections, the court gave great weight to the opinions of several chairmen of leading Obstetrics and Gynecology departments. The court stated that "it cannot be denied that the affidavits of five of the leading doctors in the field which deny general recognition creates more than a 'mere' conflict . . . [i]t is inconceivable that a drug such as this could be considered generally recognized in the face of such learned non-recognition." 15/

9/ *See, e.g., United States v. Articles of Food and Drug . . . "Coli-Trol 80"*, 518 F.2d 743, 746 (5th Cir. 1975).

10/ 62 Fed. Reg. at 18939 (citing *United States v. Articles of Drug . . . 5,906 boxes*, 745 F.2d 105, 119 n. 22 (1st Cir. 1984); *United States v. An Article of Drug . . . 4,680 Pails*, 725 F.2d 976, 990 (5th Cir. 1984); *Premo Pharmaceutical Lab. v. United States*, 629 F.2d 795, 803 (2d Cir. 1980); *Coli-Trol 80*, 518 F.2d at 746 (5th Cir. 1975); *United States v. Articles of Drug . . . Promise Toothpaste*, 624 F.Supp. 776, 782 (N.D. Ill. 1985), *aff'd* 826 F.2d 564 (7th Cir. 1987)).

11/ *See, e.g., Pails*, 725 F.2d at 990 (holding that presentation by the United States of the views of "several experts" that a drug was not generally recognized as effective showed a "severe conflict" in the expert testimony and precluded general recognition).

12/ *United States v. An Article of Drug . . . X-Otag Plus Tablets*, 441 F.Supp. 105, 113-114 (D. Colo. 1977).

13/ *Id.* at 113.

14/ *See, e.g.*, 62 Fed. Reg. at 18938-18939 (citing drug and food precedent in discussion of meaning of GRAS standard under section 201(s) of the FFDCA).

15/ *United States v. An Article of Drug . . . "Mykocert"*, 345 F.Supp. 571 (N.D. Ill. 1972).

Finally, the general quality of the evidence on which expert consensus is suggested to be based is also relevant. A lack of general recognition was found in one case where expert witnesses knew of no studies supporting a finding of general recognition and the manufacturer responded with "irrelevant or incomplete studies, expert opinions based on these tests or clinical experience (as opposed to clinical studies), and the interested opinions of . . . salesmen." 16/ The court stated that general recognition is precluded where there is a "lack of the proper reputation for ... safety of the food additive among appropriate experts" or "what reputation there is, is not based on adequate studies." 17/ Accordingly, even expert opinions lack persuasive value where the underlying evidence is weak or incomplete.

Agennix, the clear worldwide leader in research, development and production of rhLF, has consulted leading international experts on lactoferrin, glycosylation, immunogenicity, biosimilars and related subjects relevant to the safety of rhLF from transgenic cows. These experts include, among others, a pioneer in the field of lactoferrin research, a founder and director of a major center for Medical Glycobiology, and an author of more than 200 papers in peer-reviewed journals addressing biotechnology-derived therapeutic proteins (with a recent emphasis on biosimilars and the immunogenicity of therapeutic proteins). These highly qualified experts have expressed serious concern regarding the safety of rhLF from transgenic cows, demonstrating a "severe conflict" of expert opinion. Although the opinions of one or two of these experts would be compelling, the opinions of more than a dozen experts concurring in the attached scientific assessments unambiguously demonstrates a "severe conflict" that precludes GRAS status.

In summary, the clear lack of scientific consensus that rhLF is GRAS is evidenced by the published literature raising legitimate safety questions and by the views of scientific experts whose opinions are expressed in the attached scientific assessments. That so many, and such highly qualified, experts have expressed serious concern about the proposed use qualifies as a "severe conflict" of expert opinion and precludes GRAS status for rhLF from transgenic cows.

III. CONCLUSION

After carefully reviewing GRN 000189 and consulting with leading experts qualified to judge the complex safety issues raised by rhLF from transgenic cows, it is our view that Pharming presents insufficient data and information to reach any credible conclusion about the safety of transgenic cow-produced rhLF in humans. In view of the documented biologic activity of rhLF and its ability to induce clinically significant changes in immune function, we believe that transgenic cow-produced rhLF has not been shown to be safe for use in food products under the anticipated conditions of use, and that there is a severe conflict among qualified experts regarding its safety. Accordingly, we ask that FDA respond to this GRAS Notice by concluding that an adequate basis for a GRAS determination has not been provided.

16/ *"Coli-Trol 80"*, 518 F.2d at 747.

17/ *Id.* at 746.

Agennix appreciates CFSAN's consideration of this important information as Pharming's GRAS exemption claim for rhLF from transgenic cows is considered. Please do not hesitate to contact us if there are any questions or if additional information would be useful.

Sincerely,

Rick Barsky
Chief Executive Officer

Cc: Robert Merker, Ph.D. (HFS-255)
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review

Scientific Assessment

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PURIFIED FROM THE MILK OF TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

We have been asked to review data and information presented in GRAS Notice No. 000189 concerning the safety of recombinant human lactoferrin (rhLF) produced in transgenic cows. This Notice asserts that transgenic cow-produced rhLF is generally recognized as safe (GRAS) within the meaning of the Federal Food, Drug, and Cosmetic Act for use in sports and functional foods at levels not to exceed 100 mg/serving. The assertion that transgenic cow-produced rhLF is GRAS is based on (i) claimed substantial equivalence between transgenic cow-produced rhLF, other forms of recombinantly produced human lactoferrins and native human lactoferrin derived from human milk, and (ii) opinions of an expert panel that transgenic cow-produced rhLF presents no immunotoxicity or other safety concerns.

RhLF is a complex molecule with potent biological activity for which a rigorous safety assessment is warranted. We are aware of Phase II clinical trials in which rhLF from a cGMP fermentation process was found to meet efficacy endpoints for indications such as non-small cell lung cancer. The drug demonstrated promising anti-cancer activity although adverse events were observed. Considering the anti-cancer activity observed with rhLF, and its early promise as a novel and effective anti-cancer drug, extensive clinical trials will be required to evaluate its safety as a drug prior to its being made available to patients under a doctor's prescription. Post-market surveillance will also be required to adequately assess its safety following administration to a larger patient population. RhLF's use as a cancer drug involves its use under medical supervision by patients who have few alternatives available and who will receive anti-cancer therapy for a limited period of time. In contrast, allowing rhLF to be marketed in a food context will enable its consumption by a much larger number of people for unlimited periods of time and without medical supervision or post-market surveillance. Thus, an even greater assurance of safety is required than would be needed prior to rhLF's approval as an anti-cancer drug.

The conditions of use for transgenic cow-produced rhLF as described in the Notice are assumed to be comparable to likely drug uses because the identified "sports and functional food" categories are consumed for perceived effects on bodily structures or functions (as opposed to technical effects in food processing). The rhLF will be recommended for consumption at substantial dosage levels, and the Notifier has implied that the products will be marketed for express or implied benefits of a pharmaceutical nature.

In our expert opinion, the Notice raises substantial issues that preclude a finding of safety (i.e., a reasonable certainty of no harm) for the conditions of use, and that warrant further investigation, including clinical trials of appropriate size and duration. Of particular concern are the absence of adequate clinical trials with transgenic cow-produced rhLF required to assess differences of possible toxicological consequence between transgenic

cow-produced rhLF and other forms of lactoferrin, and failure of the Notice to sufficiently address a wide range of safety risks, including risks arising from long-term exposure to this highly active immunomodulatory agent. We also note that the intended daily dose substantially exceeds exposure from native human lactoferrin. This assessment addresses the following specific concerns:

1. Comparison of the intended daily dose of transgenic cow-produced rhLF to normal exposure to human lactoferrin ,
2. Absence of adequate safety studies conducted with transgenic cow-produced rhLF,
3. Specific glycosylation risks with transgenic cow-produced rhLF,
4. Potential long-term immunological risks with any rhLF, and
5. Other risks associated with extended dosing with any rhLF.

We also note what appear to be substantial safety concerns relating to the manufacture of transgenic cow-produced rhLF as described in GRAS Notice No. 000189. These concerns are addressed in detail in **Attachment A** and include the following observations:

- Genetic stability of the host organism is insufficiently characterized or controlled.
- Production and purification processes are not adequately controlled.
- Product characterization including glycosylation, secondary and tertiary structure, degradants, aggregation and contaminants is incomplete and does not provide assurances that product differences or contaminants will not pose safety risks.
- Long-term stability data are insufficient to guarantee the integrity of the product as it is intended for market.

For these reasons, we conclude that available information fails to establish transgenic cow-produced rhLF as presenting a reasonable certainty of no harm under the claimed or probable conditions of use. Specific data and information supporting this conclusion are presented in detail below.

1. Comparison Of The Intended Daily Dose Of Transgenic Cow-Produced RhLF to Normal Exposure to Human Lactoferrin

The GRAS Notice states that transgenic cow-produced rhLF is intended for use in sports and functional foods at the following doses:

Product Content:	100 mg per serving
Estimated Maximum Daily Consumption:	214 mg per person

The Notifier bases these estimates on projected 2-day consumption averages for representative food products based on USDA data collected as far back as 1994. However, these projected consumption levels may substantially underestimate the doses received by significant population sub-groups such as athletes or those who might perceive a health benefit related to greater consumption of rhLF. Since there is no

reliable means of limiting the consumption of food products containing rhLF, a safe dose must consider the potential for excess consumption by some segments of the population. The Notifier's projected consumption levels are comparable to the levels effectively administered to patients in clinical trials supporting rhLF's intended approval as a pharmaceutical drug. These pharmaceutically effective doses were as low as 250 mg/day. Additionally, the total cumulative dose received over the course of months and years must be considered.

The GRAS Notice further proposes using a bolus of transgenic cow-produced rhLF that results in a daily dose that is up to a hundred times higher than the levels of native lactoferrin that are normally consumed in saliva. It should also be noted that native lactoferrin levels in saliva from adults may be as low as 3.4 μg/mL, a level ten times lower than that assumed by the Notifier (Lentner 1981). Notwithstanding the Notifier's assertions about the safety of endogenous hLF, it must be emphasized that the oral dose of hLF consumed in saliva represents a homeostatic level and that the safety of disrupting this homeostatic equilibrium through the introduction of exogenous lactoferrin, whether hLF or rhLF, cannot be assumed.

Such large doses of transgenic cow-produced rhLF have never been adequately safety tested in humans, either in the Notifier's intended uses or for extended periods of time. The pharmacological effects of large doses of transgenic cow-produced human lactoferrin are not fully understood and could pose health risks, including those related to significant and sustained changes in immune function.

The GRAS Notice does not present adequate data to address these potential safety risks.

2. Absence Of Adequate Safety Studies Conducted With Transgenic Cow-Produced RhLF

GRAS Notice 000189 presents inadequate data from safety studies with rhLF produced in transgenic cows. The following tables summarize all of the studies conducted with the Notifiers's transgenic cow-produced rhLF cited in the GRAS Notice.

Animal Toxicology Studies with Notifier's RhLF

Species	(n=)	Duration	Dose	Reference	Location
Neonatal Rats	24 per group	14 Days	0, 10, 100, 1000 and 6000 mg/kg/day t.i.d.	**(Unpublished)** Notifier's rhLF	Page 38
Rats	20 per group	91 Days	0, 200, 600, and 2000 mg/kg/day	**(Unpublished)** Notifier's rhLF	Page 38
Acute Inhalation (Rats)	10	4 hrs	5.0 g/m^3 aerosol	**(Unpublished)** Notifier's rhLF	Page 42

Other Animal Studies with Notifier's RhLF*

Study (Species)	(n=)	Duration	Dose	Reference	Location
Allergenicity (Guinea Pig)	15	24- 48 hrs	30% or 10% rhLF in saline - topical	**(Unpublished)** Notifier's rhLF	Page 40
Dermal Irritation (Rabbits)	3	1- 72 hrs	1000 mg topical	**(Unpublished)** Notifier's rhLF	Page 41
Eye Irritation (Rabbits)	3	1- 72 hrs	12 mg topical	**(Unpublished)** Notifier's rhLF	Page 42

Human Studies with Notifier's RhLF

Species	(n=)	Duration	Dose	Reference	Location
Humans	6	24 hrs	52 mg b.i.d. for 1 day	**(Unpublished)** Notifier's rhLF	Page 43

* In addition to these studies, unpublished data was presented asserting negative results from a series of in vitro and in vivo genotoxicity assays.

The data presented in GRAS Notice 000189 are insufficient to substantiate the safety of transgenic cow-produced rhLF. While a series of unpublished preclinical studies and assays are cited, the GRAS submission references only one human study with the Notifier's transgenic cow-produced rhLF. This unpublished study had only six subjects who were administered two acute 52 mg doses over a 24 hour period. This study is not sufficient to establish the safety of a highly bioactive compound such as rhLF. The total dose level administered in this study amounts to only one half the dose the Notifier predicts may be consumed in food in a single day. These data are also inadequate to assess the safety risks of long-term rhLF administration. In fact, to demonstrate safety one should administer higher than normal doses (rather than lower than normal doses) over an extended period to large numbers of people to observe whether there might be long-term effects in a subset of the population.

The toxicity and immunogenicity studies performed by the Notifier were conducted in rats, guinea pigs and rabbits. These studies are not relevant, since these animals cannot mimic the human immune response to human glycoproteins (Descotes 2004).

The Notifier also incorrectly asserts the safety equivalence of various forms of lactoferrin including transgenic cow-produced lactoferrin, natural human lactoferrin, raw human milk, rhLF produced by cGMP fermentation techniques in *Aspergillus*, rhLF produced in rice and raw rice grain containing rhLF. There are substantial differences between these various compounds and alternate forms of lactoferrin, including differences in glycosylation that may present significant health risks. Some of the glycans on transgenic cow-produced rhLF and the plant glycans on rice-produced rhLF, for example, are known to be both highly allergenic and immunogenic in humans.

The Notifier asserts that the primary reason that so few clinical and preclinical studies have been undertaken to evaluate the safety of lactoferrin is because there is "a general consensus among experts that hLF has been shown to be safe – via natural exposure – and at such high doses that no additional safety evaluation is necessary" (Notifier's Submission page 30). This statement is not correct. There is clearly no scientific consensus on the safety of recombinant human lactoferrin. Natural hLF has a minimal history of safety testing in humans because the price, at over $3,600 per gram (Sigma-Aldrich 2006), has precluded any broad-based clinical evaluation. Furthermore, a safety claim based on native hLF (which is in itself a compound with different glycosylation than rhLF produced in transgenic cows) "via natural exposure" is irrelevant since the Notifier's intended use is based on deliberate external administration, not natural exposure. Other than suckling infants, humans normally do not consume human lactoferrin in breast milk.

Lactoferrin is a glycoprotein -- it has both polypeptide backbone and many covalently attached carbohydrates. The human lactoferrin that the Notifier is actually referring to as its product is a recombinant form made in transgenic cows and therefore has the characteristics of bovine glycosylation rather than human glycosylation. The foreign glycosylation of a *human* protein creates, in effect, a new molecule that carries the risk of immunotoxicity. Neither infants nor adult humans have ever been naturally exposed to recombinant human lactoferrin transgenically produced in cows or to any other form of rhLF.

Based on this faulty presumption of equivalence, the Notifier presents 25 additional references (see pages 59-70 of the Notifier's submission) for various clinical and preclinical studies involving alternative forms of lactoferrin. By ignoring the substantial differences between its rhLF and other lactoferrin products, the Notifier avoids the fact that extremely limited safety studies have been done with its rhLF. The data with substantially different lactoferrin products is not relevant to any safety assessment of rhLF produced in transgenic cows.

For example, 11 out of 25 references provided by the Notifier utilize pharmaceutical grade rhLF produced by Agennix, Inc. using established fermentation techniques under cGMP conditions. Four of these references (Andersen 2004, Hayes 2003, Hayes 2004, VAMC 2003) are redundant and cover data generated within the same study. An additional 7 of the 25 references (Davidson 1987, Davidsson 1994, Goldblum 1989, McMillan 1977, Spik 1982, Davidson 1990, Lindberg 1997) involve the use of natural human milk (not pure lactoferrin) and were not safety studies. Finally, the remaining studies referenced involved native human lactoferrin, rice grain expressing human lactoferrin or rice-produced rhLF, which in itself may present specific safety risks related to its plant glycosylation. Thus, these studies presented by the Notifier to assert the safety of transgenic cow-produced rhLF are inapplicable.

In spite of the Notifier's insistence of equivalence between the alternative forms of lactoferrin used to support the safety claims in its GRAS submission, substantial and material differences exist between these compounds:

- Transgenic cow-produced rhLF contains glycans not found on natural hLF (Van Berkel 2002).
- Transgenic cow-produced rhLF has up to 6 amino acid differences compared to native hLF (GRAS Notice page 12).
- Transgenic cow-produced rhLF has completely different glycosylation from rhLF produced in rice.
- Transgenic cow-produced rhLF has completely different glycosylation from rhLF produced by fermentation in *Aspergillus*.

Furthermore, as documented by the Notifier, rhLF from transgenic cows is contaminated with bovine lactoferrin (GRAS Notice page 18 and 20). The degree of contamination varies from batch to batch and could be as much as 10% with bovine lactoferrin being the major contaminant. The effect of mixing bovine lactoferrin with rhLF produced in transgenic cows as an administered drug is clouded with uncertainty, since no combination studies have been performed and the variable combination of the two may have an unpredicted result.

"Biosimilar" Therapeutic Proteins Are Not Identical

Recombinantly produced "biosimilars", such as these alternative forms of lactoferrin, are currently regarded by FDA as different molecules requiring independent safety testing and independent regulatory treatment.

The biological activities of protein therapeutics are invariably closely linked to the processes used to make them. The safety, purity, and potency of a biologic therapeutic are ensured - to this day - by maintaining the constancy of the result of each step in the production process. Analytical science continues to improve. Nevertheless, recombinant protein therapeutics cannot be completely characterized, and their behavior in human patients cannot be predicted with certainty from a comparison of chemical and biological analyses in the same way that "small molecules" can. The current regulatory frameworks established by statute are based upon and reflect these fundamental scientific differences.

Moreover, as a protein becomes larger and more complex, the structural variability and the analytical uncertainties increase, further increasing the differences between even identical products manufactured using different processes. Other than antibodies, rhLF is substantially more complex than most other recombinant therapeutic proteins (Table 1).

Table 1. Comparison between Lactoferrin and Other Therapeutic Recombinant Proteins

	Insulin	Erythropoietin	Interferon alpha	G-CSF	Growth Hormone (Somatotropin)	rhLF
Amino Acids	52	165	165	174	191	692
Molecular Weight	5,800	21,000	19,271	18,800	22,000	76,261
Disulfide Bonds	3	2	2	2	2	16
Metal coordination	No	No	No	No	No	Yes
Glycosylation	No	Yes	No	No	No	Yes

Clinical evaluation is not simply the "gold standard" for monitoring the safety and efficacy of biologics – it is the *sine qua non* of developing and commercializing a recombinant protein therapeutic. The human immune system is more sensitive than any available analytical method to subtle changes in protein products. Its behavior cannot be effectively modeled or predicted based on in vitro analytical data and bioassays.

Changes in glycosylation can have profound effects on the safety and efficacy of recombinant proteins and relevant changes can occur even between closely related host cell species. FDA has already indicated that "biosimilar" therapeutic proteins (like lactoferrin) produced in different expression systems may not be approvable (as medicines) without independent clinical evaluation (FDA Draft Guidance for Industry: Comparability Protocols - Protein Drug Products and Biological Products - Chemistry, Manufacturing, and Controls Information. September 2003, page 8).

Although definitive guidelines still need to be finalized, there is a general opinion that even where products are derived from the same gene and the same host cells using identical down-stream processing, large side-by-side clinical comparisons are essential to establish whether recombinant proteins are similar (EMEA/CPMP/3097/02 Guidelines). This position has recently been made law in the European Union and has several implications for transgenic cow-derived human lactoferrin:

- GRAS designation of a complex recombinant protein like rhLF is by definition difficult to establish because data derived from a specific product usually cannot be generalized to class level.
- Data not generated with a specific recombinant product as it will be marketed cannot typically be used to support the safety of such a product.
- A market authorization of rhLF from a specific manufacturing process should be granted only on the basis of extensive safety testing of the product as it is manufactured for marketing.

In the Notifier's comments on page 50 of the GRAS Notice, it is implied that the glycosylation differences between transgenic cow-produced rhLF and native human lactoferrin represent only a minor potential safety issue. In fact, these differences could pose a major safety issue. The nature of the glycans on transgenic cow-derived *human* glycoproteins could indeed have catastrophic consequences on the human immune system and induce a plethora of immune responses. Unfortunately, as indicated above, the Notifier does not report characterization of the glycans on its transgenic cow-produced rhLF, which is highly concerning. A few scientific examples illustrating the possible effects of transgenic cow-derived *human* glycoproteins on human immune responses and biology are provided below.

Cows glycosylate proteins in their milk in ways that are distinct from human glycosylation, such as using N-glycolylneuraminic acid (NeuGc) ("Hanganutziu-Deicher antigen" (Asaoka 1994)), which is a potent antigen, instead of N-acetylneuraminic acid. In fact, it is stated in the scientific literature that "In general, normal human tissues yield only NeuAc, while other mammals have significant levels of NeuGc. Despite the small structural difference between them (the methyl group in NeuAc being substituted by a hydroxymethyl group in NeuGc), the two derivatives do not appear to be biologically equivalent" (Moreno 1998). Humans lost the gene required for synthesizing NeuGc many millennia ago (Varki, 2001), thus creating the situation where, when attached to a *human* glycoprotein, it is considered as a "foreign" substance that invokes immunity.

Bovine glycoproteins also contain the LDN or LacdiNAc antigen and the alpha-Gal antigen (Gal(alpha)1-3Gal-R), both of which are lacking in human milk glycoproteins (Coddeville 1992, Nakata 1993). Both LDN and alpha-Gal carbohydrates are potent antigens. They are also expressed by several parasites and are involved in host immunity to parasitic infections (Die I and Cummings RD 2006). The alpha-Gal antigen is further known to cause transplant rejection of non-human derived tissues in people (Chen 2006).

The specific glycosylation of *human* lactoferrin produced in transgenic cows can have major consequences on its biological activity and stability. As the Notifier's own scientist published in 2004 (van Veen 2004), glycosylation contributes to proteolytic stability of lactoferrin, but more importantly, bovine and human lactoferrin differ in their susceptibility to proteolysis based on glycosylation.

Since human lactoferrin has demonstrated potent biologic activity in humans, it is crucial from a safety standpoint to fully understand the impact of changes in glycosylation. On page 50 of the GRAS Notice, there is a troubling misstatement by the Notifier's scientific expert who declares that "carbohydrate structures (glycans) are not generally considered to be allergens". This statement is factually incorrect. In reality, carbohydrates are considered among the strongest antigens and allergens and many published studies show that the types of carbohydrates likely to be found on transgenic cow-derived human glycoproteins are potent inducers of antibody responses, including IgE (Leino 2006, Ahrazem 2006, Chow 2005). The reason for the allergic responses to animal- and plant-derived carbohydrate antigens is beginning to be better understood, and there is

increasing concern about the safety of carbohydrate antigens of the type found on some bovine glycoproteins.

As previously stated, carbohydrate antigens are highly allergenic. For example, a recent review notes that bee venom phospholipase A2 carries an N-glycan containing the α(1,3)-linked fucose, and several T-cell clones have been identified from bee venom-sensitized individuals that proliferate in response to honey bee PLA2, but not to its non-glycosylated variants, providing evidence for the involvement of N-glycans in T-cell recognition (Die I and Cummings RD 2006). The allergenicity of carbohydrate moieties has been documented, and the Notifier's expert's statement is contradicted by a wealth of scientific publications (Breiteneder 2005, Fotisch 2001, Betenbaugh 2004). Carbohydrate structures on N-glycans and O-glycans that occur on many glycoproteins from non-human origins are associated with significant human immune responses and are also probably important in disease pathogenesis in the case of microorganisms (Andersson 2003, Die I and Cummings RD 2006, Foetisch 2003, Malandain, 2005, Nyame 2004, Showalter 2001).

Besides being antigenic and allergenic, carbohydrate moieties of glycolipids and glycoproteins in plants, animals and humans have very high biological activity and are involved in a tremendous range of biological processes including cell-cell adhesion, cell-cell signaling, immune regulation, innate immunity, and cell biological phenomena including organelle biosynthesis (Bertozzi 2001, Engering 2002, Feizi 2000, Freeze 2005, Gu 2004, Helenius 2004, Rudd 2004, van Kooyk 2004).

Thus, the presence of bovine carbohydrate antigens on rhLF produced transgenically in cows raises the possibility that they may not only induce immune responses, but may in fact interfere with and act as antagonists in regard to the biology of human cells involving endogenous carbohydrate moieties.

Finally, and contrary to the Notifier's conclusions, carbohydrate moieties have increasingly been implicated in the immunogenicity of recombinant proteins (Die I and Cummings RD 2006, Hermeling 2004, Schellekens and Casadevall 2004). It has been shown that there are significant issues to address in regard to immunogenicity and antibody formation with recombinant proteins, such as insulin, interferon, epotin alfa and others. Interestingly, the immunogenicity to recombinant proteins is independent of the route of administration (Schellekens 2003).

Given that the role of carbohydrates as recognition elements in biology is well understood, there is no justification to ignore evidence that foreign glycoforms will have an effect on transgenic cow-produced rhLF's safety. To summarize, dismissal of the safety risks relating to glycosylation is improper for the following reasons:

1. Carbohydrate moieties have increasingly been implicated in the immunogenicity of recombinant proteins (Schellekens 2004, Hermeling 2004, Die I and Cummings RD 2006). Immunogenicity and antibody formation have been noted with proteins and glycoproteins, including insulin, interferon, epotin alfa and

other recombinant proteins. Additionally, immunogenicity to recombinant proteins is independent of the route of administration. There have been no published cases where a change in route of administration completely negated immunogenicity (Schellekens 2003).

2. The GRAS Notice treats the glycans on transgenic cow-produced rhLF as part of a food product rather than structural components of a therapeutic protein vital to immune system recognition. RhLF is a biologically active immunostimulatory drug that interacts directly with receptors in the gut responsible for regulating immune response and inducing maturation of dendritic cells (Varadhachary 2006, Varadhachary 2005). Through its effect on the Gut Associated Lymphoid Tissue (the largest immune organ in the body), lactoferrin may actually serve as a vector to deliver cross-reactive glycans directly to activated antigen presenting and immune effector cells. Additional clinical studies are needed to assess these risks before any broad safety claims can be made.

3. The emergence of an antibody response and the breaking of B-cell tolerance require prolonged exposure to a recombinant protein, and generally antibodies can appear up to one year after chronic treatment (Schellekens 2004). The induction of anti-lactoferrin antibodies in people receiving exogenous lactoferrin has been documented (Brock 1998) and anti-lactoferrin antibodies have been associated with serious autoimmune disease. The induction of anti-lactoferrin antibodies could have tremendous consequences by "neutralizing" many other vital functions of ***endogenous*** lactoferrin, which is a degranulation product of neutrophils involved in, among other things, the regulation of inflammation and protection against the development of cancer.

Until robust clinical studies are conducted to determine the effects of long-term exposure to bovine glycans delivered by recombinant *human* lactoferrin to immune cells in the gut, no general conclusions can be reached about the safety of transgenic cow-produced rhLF as described in GRAS Notice 000189.

4. Potential Long-Term Immunological Risks with any rhLF

The Notifier acknowledges a wide variety of biologic activities for human lactoferrin and references several studies that elaborate on its anti-viral, anti-microbial, anti-inflammatory and immunomodulatory properties (Notifier's Submission page 28). No discussion is included regarding the potential physiological consequences of these activities in humans. The Notifier concludes that "most of the biological actions of hLF are mediated by the sequestration of iron or by the previously mentioned positively charged domain located in the N-terminus" (Notifier's Submission p. 29). This conclusion is incorrect because it pertains only to part of human lactoferrin's anti-microbial properties.

Until recently there has been little understanding of the exact mechanism by which human lactoferrin mediates its profound anti-inflammatory and immunomodulatory activity. Over the last few years, proprietary research conducted with rhLF produced by fermentation in *Aspergillus*, which is under development as an FDA regulated pharmaceutical drug for the treatment of non-small cell lung cancer, has revealed that human lactoferrin's mechanism of action is far more complex than previously understood and involves all aspects of the molecule's structure.

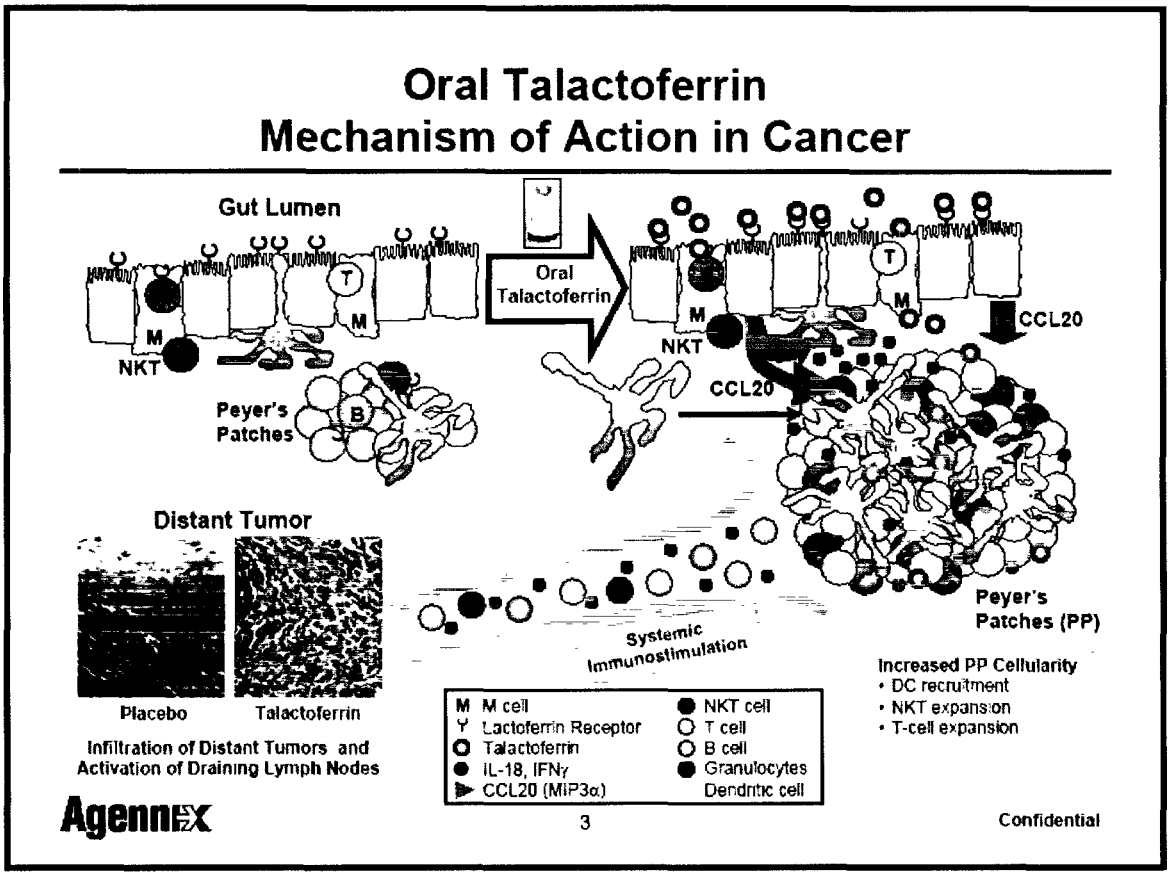
Based on this research, it has been shown that rhLF is an orally active immunomodulatory protein that is not absorbed or systemically bioavailable but that acts via the gut and Gut Associated Lymphoid Tissue (GALT) (Figure 1).

Orally administered rhLF specifically binds receptors on cells lining the upper gastrointestinal tract, and induces the production of immunomodulatory cytokines and chemokines within the small intestinal Peyer's Patches (PP), initiating an immunostimulatory cascade in the GALT. RhLF induces dendritic cell (DC) maturation, and induces the production of key chemokines by enterocytes, including MIP3-alpha/CCL20, an important chemokine for attracting immature DCs. RhLF also acts directly as a chemokine, binding chemokine receptors including IL-8RB (CXCR2) and CCR2 and attracting immune cells such as lymphocytes and antigen presenting cells (APCs). Oral administration of rhLF also results in the production of key cytokines including IL-18 and IFN-gamma in the gut. These cytokines, in concert with the influx of immune cells into the PPs, play an important role in stimulating both the innate and the adaptive immunity.

Oral administration of rhLF in preclinical experiments produced an increase in the total cellularity of small intestinal Peyer's Patches, including an increase in the numbers of Natural Killer-T cells and CD8+ T-lymphocytes. RhLF induces the migration of DCs to the intestinal Peyer's Patches and induces DC maturation. The systemic immunostimulation induced by orally administered rhLF is demonstrated by an increase in NK and CD8+ lymphocyte mediated cytotoxicity, an activation of tumor-draining lymph nodes, and immune infiltration into distant tumors. RhLF's anti-tumor effects are lost in animals lacking NK-T or CD8+ cells demonstrating the importance of these effector cells in rhLF's anti-cancer activity.

Simply stated, oral rhLF binds to specific receptors on gut enterocytes and immune cells, resulting in the attraction and activation of key immune cells and the production of key immunomodulatory cytokines. This results in the systemic activation of both the innate and the adaptive immunity, and the killing of cancer cells.

Figure 1: RhLF Mechanism of Action in Cancer



The induction of these cytokine and chemokine cascades as well as the systemic activation of immune cells has profound implications on the potential safety of long-term administration of rhLF, including the possibility of various immune reactions or impacts on autoimmunity which can only be assessed by long-term human clinical trials.

4.1 Immunotoxicity Risks

Immunomodulatory agents present a distinct risk of immunotoxic effects that require careful preclinical and clinical evaluation. A recent review by a noted immunotoxicologist summarized these concerns:

> "Immunotoxic effects are divided into four categories: immunosuppression, immunostimulation, hypersensitivity and autoimmunity. Each category is associated with distinct adverse effects."
>
> Historically, concern has primarily focused on immunosuppression and hypersensitivity (allergenicity). "However, immunostimulation is also a key issue, especially with pharmaceuticals. It is not known whether function assays used to predict immunosuppression or hypersensitivity are applicable to the

prediction of immunostimulation. Most available animal models and assays are not valid to assess the potential of immunostimulation, and autoimmunity is not predictable at all. Conflicting guidelines and the lack of human data contribute further to this situation."

"Due to the redundancy of the immune system, a single change is not necessarily sufficient evidence for immunotoxicity so that a global assessment of all preclinical findings is advisable. Genetic factors also play a major role in immune responses. As wide inter-individual variability is unavoidable, sufficient numbers of animals, a cautious comparison with both study and historical controls, and the possible use of inbred or genetically modified animals are to be considered. Marked interspecies differences in the immune system [should] lead to the use of more than one animal species and to confirm animal data during clinical trials. The inclusion of certain immune endpoints applicable to animals and man is therefore essential."

"Due to the many adverse effects reported in man, the immunotoxicity potential of every new molecular entity should be systematically and specifically evaluated." (Descotes 2004).

The long-term effects of immunostimulation by rhLF (from any source) have never been evaluated, either in animals or in human clinical trials. Given the evolving scientific understanding of the consequences of immune system manipulation and the long-term potential for unknown immunotoxic effects with immunostimulatory agents, caution is warranted. Published FDA Guidelines support this approach stating:

"Change in an immune function or level of immunological mediator may not necessarily appear as an "adverse effect", but rather as immunostimulation. Caution must be exercised in such cases, because a non-specific enhancement of the immune response that might be interpreted as a beneficial effect may result in suppression of specific immunity against a particular infection. A decision on whether a material/device is immunotoxic must rely on the available evidence from pre-clinical test results and clinical evaluation, as well as prior history of use (Guidance for Industry and FDA Reviewers - Immunotoxicity Testing Guidance, May 6, 1999).

TeGenero Case

The potential risks to humans posed by immunomodulatory recombinant proteins, and the need for robust clinical testing before asserting claims of safety, was recently demonstrated in the tragic case of TGN1412 (TeGenero Immuno-Therapeutics).

TGN1412 is an immunomodulatory antibody that (like rhLF) stimulates T-cell expansion and activation. In spite of a strong preclinical safety record, including testing in primates, TGN1412 produced severe and unexpected reactions in a Phase I clinical trial involving seven healthy volunteers. On March 13, 2006, six of the volunteers who received the

active drug suffered organ failure and violent, life-threatening side-effects after being administered TGN1412.

A follow-up investigation by the UK's MHRA (Medicines and Healthcare products Regulatory Agency) determined that:

- TeGenero observed the highest standards in developing this drug and that these symptoms were both unexpected and unforeseeable. Data previously released showed that there was no sign of risk from the pre-clinical tests of TGN1412.
- Animal study results showed that there were no drug related deaths in the preclinical testing of TGN1412, with just one animal having to be euthanized during the trial after suffering severe diarrhea caused by an unrelated bacterial infection.

In a press release on March 17, 2006, TeGenero stated, "We are shocked about the symptoms we have seen in the (clinical trial) volunteers. Extensive pre-clinical tests showed no sign of any risk. We observed strict standards for this clinical test and we obtained all required approvals both in Germany and Great Britain. The drug was tested extensively in laboratories and has been tested on rabbits and monkeys. We saw no drug related adverse events and there were no drug related deaths."

This case illustrates that the "consequence of interspecies differences is that no one can assure that negative (immunotoxicology) results obtained in rats or dogs or both, will also prove negative in man. Thus, animal results have to be confirmed in human beings" (Descotes 2004). While this appears to be an extreme case of acute immune reaction, it serves as a cautionary note, especially when considering the long-term risks of immunostimulation by rhLF, which may not surface in such an immediate or startling fashion.

4.2 Risks of Exacerbating Auto-Immune Disease with RhLF

A specific immunotoxicological risk of long-term administration of rhLF in humans is the potential induction or exacerbation of autoimmune disease and the generation of anti-lactoferrin antibodies, which are often present in patients with autoimmune disease:

- Anti-lactoferrin antibodies have been associated with autoimmune liver disease (Ohana 1998).
- Anti-lactoferrin antibodies have been associated with inflammatory bowel disease (IBD) (Roozendaal 1999).
- Anti-lactoferrin antibodies have been associated with Wegener's granulomatosis (van der Woude 1985).
- Anti-lactoferrin antibodies have been associated with rheumatoid arthritis (Locht 2000).

- Anti-lactoferrin antibodies have been associated with systemic lupus erythaematosus (Galeazzi 1998).

- Anti-lactoferrin antibodies have been associated with autoimmune pancreatitis (Okazaki 2000).

The presence of non-human glycosylation on transgenic cow-produced rhLF raises the troubling possibility that the carbohydrates may in fact stimulate immunity to the protein itself, thereby generating neutralizing and/or inhibitory antibodies that could block functions of the protein. In fact, conjugation of such carbohydrates to so-called carrier proteins is the modern way to induce protective immunity to parasites and bacteria carrying unusual carbohydrates. This is well documented in a recent review citing specific examples of vaccine development using carbohydrate-conjugates to proteins (Nyame 2004), and lactoferrin is an extremely effective carrier protein.

Evidence suggests that administration of bovine lactoferrin in mice can produce a systemic immune response (Debbabi 1998, Sfeir 2004), and that oral administration of human milk proteins containing 40% lactoferrin resulted in the production of IgG, IgM, IgA, and anti-hLF antibodies with spleen sensitization (Yuki 1998). Another published study showed that ingestion of human lactoferrin by breast-fed human infants produced IgG and anti-hLF antibodies in those subjects (Brock 1998).

The Notifier's evidence that transgenic cow-produced rhLF will not elicit a similar immune response is grossly inadequate. The Notifier relies on a single unpublished human safety study conducted by the Notifier in which only six adults were administered two 52 mg doses of transgenic cow-produced rhLF in a single 24 hour period. Any conclusion of safety based on such minimal clinical data is unwarranted and lacks credibility.

In view of the evidence presented demonstrating that lactoferrin can induce an immune response in animal models, as well as in human studies involving infants, the safety position taken in the GRAS Notice is unsupportable. Data are not presented on the impact of transgenic cow-produced rhLF in non-healthy subjects, who would also likely consume rhLF from common consumer products. Nor does the Notice address the potential impact on patients with conditions that are known to be associated with anti-lactoferrin antibodies (autoimmune liver disease, inflammatory bowel disease, Wegener's granulomatosis, rheumatoid arthritis, systemic lupus and autoimmune pancreatitis). Credible evidence to mitigate the known safety concerns relating to lactoferrin and autoimmunity is not presented.

Regarding the pathological significance of anti-lactoferrin antibodies, no evidence is presented contradicting the known risks. Anti-lactoferrin antibodies may be associated with inflammation of the colon (Roozendaal 1999). In a mouse model of rheumatoid arthritis, collagen-induced arthritis was exacerbated in transgenic mice expressing human lactoferrin (Guillen 2002). As indicated above, administration of transgenic cow-produced rhLF might induce anti-lactoferrin antibodies due to the carbohydrates acting as

adjuvants. Thus, there is a real concern about the potential complications of oral administration of rhLF containing non-human carbohydrates.

While there is evidence that orally-ingested lactoferrin can induce a systemic immune response and that antibodies to lactoferrin could theoretically be involved in disease progression, no scientific data with the Notifier's rhLF has been presented to mitigate these concerns. Rather, the facts, as revealed in published literature indicate that:

1) Lactoferrin is a potent immunostimulatory molecule that has been shown to induce a systemic immune response in both animals and humans, including the induction of anti-lactoferrin antibodies;

2) Anti-lactoferrin antibodies are associated with a host of serious human autoimmune diseases;

3) There is animal evidence that lactoferrin might indeed exacerbate autoimmune disease.

No data are presented to rebut these safety concerns. An unpublished, non-statistically significant "safety" study in six subjects cannot be considered indicative, much less conclusive.

Despite the known association of lactoferrin antibodies with serious human autoimmune diseases, the GRAS Notice fails to address the potential consequences of long-term consumption of transgenic cow-produced rhLF in people with these diseases. Moreover, while the potential risk of administering rhLF in a high dose oral bolus is real, no data are presented on the effects of such administration in humans. Given the documented risk of rhLF-induced autoimmune reactions, a conclusion of safety broad enough to authorize mass consumption by the general population in unlimited doses without medical supervision can only be based on properly controlled, definitive, long-term clinical trials in humans. No such trials are included in the GRAS Notice.

4.3 Induced Changes to Immune Function

Studies show that lactoferrin can induce a change in immune system function (Zimecki 2001), including the induction of a TH1 shift. Transgenic mice over-expressing lactoferrin show a prominent TH1 immune shift (Guillen 2002). *In vitro* studies with lactoferrin indicate that it suppresses IL-4 and IL-10 production in respiratory epithelial cells obtained from human patients (Abraham 1992). *In vivo* rodent studies show that orally administered lactoferrin is a potent stimulator of IL-18 production in the gut, and is thus a stimulator of IFN-g production (Kuhara 2000, Varadhachary 2004). Enhanced IFN-g production is associated with the induction of a TH1 immune response.

Because of this potential to inhibit IL-4 and IL-10 and stimulate IL-18 / IFN-g, which shifts the immune balance from a TH2 to TH1 response, lactoferrin represents a potential

risk for people with serious TH1-associated diseases like multiple sclerosis, type 1 diabetes and chronic obstructive pulmonary disease (COPD), among others.

It has also become clear that oral lactoferrin exerts a potent immunostimulatory effect including the production of key cytokines in the gut and in systemic circulation, an increase in circulating CD4+ and CD8+ cells and an increase in NK cell activity (Varadhachary 2004). Lactoferrin has been shown to be effective in stimulating the proliferation of a broad range of immune cells in animals (Artym 2005). In vitro, lactoferrin has been shown to activate macrophages (Edde 2001) and has been shown to induce immune maturation and proliferation of a range of immune cells (Legrand 2005, Shau 1992, Dhennin-Duthille 2000). In confidential research conducted by Agennix, Inc. and submitted to FDA (IND BB-11728), it was demonstrated that rhLF is extremely effective at inducing maturation of Dendritic Cells, the most important class of antigen presentation cells. These biological effects are important for pharmacological applications of lactoferrin (including the treatment of cancer). However, the long-term effect of chronic immunostimulation, including the possible induction of autoimmune diseases, is not known. There have been several recent examples of serious adverse events resulting from long-term or acute administration of immunomodulatory agents, including most recently Tysabri (Drazen 2005).

5. Other Risks Associated with Extended Dosing with Any RhLF

There are numerous other potential risks associated with the consumption of pharmacologically relevant doses of rhLF for extended periods of time by the general population, without the necessary premarket clinical testing or postmarket surveillance.

5.1 Toxicity in Individuals with Iron Overload

As articulated in a standard hematology textbook (Hoffman 1998), "Iron overload denotes an excess in total body iron resulting from an iron supply that exceeds requirements. Because requirements are limited and humans lack a physiological means of excreting excess iron, any sustained increase in intake may eventually result in accumulation of iron. ... When the accumulation overwhelms the body's capacity for safe storage, potentially lethal tissue damage is the result." The most common form of iron overload in the US is hereditary hemochromatosis, occurring in as much as 0.5% of the population or as many as 1 million individuals (Edwards 1993, Edwards 1988). Other forms, which also affect thousands of patients with substantially higher prevalence within specific population subgroups, include thallasemias and refractory anemias. Iron overload may also follow increased absorption of *dietary* iron in some patients with chronic liver disease, including those with alcoholic cirrhosis and portacaval shunting (Jakobvits 1979). Iron overload can proceed asymptomatically for years, with the patient often presenting only after severe tissue damage has already occurred. Liver disease is the most common complication of iron overload resulting in hepatomegaly, functional abnormalities, fibrosis and eventually cirrhosis (Scheur 1962). Hepatocellular carcinoma can be an ultimate complication. Diabetes mellitus is a common complication of all

forms of systemic iron overload (Stremmel 1988) occurring in 48% of patients. A variety of other complications have been reported including such fatal ones as iron-induced cardiac disease causing cardiomyopathy with heart failure, arrythmias or both (Model 1984).

The daily requirement of iron is only ~ 1 mg in adult men and ~ 2 mg in pre-menopausal adult women. In hereditary hemochromatosis, dysregulation of intestinal iron absorption occurs, wherein iron continues to be efficiently absorbed even in the face of substantial elevation of body iron stores eventually leading to the major morbidities and mortalities associated with the disease. Treatment for patients diagnosed with iron load disorders involves iron chelation by either regular phlebotomy or with chelating agents. Patients are also counseled to avoid foods rich in iron and avoid any iron containing supplements. The dietary concern is of course even more critical in the substantial number of patients with undiagnosed disease.

5.2 Iron Delivery to Iron Constrained Pathogens

Lactoferrin binds iron with a high avidity across a broad range of pH concentrations and its ability to deliver iron is an important biological property of this molecule. In experiments with human duodenal mucosa, unlike serum transferrin and ovotransferrin, lactotransferrin was able to yield its iron to intestinal tissue in a receptor-mediated process (Cox 1979). As has been discussed in the literature as a potential concern, administration of lactoferrin with its highly bioavailable iron can accelerate growth by pathogenic bacteria and protozoa (Weinberg 1978). An outstanding example of this involves infections caused by the enteric pathogen, *Vibrio vulnificus*, most often acquired by eating raw shellfish. When ingested by humans with iron-overload, this organism can cause rapidly progressing and fatal bacteremia (Wright 1981). In mice, the LD^{50} for an inoculum of *V. vulnificus* drops from 10^6 in normal mice to an estimated 1.1 organisms in iron-loaded animals, an impressive 6 log difference. Similar 5 to 6 log differences also have been reported for certain strains of *E. coli* (Eaton 1982). In humans, trauma-associated sepsis, which has often been linked to the ability of otherwise normal commensal bacteria to invade and penetrate the gut mucosal barrier, appears to involve catecholamine mediated iron removal from lactoferrin and its acquisition by bacteria (Freestone 2002).

Microbial colonies tend to be iron constrained (Andrews 2003), and access to a source of iron can induce infectious flare-up. In fact, a variety of bacteria have evolved a mechanism for acquiring iron directly from human lactoferrin. This mechanism involves surface receptors capable of specifically binding LF from the host, removing iron and transporting it across the outer membrane. The iron is then bound by a periplasmic iron-binding protein, FbpA, and transported into the cell via an inner membrane complex comprised of FbpB and FbpC (Elkins 2004). Iron availability is also critical to the virulence of *M. tuberculosis* and other mycobacteria that have also evolved a mechanism to acquire iron from lactoferrin (Ratledge 2004, Purdey 2006), as well as a variety of other pathogenic organisms.

The importance of iron levels in regulating bacterial growth is best expressed in the following words of an editorial comment (Shock 2002):

> "We all carry around a dangerous sack of goods—intestines filled with so many bacteria that they actually outnumber our own somatic cells. A dab of these lively intestinal contents released into the body is sufficient to kill any of us. For this reason, the guts are a site of constant and vigilant surveillance. ... The aerobes require iron and this requirement is their Achilles' Heel and, in some cases, our major protection. This is because iron is an exceedingly scarce commodity in normal mammalian body fluids... Some crafty bacteria have evolved iron-binding siderophores, such as desferrioxamine or enterobactin, which enable them to steal iron from normally safe iron reservoirs, in some cases even from the iron-binding proteins, transferrin and lactoferrin. Once this theft has occurred, bacterial growth is enabled and, clinically speaking, it is Katy, bar the door."

5.3 Iron Delivery to Tumors

The role of iron as a growth-regulating factor applies more broadly beyond micro-organisms. The growth of tumors is also known to be iron regulated (Weinberg 1983), and increased *dietary* iron has been shown to promote colon tumors in mice (Ilsley 2004, Hann 1991). Tumor cells are also known to over-express receptors that bind lactoferrin with a high affinity. These lactoferrin receptors have been shown to be up-regulated in the presence of iron chelators and to deliver iron to the interior of colon carcinoma tumor cells (Mikogami 1995). Thus, there is a risk that pre-cancerous or early stage GI tumors could also access iron from lactoferrin to accelerate their growth and metastasis.

5.4 Risks of Systemic Amyloidosis Caused by Lactoferrin Variants

In recent studies, lactoferrin variants have been linked to systemic amyloidosis. Amyloidosis is an acquired or hereditary disorder related to protein folding. Fragments of proteins that are normally soluble are deposited extracellularly where they accumulate and form deposits that interfere with the function of effected tissues or organs (Pepys 2001). These amyloid deposits have been implicated in the pathogenesis of diseases such as Alzheimer's disease, various prion diseases and type II diabetes.

Lactoferrin fragments have specifically been implicated as a cause of amyloidosis accompanied by trichiasis (a common vision threatening condition of the eyelid). The lactoferrin fragment responsible results from a single change from glutamic to aspartic acid near the end of the protein molecule (Ando 2003). The association of lactoferrin fragments with amyloidosis is of particular concern because all recombinant forms of lactoferrin will contain variants and protein fragments that cannot be fully characterized or isolated given current technology (see **Attachment A**). Given that the cited production standards for transgenic cow-produced rhLF are already far below cGMP pharmaceutical norms, adequate assurance that potentially pathogenic mutant proteins are not present cannot be provided.

5.5 Induction of Antibiotic Resistance

Another of lactoferrin's biological properties is its ability to interact with microbial membranes resulting in a variety of effects including depolarization. A recently published study demonstrated that exposure to rhLF can induce antibiotic tolerance in *Pseudomonas aeruginosa*, an important pathogen responsible for numerous hospital infections (Andres 2005). Other negative bacteriological effects may also be associated with lactoferrin. For example, it was recently described that exposure of pathogenic streptococci to lactoferrin results in the induction of the streptococcal pyrogenic exotoxin A (Kansal 2005).

5.6 Viral Activation

Oral lactoferrin may be involved in viral transmission and the facilitation of viral replication. Lactoferrin has been shown to facilitate replication of HTLV-I by up-regulating viral expression (Moriuchi 2001). Human lactoferrin in saliva has also been shown to act as a ligand for HHV-8, which suggests that orally administered rhLF could serve as a carrier for viral particles (Grange 2005). Moriuchi et al show that rhLF facilitates the replication of HTLV-1 in lymphocytes derived from asymptomatic HTLV-1 carriers and enables viral transmission to cord blood mononuclear cells (Moriuchi 2001). Transient expression assays revealed that lactoferrin can transactivate the HTLV-1 long terminal repeat promoter. Thus, lactoferrin may enhance vertical transmission of this milk-borne retrovirus, which could affect an extremely vulnerable population where even a slight risk is unacceptable.

Conclusion

This scientific panel has reviewed the evidence and arguments presented in GRAS Notice No. 000189 and concludes that the safety of transgenic cow-produced rhLF has not been established. Arguments and data presented by the Notifier's GRAS submission highlight—rather that eliminate—known safety concerns. From published data, including data referenced in the GRAS Notice, the following may be reasonably observed:

- Proposed doses of rhLF are far in excess of those naturally occurring in saliva. Numerous published studies and recent clinical trials show that, in large doses, rhLF has a potent immunostimulatory effect. Notifier has neither conducted studies nor published data showing that large doses are safe in humans. Studies with Agennix's cGMP fermentation produced rhLF, which is a distinct product with a completely different glycosylation, are not relevant to the establishment of safety of rhLF produced in transgenic cows.

- Published data show that glycans present on transgenic cow-produced lactoferrin can induce a systemic immune response, including the generation of IgE

antibodies. IgE mediated immune responses are a serious health risk and, in extreme cases, can lead to anaphylaxis, or even death.

- RhLF's mechanism is far more complex than previously understood and results in broad changes to systemic immune function. The safety impact of these immune system changes has not been fully evaluated and precludes an assumption of safety until long-term clinical trials have been conducted.

- According to published studies, IgE antibody responses and the breakdown of B-cell tolerance can take up to a year of chronic exposure to an allergen to develop, making short-term clinical trials completely inadequate for detecting induced allergic sensitization or antibody development. Human exposure to transgenic cow-produced rhLF has been extremely limited to date and there is no clinical data whatsoever on the effects of long-term consumption.

- Anti-lactoferrin antibodies are known to be associated with, and potentially exacerbate, a wide range of serious human autoimmune diseases. Furthermore, rhLF is known to induce a potent TH1 immune response. No studies have been conducted to determine the consequences of long-term immunostimulation by rhLF in people with autoimmune disease.

Additionally, since lactoferrin is believed to directly interact with immune cells in the gut-associated lymphoid tissue, it is possible that, through receptor binding, transgenic cow-produced rhLF may act as a vector to deliver allergenic bovine glycans directly to immune cells in gut associated lymphoid tissue. Lastly, there are other potential risks, as described above, associated with long-term administration of any rhLF, particularly in compromised patient populations.

Transgenic cow-produced rhLF presents numerous documented risks. These risks should be thoroughly and scientifically evaluated. The potent immunostimulatory activity of rhLF warrants large, controlled, long-term clinical safety studies before broadly exposing the public to potentially unlimited consumption. In fact, given the potential effects on lactoferrin-associated autoimmune diseases and the long period required to develop antibodies, rhLF from any source should be administered only under medical supervision. In our opinion, to expose the general public to these well-documented risks, without credible clinical safety data on the prolonged use of transgenic cow-produced rhLF, is both inappropriate and unnecessary. The designation of transgenic cow-produced rhLF as GRAS is inappropriate until it has been shown that the known risks (including, without limitation, the risks described in this scientific assessment) pose no threat to public safety—i.e., a reasonable certainty of no harm.

Note: None of the experts listed below are affiliated with Agennix or other parties with a commercial interest in recombinant human lactoferrin, but they share the concern that a GRAS listing prior to the adequate establishment of rhLF's safety in the larger population is inappropriate, and that unsupervised long-term administration of rhLF poses a significant risk to the general population.

Richard D. Cummings, Ph.D.
Professor of Biochemistry and Molecular Biology, Ed Miller Endowed Chair, Director - Oklahoma Center for Medical Glycobiology, University of Oklahoma Health Sciences Center

Michael Pierce, Ph.D.
Professor of Biochemistry and Molecular Biology, Director - University of Georgia Cancer Center, Director - Complex Carbohydrate Research Center, University of Georgia

Dr. Arno Kromminga, M.D.
Director, Immunology
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Sidney E. Grossberg, M.D.
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Huub Schellekens, M.D.
Professor - Faculty Pharmaceutical Sciences,
Utrecht University Central Laboratory,
Animal Institute and Department of Innovation Studies, Utrecht University
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SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: Richard D. Cummings

Name: RICHARD D. CUMMINGS

Title: PROFESSOR
UNIVERSITY OF
OKLAHOMA
HEALTH SCIENCES
CENTER

CHAIR AND
PROFESSOR
AS OF JULY 1, 2006
EMORY UNIVERSITY
SCHOOL OF
MEDICINE

000155

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

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Signature: _____

Name: JAMES MICHAEL PIERCE

Title: PROFESSOR OF BIOCHEMISTRY & MOLECULAR BIOLOGY

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The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: _____

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SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

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Signature: _____

Name: Sidney E Grossberg, M.D.
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The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: _____

Name: Marco van de Weert

Title: PhD, Associate Professor

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The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of.

Signature: _____

Name: [illegible]

Title: Director.

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Signature: _____

Name: DR DAVID GOLDSMITH

Title: CONSULTANT NEPHROLOGIST
GUY'S HOSPITAL
LONDON

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: _____

Name: Irma van Die

Title: PhD, Associate Professor in Glycoimmunology

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: S. D. Roger 23 June 2006

Name: A/Prof Simon D Roger MD FRACP

Title: Director of Renal Medicine
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SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

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The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: N. S. Casadevall

Name: Nicole CASADEVALL

Title: Profesor of Hematology.

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

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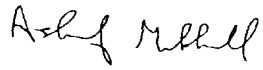
Signature:



Name: Dr. Ashraf Mikhail
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The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: Eugene D. Weinberg, PhD

Name: Eugene D. Weinberg, PhD

Title: Professor Emeritus of Microbiology

Indiana University
Bloomington, IN 47405

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of

Signature Wolfgang Jelkmann

Name Wolfgang Jelkmann, M.D.

Title Professor of Physiology, Director of the Institute of Physiology, University of Luebeck, D-23570 Luebeck, Germany

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

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Signature: _____

Name: Dr. Huub Schellekens

Title: Professor - Faculty Pharmaceutical Sciences,
Utrecht University Central Laboratory,
Animal Institute and Department of Innovation Studies, Utrecht University
Netherlands

Dr. Schellekens is currently traveling. We will provide his signature page upon his return.

Attachment A to the Scientific Assessment

Production and Purification Issues

As the use of recombinant proteins continues to increase, a debate has begun concerning the analytical tools used to characterize proteins and the comparability of products produced by different manufacturers and processes. Lactoferrin is a complex protein, even when compared to products such as insulin, growth hormone and other recombinant proteins. In contrast with small molecules, analytical tools are not available for the full characterization of complex proteins. It is also increasingly recognized that the biological properties of a protein product are highly dependent on the process of production. Consequently, a consensus is emerging that efficacy and safety cannot be established exclusively by physical and chemical characterization and should always include data obtained from studies in humans.

Complex proteins like lactoferrin differ from small molecules in that they never consist of a single type of molecule, but are always heterogeneous mixtures of different isoforms and other variants caused by clipping, mis-folding, etc. Complex proteins are also relatively unstable. During storage, modifications such as oxidation, de-amidation and aggregation are common. These modifications can have a major effect on product safety.

Because even the most detailed physical chemical characterization leaves uncertainties concerning the biological activities of a protein, the safety of these products needs to be ensured by showing:

1. The genetic stability of the host organism
2. Consistency of production
3. Detailed product characterization
4. Purity of the final product and lack of viral or prion contamination
5. Stability of the product through production and distribution

In these aspects the Notifier's GRAS petition is highly deficient and this raises serious safety concerns.

Genetic Stability

Recombinant DNA derived proteins intended for human use can be produced from microorganisms, tissue culture or transgenic plants or animals. For fermentation and tissue culture production systems, product stability is ensured by using master and working cell banks, which allows production by well characterized identical cells.

Moreover, these cells are further analyzed after the production run to guarantee the genetic stability of the production system.

The Notifier's recombinant human lactoferrin is produced in transgenic cows. These animals have not been cloned and each animal is genetically unique. Consequently, genetic stability within generations or through successive generations cannot be assumed.

The Notifier asserts that only the founder bull ("Max") has been genetically analyzed, and only at the level of lactoferrin m-RNA. There is no genetic evaluation of this animal's offspring and no criteria that the related offspring animals must meet to qualify as production animals. Due to the at-random insertion of the human lactoferrin gene into the bovine genome, induced changes in protein expression of the milk must also be evaluated to assure the integrity of the derived protein.

The inherent instability of the Notifier's expression system combined with an inadequate level of process controls creates an unacceptable level of production risk for a highly biologically active protein such as rhLF.

Consistency of Production

Because the biological characteristics of rhLF are highly dependent on the production and purification process, consistency must be shown between successive batches. Batch testing must encompass the actual batches that will be used for marketing. Batches should also be produced and purified at the same scale used for marketing. In general, at least five different production batches need to be analyzed using accepted methods for protein characterization in order to confirm product comparability and safety.

The Notifier presents very rudimentary batch characterization methods and it is unclear how and at what scale batches are produced. Based on the data and methods presented, it is not clear that the Notifier is able to produce human lactoferrin in a consistent manner. Without testing well characterized representative batches, an adequate determination of product safety is not possible.

The criticality of process and formulation to product safety has been illustrated many times with recombinant proteins. In one well known example, a relatively minor change in the formulation of recombinant human erythropoetin (EPO) resulted the generation of antibodies against EPO and the occurrence of potentially fatal pure red cell aplasia[1] (Casadevall 2005).

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[1] Responding to regulatory concerns from EMEA relating to the use of materials of human origin, J&J changed its formulation for EPREX by substituting one well-known, thoroughly characterized expander (sorbitol) for another (human serum albumin). Routine testing of the new formulation in biological and chemical assay systems revealed no cause for concern. After the reformulated product had been marketed, some 200 patients developed pure red cell aplasia (PRCA) as a result of neutralizing antibodies they generated against the erythropoietin protein in the reformulated product - meaning that their bodies cannot produce new red cells in response to the erythropoietin they produce, and they are now dependent on

In a submission to the FDA (Submission to Docket No. 2004N-0355 dated November 11, 2004) Genentech describes their experience with recombinant human growth hormone. Following a relatively minor process change that did not even involve changes in the vector or expression strain, the antibody response to the protein changed by over ten-fold. Genentech notes that due to complexity of proteins, analytical testing alone could not have identified the differences between products despite their immunogenicity varying so substantially.

As Genetech points out:

> "A survey of prescribing information for approved therapeutic proteins shows wide variability in their immunogenicity rates, ranging from 0.1% to >50%. There is similarly a wide range in the clinical sequelae associated with immunogenicity, ranging from no consequence, to anaphylactoid responses, to loss of effectiveness, to autoimmune disease. Immunogenicity is also likely to vary with type of disease and the co-medications given to patients. People with autoimmune diseases, for example, may be expected to have a more prevalent immune response to therapeutic proteins than oncology patients who may be on other immunosuppressive drugs. ... Consequently, individual patient populations must be separately evaluated for the potential immunogenicity of a drug. A low percentage of seroconverting patients treated for one disease is not predictive of the seroconversion rate in another patient population, or in the same patient population treated with different co-medications."

Physical chemical characterization

Based on the Notifier's described production process, it cannot be assumed that the biological characteristics of Notifier's rhLF will not change during production scale-up and transition from development to final manufacture.

The efficacy and safety of biologic proteins are highly dependent on the manufacturing process. Both FDA and EMEA (European Medicine Evaluation Agency) have established detailed guidelines for the evaluation of recombinant proteins following manufacturing changes. The Notifier's manufacturing and quality control processes are rudimentary and inadequate for a proper evaluation of complex protein characteristics, including biologic activity. Major flaws in the Notifier's characterization processes include the lack of amino acid sequencing, lack of glycosylation pattern identification and the failure to characterize isoforms, contaminants and impurities. The Notifier fails

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transfusions. To its credit, J&J moved aggressively, in cooperation with regulatory authorities and academic scientists, to determine the cause of the adverse reactions. Yet some years after the relationship between the reformulated EPREX and the increased incidence of PRCA was established, the reason the reformulated product led to neutralizing antibodies in some patients has not been finally established. Only clinical experience was able to reveal and characterize the enhanced risk associated with the reformulated product.

to employ adequate testing to assure protein integrity. Additionally, the Notifier has not reported any biological characterization of the product.

The Notifier has not performed much of the typical product characterization that is routinely required to establish the safety of recombinant proteins. Provided below are some of the product characterization requirements that the FDA has discussed confidentially with Agennix:

- **Glycosylation.** RhLF has a higher glycosylation content than native human lactoferrin and its carbohydrate content is significantly skewed towards mannose relative to that of normal human LF. To assure safety, complete characterization of the glycosylation is required, including site occupancy, monosaccharide analysis and chain-length analysis, and batch to batch consistency demonstrated in order to evaluate immunogenicity risks.

- **Aggregates.** Protein aggregation should be characterization and a consistent lack of protein aggregation established. Aggregation is an important cause of immunogenicity since protein aggregates are more rapidly cleared, predisposing aggregates to increased phagocytosis and antigen presentation. This includes the risk of presentation of epitopes which are normally folded and hence may be not be recognized as self-antigens, triggering autoimmunity.

- **Host cell protein and DNA.** In order to ensure safety, recombinant proteins must have very low levels of host-cell protein and host-cell DNA. Typically any contaminants above 1% must be fully characterized and their safety established independently.

- **Disulfide bond formation.** A sensitive metric to detect and demonstrate the extent of disulfide bond formation is required. This is particularly relevant in the case of rhLF, since human LF has 16 disulfide bonds. Errors in the disulfide linkages can result in alterations in the tertiary structure of the protein potentially leading to increased immunogenicity including antibodies against previously masked epitopes.

- **Oxidation and Deamidation** are important determinants of protein stability and the risk of conformational changes that can lead to immunogenicity.

Although these immunogenicity issues are most often discussed in the context of parenterally administered proteins, they are critical for orally administered rhLF as well for the following reasons:

- The gut has a very active mucosal immune system which includes the gut associated lymphoid tissue (GALT), the largest immune organ in the body. The GALT plays a critical role in the immune surveillance of ingested proteins.

- Lactoferrin has been shown to stimulate immune cells in the GALT as part of its role in modulating the immune system.

- Dendritic cells, which are the most important antigen presenting cells in the body, are known to interdigitate between the gut enterocytes in order to access the gut lumen and sample luminal contents including ingested proteins.

- Orally administered lactoferrin has been described as being transported intact through the gut wall, making it available to the systemic immune system.

Thus, in the absence of adequate characterization of transgenic cow-produced rhLF, these immunological safety risks cannot be adequately evaluated and argue against any use of rhLF as a food additive. This in itself is sufficient reason to deny GRAS status.

Purification

Because of the uncertainties concerning rhLF's biological effects, and to avoid effects due to impurities or protein variants, the purity of the final product is highly important. The Notifier's specification currently allows for up to 10% impurities. This is an unacceptably high level for a bioactive recombinant protein. It is also essential that these impurities be identified and characterized to the fullest extent possible by current analytical methods. The Notifier presents no plan for a validated characterization of impurities and merely assumes the impurities to be bovine lactoferrin and other whey proteins. Such assumptions are not acceptable given the source of the product and the non-aseptic condition of harvesting. Since the Notifier's transgenic cow-produced lactoferrin is not aseptic, which is unusual for recombinant proteins, microbial contamination remains a significant risk. Further, Notifier does not report LPS levels in the rhLF. Since LPS can cause morbidity or even death at high levels in vulnerable populations, levels of contaminating LPS are routinely measured in recombinant proteins with a requirement that the manufacturing process be controlled to ensure low endotoxins.

Viral Contamination and BSE

Although cow's milk is now considered safe regarding the potential transmission of BSE, this is the first time genetically modified cows have been used for the production of a recombinant human protein intended for human consumption. Due to the random insertion of a human gene, including a promoter to direct expression in the mammary gland that may alter the excretion function of the gland cells, precautions to avoid prion contamination should be evaluated and implemented. The purification process should therefore be validated for the removal of possible prion contaminants, as is standard for the production of proteins from animal and human sources intended for human use. Additionally, because living animals are employed as the source of production, a

validated method for the removal of conventional viruses is needed. The Notifier's described methods fail to control for these forms of potential product contamination.

Long-Term Stability

Recombinant proteins are usually unstable at room temperature. Stability studies are essential, not only to support any claim of biological activity, but also to exclude the presence of harmful degradation products that have been widely described to occur in recombinant proteins during storage. Long term stability testing is particularly vital in the Notifier's case because production and storage are not under sterile conditions and because a cold chain is not strictly controlled. Without these controls, there is a significant risk that microbial contaminants may destroy or modify the Notifier's rhLF. Stability should not only be tested in real time but also under accelerated conditions. Stability analyses should include assays that detect alterations to the protein including changes such as aggregation, degradation, structural changes, oxidation and deamidation, as well as assays for biological activity. Without proper stability studies, the integrity and safety of the Notifier's marketed product cannot be assured.

The Notifier only mentions that the product is stable, but provides no definition of stability and the techniques to evaluate stability are not elaborated. The lack of these data present serious safety questions.

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Expert letters

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RE: GRAS Application 000189 for human lactoferrin produced in transgenic cows

Dear Dr. Tarantino,

The scientific rebuttal to GRAS Notice 000189 raises many questions about the safety of transgenic cow-produced human lactoferrin as a food ingredient. I am writing to independently emphasize and expand upon a few of the points made in the scientific assessment.

First, the impact of glycosylation on proteolytic activity is of major importance and should be emphasized. The alteration in glycosylation profiles between the different entities may impact on the on metabolic clearance rates and hence physiological activity profiles.

Second, the issue of delay in the development of immunogenicity is of significant medical concern. The fact that the Notifier's transgenic cow-produced human lactoferrin was only given in 2 doses within 24 hours gives no credence to the concept that it is not immunogenic. In the case of erythropoietin, the development of pure red cell aplasia due to anti-erythropoietin antibodies occurred months after the initiation of treatment. Delayed immunogenicity is a serious safety concern that is now being considered in the safety assessment of biosimilar recombinant proteins in Europe, Australia and the US.

Lastly, the issue of side affects in healthy volunteers versus disease specific individuals is very important. In another example related to erythropoietin, it has been observed that both erythropoietin induced hypertension and pure red cell aplasia are only found in patients with chronic kidney disease and not other patients taking this drug (e.g. AIDS, rheumatoid arthritis, multiple myeloma and other oncology patients). Given the immunomodulating activity of human lactoferrin, an assessment of safety cannot be made without conducting a substantial clinical program that includes both healthy and at-risk populations.

The inclusion of transgenic cow-produced human lactoferrin in food as a nutritional ingredient is not appropriate given the very limited data available on its safety.

S. D. Roger

A/PROF SIMON ROGER MD FRACP
Renal Physician

000187

VU medisch centrum **Department of Molecular Cell Biology & Immunology**

Datum: June 20, 2006 | Uw brief van | Telefax +31 20 444 8144 | Bijlage(n)

Ons kenmerk | Uw kenmerk | Telefoon +31 20 444 8157

Postadres: Van der Boechorststraat 7, 1081 BT Amsterdam, the Netherlands

Laura M. Tarantino, Ph.D. (HFS-200)
Director, Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Room 3044
University Station
5100 Paint Branch Parkway
College Park, Maryland 20740

RE: GRAS Notice 00189

Dear Dr. Tarantino,

As a contributor to the rebuttal document submitted in response to GRAS Notice 000189, I am writing independently to emphasize my agreement with the conclusions of this scientific assessment.

I would also like to provide further support to the arguments made about the importance of (lactoferrin) glycosylation. Specifically, I would like to bring to your attention an additional example where human and bovine sources of lactoferrin differentially interact with immune cells, which may be highly relevant to a safety assessment of human lactoferrin produced in transgenic cows.

Naarding et al., described differences in the interaction of human versus bovine lactoferrin with DC-SIGN, a major lectin receptor on immature dendritic cells that specifically binds glycans. Whereas human lactoferrin (from SIGMA) shows no interaction with DC-SIGN, bovine lactoferrin (from SIGMA) clearly binds to dendritic cells via DC-SIGN. Although the authors do not explain the differences in binding, this most likely is the result of binding of DC-SIGN to glycans of bLF.

DC-SIGN is known to contribute to controlling the balance between immune activation and tolerance induction (Van Kooyk 2004, Caparros, 2006). If DC-SIGN indeed recognizes the glycans on bLF and triggers DC-SIGN function, such an interaction may have severe immunological consequences.

e-mail: im.vandie@vumc.nl

Given the demonstrated biologic activity of human lactoferrin and its affects on immune function, its inclusion in food products without rigorous clinical testing is not advised.

Respectfully,

Irma van Die, PhD
Dept. of Molecular Cell Biology & Immunology
VU University Medical Center
Van der Boechorststraat 7
1081 BT Amsterdam, the Netherlands

References

Caparros E, Munoz P, Sierra-Filardi E, Serrano-Gomez D, Puig-Kroger A, Rodriguez-Fernandez JL, Mellado M, Sancho J, Zubiaur M, Corbi AL (2006). DC-SIGN ligation on dendritic cells results in ERK and PI3K activation and modulates cytokine production. Blood 107: 3950-8.

Naarding MA, Ludwig IS, Groot F, Berkhout B, Geijtenbeek TB, Pollakis G, Paxton WA (2005). Lewis X component in human milk binds DC-SIGN and inhibits HIV-1 transfer to CD4+ T lymphocytes. J Clin Invest. 115: 3256-64.

van Kooyk Y., A. Engering, A.N. Lekkerkerker, I.S. Ludwig, and T.B. Geijtenbeek.. "Pathogens use carbohydrates to escape immunity induced by dendritic cells." Curr Opin Immunol. 2004 16:488-93.

e-mail: im.vandie@vumc.nl

June 20, 2006

Laura M. Tarantino, Ph.D. (HFS-200)
Director, Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Room 3044
University Station
5100 Paint Branch Parkway
College Park, Maryland 20740

RE: Recombinant Human Lactoferrin: A Pharmaceutical Product, Not a Nutrient

Dear Dr. Tarantino,

Lactoferrin, a powerful iron-scavenging defense protein, is present constitutively in exocrine secretions that are constantly exposed to microbial flora: milk, tears, tubotympanum and nasal exudate, saliva, bronchial mucus, gastrointestinal fluids, cervical-vaginal mucus, and seminal fluid. Additionally, lactoferrin is promptly delivered by circulating neutrophils to sites of microbial invasion. In only two of the fluids listed above (milk and tears) is lactoferrin continuously maintained at high concentration. In each of the other fluids listed above lactoferrin is maintained at quite low amounts until an actual invasion occurs.

The high concentration of lactoferrin in human milk suppresses growth in the infant gut of such iron-dependent bacteria as *Bacteroides, Clostridium, Escherichia, Salmonella* and *Staphylococcus* while permitting abundant growth of the relatively harmless iron-abstaining *Lactobacillus*. In humans above the age of infancy, the immune lymphatic system has matured so that elevated amounts of the iron-trapping lactoferrin are neither necessary nor natural in the intestine.

In tears, however, moderately high concentrations of lactoferrin are needed throughout life to inhibit (together with lysozyme) a broad spectrum of bacterial species. Accordingly, the need for antibodies to inhibit ocular bacteria is markedly lessened, thus resulting in a decrease in vision-obscuring scarring.

The proposed inclusion of 100 milligrams of lactoferrin per product serving specified in GRN 00189 would have no natural function in the gastrointestinal tracts of humans above the age of infancy. But its inclusion might have harmful effects in some proportion of consumers. Several potential risks that have not been evaluated should be considered.

1) Orally administered lactoferrin is known to increase total cellularity of the small intestine Peyer's patches, including an increase in the numbers of natural killer-T cells and CD8+ T lymphocytes. Possible adverse effects of such immunostimulation by the continual ingestion of large amounts of lactoferrin over long periods of time remain to be determined.

2) Those persons who produce a polymorphic variant of lactoferrin would be expected to regard the ingested protein as a foreign antigen. Antibodies to lactoferrin have been reported in persons who have such autoimmune conditions as lupus, rheumatoid arthritis, type 1 diabetes, primary sclerosing cholangitis, inflammatory bowel disease, and pancreatitis.

3) Some persons have latent or overt infections caused by virulent microbes that express receptors to lactoferrin that enable the pathogens to acquire growth-essential iron. Among such pathogens is *Helicobacter pylori*, an important cause of gastric ulcers and possibly gastric cancer. (The discoverers of this pathogen were awarded the 2005 Nobel Prize in Medicine.) Prior to mass medication of foods and beverages with lactoferrin, it will be necessary to determine if persons negative for the *Helicobacter pylori* breath test convert to positive when fed the protein over a period of time.

Recommendation

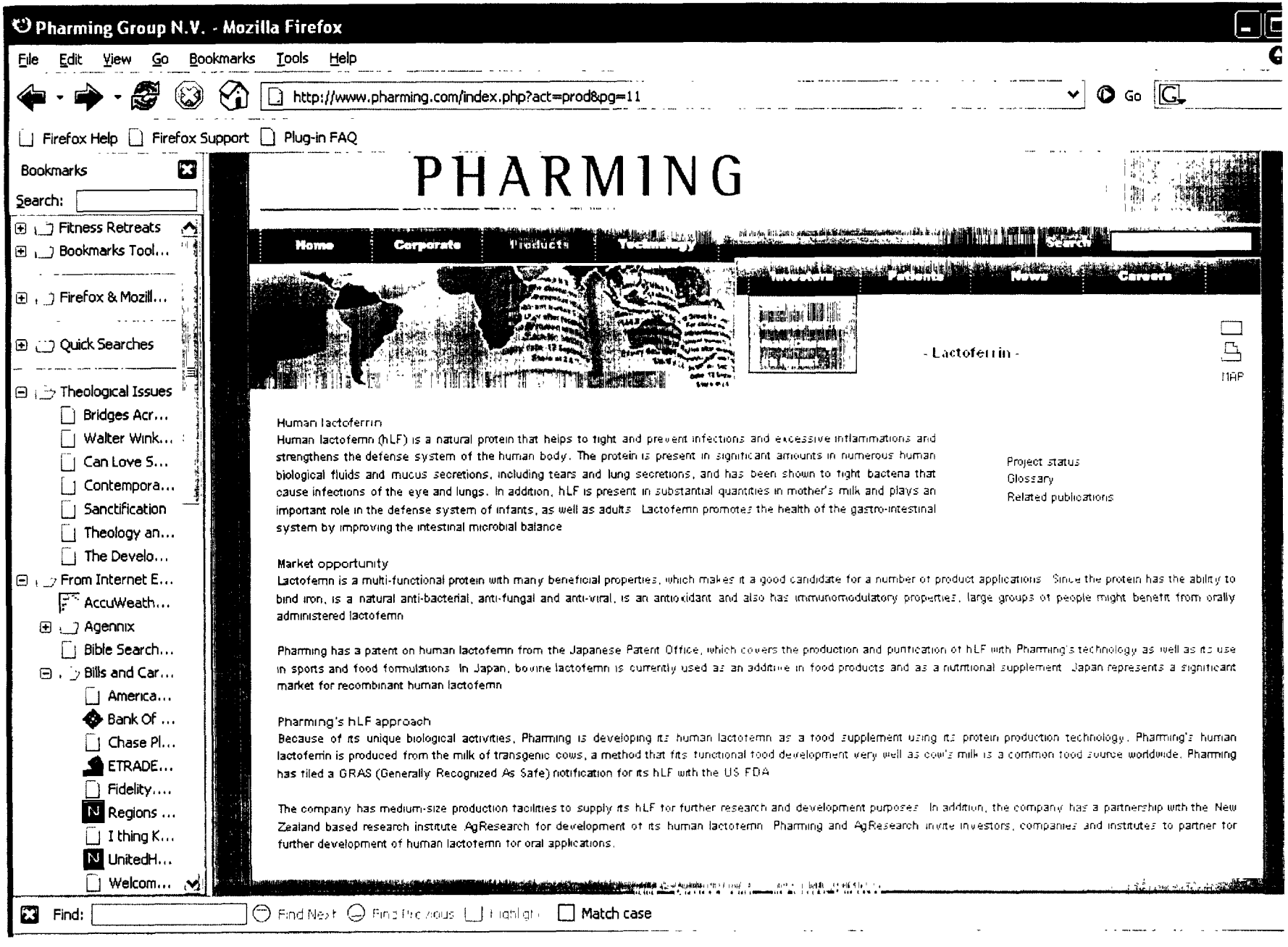
Recombinant human lactoferrin, a powerful pharmaceutical product, should not be distributed to general, non-selected populations in the absence of rigorous tests for safety. These tests should ascertain that use of the product will not result in an unacceptable rate of toxicity including (1) unbalancing the innate and adaptive immune components of intestinal lymphatic tissue, (2) development of allergic hypersensitivities, and (3) overgrowth of specific pathogenic microbes such as *Helicobacter pylori*.

Respectfully,

Eugene Weinberg, PhD

Dr. Eugene Weinberg
Professor Emeritus
Indiana University
Department of Biology
Jordan Hall Room 142
1001 East Third Street
Bloomington, IN 47405

Pharming Web Page



BEST ORIGINAL COPY

Agennix Incorporated

July 6, 2006

Robert Merker, Ph.D. (HFS-255)
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Food and Drug Administration
Room 3044
University Station
4300 River Road
College Park, Maryland 20740

Re: Correspondence of June 27, 2006 - Safety Concerns Raised by Recombinant Human Lactoferrin from Transgenic Cows (GRN No. 000189 Submitted by Pharming Group N.V.)

Dear Dr. Merker:

Please find enclosed the signature page for Dr. Huub Schellekens supporting our scientific assessment in response to GRAS Notice No. 000189. Per our correspondence of June 27, 2006, we mentioned that Dr. Schellekens had been away on travel and that we would submit his signature page upon his return.

Please do not hesitate to contact us if there are any questions or if additional information would be useful.

Sincerely,

Rick Barsky
Chief Executive Officer

Cc: Laura M. Tarantino, Ph.D. (HFS-200)
Director, Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

Eight Greenway Plaza Suite 910 Houston Texas 77046 Telephone 713/552-1091 Facsimile 713/552-0795

SAFETY CONCERNS RAISED BY RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN TRANSGENIC COWS: SCIENTIFIC ASSESSMENT OF GRAS NOTICE NO. 000189 SUBMITTED BY PHARMING GROUP, N.V.

The preceding scientific assessment of safety issues concerning recombinant human lactoferrin produced in transgenic cattle has been provided by and reflects the opinion of:

Signature: 

Name: Prof. H. Scheffelaars MD PhD.

Title: Professor in Medical Biotechnology
UTRECHT UNIVERSITY THE NETHERLANDS

Fasano, Jeremiah

From: Fasano, Jeremiah
Sent: Thursday, July 20, 2006 3:17 PM
To: 'Charles Morin'
Subject: RE: Conference telephone call concerning Pharming's hLF

Mr. Morin-

Of the three dates you proposed for a rescheduled discussion, September 1st (10 am EST) is the only one that would work well for FDA. Is this date still workable for you and your client?

Regards-

-Jeremiah Fasano

_____
Jeremiah Fasano, Ph.D.
Consumer Safety Officer
DBGNR/OFAS/CFSAN/FDA

jfasano@cfsan.fda.gov
Phone: 301-436-1173
Fax: 301-436-2964

HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

From: Charles Morin [mailto:charleslmorin@earthlink.net]
Sent: Wednesday, July 12, 2006 11:23 AM
To: Fasano, Jeremiah
Subject: Re: Conference telephone call concerning Pharming's hLF

Dear Dr. Fasano,

Thanks very much for your communication concerning postponement of Thursday's meeting. We look forward to receiving at your convenience the date and time of the rescheduled meeting.

Thanks again for your continuing support.

Charles L. Morin
Morin & Associates
388 Market Street, Suite 500
San Francisco, CA 94111
US

Phone: (415) 957-0101
Fax: (415) 957-5905

Email: charleslmorin@earthlink.net

----- Original Message -----
From: Fasano, Jeremiah
To: 'Charles Morin'
Sent: Tuesday, July 11, 2006 8:16 AM
Subject: RE: Conference telephone call concerning Pharming's hLF

Mr. Morin-

I've notified the appropriate FDA personnel of the postponement. I will contact you again soon about a date for the rescheduled discussion.

Regards-

-Jeremiah Fasano

_____
Jeremiah Fasano, Ph.D.
Consumer Safety Officer
DBGNR/OFAS/CFSAN/FDA

jfasano@cfsan.fda.gov
Phone: 301-436-1173
Fax: 301-436-2964

HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

From: Charles Morin [mailto:charleslmorin@earthlink.net]
Sent: Monday, July 10, 2006 2:40 PM
To: Fasano, Jeremiah
Subject: Conference telephone call concerning Pharming's hLF

Dear Dr. Fasano,

With regard to GN number 189 (concerning use of Pharming's rhLF in certain foods), currently we are scheduled to meet at 10:00 a.m. (E.S.T.) on July 13th to preliminarily discuss CFSAN's initial questions concerning potential immunogenicity. Pharming has been very busy over the last several weeks identifying and communicating with potential experts (all of whom are associated with academic institutions) to be added to its expert panel who can address CFSAN's questions. Pharming has made significant progress with some of them, but through no fault of anyone has not been able to get to the desired point with all of them. Part of the problem faced by Pharming is that experts' schedules and vacations and availability were all established in early January for 2006 and some are not quickly available. Thus, Pharming is not yet sufficiently prepared to have the initial conference telephone call (on Thursday). Accordingly, it respectfully asks that it be postponed. In order to work within the above-identified limitations and within the vacation times also set in January for Pharming's employees (please remember that Europe basically shuts down the last two weeks of July and all of August) **and** to assure that Pharming will not have to ask for yet another postponement, Pharming respectfully requests that the conference telephone call be postponed to (in order of preference):

1. August 31;
2. August 30; or

3. September 1.

We apologize for any inconvenience caused by the requested postponement.

Please let me know that the conference telephone call has been postponed and which date can be accommodated by CFSAN's schedule.

Thank you for you continuing assistance.

Charles L. Morin
Morin & Associates
388 Market Street, Suite 500
San Francisco, CA 94111
US

Phone: (415) 957-0101
Fax: (415) 957-5905

Email: charleslmorin@earthlink.net

Questions Intended To Clarify Questions Posed By CFSAN

1. When CFSAN uses the term "biological response modifier" does it mean anything more (or different) than – in this case – whether oral consumption of lactoferrin does, in fact, specifically or nonspecifically increase or decrease immune response?

2. When CFSAN uses the term "adverse effects" does it mean anything more or different than harmful to humans?

3. When CFSAN uses the term " susceptible individual" it suggests there are those more likely to be affected. Does this include everyone or some subset of everyone – all of which have an inherent vulnerability? If the latter, please specifically identify the subset(s).

4. CFSAN questions reference – in the context here in question – the utility of short term and long term animal (preclinical) studies. Given that it is most likely that recombinant human proteins will be immunogenic in animals **and** that the induction of antibody formation in animals is not predictive of a potential for antibody formation in humans **and** that there are currently no reliable animal models and no standard test for predicting the immunogenicity of proteins in humans, what is the value here to be provided by use of preclinical studies to predict immunogenicity?

5. A CFSAN question raises the subject of the availability of clinical evidence. Is this matter being raised merely to obtain, if available, any already existing, relevant, clinical evidence **or** to indicate the possibility of CFSAN requiring "long term" or other clinical trial(s). If the latter, does CFSAN believe that it has legal authority to require any clinical trial in the context of the matter here in question? If so, please identify such authority.

6. What published information and direct evidence is known to CFSAN that indicates that "lactoferrin has been shown to enhance Type 1 T helper (Th1) cell activity, as well as the release of specific cytokines in the gut and systemically following oral administration?

7. What published information and direct evidence leads CFSAN to be concerned – assuming for the moment oral consumption of lactoferrin has an effect on Th1 cells – that such an effect potentially might exacerbate pro-inflammatory responses?

8. What published information and direct evidence leads CFSAN to believe that "Chronic pro-inflammatory Th1 – mediated immune responses might result in the promotion of autoimmune or other inflammatory disorders, in the gut or elsewhere, in individuals predisposed to such"?

9. What published information and direct evidence leads CFSAN to believe that "Even small structural or biological differences between the native and modified form of a particular protein may have a significant impact on that protein's recognition by the immune system and subsequent response"?

10. What published information and direct evidence leads CFSAN to believe that Pharming's exogenous human lactoferrin may evoke a nonallergenic immune response in susceptible individuals that disrupts previous tolerance to endogenous lactoferrin through determinant spreading from alloepitopes, the potential for enhanced pro-inflammatory Th1 responses mentioned above, and increased uptake by antigen-presenting cells via the mannose receptor?

11. When CFSAN uses the term "determinant spreading from alloepitopes"- as it does above - exactly what does it mean?

12. When CFSAN uses the term "adult" should Pharming believe that CFSAN does not mean a human who is merely some specific age (like 18 or 21) but rather means a human with a mature gut and immune system?

13. When CFSAN uses the term "immunological safety" does it mean anything more (or different from) than that oral consumption of lactoferrin (at the maximum level here in question) does not cause adverse, non-allergenic responses by the adaptive immune system?

Fasano, Jeremiah

From: Fasano, Jeremiah
Sent: Thursday, August 17, 2006 12:53 PM
To: 'Charles Morin'
Subject: RE: Details of conference telephone call with CFSAN

Mr. Morin-

We're currently expecting the following people on the call:

-myself
-Supratim Choudhuri, Toxicology Reviewer
-Alison Edwards, Chemistry Reviewer
-Robert Merker, Supervisory Consumer Safety Officer
-Jeanette Glover Glew, Supervisory Consumer Safety Officer
-Ron Chanderban, Supervisory Toxicologist
-Mike DiNovi, Supervisory Chemist
-Bob Martin, Deputy Division Director, DBGNR
-Toni Mattia, Division Director, DBGNR
-Stefano Luccioli, OFAS Medical Officer
-Kathleen Jones, CFSAN Biotechnology Coordinator

Regards-

-Jeremiah Fasano

_____
Jeremiah Fasano, Ph.D.
Consumer Safety Officer
DBGNR/OFAS/CFSAN/FDA

jfasano@cfsan.fda.gov
Phone: 301-436-1173
Fax: 301-436-2964

HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

From: Charles Morin [mailto:charleslmorin@earthlink.net]
Sent: Wednesday, August 16, 2006 3:02 PM
To: Fasano, Jeremiah
Subject: Details of conference telephone call with CFSAN

Jeremiah Fasano, Ph.D.
Consumer Safety Officer

DBGNR/OFAS/CFSAN/FDA
HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

Re: Pharming
rhLF
Details of conference
telephone call with CFSAN

Dear Dr. Fasano,

With regard to the upcoming conference telephone call (on Friday, September 1 at 10:00 a.m. EST) between CFSAN and Pharming, please find below the details I promised.

Call in directions

Call in number: (866) 448-6761
Code: 940357

Pharming participants

Those individuals who will be participating on behalf of Pharming include:

1. Frans de Loos, PhD
   Project Director (rhLF);
2. Sandra van Wetering, PhD
   Scientist, Molecular Biology and Immunogenics;
3. Bertjan Ziere, PhD
   Senior Director, Preclinical;
4. Harrie van Veen
   Muscle Scientist;
5. Erik Doevendans
   Director, Product Registration;
6. Anurag Relan, MD
   Director Corporate Development; and
7. myself.

Please let me know (e-mail is OK) who will be participating on behalf of CFSAN.

Questions for CFSAN

Please find attached a copy of those thirteen questions Pharming intends to ask CFSAN during the teleconference. They are intended to clarify the questions CFSAN has asked Pharming so that Pharming's response can be as on point and complete as possible.

After you have had an opportunity to review the forgoing information if you have questions or need additional information, please let me know.

We look forward to a candid and helpful exchange.

Best regards.

Charles L. Morin
Morin & Associates
388 Market Street, Suite 500
San Francisco, CA 94111
US

Phone: (415) 957-0101
Fax: (415) 957-5905

Email: charleslmorin@earthlink.net

Fasano, Jeremiah

From: Charles Morin [charleslmorin@earthlink.net]
Sent: Wednesday, August 30, 2006 2:22 PM
To: Fasano, Jeremiah
Subject: Details of conference telephone call with CFSAN

Dear Dr. Fasano,

On Wednesday, August 16th, I sent you an e-mail concerning those Pharming individuals who will participate in Friday's conference telephone call. Unfortunately, I somehow got some of the titles wrong; mea culpa!

Following are the individuals and their correct titles:

1. Frans de Loos, PhD
   Director, Business Development Products (rhLF);

2. Sandra van Wetering, PhD
   Scientist, Biochemistry and Immunochemistry;

3. Bertjan Ziere, PhD
   Senior Director, Preclinical;

4. Harry van Veen
   Scientist, Process Development;

5. Erik Doevendans
   Director, Regulatory Affairs (including product registration);

6. Anurag Relan, MD
   Director, Corporate Development; and

7. myself.

Looking forward to a productive meeting!

Best Regards.

Charles L. Morin
Morin & Associates
388 Market Street, Suite 1460
San Francisco, CA 94111
US

Phone: (415) 957-0101
Fax: (415) 957-5905

Email: charleslmorin@earthlink.net

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: September 1, 2006

TIME: 10:00 AM

LOCATION: US 2013

PARTICIPANTS:

FDA

Jeremiah Fasano	HFS-255
Supratim Choudhuri	HFS-255
Anna Brown	HFS-255
Jeanette Glover Glew	HFS-255
Alison Edwards	HFS-255
Robert Martin	HFS-255
Stefano Luccioli	HFS-205
Mary Ditto	HFS-255
Michael DiNovi	HFS-255
Carrie Hendricksen	HFS-255
Ronald Chanderbhan	HFS-255
Robert Merker (by phone)	HFS-255

External

Frans de Loos	Pharming
Sandra van Wetering	Pharming
Bertjan Ziere	Pharming
Harry van Veen	Pharming
Erik Doevendans	Pharming
Anurag Relan	Pharming
Charles Morin	Morin and Associates

SUBJECT: Discussion of GRN 189 status

Members of the Office of Food Additive Safety met with representatives of Pharming, Inc. (Pharming), including Pharming's agent (Mr. Morin) to discuss the status of GRN 189 for the use of recombinant human lactoferrin from bovine milk in various foods consumed by adults. The specific purpose of the meeting was to ensure that our questions, previously conveyed to Pharming in writing, were clearly understood.

FILE COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

Pharming had provided some written questions in response, which were also discussed at this meeting.

FDA staff reviewed some general information about food ingredient safety assessment, as well as questions posed to the notifier in our correspondence of May 17, 2006. We then discussed the specific questions posed by the notifier in their correspondence of August 16, 2006.

The meeting concluded with an agreement by Pharming to consider the issues raised by FDA staff and follow up with the agency.

Jeremiah Fasano

Drafted:HFS-255:10/12/2006
F/T:HFS-255:1/16/2007

To: Pharming rhLF file

From: Charles Morin

Re: Teleconference with CFSAN

On Friday, September 1, 2006 the following personnel from CFSAN[1], i.e.,

1. Brown, Anna Marie (HFS-820)
   Division of Research and Applied Technology (ONPLDS),
2. Chanderban, Ron PhD (HFS-255)
   Supervisory Toxicologist,
3. Choudhuri, Supratim PhD (HFS-255)
   Toxicology Reviewer,
4. Diho, Mary PhD (HFS-255)
   Consumer Safety Officer,
5. DiNovi, Mike PhD (HFS-255)
   Supervisory Chemist,
6. Edwards, Alison PhD (HFS-255)
   Chemistry Reviewer,
7. Fasano, Jeremiah PhD (HFS-255)
   Consumer Safety Officer,
8. Glew, Jeanette Glover (HFS-255)
   Supervisory Consumer Safety Officer,
9. Hendrickson, Carrie PhD (HFS-255)
   Consumer Safety Officer,

[1] Although Toni Mattia, PhD (HFS-255), Division Director and Kathleen Jones, PhD (HFS-013), Biotechnology Coordinator had originally planned to participate in the teleconference, due to conflicts both were unable to attend.

10. Luccioli, Stefano MD (HFS-255)
Medical Officer,
11. Martin, Bob PhD (HFS-255)
Deputy Division Director, and
12. Merker, Robert PhD (HFS-255)
Supervisory Consumer Safety Officer,

met – via telephone – with the following personnel from Pharming, i.e.,

1. Frans De Loos, PhD
Senior Director, Business Development Products (rhLF),
2. Sandra van Wetering, PhD
Scientist, Biochemistry and Immunochemistry,
3. Bertjan Ziere, PhD
Senior Director, Preclinical,
4. Harry van Veen, MSc
Scientist, Process Development,
5. Erik Doevendans, MSc
Director, Regulatory Affairs (including product registration),
6. Anurag Relan, MD
Director, Corporate Development,
7. Mourad Salaheddine, PhD
Senior Director, Animal Health and Production, and
8. myself,

for the sole purpose of clarifying the substance and scope of the concerns/questions posed in CFSAN's email of May 17, 2006. The teleconference began at 10 a.m. (EST) and ended at 10:51 a.m. (EST).

The meeting began with Morin indicating to CFSAN that all seven individuals representing Pharming were on the line. Dr. Fasano – who coordinated and chaired the meeting and who is

coordinating the overall GRAS evaluation of GN 189 – then asked each CFSAN attendee to self identify, which each did.

Dr. Fasano then offered numerous introductory remarks, which included:

1. that the purpose of the meeting was to identify to Pharming certain questions which CFSAN has and to assure that Pharming understands the substance and scope of the questions;

2. that with regard to food ingredient safety evaluation:
   a. the FD&C Act and certain CFR regulations set forth the basic requirements;
   b. safety means that there is a **reasonable certainty** in the minds of qualified experts that a specific substance is not harmful **under the intended conditions of use** (see 21 CFR § 170.3(i));
   c. **general recognition** of safety based upon **scientific procedure** requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive (see 21 CFR § 170.3(b));
   d. the difference between determining safety of a **food additive** and a **GRAS substance** is that in the former instance CFSAN decides what is safe and in the latter instance safety is determined by qualified experts based on publicly available information;
   e. general recognition requires a **consensus** by qualified experts based on common knowledge throughout the pertinent scientific community;
   f. **intended use** is a key consideration both with regard to general recognition and determining safety;
   g. safety is evaluated (per intended use) via daily consumption (i.e., exposure), assuming **lifetime** (i.e., everyday) **exposure**;
   h. potential benefits do not weigh into a safety evaluation at all (no risk/benefit analysis takes place);

i. traditionally, when dealing with essentially small molecules which are intended to have an effect in the food (and not in the consumer), safety could be assessed via use of an evaluation which substantially depended on a set of testing that emanated from, for example, the "Red Book"; however, when the substance may (or is intended to) have an effect in the consumer, then such use may result in the asking of different questions (although asked pursuant to the same reasonable certainty standard);
j. no "blind check list" of standard tests applies (in each case, one must use all tests necessary to demonstrate safety; the actual sets of tests used in any one instance may vary from another instance); and
k. the identity and nature of the substance (within the context of the intend use) is what drives the testing;

3. that with regard to the GRAS program itself:
   a. if a substance and its intended use(s) are not listed in the CFR (as having been approved or GRASed by CFSAN), then one needs to assess whether such substance and use(s) are GRAS;
   b. if a substance has been in broad use for some time then it may be GRAS based on common use in food;
   c. if a substance is – in essence – new, then GRASness must be based on scientific procedures;
   d. while one can independently assess GRASness (and not interface with CFSAN), CFSAN does operate a voluntary program currently known as the GRAS Notification process (which is not an approval process and which emanates from a rule proposed in 1997) which permits one to obtain a written opinion from CFSAN as to whether CFSAN believes one has demonstrated in a given instance GRASness (via the information conveyed to CFSAN in the GN);
   e. CFSAN will respond either that it has "no questions" concerning the petitioner's determination of GRASness or that it believes that the evidence submitted does not demonstrate (or support) a GRAS determination;

f. in any safety evaluation, CFSAN considers all of the information in the GN, as well as all other information that may be available to it;
g. CFSAN expects that a GN will disclose all matters (i.e., the good, the bad and the ugly) that may be pertinent to a GRAS determination, especially any problematical matters;
h. use of an expert GRAS panel can often be helpful, especially in serving as a "proxy" for the expert community as to whether a general consensus exists;
i. an expert panel not only can offer technical advice but also serve to provide insight into what the expert community is thinking;
j. the GN evaluation process is generally not an interactive process (usually the decision rests solely on the information provided in the GN), but in this case, due to the novelty of the substance and its intended use(s), it is productive and helpful to have a certain amount of back and forth (thus CFSAN's questions and this meeting); and
k. GRASness can be time specific, i.e., information can become available which changes a not GRAS status to a GRAS status;

4. with respect to CFSAN's list of questions:
   a. lactoferrin is clearly a molecule with multiple modes of action – some of which appear to be immunomodulatory;
   b. use of the term "biological response modifier" is intended to convey the thought that LF may have – via its motive capability – an effect on one or more of the many components of the human immune system (and not just an on or off effect on the entire system as a whole);
   c. oral consumption of lactoferrin appears to produce effects both in the gut and systemically;
   d. some of these effects appear to be anti-inflammatory, others pro-inflammatory (e.g., Th1 cell activity is enhanced, some cytokines (e.g., IL-18, IFNγ, and IL-12) are increased in the gut and systemically and may be pro-inflammatory or linked to Crohn's disease or arthritis, and natural killer cell activity may be increased;

e. there is some evidence in support of species specific interactions – but no clear picture has emerged;
f. there is very limited evidence from some comparative studies on affinity of receptors in the small intestine (e.g., bLF is a poor competitor for such receptors vis-à-vis hLF in the human gut, whereas the mouse receptor appears to be equally receptive to either bLF or hLF – all of which complicates interpretation of this type of scientific information);
g. there exists some good papers from Pharming concerning characterization of rhLF (as expressed in cows); clearly Pharming's product is human lactoferrin; differences appear to include differences in glycosylation (i.e., different glycans are attached than to rhLF) and differences that exist between the amino acid sequence of rhLF and the different amino acid sequences that exist naturally due to allelic variation in humans (do all of these variations actually exist in nature?);
h. with regard to human exposure, the ADI was calculated based on exposure to infants (the most extreme exposure) which exposure is very large; however, adults and infants vary with respect to the strength and profile of Th1 and Th2 responses which difference may be important (and, thus, needs to be considered); in addition, the structure of the gut differs in the infant (which may have a more "leaky" gut) and adult (e.g., with regard to lymphatic tissues and porosity) and this too needs to be addressed;
i. because we are talking about the maturation of multiple components (i.e., from infant to adult) – both with regard to the gut and the immune system – we are talking about a continuum – with the infant at one end and the adult at the other;
j. with regard to the preclinical studies submitted, they amount to classical tox studies which do not focus on the immunomodulatory endpoints here at issue; thus, if other pertinent information is available, that would be helpful;
k. no real validated preclinical models for evaluating potential immunogenicity currently exist, but there may be animal models for evaluating activity of hLF (is Pharming aware of any such information?);

l. with regard to the clinical studies, they were short term and on small populations and not really related to the immunomodulatory endpoints here at issue; thus, if other pertinent information is available – especially with regard to long term exposure – that would be helpful;

m. some of the effects of LF in infants may be different then in adults; therefore, there may be a problem with using infant data to establish safe levels in adults (do you have any information that could help resolve this situation and indicate whether it is appropriate to use the infant data for establishing the safe consumption level for all consumers – especially as it relates to functionality);

n. it is important to consider – given the immunomodulatory effects of lactoferrin – whether chronic consumption of LF might cause a pro-inflammatory response or exacerbate autoimmune disorders or whether tolerance can be broken by continuous exposure to exogenous lactoferrin (especially via, for example, a Th1 increase, since there are models in which an increase by a single, type molecule leads to an event – such as a Th1 increase (a bias) or alloepitope spreading (i.e., tolerance to one kind of allelic hLF and exposure to another);

o. also consider the difference in glycosylation and the possible increased uptake of lactoferrin by mannose receptors in dendridic cells whereby there can result an increased presentation to the immune system; and

p. generally need information concerning "likelihood" that these concerns will or will not occur;

5. with regard to Pharming's thirteen specific questions pertinent to clarification:

a. in response to Morin's general inquiry concerning the sources of CFSAN's concerns – so that all such sources can be responded to – Dr. Fasano indicated that all such concerns flow from various (not just one seminal article), pertinent, published, scientific articles; thus, an appropriate literature search should reveal all such articles (CFSAN would be willing to examine our combined references list and let us know if these are important references that have been left off); and

b. as to the thirteen questions and CFSAN's remarks they follow (for the sake of clarity, the questions are set forth and immediately followed by CFSAN's comments):

Question 1

When CFSAN uses the term "biological response modifier" does it mean anything more (or different) than – in this case – whether oral consumption of lactoferrin does, in fact, specifically or nonspecifically increase or decrease immune response?

Response

No, provided that there is an understanding that the human immune system consists of numerous components and, thus, one would be looking to see whether oral consumption of lactoferrin – at the levels in question – produces any effect on any one or more of these components (and not merely an on or off effect on the system as a monolithic whole, which it isn't).

Question 2

When CFSAN uses the term "adverse effects" does it mean anything more or different than harmful to humans?

Response

No.

Question 3

When CFSAN uses the term "susceptible individual" it suggests there are those more likely to be affected. Does this include everyone or some subset of everyone – all of which have an inherent vulnerability? If the latter, please specifically identify the subset(s).

Response

CFSAN is particularly concerned about evoking or exacerbating any disorder in individuals that have a genetic predisposition to autoimmune disorders. This may involve 5-10 percent of the general population. These individuals are difficult to identify, whether via screening or otherwise, due to the complex nature of their predisposition.

Question 4

CFSAN questions reference – in the context here in question – the utility of short term and long term animal (preclinical) studies. Given that it is most likely that recombinant human proteins will be immunogenic in animals **and** that the induction of antibody formation in humans **and** that there are currently no reliable animal models and no standard test for predicting the immunogenicity of proteins in humans, what is the value here to be provided by use of preclinical studies to predict immunogenicity?

Response

CFSAN "absolutely agrees." However, CFSAN wonders whether Pharming has any additional information concerning the above-noted effects of lactoferrin.

Question 5

A CFSAN question raises the subject of the availability of clinical evidence. Is this matter being raised merely to obtain, if available, any already existing, relevant, clinical evidence **or** to indicate the possibility of CFSAN requiring "long term" or other clinical trial(s). If the latter, does CFSAN believe that it has legal authority to require any clinical trial in the context of the matter here in question? If so, please identify such authority.

Response

CFSAN – being aware of some relevant, preexisting, clinical information – merely wonders whether Pharming has any such data that speaks to any of the above – noted concerns.

Questions 6 – 10

What published information and direct evidence is known to CFSAN that indicated that "lactoferrin has been shown to enhance Type 1 T helper (Th1) cell activity, as well as the release of specific cytokines in the gut and systemically following oral administration?

What published information and direct evidence leads CFSAN to be concerned – assuming for the moment oral consumption of lactoferrin has an effect on Th1 cells – that such an effect potentially might exacerbate pro-inflammatory responses?

What published information and direct evidence leads CFSAN to believe that "Chronic pro-inflammatory Th1 – mediated immune responses might result in the promotion of autoimmune or other inflammatory disorders, in the gut or elsewhere, in individual predisposed to such"?

What published information and direct evidence leads CFSAN to believe that "Even small structural or biological differences between the native and modified form of a particular protein may have a significant impact on that protein's recognition by the immune system and subsequent response"?

What published information and direct evidence leads CFSAN to believe that Pharming's exogenous human lactoferrin may evoke a nonallergenic immune response in susceptible individuals that disrupts previous tolerance to endogenous lactoferrin through determinant spreading from alloepitopes, the potential for enhanced pro-inflammatory Th1 responses mentioned above, and increased uptake by antigen-presenting cells via the mannose receptor?

Response

Such evidence all emanates from the published literature.

With regard to question 10, CFSAN is not aware of any direct evidence that indicates that lactoferrin will cause the tolerance breakdown referenced above. Its concern arises from certain models – such as a mouse model pertinent to experimental auto-immune encephalomyelitis and

other information (such as that pertinent to the flooding of numerous receptors) – which generally indicate that a substance can cause such a breakdown.

Question 11

When CFSAN uses the term "determinant spreading from alloepitopes" – as it does above – exactly what does it mean?

Response

Since there are in nature various slightly different hLFs resulting from different alleles for hLF which will activate different T cell epitopes, will receptors for lactoferrin be tolerant to all such resulting epitopes or will any one slightly different epitope – which may vary from the epitope associated with endogenous lactoferrin – cause a non-tolerant effect.

Question 12

When CFSAN uses the term "adult" should Pharming believe that CFSAN does not mean a human who is merely some specific age (like 18 or 21) but rather means a human with a mature gut and immune system?

Response

Yes. CFSAN merely means that since there are known differences between the gut and immune systems of an infant and adult (for example, an infant's gut is a leaky one and its immune system is biased towards Th2) and since Pharming has relied on exposure confirmation from infants to calculate an ADI for adults, do the known differences make any difference. If so, perhaps the ADI needs to be recalculated.

Question 13

When CFSAN uses the term "immunological safety" does it mean anything more (or different from) than that oral consumption of lactoferrin (at the maximum level here in question) does not cause adverse, non-allergenic responses by the adaptive immune system?

Response

No. The focus here is not potential allergenicity; rather, it deals with the issues unique to this substance and its intended use.

****

Dr. de Loos indicated that the clarifications and questions are clear and very helpful.

Morin inquired into the schedules of events – including the filing of Pharming's response, CFSAN's review of Pharming's response, and a face-to-face meeting at CFSAN – that need to take place prior to CFSAN making its decision. Someone at CFSAN indicated that CFSAN would be looking for all information to be in the written response and that no meeting would likely take place. However, Morin pointed out that such remark differs from the schedule of events – including a second meeting – set forth in Dr. Fasano's email and discussed with and agreed to with Dr. Mattia before the filing of Pharming's GN. It was determined that such prior agreed to schedule should prevail and that a scientific meeting could prove very useful. Dr. Fasano raised the question of whether such meeting should take place before or after Pharming filed its response.

Finally, Ms. Glew encouraged Pharming to contact Dr. Fasano if it had any follow up questions.

Dr. Fasano encouraged Pharming to tie all the pieces of its response together so as to set forth a unified, compelling story. Also, he encouraged Pharming to carefully tie its response to intended use(s).

Fasano, Jeremiah

From: Charles Morin [charleslmorin@earthlink.net]
Sent: Monday, September 11, 2006 6:41 PM
To: Fasano, Jeremiah
Subject: Details of conference telephone call with CFSAN
Follow Up Flag: Follow up
Flag Status: Completed
Attachments: fasano rhLF_Draft_1[1][1].doc

Dear Dr. Fasano,

Again, thank you for organizing and chairing the meeting concerning CFSAN's questions about Pharming's rhLF. Please find attached a copy of my memorandum of the meeting. Although I do not believe you are required to do so, if you choose to review the memo and find any errors or omissions, I would appreciate learning of same.

Best regards.

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San Francisco, CA 94111
US

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December 22, 2006

Antonia Mattia, PhD (HFS-255)
Director
Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied
Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

ORIGINAL

Re: Pharming Group N.V.
Notice of GRAS exemption for human lactoferrin derived from the milk of transgenic cows expressing a human gene encoding human lactoferrin
GRN No. 000189
Response to request for additional information

Dear Dr. Mattia:

On December 29, 2005 Pharming forwarded a GRAS Notification to CFSAN concerning the above-referenced lactoferrin. Such notice was

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received on January 3, 2006 and filed on January 12, 2006. It has been designated GRN No. 000189.

On May 17, 2006 Dr. Fasano forwarded an email to me indicating that CFSAN had completed its "preliminary evaluation" of GN 189 and was forwarding a list of those concerns/questions considered "significant". Such concerns/questions seek additional specific information. All such concerns/questions involve certain specified aspects of Pharming's hLF's potential to induce any adverse, non-allergic response by the adaptive immune system.

In order to adequately respond to such concerns/questions and to assure that such response is complete, accurate and represents – at a minimum – the consensus view of qualified experts, Pharming (among other things):

1. participated in a telephone conference with CFSAN personnel on September 1, 2006 during which certain clarifications were provided to assure that Pharming fully understood the concerns/questions CFSAN is seeking responses to; and
2. added additional pertinent expertise to its expert panel to help address CFSAN's concerns/questions.

Such latter, total, pertinent expertise – that is, the individuals who are expert in immunology (and related matters) – now include those who have already contributed to Pharming's GN, i.e.,

1. Jeremy H. Brock, ScD, PhD, MSc;
   Senior Research Fellow
   Department of Immunology
   University of Glasgow

(Dr. Brock is a chemist/ micro-biologist/immunologist who is currently a Senior Research Fellow at the University of Glasgow in the Department of Immunology and who has a long and distinguished research career concerned with iron-binding proteins – especially with regards to infection and immunity – which includes the study and publication of much of what is known about lactoferrin) and

2. André H. Penninks, PhD
Senior (Immuno) Toxicologist
Division of Experimental Immunology
Department of Toxicology and Applied Pharmacology
TNO; and
Department of Immunotoxicology
University of Utrecht

(Dr. Penninks is trained in experimental immunology and immunotoxicology, teaches immunotoxicology and cell pathology, and has spent a lifetime researching the effects of compounds, especially food-related substances),

as well as (new members):

1. Charles O. Elson, MD
Professor of Medicine and Microbiology
Vice-Chair for Research
Department of Medicine
Director, Inflammatory Bowel Disease Center
Senior Scientist, Multipurpose Arthritis Center
University of Alabama at Birmingham

(Dr. Elson is trained in medicine and, in particular, gastroenterology (especially mucosal immunology) and has a

long and distinguished research career involving numerous aspects of the gastrointestinal immune system (especially as that relates to IBD and oral tolerance) and is associated with numerous professional associations and committees and editorial boards);

2. Cathryn R. Nagler, PhD
Associate Professor of Pediatrics (Immunology)
Center for Immunology and Inflammatory Disease
Division of Rheumatology, Allergy and Immunology and
Center for the Study of Inflammatory Bowel Disease
Massachusetts General Hospital and Harvard Medical School

(Dr. Nagler is an expert in immunology specializing in various immunology-related research topics, including inflammatory bowel disease, Crohn's disease, ileitis and colitis, immunological related mechanisms and autoimmunity. She has studied the immune response, induction of tolerance, and the consequences of breaking tolerance as induced by dietary agents for years. She is associated with various professional, committee and editorial entities); and

3. Hubertus F.J. Savelkoul, PhD
Professor and Chairman
Department of Cell Biology and Immunology
Wageningen University

(Dr. Savelkoul is a cell biologist/immunologist who specializes in basic and applied immunology, immunoregulation, immune assays, regulatory T cells, cytokines and allergy. In addition to chairing his department, he has published 255 peer-review articles

and serves on various boards, in societies and on editorial boards).

All of these individuals together with numerous Pharming personnel – including those with an expertise in immunology – have worked together for several months to prepare the attached response to CFSAN's questions. It concludes – after considerable discussion of pertinent information – that Pharming and its experts:

> are of the opinion that when all of the pertinent, direct, scientific evidence is considered as a whole, a fair evaluation of it demonstrates to a reasonable certainty that Pharming's exogenous lactoferrin will **not** induce any adverse, non-allergic response by the adaptive immune system and – when combined with the information in Pharming's GN – demonstrates to a reasonable certainty that Pharming's product is not deleterious and generally recognized as safe for human consumption at 100 mg per product serving.

After you and your colleagues have had an adequate opportunity to review the attached information, if you determine that CFSAN has no further questions regarding Pharming's and its expert panel's determination, i.e., that the above-referenced lactoferrin is GRAS under the intended conditions of use, please forward to me an "Agency Response Letter". If, however, you have additional questions (including concerns), please let me know and we can arrange – pursuant to my conversation/agreement with you – to meet with you and your colleagues to discuss such remaining questions/concerns.

Finally, as agreed to at the start of this project, Pharming has not attached copies of all of the references. However, if you need any of them, (and they are all set forth on the reference list), please let me know and I can have them to you by next day (if not before via email).

Thank you in advance for your and your colleagues' efforts.

Sincerely,

Charles L. Morin

cc: Frans de Loos, PhD
Senior Director, Business Development
Pharming Group N.V.

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RESPONSE

Pharming's Response
To CFSAN's Concerns
Regarding
Its Lactoferrin's Potential
To Induce
Any Adverse, Non-allergic Response
By The
Adaptive Immune System

I. Introduction

Before and after the September 1, 2006 teleconference with CFSAN (to assure that Pharming fully understood the concerns of CFSAN that Pharming needed to address with regard to the potential of its exogenous lactoferrin to cause any adverse, non-allergic, immunological response by the adaptive immune system), Pharming did a number of things to assure that this document is fully and professionally responsive. Such things are discussed below in detail (in highlighted subparts).

A. Pharming sought out direct evidence

Dr. Tarantino, Director, Office of Food Additive Safety, has correctly indicated (see, e.g., Tarantino response – dated November 28, 2005 – to a scientific question raised in connection with GRN000049), that any decision (whether made by CFSAN or a notifier) made with regard to a GRAS

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Notification is legally required to be based on "direct evidence" and **not** on mere "hypothesis" or on mere "speculation". Such a requirement is intended to assure that such decision has an adequate informational (including being based – when pertinent – on established science[1]) – and, therefore, legal – basis and is not founded on information which is inherently inadequate due, for example, to its poor quality, inconclusiveness, unscientific nature, or insufficiency. In short, mere conjecture will not suffice as an adequate basis for any decision pertinent to a GRAS determination.

To this end, Pharming – with the assistance of its experts – obtained and reviewed all pertinent, published (including peer-reviewed), scientific evidence which it could locate and which was directly related – regardless of whether pro or con – to the CFSAN concerns to be addressed[2].

B. Pharming consulted with pertinent expertise

Notwithstanding that Pharming has in-house expertise in immunology, Pharming also sought out and utilized additional, qualified expertise in immunology in preparing this response and to serve on its expert panel (as expanded). Such total expertise was used to assure that the information referred to above was as complete as possible and to provide the best possible expert insight into and opinion concerning the meaning of such information.

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[1] General recognition based upon **scientific procedures** – as is the case here – requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive. (21 CFR §170.30(b)).

[2] The list of references initially considered was much longer than the list of references attached to this response. The initial list included all – even remote – possibilities; the final list includes only those determined – after careful review – to be really pertinent.

C. Pharming utilized appropriate, professional opinion

When preparing this response, Pharming required – pursuant to pertinent legal requirements – that certain proof requirements be met. **First**, it required that this response be primarily and adequately based – as the law requires – on publicly available (i.e., both peer-reviewed and published), established, direct, scientific evidence.[3] (21 CFR §170.30(b)). As the law mandates, use of, for example, secret information is – by definition – unacceptable. Of course, other information might be used as secondary, supplemental, corroborating evidence – but not as direct evidence. **Second**, qualified experts (as a result of their pertinent training and expertise) were consulted to assure that all pertinent, direct evidence was identified and considered. Such experts were also utilized to review the pertinent evidence and to determine its meaning and to help formulate and confirm the conclusions that could reasonably and accurately be derived from it. **Third**, Pharming required – as does the law – that such meanings and conclusions emanate from a general recognition, i.e., a consensus, of the experts.[4] (21 USC §321(s)). **Finally**, Pharming required that meanings and conclusions – including with regard to safety – be based (as the law requires) on reasonable certainty.[5] As pertinent regulations make unequivocally clear with regard to the meaning of reasonable certainty:

[3] General recognition of safety through scientific procedures shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and confirmation. (21 CFR §170.30(b)).

[4] The law also requires that the safety factors utilized to evaluate safety also be generally recognized. (21 CFR §170.3(i)(3)).

[5] Safety means that there is a **reasonable certainty** in the minds of competent scientists, i.e., qualified experts, that a specific substance is not harmful under the intended conditions of use. (21 CFR §170.3(i)).

> It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance.

(21 CFR §170.3(i)). Thus, absolute certainty is not required. Nor is proof beyond a reasonable doubt. Nor is one required to prove a negative (which, of course, is impossible). Nor does reasonable certainty amount to mere gut feeling. Rather, reasonable certainty is achieved when qualified experts determine in consensus fashion – pursuant to generally recognized principles of safety – that it is generally recognized among such experts that a fair evaluation of the direct evidence indicates that there is convincing evidence a substance is not harmful under the intended conditions of use.

Following, then, is Pharming's response – including the identification and meaning of, and the conclusions emanating from the pertinent, direct evidence. For the convenience of the reader, such response is set forth in the following format – CFSAN's concerns are individually set forth in the order in which they appear as bullet points in CFSAN's email (with one exception)[6] and then immediately followed by Pharming's response (usually in highlighted subparts). In addition, since Pharming cannot practically know the extent to which any reader of this response may or may not be a qualified expert on the subject matter **and** in the spirit of attempting to make this response as useful to all readers as possible (including, especially, any non-expert), Pharming has, from time to time, throughout the response included some very basic information, especially as it relates to definitions of terms. Pharming trusts that the inclusion of such information will not offend anyone, especially any qualified expert.

[6] CFSAN's concern about the identity of "Pharming's lactoferrin" (i.e., CFSAN's second bullet point) is set forth first since it is important to the response to clearly establish at the outset exactly what substances we are all discussing.

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II. Concerns And Responses

Following are CFSAN's concerns (as set forth in its email of May 17, 2006) and Pharming's responses.

A. CFSAN's concern (its second bullet point):

Pharming's lactoferrin is distinct from the endogenous lactoferrin of individual consumers with respect to expected differences between the amino acid sequence of the exogenous lactoferrin and the polymorphic endogenous lactoferrin alleles present in the general population, and the modification of some species of the exogenous lactoferrin with oligomannose glycans not found on endogenous forms. Even small structural or biological differences between the native and modified form of a particular protein may have a significant impact on that protein's recognition by the immune system and subsequent response. We are concerned that Pharming's exogenous human lactoferrin may evoke a nonallergic immune response in susceptible individuals that disrupts previous tolerance to endogenous lactoferrin through determinant spreading from alloepitopes, the potential for enhanced pro-inflammatory Th1 responses mentioned above, and increased uptake by antigen-presenting cells via the mannose receptor.

B. Pharming's response:

1. The substance In question

At the outset, it seems important to emphasize that this entire discussion should be about the substances which are the focus of Pharming's GRAS Notification – that is, Pharming's and native human

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lactoferrin. Thus, much of what is in the GRAS Notification and this response is only about human lactoferrin (sometimes referred to in this response as hLF). However, Pharming recognizes that human lactoferrin is only one in a broad set of mammalian lactoferrins (including, especially, bovine lactoferrin – sometimes referred to in this response as bLF)[7]; accordingly, Pharming has also – from time to time – included in its response information about other lactoferrins because such information is helpful in establishing a broader context of safety of human lactoferrin.

2. Endogenous and exogenous human lactoferrin

CFSAN has expressed a concern that Pharming's exogenous lactoferrin is structurally significantly different from the polymorphic, endogenous lactoferrin produced naturally by the individual consumers comprising the U.S. population **and** that such structural differences may have a significant impact on the way in which such exogenous lactoferrin is recognized and responded to by the human immune system. Accordingly, what follows is a discussion of the extent to which both lactoferrins are identical, the extent to which both lactoferrins are different, and the importance of any difference.

a. Both lactoferrins are almost entirely the same

As discussed in considerable detail in Pharming's GRAS Notification (please see, e.g., pages 4-5, 12-13, and 32-34), both Pharming's exogenous

[7] To the extent hLF is considered to be a "known biological response modifier" (KBRM) of the human immune system, bLF must also be considered a KBRM. bLF has been determined to be GRAS and at a level equivalent to the level being requested in Pharming's GN.

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lactoferrin ("rhLF") and endogenous lactoferrin ("hLF") are overwhelmingly identical. As a reminder, such identicalness extends to the fact that both lactoferrins:

1. are the same metal-binding, glycoprotein, i.e., hLF (Thomassen, 2005; van Berkel, 2002; Anderson, 1989);
2. have the same amino acid sequence and composition based on the nucleic acid sequence pertinent to the allelic variation seen in the normal population (see, GN, subsection III(C)(1)(e));
3. have the same N-terminal protein sequence (van Berkel, 2002);
4. have the same three-dimensional, protein structure (Thomassen, 2005);
5. are N-glycosylated (van Berkel, 2002);
6. have the same number and location of glycosylation sites (van Veen, 2004);
7. show the same chromatographic profiles upon analytical Mono S analysis (van Berkel, 2002);
8. have the same core-molecular weight (although overall molecular weight slightly differs – Pharming's hLF is slightly lower – due to the differences in the carbohydrate moieties attached to the lactoferrin core) (van Berkel, 2002);
9. show the same tryptic degradation kinetics, i.e., digestibility (van Veen, 2004);
10. have the same iron-binding and iron-release properties (van Berkel, 2002); and
11. are equally effective against experimental infections with multidrug-resistant S. aureus and K. pneumoniae in mice (van Berkel, 2002).

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Thus, from the point of view of considering any difference which actually makes any significant difference, Pharming's lactoferrin is identical to endogenous lactoferrin – except for the difference that is discussed below.

b. The extent to which both lactoferrins are different

CFSAN questions whether Pharming's lactoferrin differs from endogenous lactoferrin in two, different ways. Each is discussed below.

(1.) With regard to respective amino acid sequences

CFSAN first questions whether the amino acid sequence of Pharming's exogenous lactoferrin is structurally different from that of endogenous lactoferrin.[8] As Pharming's GN explains (see pages 12-13) and as discussed below, the two lactoferrins are **not** really different.

Careful comparison of the ten, published, amino acid sequences of endogenous lactoferrin demonstrates that such naturally-occurring sequences may naturally differ from one another in six instances[9], i.e., in amino acid positions 4, 11, 14, 29, 413, and 561. In each such natural instance, the amino acid present is one of only two possibilities. Thus, there exists a well-known and well-documented naturally-occurring **range** of amino acid variation in endogenous lactoferrin.

---

[8] Specifically, CFSAN asserts that "Pharming's lactoferrin is distinct from the endogenous lactoferrin of individual consumers with respect to expected differences between the amino acid sequences of the exogenous lactoferrin and the polymorphic endogenous lactoferrin alleles present in the general population."

[9] Only four of these instances have been scientifically confirmed, i.e., at amino acid positions 4, 11, 29 and 561. (Van Veen, 2004). The other two, i.e., positions 14 and 413, have not yet been confirmed. Pharming's lactoferrin has – with regard to these latter two amino acid positions – the same amino acids as reported in 9 of the 10 above-referenced amino acid sequences. (See pages 12 and 13 of Pharming's GN). It is possible that the latter two differences may not be real.

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Pharming's lactoferrin does **not** differ from but rather exactly duplicates this range, i.e., it is no more different from such range than any one of the ten, known endogenous lactoferrins. In each of the six, above-referenced amino acid positions, Pharming's lactoferrin incorporates exactly the same one of two possible amino acids, as does any one of the ten endogenous lactoferrins. Thus, there exists **no** real difference here between what occurs endogenously and what occurs exogenously. (Please note that with regard to all other amino acid positions, they are all identical).

There is **no** scientific evidence whatsoever that an individual producing one of the above-referenced endogenous lactoferrins reacts – immunologically speaking – differently when exposed to any one of the other above-referenced endogenous lactoferrins. Indeed, extensive and long-term human experience demonstrates just the opposite. For example, infants when consuming mother's milk are exposed – in the vast majority of instances – to an endogenous hLF variety that differs from their own and all without adverse, immunological reaction, probably due to oral tolerance[10] and anergy[11]. It should be noted that these daily exposure levels far exceed the daily exposure level being requested in Pharming's GRAS Notification, i.e., 100 mg/product serving.[12] In addition, patients – of all varieties and ages – who receive transfusions of blood products, e.g., fresh, frozen plasma, are routinely exposed to an endogenous lactoferrin that differs from

[10] The term "**oral tolerance**" is defined as the suppression of systemic humoral and cell-mediated immune responses to an antigen after the oral administration of that antigen, due to anergy of antigen-specific T cells or the production of immunoregulatory mediators such as transforming growth factor-β or interleukin-10. Oral tolerance is a physiological mechanism for preventing immune responses to food antigens. For a thorough discussion of how tolerance is established, etc., see Iweala , 2006 or Faria, 2005.

[11] The term "**anergy**" is defined to mean a state of unresponsiveness to antigenic stimulation. Lymphocytic anergy (also called clonal anergy) is the failure of clones of T or B cells to react to antigen, and this may be a mechanism of maintaining immunologic tolerance to self antigens. In clinical practice, anergy refers to a generalized defect in T cell-dependent cutaneous delayed-type hypersensitivity reactions to common antigens. (Abbas, 2006).

[12] The issue concerning the impact of the differences between the adult and infant gut and immune system are discussed in a later section. (See, section J).

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their own. This lactoferrin – present in the plasma at varying concentrations from 42-202 µg/ml – is predominantly derived from degranulating neutrophils. (Scott, 1989). Moreover, patients who receive such transfusions commonly have ongoing inflammatory reactions, e. g., trauma. Even so, such very numerous, systemic exposures to these exogenous lactoferrins in these patients have not been reported to have led to any known, adverse, immunological event. Oral exposure to human lactoferrin should be even less potentially immunogenic than this type of exposure. Since Pharming's exogenous human lactoferrin only duplicates endogenous, human lactoferrin (with regard to amino acid sequence), one would also expect such exogenous lactoferrin not to induce any adverse, immunological event (as a result of its amino acid sequence). And there in **no** evidence that it could or does.

(2.) With regard to glycosylation

As CFSAN correctly notes, Pharming's exogenous lactoferrin does differ from endogenous lactoferrin with regard to the type of carbohydrate structures that are attached at each of the three glycosylation sites. However, that is the extent of their structural differences (as Pharming's GN discusses at pages 4 and 33), since both lactoferrins incorporate the same number and location of glycosylation sites and both utilize these glycosylation sites in the same fashion. (van Veen, 2004).

With regard then to the specific glycans attached at each of the glycosylation sites, the only glycans attached to the glycosylation sites of natural hLF (from human milk) are N-linked, complex-type glycans. (van Berkel, 2002; Spik, 1982). In addition to the complex, N-linked glycans that

are attached to the endogenous lactoferrin glycosylation sites, Pharming's exogenous lactoferrin also bears oligomannose and/or hybrid-type, N-linked glycans (van Berkel, 2002) – as one would expect, since the distribution and structures of attached glycans is species-, tissue-, cell type- and protein-specific. (James, 1995; Opdenakker, 1993). Furthermore, the complex, N-linked glycans of Pharming's hLF contain N-acetylgalactosamine next to galactose, which is typical for N-linked glycoproteins produced in bovine milk, such as bovine lactoferrin. (Van den Nieuwenhof, 1999; Coddeville, 1992). Finally, the glycans of Pharming's hLF contain less fucose compared to natural hLF. (van Berkel, 2002). However, as a result of crystallography studies, it has been determined that – despite the differences in N-linked glycosylation – the three-dimensional structure of Pharming's hLF and natural hLF are identical. (Thomassen, 2005).

Thus, the attached glycan-related differences then are the only **known** structural differences that exist between endogenous lactoferrin (from human milk) and Pharming's exogenous lactoferrin.

c. The importance of the single difference

At this point, the key question becomes: Does the above-described difference (with regard to exactly what glycan is attached at each of lactoferrin's three glycosylation sites) make any real difference with regard to the ability of Pharming's exogenous lactoferrin to disrupt previous tolerance to endogenous lactoferrin? The direct evidence indicates that it does not.

The mere fact that a difference exists – as here – between two forms of a molecule (one of which naturally occurs – in this case in human milk – and the other of which differs from that naturally-occuring form only with

regard to the kinds of glycans attached at each of the glycosylation sites) does **not** – by itself – amount to direct evidence that such difference **will** affect the latter molecule's potential immunogenicity. For example, please note that endogenous hLF (from human milk) and endogenous hLF (from human neutrophils) also differ in their respective glycosylation patterns. (Derisbourg, 1990). The glycan associated with neutrophilic hLF is not fucosylated – thus, it resembles the glycan pattern of human serum transferrin. (Spik, 1994). However, such difference in glycosylation pattern does **not** affect hLF's function with respect to isoelectric point, stability of the iron-saturated form, rate of clearance, or antigenicity. (Derisbourg, 1990; Moguilevsky, 1985).

And there exists another, even more relevant, well-known example, which demonstrates that consumption of a differently glycosylated lactoferrin does **not** lead to any adverse consequences with regard to immune response or any interruption of tolerance. The example, of course, involves the human consumption of bLF[13] which is long known to be safe (and at levels far exceeding the level here at issue, i.e., 100 mg per product serving) as a result of a long and well-documented history of safe use (and by humans of every variety, including age, race and ethnic background).

Since Pharming's exogenous lactoferrin and bLF are both produced by the bovine mammary gland which determines the type of glycosylation (in this case, a mammalian type of glycosylation) **and** since, similarly to Pharming's hLF, bLF bears oligomannose-type glycans and complex-type

[13] Bovine lactoferrin (bLF) is also – like hLF – an iron-binding glycoprotein (of about 80 kDa) which is similar in structure and function compared to its human homologue. (Nuijens, 1996). The amino acid sequence of bLF (which contains 689 amino acids) shows 69% homology with hLF. (Pierce, 1991). The sequence of bLF contains five possible N-linked glycosylation sites. Four sites, i.e., Asn 233, 368, 476, and 545, are always utilized (Spik, 1994) while the fifth (Asn 281), located in the N-lobe, is glycosylated in about 30% and 15% of the molecules in bovine colostrums and mature milk, respectively. (van Veen, 2002; Wei, 2000; Yoshida, 2000).

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glycans with N-acetylgalactosamine next to galactose (Coddeville, 1992) **and** since historical human consumption of bLF at or exceeding the level of consumption of hLF being proposed in Pharming's GN has not resulted in any reported, adverse, immunological events, one would not expect that consumption of Pharming's exogenous lactoferrin would induce any adverse, immunological event. And there is **no** direct evidence that it does – absolutely none.

Of course, under certain circumstances, it may be possible that a specific difference in glycosylation pattern may make a significant difference in the way in which a specific glycosylated protein will be recognized by the human immune system. (Cobb, 2005). But in the specific instance at hand, the single difference that exists between Pharming's human lactoferrin and endogenous human lactoferrin is hardly a difference which might lead to an adverse effect. Finally and not least importantly, neither Pharming nor its experts are aware of any direct evidence that indicates that there is any protein to which humans are tolerant – including bLF and hLF – which will induce any adverse, immune response merely as a result of a difference in glycosylation. Therefore, it is extremely unlikely that such difference will alter the normal way in which Pharming's hLF is recognized and processed.

3. Determinant spreading

With regard to the potential for determinant spreading from alloepitopes, Pharming and its experts believe that such event is unlikely to occur in the situation involving consumption of Pharming's hLF. An epitope is any molecular structure that can be recognized by the immune system. Epitopes, or the antigen from which they are derived, can be composed of

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protein, carbohydrate, lipid, nucleotide, or a combination thereof. (Abbas, 2006). It is through recognition of foreign, or non-self, epitopes that the immune system can identify and destroy pathogens. T-cells are known to respond only to linear epitopes, i.e., peptide fragments (usually 8 or 20 amino acids in length) digested from the native protein, that are presented in association with major histocompatability complex (MHC) molecules.[14] (Abbas, 2006). An epitope is considered linear, if the target of the immune response is apparent in the series of adjacent amino acids without any requirement for secondary or tertiary structure (folding) as would occur in a native protein. Thus, any discussion of glycosylation is irrelevant to linear peptide fragments which are the only entity which determines T-cell response and, thus, T cell tolerance. Moreover, neither Pharming nor its experts are aware of any evidence showing that a mere difference in glycosylation would alter epitope spreading or that oral tolerance can be disrupted by the introduction of a differently glycosylated version of the same, native protein.

In addition, although single amino acid substitutions have been reported to alter epitope spreading resulting in increased immune response, the amino-acid substitutions in Pharming's lactoferrin mirror those in endogenous lactoferrin in the general population.

Therefore, while it is true that polymorphisms present in Pharming's lactoferrin can differ from those in the endogenous lactoferrin **for a given individual**, such naturally-occurring, amino acid substitutions – which fall within the range of variation that can be found in a normal population – are

[14] A **major histocompatibility complex** molecule is defined to mean a heterodimeric membrane protein encoded in the major histocompatibility complex (MHC) locus that serves as a peptide display molecule for recognition by T lymphocytes. Two structurally distinct types of MHC molecules exist. Class I MHC molecules are present on nucleated cells, bind peptides derived from cytosolic proteins, and are recognized by $CD8^+$ T cells. Class II MHC molecules are restricted largely to professional antigen-presenting cells, macrophages, and B lymphocytes, and bind peptides derived from endocytosed proteins, and are recognized by $CD4^+$ T cells. (Abbas, 2006).

considered not to be immunogenic and, therefore, of little or any risk. Moreover, since T cells recognize only linear peptide epitopes, the concern about the effect, if any, of glycosylation is likely to be irrelevant to the discussion of T cell tolerance.

4. Enhanced pro-inflammatory Th1 response

As indicated in Pharming's GN, there is already a fairly sizeable endogenous lactoferrin production that occurs in humans as a result of human lactoferrin being produced in salivary glands and in intestinal mucosa (and elsewhere). Therefore, ingestion of Pharming's human lactoferrin would simply supplement an already existing endogenous protein. Humans are already tolerant to human lactoferrin and bovine lactoferrin and once mucosal tolerance is established, it is quite difficult to "break" it. For example, a recent study looking at chronic ingestion of foreign proteins by humans (Zivny, 2001) showed that the major response to chronic antigen feeding is T-cell anergy (the major mechanism of tolerance to chronic antigen feeding) even though there are low titers of antibodies to dietary proteins present in secretions and serum, such as ovalbumin, bovine gammaglobulin and soy proteins. These anergic, antigen-specific T cells actively contribute to maintenance of homeostasis in the intestine in the face of massive antigen challenge. (Zivny, 2001). This is why significant consumption of bovine lactoferrin does not result in any breakage of tolerance to bLF and why the same significant consumption of Pharming's lactoferrin will not disrupt any tolerance to endogenous lactoferrin. Indeed, one would expect Pharming's lactoferrin to be even less immunostimulatory and more tolerogenic than bovine lactoferrin.

Finally, Pharming and its experts believe that it is very unlikely that consumption of Pharming's lactoferrin would result in perturbation of intestinal barrier function. (Dickenson, 1998).

5. Increased uptake by antigen-presenting cells via the mannose receptor

Although it may be theoretically possible that the differences in glycosylation between Pharming's lactoferrin and endogenous lactoferrin could result in increased lactoferrin uptake by an antigen presenting cell (APC) via mannose receptors in such a manner that the Th1 response is potentiated, Pharming is not aware of any direct evidence to support this. On the contrary, uptake by a mannose receptor appears to lead to an anti-inflammatory response, rather than a Th1 response. (Chieppa, 2003). Furthermore, the mannose-type glycans present in Pharming's lactoferrin are also present in bovine lactoferrin, which is already GRAS and is not reported to give rise to harmful, Th1 responses. Finally, Pharming is not aware of any direct evidence demonstrating that differential glycosylation alters antigen uptake and potentiates immune reactivity for native proteins. In conclusion, the risk of disruption of previous tolerance to endogenous lactoferrin via any increased uptake of Pharming's lactoferrin by APCs via the mannose receptor is considered remote.

C. CFSAN's concern (its first bullet point):

Lactoferrin has been shown to enhance Type 1 T helper (Th1) cell activity, as well as the release of specific cytokines in the gut and

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systemically following oral administration. We are concerned about lactoferrin's ability, through effects on Th1 cells, to potentially exacerbate pro-inflammatory responses by this arm of the adaptive immune system. Chronic pro-inflammatory Th1-mediated immune responses might result in the promotion of autoimmune or other inflammatory disorders, in the gut or elsewhere, in individuals predisposed to such disorders.

D. Pharming's response:

1. Background information concerning the adaptive immune system and Th1 and Th2 cells

Since the term "adaptive immune system" and, in particular, an understanding of the activities engaged in by Th1 and Th2 cells are critical to CFSAN's concerns and Pharming's response, it seems appropriate – at this specific point – to provide some helpful background information[15] concerning what such term and activities entail – so as to promote common understanding. Since such information is quite basic and, therefore, not particularly helpful to a "qualified expert", it has been set forth in a stand-alone attachment. (**See, Attachment 1**). Notwithstanding its basic nature,

[15] Such information – over 6 pages of it – is not set forth with numerous quotes because almost all of it comes from two, authoritative, sources, i.e., two widely-respected and widely-used medical school textbooks by two widely-respected immunologists – specifically, that by Abbas, Abul K. (at UCSF Medical School) and Lichtman, Andrew H. (at Harvard Medical School) entitled: Basic Immunology: Functions and Disorders of the Immune System, Second Edition, Saunders Elsevier (Phil, PA) (2006) and that by Abbas, Abul K. (at UCSF Medical School) and Lichtman, Andrew H. (at Harvard Medical School) entitled: Cellular and Molecular Immunology, Fifth Edition, Saunders Elsevier (Phil, PA) (2005). These sources were used because they are widely respected and represent the consensus, established viewpoint of qualified experts. The authors are to be credited for the information presented – including that appearing in many of the footnotes (in particular, the definitions).

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however, the information is important and is, therefore, intended as a part of Pharming's response.

2. Enhancement of Th1 cell activity and release of specific cytokines

a. Th1 cell activity

Recent studies inconsistently suggest that lactoferrin has immunoregulatory properties influencing both innate and acquired immunity. (See, review by Fischer, 2006). In particular, it has been suggested that lactoferrin influences T cell maturation, proliferation and differentiation into T-helper 1 (Th1) or T-helper 2 (Th2) cells. Th1 and Th2 cells are two functional subsets of Th- or CD4-positive T cells, whose function depends upon the specific types of cytokines that are generated. (Rafiq, 2000; Mosmann, 1996; Abbas, 1996). CD4-positive Th1 cells produce IFNγ and IL-2, but not IL-4 or IL-5, and drive cellular immunity to attack viruses and other intracellular pathogens; conversely, CD4-positive Th2 cells produce IL-4, IL-5 and IL-13, but not IFNγ or IL-2, and drive humoral immunity that up-regulates antibody production to attack extracellular organisms. Whereas Th1 cells are known as important producers of IFNγ, other cell types are also able to produce IFNγ, including (in particular) NK cells and nonpolarized memory T cells. (Ye, 1995; Biron, 1999). It is important to note that increased IFNγ production does not necessarily reflect increased Th1 cell activity.

The establishment of the Th1/Th2 balance is determined early during immune responses and depends on many factors including antigen structure, the functional status of antigen-presenting cells (APCs), the strength of T cell activation, the presence of cytokines, co-stimulatory signals

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and the microenvironment. (Rafiq, 2000). Both Th1 and Th2 cells negatively cross-regulate the function of one another through their respective cytokines. (Romagnani S, 1994; Maggi, 1992). Furthermore, it should be noted that IL-18, frequently reported as being upregulated upon lactoferrin oral administration, does not skew Th responses towards a Th1 response. Rather, Th1 responses are highly dependent on and stimulated by IL-12. Once Th1 cells are polarized, then IL-18 can act on them to enhance IFNγ production. IL-18 also enhances IFNγ production of NK cells. Thus, production of IL-18 does not correlate to induction of Th1 responses. (Nakanishi, 2001; Okamura, 1998).

Regarding oral administration of lactoferrin, most of the data comes from orally administrated bovine lactoferrin (bLF) rather than human lactoferrin (hLF). Since there is sufficient evidence indicating that both proteins are comparable in structure and function (Baker, 2000; Nuijens, 1996), the effects observed on the immune system as a result of either bLF or hLF administration have been used as model for oral administration of Pharming's hLF.

Review of the available, scientific literature[16] concerning oral administration of lactoferrin indicates that there are contradictory results with respect to the evidence showing that lactoferrin affects proliferation and differentiation of T cells into Th1 and Th2 cells[17]. The induction of either Th1 or Th2 biased immune responses by lactoferrin is complex as the observed

[16] These preclinical and clinical studies – involving **oral administration** of lactoferrrin – include those by Artym (2003), Haversen (2003), Hayes (2005), Iigo (2004), Ishii (2003), Kruzel (2006), Kuhara (2006), Kuhara (2000), Nakajima (1999), Sfeir (2004), Takakura (2006), Takakura (2004), Tanaka (1999), Togawa (2002), Ueno (2006), Varadhachary (2004), Wakabayashi (2006), Wakabayashi (2004), Wakabayashi (2003), Wakabayshi (2002), Wang (2000), Zimecki 2006), Zimecki (2005), Zimecki (1998) and Zimecki (1995). Other, older studies also have been conducted. Their findings are all referred to in one or more of the studies referred to in the above-referenced list. All of the above-referenced studies are fully cited and summarized in **Attachment 2**.

[17] However, please note that Zimecki, et al. reported that lactoferrin inhibits proliferation and cytokine production by Th1 cells – but not Th2 cells. (Zimecki, 1996).

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effects appear to be, at least in part, dependent on the mode of lactoferrin delivery and on whether any ongoing inflammatory or immune response is occurring. (For a review of all pertinent studies, see Fischer, 2006). Based on the available data, Pharming and its expert panel concluded that the evidence for orally administered lactoferrin eliciting a positive $CD4^+$ Th1 biased response is not convincing. This is because most studies suggest a change in Th1 cell activity based on alterations in cytokine levels, in particular IFNγ levels, but did not identify the cell-type responsible for the cytokine production. As mentioned above, increased IFNγ production does not specifically indicate increased Th1 cell activity. More likely, it indicates enhanced NK cell activity. In addition, the information is not convincing because some papers show potential Th1 responses (i.e., IFNγ secretion) within a few days. However, there is a critical time element involved in that it takes weeks for Th1 and Th2 cells to become firmly polarized. (Murphy, 1996). Even in culture, where one can create an optimal environment, it takes at least a week – and usually 2-3 weeks – to generate $CD4^+$ Th1 and Th2 cells. (Perez, 1995).

Finally and not least importantly, even if – for sake of argument – oral consumption of human lactoferrin were to enhance Th1 responses, that would not necessarily be deleterious. First of all, there is nothing in the direct evidence that demonstrates that lactoferrin **given orally** enhances any pathologic Th1 responses[18]. On the contrary, there is evidence from a rat colitis model and other rat and mouse studies that demonstrate that oral

[18] Guillen (2002) did report increased severity of collagen-induced arthritis in transgenic mice expressing human lactoferrin associated with an apparently enhanced Th1 response. However, this conclusion was based on cytokine levels which, as argued elsewhere, do not automatically imply a Th1 response, and the continuous and chronic systemic exposure in this model is quite different from the oral exposure envisaged in humans. In contrast to these results, the same group earlier demonstrated that periarticular injection of hLF in mouse models of autoimmune arthritis and septic arthritis demonstrated significant treatment benefits. (Guillen, 2000).

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consumption of lactoferrin inhibits a pathologic Th1 response via upregulation of IL-10 and inhibition of IFN-γ. (Zimecki, 2006; Takakura, 2006; Togawa, 2002).

b. Release of specific cytokines

With respect to increased release of specific cytokines in the gut and/or systemically following oral administration of lactoferrin, various animal studies generally reported only local changes in the expression/production of both Th1 (e.g., IFNγ, IL-2) and Th2 (e.g.,IL-4, IL-10) cytokines. (Wang, 2000; Kuhara, 2000; Iigo, 2004; Wakabayashi, 2006; Varadhachary, 2004). In addition, various animal studies indicate that oral lactoferrin administration might increase both local and systemic IL-18 levels. (Iigo, 2004; Wakabayashi, 2004; Kuhara, 2006; Hayes, 2005). Pharming's expert panel believes, however, that the effect of IL-18 will occur locally and not systemically. Regarding the systemic levels of IL-18, oral administration of lactoferrin at doses up to 9 gram per day in human adults with solid tumors only resulted in a 15% increase of circulating IL-18, which is considered very low. (Hayes, 2005). More importantly, in this study no serious adverse events were reported and lactoferrin was well-tolerated by all subjects at a dosage of 150 mg/kg/day – which is very significantly higher than the level of maximum daily consumption that Pharming proposes in its GRAS notification. In another study, a transient increase of IL-18 was observed in serum of hepatitis C patients receiving lactoferrin at an oral dosage of 600 milligrams per day for 12 months. (Ishii, 2003). However, the data showed large variation and the observed increase of IL-18 decreased again after 3 months to baseline levels. Taking all such information into account, Pharming and its experts believe that to the extent cytokines are reported to

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be released upon oral administration of lactoferrin, such reports do not indicate a consistent pattern of enhancement.

In conclusion, it is Pharming's and its experts' opinion that, based on the available data, there is not convincing evidence that demonstrates to a reasonable certainty that lactoferrin specifically enhances Th1 responses or can significantly increase systemic cytokine levels over time. In contrast, there is sufficient evidence that lactoferrin enhances innate immune responses in the gut, e.g., by increasing IL-18 production[19] (most likely locally, not systemically) and by increasing NK cell activity, both of which are considered beneficial rather than deleterious. Indeed, there is no direct evidence that increasing innate function is in any way detrimental; rather, such increased function is considered beneficial.

3. Effect of oral administration of lactoferrin on autoimmune or other inflammatory disorders

a. Autoimmunity

T cell responses to antigens are classified on the basis of the amount and kind of cytokines produced. Using this classification, T cell responses in MHC-class-II-restricted autoimmune diseases appear to be predominantly of the Th1 type. (Rosloniec, 2002). Thus, Pharming understands CFSAN's concern to be about whether oral administration of lactoferrin enhances Th1 responses and, thus, whether same could lead to the onset or enhancement of autoimmune diseases. Although the mechanisms of autoimmunity are not

[19] Lactoferrin has been shown to enhance IL-18 production by intestinal epithelial cells, thus enhancing the innate immune response. Human intestinal epithelial cells have been shown to condition human dendritic cells along a non-inflammatory Th2-like pathway, rather than towards Th1 responses. (Rimoldi, 2005).

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yet sufficiently understood, the concern of CFSAN is considered possible but highly unlikely by experts consulted by Pharming. **First** (and, perhaps, most importantly), there is a growing body of scientific evidence that indicates that orally administered lactoferrin significantly inhibits and/or diminishes and/or improves (rather than initiates or enhances) autoimmune diseases. (See, e.g., Kruzel, 2006 (orally administered lactoferrin causes reduction of clinical signs of multiple sclerosis in patients – in parallel to normalization of cytokine production by peripheral blood cells); Zimecki, 2006 (orally administered lactoferrin significantly diminished the clinical symptoms of experimental autoimmune encephelomyelitis in Lewis rats); and Togawa, 2002 (oral administration of lactoferrin significantly reduced colitis in rats))[20]. **Second**, it is very possible that Th1 cells are not even involved in autoimmune diseases. Rather, such diseases may well be induced by the recently discovered T-helper 17 subset. (Hue, 2006; Yen, 2006). **Third**, as already discussed above, the evidence that orally administered lactoferrin elicits a Th1 biased response or potentiates a pre-existing Th1-mediated immune response is considered not well-established. **Fourth**, hLF is naturally expressed in saliva and the gastro-intestinal tract; thus, humans have a significant daily naturally-occurring exposure to hLF[21]. For instance, the intake of lactoferrin from saliva alone is about 20 mg/day. (Tanida, 2003). Consequently, humans are tolerant to hLF. Once oral tolerance has been established, it is very hard to disrupt, even in patients with chronic

[20] See also, two other studies showing similar results, i.e., Guillen, 2000 (which study demonstrated that periarticular injection of hLF in mouse models of autoimmune arthritis and septic arthritis demonstrated significant treatment benefits) and Zimecki, 1995 (which study demonstrated that intraperitoneal injection of bLF in mice significantly inhibited autoimmune hemolytic anemia).

[21] Such daily, natural exposure also emanates from lactoferrin produced and released by or in, for example, mothers' milk, neutrophils and various mucosa. Indeed, as Pharming's GN indicates (at pages 26 and 28), human lactoferrin is virtually ubiquitous throughout the human body.

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stimulation of the immune system. (Zivny, 2001). Moreover, the oral administration of an autoantigen has been shown to be beneficial in the treatment of various experimental, autoimmune diseases and this method of inducing immune non-responsiveness has currently been applied to the prevention and treatment of human autoimmune diseases. (See reviews by Wardrop, 1999; and Sosroseno, 1995). **Fifth**, although there is extensive reporting on the presence of autoantibodies against lactoferrin, there is no evidence that these antibodies play any role in the pathology of autoimmune diseases. There is a large body of scientific literature on antilactoferrin auto-antibodies as a component of antineutrophil cytoplasmic antibodies (ANCA). (See review by Malenica, 2004). In addition, individuals with a wide range of autoimmune conditions have anti-lactoferrin autoantibodies. Despite this large body of scientific literature on these antibodies, there is no evidence showing them to have any role in the etiology of autoimmune disease, and there is a general consensus among qualified experts that they are an epiphenomenon. Furthermore, all individuals possess low but detectable amounts of circulating and mucosal human lactoferrin. Therefore, it is considered highly unlikely that oral administration of human lactoferrin, even to an individual with an ongoing autoimmune disease, would increase autoantibody levels. Even if oral lactoferrin were to increase the level of such antibodies, it would be clinically irrelevant, i.e., unlikely to have any impact on disease pathogenesis.

Anti-lactoferrin autoantibodies have **not** been shown to be involved in the pathogenesis of any disease. In contrast, there is data that autoantibodies in general may help clear and degrade autoantigens, thus reducing T cell sensitization to them. (Mizoguchi, 1997). It should also be pointed out that there have been multiple trials in which autoantigens were

fed to patients with autoimmune diseases to see if this might ameliorate the disease. For example, these trials have fed human insulin to autoimmune diabetics, collagen to rheumatoid arthritis patients, and myelin proteins to patients with multiple sclerosis. These trials have not shown any consistent benefit to the patients; however, there were no deleterious effects from autoantigen feeding and this was done in substantial numbers of individuals. (Faria, 2005).

In conclusion, Pharming and its experts believe that it is highly unlikely that oral consumption of Pharming's lactoferrin at the level here in question would lead to the development or the perpetuation or enhancement of an autoimmune response.

b. Inflammatory disorders

As discussed above, there is sufficient evidence that lactoferrin enhances innate immune responses in the gut. It is CFSAN's concern that this may lead to promotion of inflammatory disorders in the gut. Pharming understands this concern, particularly as it relates to inflammatory bowel disease (IBD), a term which commonly incorporates ulcerative colitis (UC) and Crohn's disease (CD). Both diseases are chronic inflammatory conditions of the gut in which Crohn's disease may affect any part of the gastrointestinal tract, whereas UC mainly affects the colon. In IBD, there appear to be multiple levels of immune responses, including innate, adaptive and regulatory immune responses. There is emerging literature that innate immune defects can contribute to the development of IBD. (See, e.g., Beckwith, 2005; Hugot, 2001; Ogura, 2001). However, neither Pharming nor its experts are aware of any scientific evidence that supports the idea that a

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low-level Th1 response or enhancement of the innate immune response, even on a chronic basis, would be detrimental or trigger IBD. In contrast, lactoferrin has been repeatedly shown to enhance the production of IL-18 by intestinal epithelial cells (see Attachment 2), thereby increasing innate immunity, which is considered beneficial rather than deleterious for susceptible individuals. This beneficial enhancing of innate immunity has been confirmed in a recent open label trial in patients with Crohn's disease who received granulocyte-macrophage colony-stimulating factor (GM-CSF). (Dieckgraefe, 2002). GM-CSF is a cytokine involved in enhancement of the qualitative function of various immune cells, and stimulates the expansion and differentiation of haemopoetic progenetors. (Armitage, 1998). The results showed an enhancement of the intestinal innate immune response resulting in an amelioration of the disease.

Even with regard to individuals who have a "leaky" gut[22], such as can be found in inflammatory bowel disease, orally administered exogenous lactoferrin is simply supplementing large endogenous production of lactoferrin in alimentary secretions. There are low levels of antibodies to various foods in intestinal secretions and serum, but there is no evidence that these have any detrimental effect. There is also no evidence that immunologic reactions to food have any adverse effect in inflammatory bowel disease or that any foods exacerbate inflammatory bowel disease.

In contrast to the concern that orally administered lactoferrin may impact negatively on inflammatory bowel disorders, there is a growing body of scientific evidence – as Zimecki et al. point out – that demonstrates just the opposite, i.e., that orally consumed lactoferrin exhibits "distinct anti-inflammatory properties." (Zimecki, 2006). Such conclusion – the authors

[22] To the extent that the "leaky" gut concept exists – and such concept is **not** generally recognized – it generally refers to the movement of molecules with a molecular weight of less than 1000 daltons.

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indicate – is supported by a growing number of studies incorporating a number of models "including experimentally induced bowel inflammation in rats (Togawa et al., 2002), autoimmune disorders in mice (Zimecki et al., 1995; Guillen et al., 2000), experimental endotoxemia in mice (Kruzel et al., 2002), and inflammatory reactions to Mycobacterium bovis (Zimecki et al., 1994)." (Zimecki, 2006; see also Haverson, 2003 which reported on the anti-inflammatory effects of hLF in an experimental colitis model in mice). In all such models, lactoferrin exhibited significant anti-inflammatory properties.

Moreover, lactoferrin induces TGF-β production which is widely considered an anti-inflammatory cytokine. (Zimecki, 2005; Ward, 2002). Since TGF-β is an anti-inflammatory cytokine associated with the induction of antigen-specific regulatory T cells and such cells produce TGF-β or IL-10, these cells can inhibit the induction of inflammatory responses. In particular, these cytokines suppress IFN-γ production and activity from activated Th1 cells. Lactoferrin can even exhibit strong anti-inflammatory effects in dexamethasone-induced acute colitis in a mouse model. (Haverson, 2003).

In further contrast to suggesting that human lactoferrin – a substance native to humans – might be responsible for either autoimmune or other inflammatory disorders, there is a growing body of scientific evidence showing that **defects** in innate immunity can lead to an abnormal adaptive immune response, some of which are manifest by autoimmune disease. A good example of this is the non-obese diabetic (NOD) mouse, which has some well-defined defects in innate immune responses. Stimulation of the NOD innate system by a variety of means blocks the development of the autoreactive T cell response to islet cells and, thus, prevents diabetes. In inflammatory bowel disease there is emerging literature that innate immune defects can contribute to the development of IBD. (Korzenik, 2006). For

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example, a colitis susceptibility gene has been identified which appears to function by regulating innate immunity. (Beckwith, 2005; Hugot, 2001; Ogura, 2001). In addition (and as mentioned above), there is a trial in which GM-CSF has been administered to patients with Crohn's disease to enhance their innate immunity and, thus, ameliorate their disease. (Diekgraefe, 2002). Thus, autoimmune or chronic inflammatory diseases are more likely to result from deficient innate immune cytokine production or function.

Of course, there is no scientific evidence that suggests – let alone demonstrates – that orally consumed human lactoferrin induces any **deficiency** in any innate immune mechanism. In fact, orally consumed human lactoferrin does just the opposite, i.e., it enhances innate immunity, which is deemed beneficial.

E. CFSAN's concern (its third bullet point):

The notice states that lactoferrin is known for its immunomodulatory properties. However, the preclinical studies presented in the notice do not address the immunomodulatory activities of lactoferrin. What preclinical evidence supports the safety of exogenous lactoferrin for its intended use given its activity as a biological response modifier of the immune system?

F. Pharming's response:

Fortunately, there are numerous, published, preclinical studies and other published information which are pertinent to and evaluate the potential immunogenicity of lactoferrin. Most of the preclinical studies have already been discussed above. Ordinarily, Pharming would review all such studies

at this juncture; however, because almost all[23] have been recently reviewed by Fischer, 2006 (the review was published in June), it need not do so again here. (However, all such studies are cited and summarized in Attachment 2. Such attachment is intended as a part of Pharming's response).

Fischer et al. reviewed 80 different studies and related publications pertinent to lactoferrin's potential immunoregulatory properties – especially as they relate to lactoferrin's ability to regulate Th1 and Th2 responses. After discussing the findings of all such studies and information, Fischer et al. concluded that lactoferrin does **not** induce any adverse, non-allergic immune responses – via either the innate or adaptive immune defense mechanisms. More specifically, the authors concluded that:

1. lactoferrin causes a Th1 polarization in diseases in which the ability to control infection or tumor relies on a strong Th1 response;
2. lactoferrin also reduces the Th1 component to limit excessive inflammatory response; and
3. lactoferrin provides protection against Th1- or Th2-induced diseases, such as autoimmune or allergic diseases, through correction of the Th1/Th2 imbalance.

Thus, consistent with Pharming's and its experts' assessment, Fischer et al. also concluded that the available information indicates that oral consumption of lactoferrin – even at levels exceeding the level of use here at issue – results in only beneficial immunological effects.

Finally, since it is very difficult – despite numerous attempts – to find a mucosal adjuvant among substances likely to be orally consumed, Pharming

[23] There are a few preclinical studies on Pharming's reference list which do not appear on the reference list attached to the Fischer article. Since such studies supply only information like that already reviewed by Fischer, they do not alter the scope of the substance discussed or the conclusions reached by Fischer, et al.

and its experts believe that it is very unlikely that further preclinical testing of Pharming's lactoferrin at the daily level here at issue and even for longer periods of exposure would result in any demonstration that Pharming's lactoferrin is able to induce – via oral consumption – any adverse immunomodulatory effect.

G. CFSAN's concern (its fourth bullet point):

The primate and human studies of oral lactoferrin administration cited in the notice are in small populations for relatively short periods of time. Most of the studies with recombinant human lactoferrin focus on efficacy rather than safety, and many of the human studies involve subjects with pre-existing medical conditions. Where safety endpoints are included, they do not appear relevant to the effects of lactoferrin as a biological response modifier of the immune system. Is there clinical evidence that supports the immunological safety of long-term exogenous lactoferrin administration at the proposed use level in the general population?

H. Pharming's response:

To date, no clinical studies have been performed in healthy volunteers in whom the long-term safety of exogenous applied lactoferrin has been investigated. The primary reason for this is that there is general consensus among experts that hLF has been shown to be so safe – via natural exposures – and at such high doses that no additional safety evaluation is necessary. However, there are a few clinical studies that have investigated the immunological consequences of long term oral administration of

lactoferrin in diseased adults. In one study, 36 patients with chronic hepatitis C and orally administered bLF (600 mg/day for 12 months) showed a transient increase in serum IL-18 levels (pg/ml range) that peaked after 3 months and gradually returned to base-line. (Ishii, 2003). The authors concluded that the effect of bLF administration was limited to 3 months which suggest that prolonged administration results in adaptation. During this study lactoferrin was co-administered with active (live) *Bifidobacterium longum*. These bacteria are known to be potent immunomodulatory "probiotic" bacteria, which makes it impossible in this study design to distinguish the effects of lactoferrin, if any, from those of the bifidobacteria. In another study, 199 subjects were evaluated for safety and efficacy in a randomized, double-blind, placebo-controlled trial using bovine lactoferrin fed orally (1.8 gram/day for 12 weeks) to chronic hepatitis C patients. The authors concluded that bLF treatment was well-tolerated and no serious toxicities were observed. (Ueno, 2006).

Taken together, based on these data and the fact that exogenous administration of lactoferrin would supplement an already substantial amount of endogenous lactoferrin, it is Pharming's and its experts' opinion that it is extremely unlikely that long-term, exogenous lactoferrin administration (at the level here in question) would be detrimental when consumed by either the general population or by individuals with pre-existing conditions.

I. CFSAN's concern (its fifth bullet point):

The notice provides an acceptable daily intake (ADI) based on the maximal consumption of lactoferrin in human milk by infants. The infant immune system and gut are different from that of the adult, for example in

the infant bias towards Th2 responses relative to Th1. Given this, what evidence supports the use of exposure data derived from infants in setting an ADI for adults that takes into account lactoferrin's activity as a biological response modifier of the immune system?

J. Pharming's response:

The infant immune system and gut are, indeed, different from those of an adult – in both instances the infant system is less mature than that of the adult. Both situations should result in an infant being significantly more – not less – vulnerable to the deleterious effects of orally consumed substances than adults who are able to tolerate significantly more. Indeed, infants tolerate very large quantities of bLF with no problem. With regard to those adults who are deemed "predisposed" or "susceptible", please see the discussions set forth above in Sections II B and D.

The notice actually provides not one but various approaches to determine, based on safety parameters, the maximum daily exposure of human lactoferrin that is safe for oral consumption, one of which is indeed based on the maximal consumption of natural lactoferrin in human milk calculated for infants. (GRN000189 subsection V(A)(3)). Other approaches are based on preclinical studies, mainly in rats and rhesus monkeys, and clinical studies in both infants and adults with natural or recombinant human lactoferrin. (See subsections V(A)(6), V(H)(1)(b)(1-3), V(H)(2)(a-b)).

The maximum acceptable daily oral exposure of humans to human lactoferrin found in the evaluation of available data was obtained from infants – which consumption level was equivalent to, at least, 266 mg (and perhaps

as high as 3077) hLF/kg/day which was based on a daily consumption of at least 2 gram hLF.

In this respect, it should be noted that such values have also been obtained for adults, as the highest oral consumption of hLF given to adults was 250 mg hLF/kg/day which corresponded to an oral dosage of 15 gram hLF. (Andersen, 2004). In animals, even higher oral dosages of hLF were tested; the highest dose evaluated and considered safe was 6000 mg/kg/day. (See, section V(H)(1)(b)(1)).

More importantly, the oral safety of Pharmings hLF, as proposed for use as an ingredient in sports and functional foods at 100 mg per product serving, is based on the NOAEL of 2000 mg of Pharming's hLF per kg BW as described in section V(I)(2). Based on this NOAEL, all estimated daily intakes (see section GRN000189 V(I)(2)) are all well under 1/100th of the NOAEL – even at the 90th percentile consumption level. Accordingly, the consumption levels should not pose any safety risk to any consumer. In addition, the safety level is fully supported by the safety levels emanating from the natural exposure to native hLF and from the preclinical and clinical studies of various hLF products (as discussed in subsections V(H)(1)(b)(3) and V(H)(2)(b)), which include both adults and infants.

K. CFSAN's concern (its sixth bullet point):

The notice provides an assessment of the potential allergenicity of Pharming's lactoferrin and states that there is no evidence to date that anti-lactoferrin antibodies are associated with autoimmune pathology. Other than this statement, the notice does not address the potential for adverse non-allergic responses to Pharming's lactoferrin by the adaptive immune system

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as described above. To what extent has Pharming evaluated this risk, and what evidence was used in the evaluation?

L. Pharming's response:

The extent to which Pharming has considered and evaluated the risk, if any, that oral consumption of Pharming's lactoferrin – at the level proposed in its GN – might induce some adverse response by the adaptive immune system has been thoroughly discussed in the preceding responses to bullet points numbers 1-5. Such responses indicate that any such risk is, at worst, very unlikely, and at best, practically non-existent.

III. Conclusion

Pharming and its experts are of the opinion that when all of the pertinent, direct, scientific evidence is considered as a whole – and as specifically discussed above in this response and in Attachments 1 and 2 – a fair evaluation of such evidence demonstrates to a reasonable certainty that Pharming's exogenous lactoferrin will **not** induce any adverse, non-allergic response by the adaptive immune system. This discussion and conclusion – when combined with the information in Pharming's GN – demonstrate to a reasonable certainty, Pharming and its experts believe, that Pharming's human lactoferrin product is not deleterious and generally recognized as safe for human consumption by all individuals at 100 mg per product serving.

IV. Expert Panel's Statement

We, the undersigned, are the qualified experts asked by Pharming to participate in responding to CFSAN's concerns (as set forth in its May 17, 2006 email to Charles L. Morin, Pharming's regulatory counsel). Copies of our curriculum vitae are attached.

In response to Pharming's request, we (among other things):

1. agree to participate as qualified experts;
2. assisted in identifying pertinent information, especially direct scientific evidence;
3. reviewed much of such information;
4. reviewed Pharming's GRAS notification;
5. provided initial assessments of CFSAN's concerns;
6. responded to numerous written and oral questions presented by Pharming;
7. reviewed and commented on non-final drafts of Pharming's response to CFSAN; and
8. reviewed and accepted Pharming's final draft of its response to CFSAN.

Accordingly, that which is in the response as received by CFSAN amounts to not only Pharming's response, but also our expert view.

More specifically, we believe, in summary:

1. that with regard to the amino acid sequence of Pharming's exogenous lactoferrin there is **no** scientific evidence whatsoever that an individual producing one of the above-referenced endogenous lactoferrins reacts – immunologically speaking –

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differently when exposed to any one of the other above-referenced endogenous lactoferrins; consequently, since Pharming's human lactoferrin only duplicates endogenous lactoferrin (i.e., falls within the range of variation that can be found in a normal population), we believe that a reasonable, qualified expert would also expect such exogenous lactoferrin **not** to induce any adverse, immunological event and we believe that there is virtually no risk that it will;

2. that with regard to the specific glycosylation pattern (including any attached oligomannose glycans) of Pharming's exogenous lactoferrin there is no demonstrated reason to believe that such pattern of glycans – either individually or collectively – would induce any adverse immunological event and we think it very unlikely that it would;

3. that with regard to previous tolerance being disrupted via determinant spreading from alloepitopes we are not aware of any evidence showing that a mere difference in glycosylation would alter epitope spreading or that oral tolerance can be disrupted by the introduction of a differently glycosylated version of the same, native protein; therefore, we believe it is very unlikely that Pharming's lactoferrin could or would induce such an event;

4. that with regard to previous tolerance being disrupted via enhanced pro-inflammatory Th1 responses there is nothing in the direct evidence that demonstrates that lactoferrin given orally

– especially at the fairly small level here in question, i.e., 100 mg/product serving – enhances any pathologic Th1 responses and we believe it very unlikely that it could or would;

5. that it is very unlikely that consumption of Pharming's lactoferrin would result in perturbation of intestinal barrier function;

6. that with regard to previous tolerance being disrupted via increased uptake by antigen-presenting cells via the mannose receptor we are not aware of any direct evidence demonstrating that differential glycosylation alters antigen uptake and potentiates immune reactivity for native proteins; thus, we believe the risk of disruption of previous tolerance to endogenous lactoferrin via any increased uptake of Pharming's lactoferrin by APCs via the mannose receptor to be remote;

7. that it is highly unlikely that oral consumption of Pharming's lactoferrin at the level here in question would lead to the development or the perturbation or enhancement of an autoimmune response – either in the general population or in one predisposed to inflammatory disorders;

8. that there is no scientific evidence that immunologic reactions to food have any adverse effect on inflammatory bowel disease or that any foods exacerbate inflammatory bowel disease; thus, we think it very unlikely that oral consumption of Pharming's

lactoferrin at the level here in question could or would lead to the development, perturbation or enhancement of any inflammatory disorder;

9. that there is no scientific evidence that suggests – let alone demonstrates – that orally consumed human lactoferrin induces any deficiency in any innate immune mechanism; in fact, orally consumed human lactoferrin does just the opposite, i.e., it enhances innate immunity, which we deem beneficial;

10. that to the extent oral consumption of lactoferrin at the level here at issue might induce local production of cytokines we believe such production would result in beneficial – not adverse – effects;

11. that the available scientific evidence indicates that oral consumption of lactoferrin – even at levels exceeding the level here at issue (i.e., 100 mg/serving) – results in only beneficial immunological events;

12. that it is very unlikely that further preclinical testing of Pharming's lactoferrin would result in any demonstration that Pharming's lactoferrin is able to induce – via oral consumption (especially at the level here at issue) – any adverse immunomodulatory effect; and

13. that it is extremely unlikely that long-term, exogenous lactoferrin consumption (at the level here in question) would be detrimental when consumed by either the general population or by individuals with pre-existing conditions.

Accordingly, we are of the opinion that when all of the pertinent, direct, scientific evidence is considered as a whole, a fair evaluation of it demonstrates to a reasonable certainty that Pharming's exogenous lactoferrin will not induce any adverse, non-allergic response by the adaptive immune system and – when combined with the information in Pharming's GN – demonstrates to a reasonable certainty that Pharming's product is not deleterious and is generally recognized as safe for human consumption at

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Attachment

1

Background Information[1]
Concerning
The adaptive immune system
and
Th1 and Th2 cells

The **human immune system** is comprised of a collection of specific cells, tissues, and molecules that mediate protection against – under normal conditions – entities (such as pathogens) deemed to be foreign, i.e., non-self. The **immune response** is the coordinated reaction of the above-referenced cells and molecules to such foreign entities.

To the extent that one is focusing – as here – on that part of the immune system that responds to and protects against foreign entities that enter the body through mucosal surfaces – such as those found in the gastrointestinal tract, i.e., the gut – one is focusing on the mucosal immune system. This system is comprised of collections of lymphocytes[2] and antigen-presenting cells[3] in the epithelia[4] and

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[1] Such information – over 6 pages of it – is not set forth with numerous quotes because almost all of it comes from two, authoritative, sources, i.e., two widely-respected and widely-used medical school textbooks by two widely-respected immunologists – specifically, that by Abbas, Abul K. (at UCSF Medical School) and Lichtman, Andrew H. (at Harvard Medical School) entitled: Basic Immunology: Functions and Disorders of the Immune System, Second Edition, Saunders Elsevier (Phil, PA) (2005) and that by Abbas, Abul K. (at UCSF Medical School) and Lichtman, Andrew H. (at Harvard Medical School) entitled: Cellular and Molecular Immunology, Fifth Edition, Saunders Elsevier (Phil, PA) (2005). These sources were used because they are widely respected and represent the consensus, established viewpoint of qualified experts. The authors are to be credited for the information presented – including that appearing in the footnotes.

[2] A **lymphocyte** is a cell type found in the blood, lymphoid tissues, and virtually all organs, that expresses receptors for antigens and mediates immune responses. Lymphocytes include B and T cells (the cells of adaptive immunity) and natural killer (NK) cells (the mediators of some innate immune responses).

[3] An **antigen-presenting cell** (APC) is a specialized cell that displays peptide fragments of protein antigens, in association with major histocompatibility (MHC) molecules on its surface, and activates antigen-specific T cells. In addition to displaying peptide-MHC complexes, APCs must also express

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lamina propria[5] of mucosal surfaces. It includes intra-epithelial lymphocytes – mainly T cells[6] – and organized collections of lymphocytes – often rich in B cells[7] – below mucosal epithelia.

Host defense mechanisms consist of "innate immunity" and "adaptive immunity". **Innate immunity** (a pre-existing or native immunity) mediates the initial protection against infectious, foreign entities (but not noninfectious foreign entities) and relies on mechanisms that exist before infection. These mechanisms are capable of rapid responses and react in essentially the same way to repeat infections. The innate immune system includes – as its first line of defense against invading microbes – epithelial barriers, specialized cells and natural antibiotics present in epithelia and – when a microbe does breach epithelia and enter either a tissue or the circulation – phagocytes[8] (including neutrophils[9] and macrophages[10]),

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costimulatory molecules to optimally activate T lymphocytes. Located in the epithelium, APCs serve to capture antigens and transport them to peripheral lymphoid tissues – such as regional lymph nodes.

[4] The **epithelia** are the coverings of the internal and external surfaces of the human body. They consist of cells joined by small amounts of cementing substances. Epithelium is classified into types on the basis of the number of layers deep it is and the shape of the superficial cells comprising each layer.

[5] The **lamina propria** is the connective tissue coat of a given membrane just deep to the epithelium and basement membrane.

[6] **T cells** are the cell type that mediates cell-mediated immune responses in the adaptive immune system. T lymphocytes mature in the thymus, circulate in the blood, populate secondary lymphoid tissues, and are recruited to peripheral sites of antigen exposure. They express antigen receptors (T cell receptors) that recognize peptide fragments of foreign proteins bound to self major histocompatibility complex molecules. Functional subsets of T lymphocytes include $CD4^+$ helper T cells and $CD8^+$ cytolytic T lymphocytes.

[7] **B cells** are the only cell type capable of producing antibody molecules and, therefore, the central cellular component of humoral immune responses. B lymphocytes, or B cells, develop in the bone marrow, and mature B cells are found mainly in lymphoid follicles in secondary lymphoid tissues, in bone marrow, and in low numbers in the circulation.

[8] **Phagocytic cells** are responsible for ingesting and destroying foreign matter such as microorganisms or debris via a process known as phagocytosis, a process analogous to cellular digestion, usually using lysosomes (a membrane-bound, acidic organelle abundant in phagocytic cells which contains proteolytic enzymes that degrade proteins derived mainly from the extracellular environment and which is involved in

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NK cells[11], several plasma proteins (including those made by the complement system[12]), and cytokines[13] (largely made by mononuclear phagocytes[14]) that regulate and coordinate many of the activities of the cells of innate immunity. Different mechanisms of innate immunity may be specific for molecules produced by different classes of microbes. Finally, in addition to providing early defense

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the class II major histocompatibility complex (MHC) pathway of antigen processing) which carry potent enzymes that digests cell components such as other lipids or proteins. Phagocytes are extremely useful as an initial immune system response to tissue damage.

[9] A **neutrophil** is the most abundant circulating white blood cell, also called a polymorphonuclear leukocyte (PMN), which is recruited to inflammatory sites and is capable of phagocytosing and enzymatically digesting microbes.

[10] A **macrophage** is a tissue-based phagocytic cell derived from blood monocytes, which plays important roles in innate and adaptive immune responses. Macrophages are activated by microbial products, such as endotoxin, by molecules such as CD40 ligand, and by T cell cytokines such as interferon-γ Activated macrophages phagocytose and kill microorganisms, secrete proinflammatory cytokines, and present antigens to helper T cells. Macrophages may assume different morphologic forms in different tissues, including the microglia of the central nervous system, Kupffer cells in the liver, alveolar macrophages in the lung, and osteoclasts in bone.

[11] A **natural killer (NK) cell** is a subset of bone marrow-derived lymphocytes, distinct from B and T cells, that function in innate immune responses to kill microbe-infected cells and to activate phagocytes by secreting interferon-γ. NK cells do not express clonally distributed antigen receptors like immunoglobulin or T cell receptors, and their activation is regulated by a combination of cell surface stimulatory and inhibitory receptors, the latter recognizing self MHC molecules.

[12] The **complement system** is a system of serum and cell surface proteins that interact with one another and other molecules of the immune system to generate important effectors of innate and adaptive immune responses. There are three pathways of complement activation that differ in how they are initiated. The classical pathway is activated by antigen-antibody complexes, the alternative pathway by microbial surfaces, and the lectin pathway by plasma lectins that bind to microbes. Each complement pathway consists of a cascade of proteolytic enzymes that generate inflammatory mediators and opsonins and leads to the formation of a lytic complex that inserts in cell membranes.

[13] **Cytokines** are secreted proteins that function as mediators of immune and inflammatory reactions. In innate immune responses, cytokines are produced by macrophages and NK cells and, in adaptive immune responses, mainly by T lymphocytes.

[14] A **mononuclear phagocyte** is a cell with a common bone marrow lineage whose primary function is phagocytosis. These cells function as antigen-presenting cells in the recognition and activation phases of adaptive immune responses and as effector cells in innate and adaptive immunity. Mononuclear phagocytes circulate in the blood in an incompletely differentiated form called monocytes, and once they settle in tissues they mature into cells called macrophages.

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against infection, innate immune responses enhance adaptive immune responses against infectious agents.

In contrast, **adaptive immunity** (also called acquired immunity) develops more slowly than innate immunity and mediates the later – even more effective – defense against foreign entities. When such entities actually invade tissues (i.e., pass through epithelial barriers), such invasion stimulates adaptive immunity – which, of course, adapts to the presence of any invading, foreign entity. This form of immunity is mediated by lymphocytes[15] and their products, such as antibodies. In contrast to innate immunity, adaptive immunity is characterized by exquisite specificity for distinct macromolecules (including those that are non-infectious) and "memory," which is the ability to respond more vigorously to repeated exposures to the same microbe. Whereas the mechanisms of innate immunity recognize structures shared by classes of microbes, the cells of adaptive immunity, namely lymphocytes, express receptors that specifically recognize different substances produced by microbes as well as non-infectious molecules. These substances are called antigens.[16] Adaptive immune responses are only triggered if microbes or their antigens pass through epithelial barriers and are delivered to lymphoid organs where they can be recognized by lymphocytes.

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[15] These lymphocytes, i.e., T cells and B cells, originate in the tissues of the immune system referred to above. These tissues consist of the generative (or "primary" or "central") lymphoid organs, i.e., the bone marrow and the thymus, in which T and B lymphocytes mature and become competent to respond to antigens, and the peripheral (or "secondary") lymphoid tissues, i.e., the lymph nodes, the spleen, and the mucosal and cutaneous immune systems, in which adaptive immune responses are initiated.

[16] An **antigen** is a molecule that binds to an antibody or a T cell antigen receptor (TCR). Antigens that bind to antibodies include all classes of molecules. TCRs only bind peptide fragments of proteins complexed with major histocompatibility molecules; both the peptide ligand and the native protein from which it is derived are called T cell antigens.

Adaptive immune responses generate mechanisms that are specialized to combat different types of infections. For example, antibodies function to eliminate microbes in extra-cellular fluids, and activated T lymphocytes eliminate microbes living inside cells. Adaptive immune responses often use the cells and molecules of the innate immune system to eliminate microbes, and adaptive immunity functions to greatly enhance the antimicrobial mechanisms of innate immunity. For instance, antibodies (a component of adaptive immunity) bind to microbes, and these coated microbes avidly bind to and activate phagocytes (a component of innate immunity), which ingest and destroy the microbes. By convention the terms "immune system" and "immune response" usually refer to adaptive immunity.

There are two types of adaptive immunity, called "humoral immunity" and "cell-mediated immunity", that are mediated by different cells and molecules and are designed to provide defense against extracellular microbes and intracellular microbes, respectively. **Humoral immunity** is mediated by proteins called antibodies[17], which are produced by cells called B lymphocytes. Antibodies are secreted into the circulation and mucosal fluids, and they neutralize and eliminate microbes and microbial toxins that are present in the blood and in the lumens of mucosal organs, such as the gastrointestinal tract. One of the most important functions of

[17] An **antibody** is a type of glycoprotein molecule, also called immunoglobin (Ig), produced by B lymphocytes, that binds antigens, often with a high degree of specificity and high affinity. The basic structural unit of an antibody is composed of two identical heavy chains and two identical light chains. Amino-terminal variable regions of the heavy and light chains form the antigen binding sites, whereas the carboxy-terminal constant regions of the heavy chains functionally interact with other molecules in the immune system. In any individual, there are millions of different antibodies, each with a unique antigen-binding site. Secreted antibodies perform various effector functions, including neutralizing antigens, activating complement, and promoting phagocytosis and destruction of microbes.

antibodies is to stop microbes that are present at mucosal surfaces and in the blood from gaining access to and colonizing host cells and connective tissues. In this way, antibodies prevent infections from ever getting established. Antibodies do not have access to microbes that live and divide inside infected cells. Defense against such intracellular microbes is called **cell-mediated immunity** because it is mediated by T lymphocytes (or T cells). Some T lymphocytes activate phagocytes to destroy microbes that have been ingested by the phagocytes into phagocytic vesicles. Other T lymphocytes kill any type of host cells that are harboring infectious microbes in the cytoplasm. The antibodies produced by B lymphocytes are designed to specifically recognize extracellular microbial antigens, whereas T lymphocytes recognize antigens produced by intracellular microbes and presented on the cell surface by MHC proteins. Another important difference between B and T lymphocytes is that most T cells recognize only these microbially-derived, protein antigens, whereas antibodies are able to recognize many different types of microbial molecules, including proteins, carbohydrates, and lipids.

With regard to the above-referenced helper T cells[18], they are the functional subset of T lymphocytes whose main effector functions are to activate macrophages in cell-mediated immune responses and promote B cell antibody production in humoral immune responses. These effector functions are mediated by secreted cytokines and by T cell CD40 ligand binding to macrophage or B cell CD40. Most helper T cells express the CD4 molecule.

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[18] **$CD4^+$ T** cells are called helper T cells because they help B lymphocytes to produce antibodies and help phagocytes to destroy microbes.

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Finally, two functional subsets of helper T cells are of key importance to CFSAN's concerns and Pharming's response. The first, **TH1 cells**, secrete a particular set of cytokines (discussed in subsection II D 2) and principally function to stimulate phagocyte-mediated defense against infections, especially with intracellular microbes. The second, **TH2 cells**, secrete a particular set of cytokines (discussed in subsection II D 2) and principally function to stimulate IgE and eosinophil/mast cell-medicated immune reactions and to down-regulate Th1 responses.

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Attachment

2

Preclinical And Clinical Studies
Concerning
Oral Administration Of Lactoferrin
Which Are Pertinent
To The Concerns Raised By CFSAN
Concerning Pharming's GRAS Notification

Artym, J., Zimecki, M., Paprocka, M., and Kruzel, M.L. Orally administered lactoferrin restores humoral immune response in immunocompromised mice. Immunol Lett. 89, 9 (2003).

Artym et al. reported that in cyclophosphamide immunocompromised mice (specifically with inhibited humoral immune responses) oral administration of bLF (at 0.5 percent of drinking water for five weeks) was shown to restore the humoral response, as measured by elevated T (including $CD4^+$ T cells) and B cells, macrophage content, and the proliferative response of splenocytes.

Haversen, L. A., Baltzer, L., Dolphin, G., Hanson, L. A., and Mattsby-Baltzer, I. Anti-inflammatory activities of human lactoferrin in acute dextran sulphate-induced colitis in mice. Scand. J. Immunol. 57, 2 (2003).

Haversen et al. investigated the anti-inflammatory effects of orally administered human lactoferrin in the course of experimental colitis induced in mice by giving five percent dextran sulfate in the drinking water. Mice were given hLF twice a day at a dose of 2 mg until the end of the experiment, i.e., the killing of the mice after 2 or 7 days of DX exposure. The findings reported by the authors were as follows:

1. significantly delayed and partly reduced appearance of occult blood in the stool and macroscopic rectal bleeding;
2. significantly less pronounced shortening of the colon;
3. significantly diminished IL-1β levels in the blood;
4. a significantly lower crypt score pertinent to the distal

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part of the colon;

5. significantly reduced numbers of CD4 cells, F4/AD positive macrophages and TNF-α-producing cells (detected via immunohistochemistry) in the distal colon; and
6. a reduction of IL-10 producing cells in the middle colonic submucosa.

Based on these findings, the authors concluded that hLF has significant anti-inflammatory effects in the colon.

Hayes, T.G., Falchook, G.F., Varadhachary, G.R., Smith, D.P., Davis, L.D., Dhingra, H.M, Hayes, B.P., and Varadhachary, A. Phase 1 trial of oral lactoferrin alpha in refractory solid tumors. Invest New Drugs (2005).

Hayes et al. investigated the immunomodulatory effect of rhLF in ten patients with progressive advanced solid tumors who had failed conventional chemotherapy. The patients were orally administered rhLF in doses ranging from 1.5 to 9.0 grams per day (i.e., approximately 25-150 mg/kg/day), using a 2 weeks on, 2 weeks off schedule. Following such administration, significant levels of rhLF were not detected in circulation; however, a "small" i.e., 15 percent, but statistically significant, increase in circulating IL-18 was observed. Such increase was not dose dependent and "far smaller" than the increase in intestinal IL-18. The authors indicated that these concentrations are consistent with other studies showing much lower levels of circulating IL-18 levels as compared to the increase in levels of intestinal IL-18.

This study contains **no** data indicating any effect on Th1 cells.

Iigo, M., Shimamura, M., Matsuda, E., Fujita, K., Nomoto, H., Satoh, J., Kojima, S., Alexander, D.B., Moore, M.A., and Tsuda, H. Orally administered bovine lactoferrin induces caspase-1 and interleukin-18 in the mouse intestinal mucosa: a possible explanation for inhibition of carcinogenesis and metastasis. Cytokine 25, 36 (2004).

Iigo et al. studied the effects of orally administered bLF to mice – either as a single dose (300 mg/kg) or as the same single dose for seven consecutive days. The authors concluded that bLF:

1. "markedly elevated" IL-18 levels in the small intestine;
2. "significantly increased" caspase-1 activity and IFN-γ in the small intestine (but not IFN-γ in serum); and
3. "markedly enhanced" response caspase-1 activity and IL-18 levels in peritoneal macrophages.

However, the authors did not identify the source of the IFN-γ. Finally, the authors indicated that bLF's effects seemed to be confined to the intestinal mucosa.

Ishii, K., Takamura, N., Shinohara, M., Wakui, N., Shin, H., Sumino, Y., Ohmoto, Y., Teraguchi, S., and Yamauchi, K. Long-term follow-up of chronic hepatitis C patients treated with oral lactoferrin for 12 months. Hepatol Res. 25, 226 (2003).

Ishii et al. reported in a fairly recent, "long term" study in 36 patients with chronic hepatitis C that orally administered bLF (600 mg/day for 12 months) showed a rapid, significant increase in serum IL-18 levels (in the pg/ml range) that peaked after 3 months and gradually returned to base-line (even though significant oral administration of bLF continued for another 9 months). No such effect occurred in the control group. No significant increases in IL-10 levels were observed. Also, the number of Th1 cells in the peripheral blood increased, although not significantly, and returned to baseline after 3 months.

Kruzel, M.L., Artym, J., Chodaczek, G., Kocieba, M., Kochanowska, I., Kruzel, T. and Zimecki, M. Effects of lactoferrin on stress-related immune dysfunction in mice and humans. Proceedings of the 4th International Whey Conference, Chicago, pp. 121–132 (2006).

Kruzel et al. (the same group otherwise known as Zimecki et al.) investigated the effects of orally administered bovine lactoferrin (bLF) on the cellular and humoral immune responses in mice subjected to immobilization stress (IS). Here, the

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authors demonstrated that long-term IS (5 d) induced significant suppression of cellular and humoral immune responses in CBA mice. This suppression was attenuated by bLF administered to mice in drinking water as determined by antibody-forming cells and delayed-type hypersensitivity (DTH). Conversely, bLF lowered elevated DTH responses in mice exposed to short-term IS (5 h only) on the day of elicitation of the DTH reaction. To evaluate the effect of bLF on stress-related autoimmune disorders, the production of selected cytokines in multiple sclerosis (MS) patients was evaluated. Treatment of MS patients (n = 6) with bLF (50 mg/d) administered orally for 7 consecutive days resulted in a significant increase in inducible interleukin 10 production by leukocyte cultures stimulated with lipopolysaccharide and phytohemagglutinin compared with reduced responses in placebo-treated control patients with fatigue syndrome. Conversely, interferon gamma production was reduced 4-fold in MS patients with minor changes in the placebo group. Together, these findings, the authors indicated, revealed that the medical benefit of bLF in stress-related and autoimmune disorders may be in part due to differential regulation of interleukin 10 and interferon gamma production.

Kuhara, T., Yamauchi, K., Tamura, Y., and Okamura, H. Oral administration of lactoferrin increases NK cell activity in mice via increased production of IL-18 and type I IFN in the small intestine. J Interferon Cytokine Res 26, 489 (2006).

Kuhara et al. investigated the immunomodulatory effects of bLF (suspended in saline) orally administered (via a gastric tube) to mice for 7 days at a dose of 30, 100, 300, or 1000 mg/kg body weight/day. The findings reported by the authors included:

1. a significant increase (in a dose-dependent manner) in the percentage of leukocytes that were NK cells in both peripheral blood and the spleen;
2. enhanced IFN-γ production by NK cells;
3. increased NK cell migration (after using intraperitoneal injection of poly (I:C) to in-

duce NK cell trafficking into the peritoneum);
4. an increase of IL-18 in the portal circulation; and
5. increased expression of IFN-α and IFN-β in Payer's patches and mesenteric lymph nodes.

The authors also reported that bLF – in IL-18 knockout mice – did not increase the numbers of NK cells, although NK cell cytotoxic activity and poly(I:C)-induced trafficking activity were enhanced. Collectively, these results – the authors concluded – demonstrate that "orally administered bLF stimulates intestine-associated immune functions."

Kuhara, T., Iigo, M., Itoh, T., Ushida, Y., Sekine, K., Terada, N., Okamura, H., and Tstuda, H. Orally administered lactoferrin exerts an antimestastic effect and enhances production of IL-18 in the intestinal epithelium. Nutr. Cancer 38, 192 (2000).

Kuhara et al. studied the effects of orally administered bLF to mice (in a tumor metastasis model) at doses of 100 or 300 mg/kg/day for seven consecutive days. The authors reported finding:

1. "augmented" $CD4^+$, $CD8^+$, and asialoGM1$^+$ cells in the spleen and peripheral blood;
2. "markedly increased" $CD4^+$ and $CD8^+$ cells in the small intestine epithelium;
3. "enhanced production" of IL-18 in the intestinal epithelial cells; and
4. "significantly augmented" NK activity.

Administration of bLF did not increase serum levels of bLF; thus, the authors indicated that bLF probably has its effect in the gastrointestinal tract. The authors also concluded that bLF did not have any "direct effects" in the study. Finally, the authors indicated that bLF may enhance "mucosal immunity".

This study did not measure any Th1 responses.

Nakajima, M., Iwamoto, H., Shirasawa, T., Miyauchi, H., Takatsu, Z., Yamazaki, N., Teraguchi, S., and Hayasawa, H. Oral administration of lactoferrin enhances the productions of IFN-γ and IL-10 in spleen cells cultured with concanavalin A or lipopolysaccharide. Biomed.

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Res., 20, 27 (1999).

Nakajima et al. investigated the effects of orally administered bovine lactoferrin (bLF) on cytokine productions of spleen cells. Spleen cells from BALB/c mice (female only) were cultured with concanavalin A 1 to 4 days after the oral administration of bLF (at 3 mg/mouse). Concentrations of IFN-γ in the supernatants were enhanced by bLF feeding, while those of IL-4 were not. In contrast to bLF, an oral administration of β-lactoglobulin or pepsin hydrolysate of bLF failed to show the enhancement. When stimulated by anti-CD3 antibody, IFN-γ production by CD4+ T cells fractionated from spleen cells was augmented by the oral administration of bLF. On the other hand, in response to lipopolysaccharide (LPS), spleen cells from the mice fed bLF secreted enhanced levels of IL-10 – "IL-10 being known to be an immuno-suppressive cytokine secreted by Th2 cells or monocytes which inhibits other cytokine productions such as IFN-γ, IL-2, TNF-α, IL-1 and IL-8, and suppresses the antigen-presenting capacity of monocytes through down-regulation of class II molecules". Levels of IFN-γ secretion from the spleen cells were not affected. While IL-10 production in response to LPS by CD11c+ cells from spleen cells was promoted by bLF feeding, the cytokine secretion from CD11b+ cells was not affected.

Sfeir, R.M., Dubarry, M., Boyaka, P.N., Rautureau, M., and Tome, D. The Mode of Oral Bovine Lactoferrin Administration Influences Mucosal and Systemic Immune Responses in Mice. J. Nutr. 134, 403 (2004).

Sfier et al. reported that oral administration (i.e., via gastric intubation, single buccal doses, or continuous doses of bLF in the diet, but **not** via addition of bLF to the drinking water) of bLF (100 mg/day for 4 weeks) to mice resulted in a biased mucosal and systemic T-cell response towards a Th2 response. In contrast, the "less natural gastric intubation" also promoted Th1-type responses.

Unfortunately, all that was measured in this study – with regard to Th1- and Th2-type cytokines – were the cytokines

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themselves in spleen cells. The $CD4^+$ T cells were not isolated and studied separately; thus, one cannot conclude from the information provided by this study whether – in fact – there were either $CD4^+$ Th1 and/or $CD4^+$ Th2 responses generated.

Takakura, N., Wakabayashi, H. Yamauchi, K. and Takase, M. Influences of orally administered lactoferrin on IFN-γ and IL-10 production by intestinal intraepithelial lymphocytes and mesenteric lymph-node cells. Biochem. Cell Biol. 84, 363 (2006).

Takakura et al. investigated the influences of orally administered bovine lactoferrin (bLF) on cytokine production by intestinal intraepithelial lymphocytes (IEL) and mesenteric lymph-node (MLN) cells, especially T cells. Bovine lactoferrin or bovine serum albumin (control) was administered to mice (via intragastric intubation at 500 mg/kg/day) once daily for 3 d. After 24 h from the last administration, IEL of the jejunum and ileum and MLN cells were isolated. These cells were cultured with and without the anti-T-cell-receptor antibody, and then the culture supernatants were assayed for cytokines with ELISA. Oral bLF did not affect the ratio of T cell subpopulations in IEL and MLN; however, bLF enhanced both interferon IFN-γ and IL-10 production by unstimulated IEL and by IEL stimulated with the αβ T cell receptor but not with the γδ T cell receptor. bLF also enhanced both IFN-γ and IL-10 production by stimulated and unstimulated MLN cells. The production level of IFN-γ by MLN cells was correlated with that of IL-10. The authors indicated that these results suggest that oral bLF enhances the production of both Th1-type and Th2/Tr-type cytokines in the small intestine of healthy animals.

Takakura, N., H. Wakabayashi, H. Ishibashi, K. Yamauchi, S. Teraguchi, Y. Tamura, H. Yamaguchi, and Abe, S. Effect of orally administered bovine lactoferrin on the immune response in the oral candidiasis murine model. J Med Microbiol 53, 495 (2004).

Takakura et al. investigated the effect of orally administered bLF (in drinking water – equivalent to 0.5 g/kg BW/day – administered continuously from day 1 before the

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infection) to mice "immunosuppressed with prednisolone 1 day before and 3 days after" being infected with Candida. The study lasted 7 days. The authors reported the following results:

1. bLF prevented the reduction in the numbers of peripheral blood leukocytes (PBLs) on day 1 and cervical lymph node (CLN) cells on days 1, 5 and 6;
2. increased production of IFN-γ and TNF-α by CLN cells; and
3. a significant increase in the production of IFN-γ and TNF-α on day 6.

The authors concluded that these results may indicate enhancement of the number of leukocytes and their cytokine responses in regional lymph nodes.

Tanaka, K., Ikeda, M., Nozaki, A., Kato, N., Tsuda, H., Saito, S., and Sekihara, H. Lactoferrin inhibits hepatitis C virus viremia in patients with chronic hepatitis C: a pilot study. Jpn. J. Cancer Res. 90, 367 (1999).

Tanaka et al., having recently found that in vitro administered bLF effectively prevented hepatitis C virus (HCV) infection in cultured human hepatocytes (PH5CH8), in this report investigated (in a "pilot study") the hypothesis that bLF inhibits HCV viremia in patients with chronic hepatitis C. Eleven patients with chronic hepatitis C received an 8-week course of bovine lactoferrin (1.8 or 3.6 g/day). At the end of lactoferrin treatment, a decrease in serum alanine transaminase and HCV RNA concentrations was apparent in 3 (75%) of 4 patients with low pretreatment serum concentrations of HCV RNA. However, 7 patients with high pretreatment concentrations showed no significant changes in these indices. (See, **Ueno, 2006** for a summary of the published report of the full clinical trial conducted after this pilot study).

Togawa, J., Nagase, H., Tanaka, K., Inamori, M., Umezawa, T., Nakajima, A., Naito, M., Sato, S., Saito, T., and Sekihara, H. Lactoferrin reduces colitis in rats via modulation of the immune

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system and correction of cytokine imbalance. Am. J. Physiol. Gastrointest. Liver Physiol. 283, G187 (2002).

Togawa et al. reported that, in a TNBS-induced colitis model in rats, it has been shown that oral administration of bLF reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. More specifically, oral administration of bLF (at 200 mg/kg/day for, essentially, a lifetime) resulted in decreased levels of the pro-inflammatory cytokines TNF-α, IL-18 and IL-6, whereas the anti-inflammatory cytokines IL-4 and Il-10 levels – both cytokines known to promote a Th2 response – were significantly increased. The authors concluded that bLF exerts a protective i.e., anti-inflammatory, effect against colitis in rats.

Ueno, H., Sato, T., Yamamoto, S., Tanaka, K., Ohkawa, S., Takagi, H., Yokosuka, O., Furuse, J., Saito, H., Sawaki, A., Kasugai, H., Osaki, Y., Fujiyama, S., Sato, K., Wakabayashi, K. and Okusaka, T. Randomized, double-blind, placebo-controlled trial of bovine lactoferrin in patients with chronic hepatitis C. Cancer Sci 971105 (2006).

Ueno et al. (including Tanaka, K.) investigated the efficacy of orally administered bovine lactoferrin (bLF) in patients with chronic hepatitis C. The patients with chronic hepatitis C randomly received either oral bLF at a dose of 1.8 g daily for 12 weeks, or an oral placebo. The primary endpoint was the virologic response, defined as a 50% or greater decrease in serum HCV RNA level at 12 weeks compared with the baseline. The secondary endpoint was the biochemical response, which was defined as a 50% or greater decrease in the serum alanine aminotransferase (ALT) level at 12 weeks compared with the baseline. The study involved 199 subjects/patients. bLF treatment was well-tolerated and no serious toxicities were observed. A virologic response was achieved in 14 of 97 patients (14.4%) in the bLF group, and 19 of 101 (18.8%) in the placebo group. There was no significant difference in virologic response rates between the two groups (−4.4%, 95% confidence interval −14.8, 6.1). In addition, bLF

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intake did not have any favorable effect on the serum ALT level. The virologic responses were not different between two groups in any subgroup analysis. The authors concluded that orally administered bLF does not demonstrate any significant efficacy in patients with chronic hepatitis C.

Varadhachary, A., Wolf, J.S., Petrak, K., O'Malley Jr., B.W., Spadaro, M., Curcio, C., Forni, G., and Pericle, F. Oral lactoferrin inhibits growth of established tumors and potentiates conventional chemotherapy. Int. J. Cancer 111, 398 (2004).

Varadhachary et al. demonstrated – in both tumor-bearing and normal (i.e., naïve) mice – that oral administration of rhLF (at 300 mg/kg /day) for three consecutive days resulted in (1.) significantly increased production of the active form of IL-18 in the intestinal tract mucosa, i.e., in the intestinal epithelial cells (consistent with the fact, the authors noted, that the gut is the primary site of lactoferrin activity since lactoferrin is not absorbed (systemic bioavailability < 1%) and the lactoferrin receptors are present in the gut epithelium), (2.) systemic NK cell activation, i.e., enhanced cytotoxicity of splenic NK cells, and (3.) a significant increase in the relative percentage of $CD8^+$ and $CD3^+$/ $CD8^+$ cells.

Please note that rhLF also was reported to have increased IL-18 in nude mice that lacked T cells. Please also note that the authors measured for but found no statistically significant increase in $CD4^+$ T cells. This study contains **no** data indicating any effect on Th1 cells.

Finally, this study also incorporated use of bovine lactoferrin (as a test article) exactly as rhLF had been used. The results for bLF were exactly the same as for rhLF.

Wakabayashi, H., Takakura, N., Yamauchi, K., and Tamura, Y. Modulation of Immunity-Related Gene Expression in Small Intestines of Mice by Oral Administration of Lactoferrin. Clinical and Vaccine Immunology 13, 239 (2006).

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Wakabayashi et al. initially confirmed an immunomodulatory effect of bLF by observing changes in the number of cells in the leukocyte subsets in the peripheral blood and spleens of mice 1 day after oral administration (via gavage) of bLF (in solution equivalent to 2.5g/kg BW/day for 1 day). Then the authors developed a quantitative reverse transcription-PCR method for 20 immunity-related genes of antimicrobial proteins, pattern recognition receptors, cytokines, and lymphocyte mobilization-related proteins, and assessed the expression of these genes in the small intestines of mice 2 hours after administration of water, bovine serum albumin (BSA), or bLF. Expression of the bLF gene was lower in mice administered bLF than in mice administered water or BSA, implying a negative-feedback control. Expression of gamma interferon (IFN-γ) and interleukin-10 (IL-10) was lower in both BSA- and bLF-administered mice than in water administered mice, suggesting a nonspecific effect of protein ingestion. Expression of NOD2, IFN-β, and IL-12p40 was higher with bLF administration than with water or BSA administration. The expression levels of these three genes were correlated. This study indicated, the authors concluded, that oral administration of bLF modulates the small intestinal expression of genes closely related to the host defense in a specific or a nonspecific manner.

Wakabayashi, H., Kurokawa, M., Shin, K., Teraguchi, S., Tamura, Y., and Shiraki, K. Oral lactoferrin prevents body weight loss and increases cytokine responses during herpes simplex virus type 1 infection of mice. BioSci. Biotechnol. Biochem. 68, 537 (2004).

Wakabayashi et al. reported that oral administration of bLF (a 1.5 percent solution fed ad libitum) for 10 days to mice then exposed to herpes simplex virus-1 infection resulted in significantly increased serum IL-18 levels (but only on day 9), whereas no increase was observed in non-infected animals. bLF treatment also resulted in significantly increased splenocyte production of IL-12 and IFN-γ – but only on day 5; however, the source of the cytokines was not identified. bLF

consumption did not significantly affect levels of RANTES, MIP-1a, MIP-2, TNA-α or IL-10.

This study provides no direct demonstration of any Th1 response after viral infection.

Wakabayashi, H., Takakura, N., Teraguchi, S., and Tamura, Y., Lactoferrin feeding augments peritoneal macrophage activities in mice intraperitoneally injected with inactivated Candida albicans. Microbiol. Immunol. 47, 37 (2003).

Wakabayashi et al. investigated the effect on peritoneal macrophage activities of bLF orally administered (1.5%) in water (ad libitum) to mice intraperitoneally injected with inactivated Candida albicans as a priming agent generating a local inflammation. Oral administration lasted for 14 days post priming. The authors reported that bLF administration:

1. slightly increased the number of peritoneal exudate cells;
2. significantly enhanced the production of superoxide anion and nitric oxide by peritoneal macrophages at day 7; and
3. significantly enhanced IFN-γ at day 9 and IL-12 at day day 5, but not TNF-α or IL-10.

The authors concluded that the results indicated that bLF augmented the activities of macrophages and such effects may be related to enhanced cytokine levels.

Wakabayashi, H., Takakura, N., Yamauchi, K., Teraguchi, S., Uchida, K., Yamaguchi, H. and Tamura, Y. Effect of lactoferrin feeding on the host antifungal response in guinea-pigs infected or immunised with Trichophyton mentagrophytes. J Med Microbiol 51,844 (2002).

Wakabayashi et al. investigated the effect of bLF orally (gavage) administered (twice a day at a daily bLF dose of 2.5g/kg BW) on, among other end points, the immune system in guinea pigs infected or immunized with Tryptophyton mentagrophytes. Animals received bLF for either 1 or 2 weeks. Among the results, the authors reported that:

1. bLF administration caused no significant effects on either phagocytic activity or reactive oxygen production of blood neutrophil polymorpho-nuclear leukocytes in either infected or noninfected animals; and
2. in the bromo-deoxyuridine incorporation assay, the stimulation index was significantly higher for mononuclear cells (MNC) dereived from bLF-treated animals.

The authors concluded that bLF may act by modulating MNC function.

Wang, W.P., Iigo, M., Sato, J., Sekine, K., Adachi, I., and Tsuda, H. Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. Jpn. J. Cancer Res. 91, 1022 (2000).

Wang et. al. investigated the activation of intestinal mucosal immunity in tumor-bearing mice. Mice were orally administered 300mg/kg/day of bLF from day 11 for three days. The authors indicated that their study demonstrated that oral administration of bLF resulted in:

1. "strong increases" in $CD4^+$ and $CD8^+$ T-cells, as well as asialoGM1$^+$ cells in lymphoid tissues and lamina propria of the small intestine;
2. "significantly increased" IgM^+ and IgA^+ B cells in lamina propria of the small intestine;
3. "significantly increased" $CD8^+$ cells and significantly decreased asialoGM1$^+$ cells in the colon;
4. "increased production of IL-18, IFN-γ and caspase-1 in the mucosa of the small intestine;
5. "particularly high levels" of IL-18 in the epithelial cells of and induced IFN-γ presenting cells in the small intestine; and
6. caspase-1 being induced in the epithelial cells of the small intestine.

The authors concluded that such results may be important for elevation of intestinal mucosal immunity.

Unfortunately, the authors did not indicate which cell type was producing the IFN-γ. Thus, this study does not necessarily provide evidence of any Th1 effect.

Zimecki M., Kocięba M., Chodaczek G., Houszka M., Kruzel M. Lactoferrin ameliorates symptoms of experimental encephalomyelitis in Lewis rats. J. Neuroimmuno. (2006).

Zimecki et al. investigated the effects of oral administration of lactoferrin (bLF) on experimental autoimmune encephalomyelitis (EAE) in Lewis rats. bLF was given in drinking water (as a 0.25% solution) beginning the day of elicitation of EAE or with a seven-day delay. The authors reported that lactoferrin treatment

1. led to a "significant acceleration" of the recovery process;
2. "normalized" cell number of the inguinal lymph nodes of untreated EAE rats (which were almost 3 times higher as compared with central, naive rats) after lactoferrin treatment;
3. decreased elevated serum concentrations of TNF-α and TNF-β (i.e., Th1 proinflammatory cytokines) to those values found in controls; and
4. reduced (evaluated via histological analysis of the spinal cords) the number and size of inflammatory foci.

As a consequence of the above-referenced findings, the authors concluded that – as others have shown – lactoferrin has the ability to inhibit autoimmune disorders – in this case, diminution of the clinical symptoms of EAE. This study confirmed other recent findings by the authors, i.e., that orally administered lactoferrin caused reduction of clinical signs of MS in patients – in parallel to normalization of cytokine production by peripheral blood cells. (Kruzel, 2006).

Zimecki, M., Artym, J., Chodaczek, G., Kociêba, M. and Kruzel, M. Effects of lactoferrin on the immune response modified by the immobilization stress. Pharma. Reports 57, 811 (2005).

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Zimecki et al. investigated the effects of orally administered bovine lactoferrin (bLF) on the cellular and humoral immune responses in mice subjected to immobilization stress (IS). First, the authors demonstrated that long-term IS induced significant suppression of cellular and humoral immune responses in CBA mice. Then the authors indicated that the suppression was attenuated by bLF given to mice in drinking water as determined by the number of antibody-forming cells (AFC) in the spleen and the magnitude of delayed type of hypersensitivity (DTH). On the other hand, bLF lowered the elevated DTH response in mice exposed to short-term IS (5 h only) on the day of elicitation of the DTH reaction. The authors also reported that bLF up-regulated spontaneous transforming growth factor beta (TGF-β) production in the cultures of mesenteric lymph node cells derived from short-term stressed mice. Finally, the authors indicated that this study represents the first report on the regulatory effect of bLF on the immune response modified by the psychic stress and is consistent with other reports on antinociceptive and analgesic actions of bLF in experimental animals.

Zimecki, M., Wlaszczyk, A., Cheneau, P., Brunel, A.S., Mazurier, J., Spik, G., and Kubler, A. Immunoregulatory effects of a nutritional preparation containing bovine lactoferrin taken orally by healthy individuals. Arch. Immunol. Ther. Exp. (Warsz) 46, 231 (1998).

Zimecki et al. reported on a study performed in 17 healthy volunteers during which individuals consumed 40 mg of bLF per day for ten days. Such exposure resulted in (during treatment) a 100 percent increase in the level of immature cell forms, a significant decrease in the percentages of eosinophils and monocytes, and a marked increase (from 33-42 percent) in the numbers of lymphocytes. Unfortunately, the type of lymphocytes (either Th1 or Th2 cells) was not specified; thus, this study does not necessarily provide support for any Th1 response. The authors concluded that bLF may be applied in the clinical setting "to improve the immune status of patients."

Zimecki, M.,Wieczorek, Z., Mazurier, J., Spik, G. Lactoferrin lowers the incidence of positive Coombs' test in New Zealand Black mice. Arch. Immunol. Ther. Exp. 43, 207 (1995).

Zimecki et al. indicate in this publication that New Zealand Black (NZB) mice treated for a prolonged period (i.e., 329 days) with bovine lactoferrin (bLF) (given intraperitoneally at doses of 2, 10 or 50 µg 3 times/week) exhibit a decreased frequency of positive Coombs' reaction. The authors reported that this effect was dose dependent and best pronounced at a dose of 50 µg/dose, In addition, the authors reported that incubation of peritoneal cells with bLF resulted in a decreased number of cells recognizing Hb antigen on autologous erythrocytes. Consequently, the authors concluded that the data indicated that bLF may be of therapeutic value in treatment of autoimmune disorders – in particular, in inhibiting autoimmune hemolytic anemia.

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Attachment

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CURRICULUM VITAE: J.H. BROCK 26 Oct 2005

PERSONAL DETAILS

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Education: Owen's School, London, 1952-60
Selwyn College, Cambridge, 1960-63
University of Newcastle, 1966-67

Degrees: MA(Hons), Cambridge, 1963 (Organic Chemistry)
MSc, Newcastle, 1967 (Microbiological Chemistry)
PhD, Reading, 1972 (Microbiology)
ScD, Cambridge 1998

CURRENT POST: Honorary Senior Research Fellow, Department of Immunology, University of Glasgow . Appointed 2001

PREVIOUS POSTS:
1991-2001 Reader in Immunology, university of Glasgow (Retired 2001)
1986-1991 Senior Lecturer in Immunology, University of Glasgow.
1978-1986 Lecturer in Immunology, University of Glasgow.
1974-1978 Section Head, Instituto de Investigación ULTA, Fundación Cuenca Villoro, Zaragoza, Spain.
1967-1974 Scientific Officer, National Institute for Research in Dairying, Shinfield, Reading (University of Reading)
1966-1967 Postgraduate student, University of Newcastle-upon-Tyne
1963-1966 Scientific Officer, Greater London Council [Scientific Branch]

OTHER APPOINTMENTS

1993-4 Visiting Scientist, Gene Expression Programme, European Molecular Biology Laboratory, Heidelberg
1991 Visiting Lecturer, Universidad Nacional de Chihuahua, Mexico
1989-2001 Visiting Lecturer, University of Zaragoza, (Science and Veterinary faculties)
1988 Visiting Full Professor, Dept of Cellular and Structural Biology, University of Texas

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Health Science Center, San Antonio.
1982 Visiting Lecturer, Dept of Biology, Universidad Nacional Autónoma de Mexico.

RESEARCH

Main area of research concerned with iron and iron-proteins, especially in relation to infection and immunity, and with special emphasis on lactoferrin. Earlier experience with milk protein biology and immunology, and composition of bacterial cell walls.

PUBLICATIONS – JH BROCK

Mulero V, Brock JH. Disturbances of iron homeostasis. In Anemia of Chronic Disease (Eds. G Weiss, VR Gordeuk and C Hershko) Taylor and Francis, Boca Raton (2005), pp 105-126.

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EDUCATION

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1964-1968 M.D., Washington University

POST-DOCTORAL TRAINING

1968-1969 Intern in Medicine, Cornell University Hospitals
1969-1970 Assistant Resident in Medicine, Cornell University Hospitals
1972-1973 Senior Resident in Medicine, University of Chicago Hospitals
1973-1975 National Institutes of Health, Fellow in Gastroenterology
The University of Chicago Hospitals and Clinics

MILITARY

1970-1972 Major, Medical Corps, U.S. Army Reserve
Preventive Medicine Officer, United States Army
Headquarters Area Command, Saigon, Vietnam

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ACADEMIC APPOINTMENTS

1969-1970	Fellow in Medicine, Cornell University Medical College
1975-1976	Instructor in Medicine, The University of Chicago
1975-1976	Attending Physician, University of Chicago Hospitals
1976-1978	Assistant Professor of Medicine, The University of Chicago (on leave to NIH)
1976-1977	Intergovernmental Personnel Act Appointment, Metabolism Branch, National Cancer Institute, National Institutes of Health
1977-1978	Intergovernmental Personnel Act Appointment, Lab Microbiology and Immunology, National Institutes of Dental Research, NIH
1978-1980	Expert, Immunophysiology Section, Metabolism Branch, National Cancer Institutes, National Institutes of Health
1980-1986	Associate Professor of Medicine, Med. College of Virginia, Richmond, VA
1980-1987	Attending Physician, Medical College of Virginia Hospitals
1982-1986	Associate Professor of Microbiology and Immunology, Medical College of Virginia, Richmond, VA
1986-1987	Professor of Medicine and Microbiology and Immunology, Medical College of Virginia, Virginia Commonwealth University of Virginia, Richmond, VA
1987-present	Professor of Medicine, University of Alabama at Birmingham
1987-2001	Director, Division of Gastroenterology, University of Alabama at Birmingham
1987-present	Director, Inflammatory Bowel Disease Center, University of Alabama at Birmingham
1987-present	Attending Physician, University of Alabama Hospitals
1988-1996	Senior Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham
1988-present	Senior Scientist, Multipurpose Arthritis Center, University of Alabama at Birmingham
1988-present	Senior Scientist, Center for AIDS Research, University of Alabama at Birmingham
1990-present	Professor of Microbiology, University of Alabama at Birmingham
1997-present	Basil I. Hirschowitz Chair in Gastroenterology, University of Alabama at Birmingham
2001-present	Vice Chair for Research, Department of Medicine, University of Alabama at Birmingham

CERTIFICATION

1969	Diplomate, National Board of Medical Examiners, Certificate # 100366
1974	Diplomate, American Board of Internal Medicine, Certificate # 45524
1975	Diplomate, Subspecialty of Gastroenterology, American Board of Internal Medicine, Certificate # 45524

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MEDICAL LICENSURE

1972	State of Illinois, Certificate # 36-45828
1978	State of Virginia, Certificate # 029255
1988	State of Alabama, Certificate # 13793

HONORS

1964	Alpha Epsilon Delta, Honor Premedical Fraternity
1968	Alpha Omega Alpha, Honor Medical Fraternity
1998	Fogarty International Fellowship
1998	Listed in Best Doctors in America
1998	Humanitarian Award, Crohn's and Colitis Foundation of America, Alabama Chapter
1998	Elected to Membership, Association of American Physicians
2000	R.D. McKenna Memorial Lecturer, Canadian Association of Gastroenterology
2000	Distinguished John V. Carbone Lecturer, University of California, San Francisco
2002	Elected to Fellowship, American Academy of Microbiology
2003-present	Listed in "Best Doctors in America"
2003	Sidney Truelove Lecturer, International Organization for the Study of Inflammatory Bowel Disease (IOIBD)

GRANTS/AWARDS

1975	Diplomate, Subspecialty of Gastroenterology, American Board of Internal Medicine, Certificate #45524
1981-1983	National Foundation for Ileitis and Colitis, "T Cell Regulation of Immunoglobulin Synthesis in Inflammatory Bowel Disease"
1981-present	National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, "Regulation of Intestinal Immune Responses"
1981-1986	National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, "Research Career Development Award, Gastrointestinal Immunology"
1982-1983	A.D. Williams Fund of Medical College of Virginia, Virginia Commonwealth University, "Immune Mediator Population in the Intestinal Lesions of Inflammation Bowel Disease"
1983-1990	National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, "Intestinal Immunoregulation: Inflammatory Bowel Disease"
1990-1991	National Foundation for Ileitis and Colitis "The Role of Cytokines in Acute and Chronic Colitis"
1989-1994	National Institutes of Health, "Antigen Delivery Systems for HIV and SIV Vaccine" - Project 2 in National Cooperative Vaccine Development Group for AIDS Program "Mucosal and Systemic Immunity to SIV and HIV Vaccines"

GRANTS/AWARDS (cont.)

1990-1992	The Nestle Company, "Oral Tolerance in Man"
1991-2005	National Institute of Diabetes, Digestive and Kidney Diseases, Program Project Grant, "Chronic Intestinal Inflammation: Mechanisms and Effects"
1991-1997	Investigator, Mucosal Immunology Research Group, USPHS contract AI 15128
1992-1995	Investigator, "Vaccine Adjuvant Formulations for AIDS", Emory University
1994-1999	NIAID, "Mucosal Tolerance and Immunity in Humans"
1999-2002	"Mechanisms of Oral Tolerance", Sankyo Pharmaceuticals
2002-2007	Principal Investigator, NIDDK RO1 DK60132, " Intestinal T regulatory-1 cells in mucosal homeostasis"
2003-2005	Co-investigator, "Immunoregulation", Sankyo Program for Rheumatic Diseases
2003	Principal Investigator, "Expression cloning and identification of dominant intestinal microbial antigens and modulins", Eli and Edythe L. Broad Medical Foundation
2003-2008	Investigator, NIDDK, DK-01-030, "Mucosal HIV and Immunobiology Digestive Diseases Research Development Center"
2003-2008	Associate Director, NIAID Autoimmunity Center of Excellence (ACE)
2002-2004	Investigator, Center for Biologics Evaluation and Research, FDA
2004-2009	Co-Investigator, NIAID AI57956 "Immune Regulation to Intestinal Bacterial Antigens" (C. Weaver, PI)
2005-2010	Principal Investigator, NIDDK PO1 DK 07116 "Innate and Adaptive Microbial Immunity in IBD"

PROFESSIONAL ASSOCIATIONS

Alpha Omega Alpha, 1968
American Gastroenterological Association, 1976
American College of Physicians, 1975; Fellow, 1983
American Federation for Clinical Research, 1976
American Association of Immunologists, 1983
Gastroenterology Research Group, 1983
Society for Mucosal Immunology, 1987
Clinical Immunology Society, 1988
NIH Alumni Association, 1988
American Society for Microbiology, 1990
Southern Society for Clinical Investigation, 1992
Association of Subspecialty Professors, 1994
New York Academy of Sciences, 1995
Association of American Physicians, 1998
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COMMITTEES

1981-1984	Committee on Research, American Gastroenterological Association
1982-1983	Program Selection Committee, Immunology/Microbiology Section, American Gastroenterological Association
1982-1984	University Grievance Panel, Virginia Commonwealth University
1984-1985	Research Training Awards Program Committee, National Foundation for Ileitis and Colitis
1984-1987	Member, Massey Cancer Center, Medical College of Virginia, Virginia Commonwealth University
1984-1987	Tenure and/or Promotion Committee, Department of Internal Medicine, Medical College of Virginia, Virginia Commonwealth University, Chairman 1986-1987
1985-1989	Member, Subcommittee C of the Arthritis, Diabetes, Digestive and Kidney Diseases Special Grants Review Committee, NIDDK, NIH; Chairman 1987-1989
1985-1989	Member, Grants Review Committee, National Foundation for Ileitis and Colitis
1987-1994	Member, Department of Medicine Practice Executive Committee, University of Alabama at Birmingham
1987-1997	Co-Founder, Secretary-Treasurer and Member of Governing Board, Society for Mucosal Immunology
1989-1993	Member, Research Committee of the American Gastroenterological Association
1989-present	Member, Center for AIDS Research, University of Alabama at Birmingham, Birmingham, AL
1989-1994	Chairman, Research Initiatives Committee, Crohn's and Colitis Foundation of America
1989-1994	Member, National Scientific Advisory Committee, Crohn's and Colitis Foundation of America
1991-present	Member, External Advisory Committee, Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital/Harvard University, Boston, MA; Chairman 1996-present
1991-present	Member, External Advisory Committee, NIH Program Project - "Molecular Immunopathogenesis of Demyelinating Disease" University of Alabama at Birmingham
1990-1994	Member, Program Committee, American Association of Immunologists - Co-Chairman, Block C, Regional Immunology
1993-1999	Member, External Advisory Committee, Center for Gastrointestinal Biology and Disease, University of North Carolina, Chapel Hill, NC
1993-1994	Member, Department of Medicine Quality Improvement Committee, University of Alabama at Birmingham
1996-present	Member, Gene Therapy Project Review Panel, University of Alabama at Birmingham

COMMITTEES (cont.)

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1996-1999	Chairman, Grants Council, and Member, National Scientific Advisory Committee, Crohn's and Colitis Foundation of America
1998-present	Member, Research Advisory Committee, University of Alabama School of Medicine
1999-2001	President, Society for Mucosal Immunology
1999-2002	Chairman, National Scientific Advisory Committee, and Member, Board of Trustees, Crohn's and Colitis Foundation of America
2002	DDIDC Panelist
2002	NIH Boundaries Panel, April 2002
2002	Councillor, Clinical Immunological Society
2002	FOCIS Section Leader
2002	Organizer, 10th International Congress of Mucosal Immunology
2003	Chairman, NIH Concensus Panel on Celiac Disease
2003	Chairman, Strategic Planning Committee of the Crohn's and Colitis Foundation of America
2005-present	Ex-Officio Member, Internal Advisory Board, U.A.B. Nephrology Research and Training Center (NRTC)
2002-2005	Member, Board of Trustees, Crohn's and Colitis Foundation of America (Nominations, Finance, Government Affairs Committees)
2006-present	Member, Internal Advisory Committee, Recessive PKD P30 Core Center, University of Alabama at Birmingham

EDITORIAL ACTIVITIES

1986-1990	Member, Editorial Board, Gastroenterology
1986-1991	Member, Editorial Board, Viewpoints in Digestive Disease
1989-1995	Member, Editorial Board, Infection and Immunity
1992-1998	Member, Editorial Board, Gastroenterology
1994-present	Member, Editorial Board, Inflammatory Bowel Diseases
1996-present	Member, Editorial Board, Journal of Clinical Immunology
1997-2000	Member, Editorial Board, American Journal of Physiology
1999-present	Member, Editorial Board, Clinical Immunology

COMMUNITY SERVICE

1981-1987	Member, Scientific Advisory Committee, National Foundation for Ileitis and Colitis, Richmond Chapter; Chairman 1984-1985
1989-present	Member, Scientific Advisory Committee, Crohn's and Colitis Foundation of America, Alabama Chapter
2000-present	Member, Board of Trustees, Alabama/Northwest Florida Chapter, Crohn's and Colitis Foundation of America

OTHER SIGNIFICANT SCHOLARLY AND RESEARCH EXPERIENCE

1983	Co-Chairman, Research Forum on Immunology/ Microbiology, American Gastroenterological Association/ Gastrointestinal Research Group Meeting
1983-present	Ad Hoc Editorial Consultant for Annals of Internal Medicine, Arthritis and Rheumatism, Digestive Diseases and Sciences, Gastroenterology, Journal of Immunology
1983-1984	Ad Hoc Reviewer for the National Science Foundation, National Institutes of Health, and the National Foundation for Ileitis and Colitis
1984	Convener and Chairman, American Gastroenterological Association Workshop on Intestinal Immunity and Inflammation, Fort Lauderdale, FL
1986-1987	Chairman, Organizing Committee, Society for Mucosal Immunology
1987-1988	Member, Program Selection Committee, Inflammatory Bowel Disease, American Gastroenterological Association; Chairman 1988
1987	Member, Selection Committee for Western Gastroenterological Research Prize
1984-present	Member, Ad Hoc NIH Study Sections in 1984, 1985, 1986 (two), 1988, 1992
1989	Co-Moderator, Planning Meeting for Developing Research Strategies for Inflammatory Bowel Disease for the 21st Century, NIDDK, NIH, Bethesda, MD, September 18, 1989
1989, 1993	Chairman, Task Force on Immunology for "Challenges in IBD Research: Agenda for the 1990's"; Crohn's and Colitis Foundation of America
1989-91	Member, Program Selection Committee, Intestinal Disorders, American Gastroenterological Association
1988-present	Program Review Committee, Basic IBD. American Gastroenterological Association
1994	Program Committee, Clinical Immunology Society
1994	Invited Participant and speaker, Dedication of IBD Center of the University of Chicago
1994	Co-Chairman, Minisymposium. Society for Mucosal Immunology/FASEB Meeting
1994	Participant, Astra-Draco Workshop on Asthma Pathogenesis, Paros, Greece
1994-present	Ad hoc reviewer, Crohn's and Colitis Foundation
1996	American Gastroenterological Association Meeting, Focussed Discussion "Knockout Mouse Models"; Meet-the-Professor Management of Refractory IBD
1996	Panel Discussant, IBD Symposium, Crohn's and Colitis Foundation, Chicago Chapter. "Management of Complex Problems in IBD".
1996	Discussant, NIAID Conference on Clinical Trials in Immune-Mediated Diseases, Bethesda, MD. "Inflammatory Bowel Disease"
1996	Chairman, Task Force on Animals of IBD for "Challenges in IBD Research: Agenda for the 1990's", Crohn's and Colitis Foundation of America.

1997	Moderator, Plenary Session, 9th International Congress of Mucosal Immunology, Sydney Australia
1998	Participant: NIAID Conference on Research Directions in Mycobacterium Avium Infections. Rockville, MD

CONSULTING ACTIVITIES

1993-1999	Schering Plough Research Institute, Kenilworth, NJ
1993-1994	Centocor, Inc., Malvern, PA
1994, 2003	Otsuka Pharmaceuticals, Washington, D.C.
1997	Cortecs, Inc., London, England
1998	ImmuLogic, Inc., Waltham, MA
1998	Protein Design Labs, Mountain View, CA
1998	Biocryst Pharmaceuticals, Birmingham, AL
1998	Axis Genetics PLC, Babraham, Cambridge, England
1998	Creative Biomolecules, Boston, MA
1999	Board of Scientific Advisors, Santarus, Inc.
1999	Celltech, Chiroscience, Limited
2000	Integriderm, Inc.
2000	Genetics Institute, Cambridge, MA
2000	Biogen, Cambridge, MA
2000-2004	Corixa Corporation
2000, 2002	Astra Zeneca
2000-2003	Curagen
2001	Cell Pathways
2001	Elan/Biogen
2002	Human Genome Sciences
2003	Solvay Pharmaceuticals
2003-2005	Abbott Laboratories
2004	Glaxo SmithKline
2004-2005	Schering Plough Biopharma
2005	Shire Pharmaceuticals
2005	Novartis

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BOOKS

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2. MacDermott, R.P. and Elson, C.O. (Eds.). Mucosal Immunology II: Clinical Applications. Gastro Clinics of North America. Volume 20, No. 3. W.B. Saunders, Philadelphia, PA. 1991.

BOOK CHAPTERS, REVIEWS

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2. Elson, C.O. Induction and control of the gastrointestinal immune system. Scand. J. Gastroenterol. 20(Suppl. 114):1-15, 1985.

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4. Elson, C.O. Gastrointestinal Diseases with an Immune Basis. In: Textbook of Internal Medicine. Ed. by W.N. Kelley. J.P. Lippincott Co., Philadelphia, PA, 1988, pp 572-578.

5. Mestecky, J., McGhee, J.R. and Elson, C.O. Intestinal IgA System. In Immunology and Allergy Clinics of North America, 8:3, December, 1988, pages 349-368.

6. McGhee, J.R., Mestecky, J., Elson, C.O. and Kiyono, H. Regulation of IgA Synthesis and Immune Response by T Cells and Interleukins. In J. Clin. Immunol. 9(3):175-199, 1989.

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9. Beagley, K. and Elson, C.O. Cells and cytokines in mucosal immunology and inflammation. In Gastroenterology Clinics of North America. Mucosal Immunology II:

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PEER-REVIEWED ARTICLES:

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3. Elson, C.O., Reilly, R.W. and Rosenberg, I.H. Small intestinal injury in the graft versus host reaction: an innocent bystander phenomenon. Gastroenterology 72:886, 1977.

4. Elson, C.O., Heck, J.A. and Strober, W. T-cell regulation of murine IgA synthesis. J. Exp. Med. 149:632, 1979.

5. Baker, A.L., Elson, C.O., Jaspan, J., and Boyer, J.L.: Liver failure with steatonecrosis after jejunoileal bypass. Archives of Internal Medicine 139:289, 1979.

6. Elson, C.O., Layden, T., Nemchausky, B., Rosenberg, J., and Rosenberg, I.: An evaluation of total parenteral nutrition in the management of inflammatory bowel disease. Digestive Diseases and Sciences 25:42, 1980.

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10. Elson, C.O., Graeff, A.S., James, S.P., and Strober, W.: Covert suppressor T cells in Crohn's disease. Gastroenterology 80:1513, 1981.

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15. Elson, C.O., Ealding, W., Lefkowitz, J. A lavage technique allowing repeated measurement of IgA antibody in mouse intestinal secretions. Journal of Immunological Methods. 67:101, 1984.

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17. Elson, C.O. and Ealding, W. Cholera toxin feeding did not induce oral tolerance in mice and abrogated oral tolerance to an unrelated protein antigen. J. Immunol. 133:2982, 1984.

18. Graham, M.F., Diegelmann, R., Elson, C.O., Bitar, K.N., Erlich, H.P. Isolation and culture of human intestinal smooth muscle cells. Proc. Soc. Exp. Biol. Med. 176:503, 1984.

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1986	Ph.D.	New York University, Sackler Institute of Graduate Biomedical Science New York, N.Y. (Immunology)

Postdoctoral Training:

1986-1989	Postdoctoral Fellow, MIT Center for Cancer Research
1989-1990	Postdoctoral Associate, MIT Center for Cancer Research

Academic Appointments:

1989-1991	Tutor in Biology, Harvard University
1990	Assistant Professor of Pediatrics (Immunology), Harvard Medical School
1995-	Executive Steering Committee and Co-Director, Immunology Core, Mass. General Hospital Center for the Study of Inflammatory Bowel Disease
1995-2005	Co-Director, Morphology/Tissue Culture/Immunology/Flow Cytometry Core Clinical Nutrition Research Center at Harvard
2001	Associate Professor of Pediatrics (Immunology), Harvard Medical School

Hospital Appointments:

1990	Associate Immunologist, Children's Service, Mass. General Hospital

Major Committee Assignments

1992-	MGH Subcommittee on Immunology
1995 -1998	Committee for Immunology Graduate Student Qualifying Exams, HMS
1998-	Re-elected to membership in the Committee of Immunology at Harvard Medical School
2001-	Member, Grant Review Committee, Crohn's and Colitis Foundation of America
2004-	Block Chair, Regional and Mucosal Immunology, American Association of Immunologists
2005-	Ad hoc reviewer, NIH Gastrointestinal Mucosal Pathobiology Study Section
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Professional Societies

1990-	American Association of Immunologists
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Editorial Boards:

1990- Ad Hoc Reviewer:

Journal of Immunology
Gastroenterology
International Immunology
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Clinical Immunology
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2001-2003	Associate Editor, *Journal of Immunology*
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1979	Honors in Biology, Barnard College
1981	NIH Research Scientist Training Fellowship in Viral Oncology and Immunology, New York University School of Medicine
1986	NIH Training Fellowship in Immunology, M.I.T.
1988	National Research Service Award, NIAID
1990	Career Development Award, National Foundation for Ileitis & Colitis
1990	Roche II Award for Research in Autoimmunity, Harvard Medical School
1995	NIH First Independent Research Support and Transition (FIRST) Award
2006	Member, Food Allergy Expert Panel

Part II: Research, Teaching and Clinical Contributions

A. Funding Information

Past:

1990-1992	Career Development Award, National Foundation for Ileitis & Colitis, P.I., "Cytolytic T Cell Response to Stress Proteins in IBD"
1990-1993	Harvard Medical School/Hoffman La Roche Award for Research in Autoimmunity P.I., "Autoimmunity to Stress Proteins"
1990	MGH/NERPC Center for the Study of Inflammatory Bowel Disease/Pilot Feasibility P.I. "Heat Shock Proteins and Cytolytic T Cells in the Pathogenesis of IBD"
1992-1993	MGH Interim Support Fund P.I. "Cytolytic T Cell Response to Stress Proteins in IBD"
1994	Milton Fund P.I. "Mechanisms of orally-induced immunologic non-responsiveness"
1994-1997	NIH/RO1

Co-P.I. "Inflammatory Bowel Disease in TCR mutant mice"

1994-1999 NIH/PO1
Co-Director, Tissue Culture/Morphology Core
Co-P.I. (Project 1) "Uptake of Intestinal Macromolecules"

1995-2000 NIH/R29
P.I. "Self-Reactive intestinal intraepithelial lymphocytes"

1999 NIH/CNRC at Harvard/Pilot Feasibility Project
P.I. " Influence of helminth infection on food allergy"

Current:

2005-2010 NIH/MGH Center for the Study of Inflammatory Bowel Disease
Co-Director, Immunology Core

2005-2010 NIH/RO1
P.I. "Altered responses to food proteins in enteric infection"

B. Report of Current Research Activities

Project	**Role**
Enteric infection as a mucosal adjuvant for the response to orally administered antigens	Principal Investigator
Influence of enteric infection on allergic response to food	Principal Investigator
Innate immune signaling by the commensal flora and susceptibility to food allergy	Principal Investigator

C. Report of Teaching

1. Local Contributions

a. Courses (medical school)

2000, 2001 Tutor, Immunology, Microbiology and Infectious Disease, Harvard Medical School. Tutor for a small group (8) of first year medical students. Each tutorial is 1 1/2 hrs (13.5 hrs. total). 6-7 hrs. of tutor and faculty development meetingsand 15 hrs. of preparation time.

2000, 2001, 2005 Tutor, Gastrointestinal Pathophysiology, Harvard Medical School. Tutor for a small group (8) of second year medical students for the Gastroenterology block of the Human Systems module. Each tutorial is 1 1/2 hours (4.5 hrs. total). 6-7 hrs. of tutor and faculty development meetings and 15 hrs. of preparation time.

b. Courses (graduate school)

1991, 93, 95, 97 Lecturer, Contemporary Topics in Immunobiology (200b), Harvard Medical School, Committee on Immunology . This course was attended by approximately 30 graduate students/postdoctoral fellows. Each lecture involved about 3 hrs of contact time and 6 hrs of preparation time.

c. Local Teaching

1989-1991 Tutor, Harvard University Biology Tutorial Program "The Evolutionary Development of the Immune System" This course, for which I developed the curriculum, was a semester long seminar/discussion course for 5-7 advanced Biology majors. Each seminar involved about 3 hrs. of preparation time and 2 hrs of class time.

1986-1994 Faculty sponsor, M.I.T. Undergraduate Research Opportunity Program. Students worked in the lab for a full semester on individual research projects for class credit. daily interaction.

1991-1992 Undergraduate Thesis Advisor, Department of Biology, Harvard University. Honors Thesis, Elizabeth Hsia "Stimulation of murine intestinal intraepithelial lymphocytes by *Staphylococcal* enterotoxin B"

1991-1992 Faculty sponsor, Harvard University, Biology 90R, Independent Research Study in Biology

e. Advisory/supervisory responsibilities in laboratory setting

1992- present *MGH Subcommittee on Immunology:* Organization of the MGH Immunology seminar series, invitation and hosting of speakers, fund-raising

1995-2005 *Co-Director, Morphology/Tissue Culture/Immunology/Flow Cytometry Core, Clinical Nutrition Research Center at Harvard*

Co-Director, Immunology Core, MGH Center for the Study of Inflammatory Bowel Disease

2006- *Director, Immunology Core, MGH Center for the Study of Inflammatory Bowel Disease*

Responsible for training center members (both principal investigators and fellows) in beginning and advanced techniques in cellular and molecular immunology, particularly monoclonal antibody production and flow cytometry. We assist with both the design and execution of experiments, some of which are performed by the Core technician under my supervision.

1996-2000 Thesis advisory and thesis defense committees, Katherine Silvey, Graduate School of Arts & Sciences, Program in Immunology

1997 Thesis defense committee, Lara Ausubel, Graduate School of Arts & Sciences, Program in Immunology

2001 Thesis defense committee, Wanda Coston, Graduate School of Arts & Sciences, Program in Immunology

2005- Dissertation advisory committee, Edwin Manuel Graduate School of Arts & Sciences, Program in Virology

f. Teaching leadership roles

1990- Preceptor, NIH Training Grant (T32-DK07477) Training in Pediatric Gastroenterology and Nutrition, MGH

1995-	Preceptor, NIH Training Grant (T32-AI07498) PhD Program in Immunobiology, Harvard Medical School
1998-	Preceptor, NIH Training Grant (T32-AI07529) Training in Transplantation Biology, MGH
2000-	Preceptor, NIH Training Grant (T32-DK07191) Training in Gastroenterology, MGH
2000-	Preceptor, NIH Training Grant (T32-DK07471) Training in Pediatric G.I. and Nutrition Tufts/New England Medical Center

g. Advisees/Trainees

		Current Position
Martina Siebrecht , Ph.D.	1991-93	Research Scientist, Munich, Germany
Elizabeth Hsia, M.D.	1991-92	Fellow in Rheumatology, Univ. of Pennsylvania KO-8 award, 2002
Mary Tschoi, M.D.	1992-94	Research Associate (NIH) NRSA, 2002
Christian Ingui, M.D.	1995-97	Resident in Radiology Boston, MA
Gerburg Spiekermann,M.D.	1995-97	Astra Merck Training Award, AGA, Instructor in Pediatrics
Abhijit Afzalpurkar, Ph.D.	1997-98	Senior Fellow, Medical College of Georgia
Hai Ning Shi, DVM, Ph.D.	1996-1999 1999- 2002 2001-2002 2002- 2003-	Research Fellow Instructor in Pediatrics, Research Training Award, Crohn's and Colitis Foundation of America Career Development Award, CCFA Assistant Professor of Pediatrics First Award, CCFA KO-1 Award, N.I.H.
Mohamed E.H. Bashir, Ph.D.	2000-2005	Post-doctoral fellow
Emma I. Melendro, Ph.D.	2000-2001	Visiting scientist (sabbatical) National University of Mexico
Donald Smith	2001-	Graduate Student , Biological and Biomedical Science Program
Guenolee Prioult, Ph.D.	2004-	Post-doctoral Fellow
Hidehiro Murakami, M.D.	2004-	Post-doctoral Fellow
Onyinye Iweala	2005-	M.D., Ph.D. student, Harvard Medical School

Harvard Undergraduate Honors Theses

Elizabeth Hsia	1992	"Stimulation of murine intestinal Intraepithelial lymphocytes by Staphylococcal Enterotoxin B"

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Onyinye Iweala	2002	"The effect of helminth infection on the immune response to an oral Salmonella-GFP-OVA vaccine"

2. Regional, National and International Contributions

a. Invited Presentations

1986 Invited Seminar, Ayerst Research Laboratories, Trenton, New Jersey

1989 Symposium presentation, Seventh International Congress of Immunology, Berlin, West Germany

1991 Annual Symposium Presentation, Center for the Study of Inflammatory Bowel Disease, Mass. General Hospital Boston, MA

1991 Symposium Presentation, HoffmannRoche/Harvard Medical School Collaboration, Basel, Switzerland

1992 Workshop presentation: Intraepithelial Lymphocytes: Molecular and Functional Characterization of Intraepithelial Lymphocytes: Center for the Study of Inflammatory Bowel Disease, Mass. General Hospital, Boston, MA

1993 Symposium Presentation, Hoffmann La Roche/Harvard Medical School Collaboration: Nutley, New Jersey

1993 Invited Seminar, Dartmouth Medical School Immunology Series, Hanover, New Hampshire

1994 Workshop presentation, American Association of Immunologists Annual Meeting, Experimental Biology '94: Anaheim, CA

1994 Faculty participant, The New Age of Research in Inflammatory Bowel Diseases University of Chicago Inflammatory Bowel Disease Center, Chicago, IL.

1994 Grand Rounds, Department of Pediatrics, Mass. General Hospital: Boston, MA

1995 Invited Seminar, NYU School of Medicine, Immunology Club, New York, NY

1996 Panel discussant, Sixteenth Ross Research Conference on Medical Issues: Nutritional influence in inflammation: its role in inflammatory disease management, Williamsburg, VA

1997 Invited Seminar, MGH Immunology Seminar Series, Boston, MA

1998 Workshop presentation, American Association of Immunologists Annual Meeting, Experimental Biology '98: San Francisco, CA

1998 Invited Seminar, Beth Israel/Deaconess Medical Center Immunology Series, Boston, MA

1998 Invited Seminar, Gastrointestinal Unit/ MGH, Boston, MA

1998 Invited Seminar, Institute of Parasitology, McGill University: Montreal, Canada

1998 Workshop Presentation: Lymphocytes and IBD, Current Paradigms of Disease and Treatment: Center for the Study of Inflammatory Bowel Disease, Mass. General Hospital Boston, MA

1999 Invited Seminar, Harvard Digestive Diseases Center/ Children's Hospital, Boston, MA

1999 Invited Seminar, University of Florida, Department of Pathology: Gainesville, FL.

1999 Presentation, The Pediatric Research Symposium, Mass.General Hospital for Children,

1999 Invited Seminar, Albert Einstein College of Medicine, Bronx, N.Y.

1999 Invited Seminar, Albany Medical College, Albany, N.Y.

2000 Invited Speaker, 10th International Symposium on the Immunobiology of Proteins and Peptides; Current approaches to immunotherapy and immunotechnology, Tahoe City, CA;

2001 Invited Seminar, Gastrointestinal Unit, Mass. General Hospital

2002 Invited Speaker, Keystone Symposia on Molecular and Cellular Biology Microbial - Epithelial - Lymphocyte Interactions in Mucosal Immunity, Breckenridge, CO

2002 Invited Seminar, University of Virginia, Basic Science Physiology Seminar Series Charlottesville, VA

2003 Invited Speaker, 53rd Nestle Nutrition Workshop "Allergic Diseases and the Environment" Lausanne, Switzerland

2003 Invited Seminar, Immunology and Oncobiology Training Program, Boston University Medical Center, Boston MA

2003 Invited Speaker, Federation of Clinical Immunology Societies (FOCIS), Paris, France

2003 Invited Speaker, TEDDY project: Environmental Triggers for Diabetes, Reston, VA

2003 Invited Seminar, MGH Immunology Seminar series

2004 Invited Seminar, NYU School of Medicine Immunology Seminar series, New York, NY

2004 Invited Seminar, Pediatric Grand Rounds, Mass. General Hospital, Boston, MA

2004 Invited Seminar, Thomas Jefferson University, Philadelphia PA

2004 Invited Speaker, American Physiological Society-sponsored translational conference "Pathophysiological Mechanisms of Inflammatory Bowel Disease", Snowmass, CO

2004 Invited Seminar, Department of Pathology, University of Mass. Medical School

2004 Invited Seminar, University of Alabama, Birmingham, AL

2005 Plenary lecture, "Food hypersensitivity", 12th International Congress of Mucosal Immnology, Boston, MA

2005 Invited Speaker, "Helminths as Modulators of Immunity", Hamburg, Germany

2005 Invited Seminar, Case Western Reserve University, Cleveland, Ohio

2005 Invited Seminar, Graduate Program in Immunology and Microbial Pathogenesis Weill Medical College of Cornell University, New York, NY

2006 Invited Speaker, NIAID-American Academy of Allergy, Asthma and Immunology symposium "Targeting Toll Receptors for Prevention and Treatment of Asthma and Allergic Diseases" and "Immune Regulation, Environmental Influences and Immunotherapy" AAAAI Annual Meeting, Miami, Florida

2006 Invited Seminar, Laboratory of Parasitic Diseases, NIAID, Bethesda MD

2006 Invited Seminar, Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit MI

2006 Plenary Lecture Presentation and Chair, Major Symposium "Gut reaction to symbiosis - how bugs shape the immune response" American Association of Immunologists Annual Meeting, Boston, MA

2006 Invited Lecture, US-Japan Gastroenterology Meeting, Keio University, Tokyo, Japan

b. Professional and educational leadership roles

1999 Abstract review committee for the American Gastroenterological Association Annual meeting, "Mucosal Immunity" section

2000 Co-chair, Block Symposium, "Mucosal Tolerance", American Association of Immunologists Annual Meeting (AAI 2000), Seattle, Washington

2000 Program organizer and Presenter, "Antigen Presentation at Mucosal Surfaces", 10th Annual Workshop of the MGH Center for the Study of Inflammatory Bowel Disease

2001 - Grant Review committee, Crohn's and Colitis Foundation of America

2001 - Abstract review committee for the American Gastroenterological Association Spring meeting, "Mucosal Immunology and Inflammation, Innate Immunity" sections

2002 Senior Faculty Chair, Midwest Inflammatory Bowel Disease Junior Faculty Symposium Northwestern University, Chicago, IL

2002 Program Organizer and Presenter "Microbial-Mucosal Interactions", 12th Annual Workshop of the MGH Center for the Study of Inflammatory Bowel Disease

2003 Whitehead Institute's 12th Annual Seminar Series for High School Teachers "Biological Challenge to Humanity: Emerging and Re-Emerging Pathogens", Whitehead Institute for Biomedical Research, Cambridge MA

2003 Session Chair, Workshop on "Immunogenetic Mechanisms of Intestinal Inflammation: Role of Epithelium" UVA Digestive Health Center of Excellence, Charlottesville, VA

2003 National Institutes of Health Special Emphasis Panel "Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics and Diagnostics for Biodefense, Gaithersburg, MD

2003 Program Organizer, "Regulatory T Cells" 13th Annual Workshop of the MGH Center for the Study of Inflammatory Bowel Disease

2003 National Institutes of Health Special Emphasis Panel "Immune Tolerance", Bethesda, MD

2004 National Institutes of Health Special Emphasis Panel "Silvio O. Conte Digestive Diseases Research Core Centers"

2004 - Lecturer, "Mucosal Immunology" American Association of Immunologist's Introductory Course in Immunology, Philadelphia, PA

2004 - Block Chair, Mucosal and Regional Immunology, American Association of Immunologists Annual Meeting

2005- Chair, Mucosal Immunology abstract review for American Gastroenterological Association Annual Meeting, Meeting Session Chair Digestive Diseases Week

2005 National Institutes of Health Special Emphasis Panel "Food Allergy Research Consortium"

2005 Federation of Clinical Immunology Societies (FOCIS) Abstract Review

2005 Senior Faculty Member, IBD Junior Faculty Research Day, Baltimore MD

2005 Participant, National Academies of Science brainstorming workshop, "New Directions in the Study of Antimicrobial Therapeutics: Immunomodulation" Washington, DC

2005 Session Chair, Workshop on Immunogenetic Mechanisms of Intestinal Inflammation: Leukocyte Trafficking and Adhesion Molecules, UVA Digestive Health Center of Excellence, Charlottesville, VA

2005 Lecturer, "Mucosal Immunology" American Association of Immunologist's Introductory Course in Immunology

2005 Program Organizer, "Immunological Memory" 14th Annual Workshop of the MGH Center for the Study of Inflammatory Bowel Disease, Boston, MA

2006 Participant, Atlantic Digestive Diseases Center Conference, Charlottesville, VA

2006 Invited Speaker, NIAID-American Academy of Allergy, Asthma and Immunology symposium "Targeting Toll Receptors for Prevention and Treatment of Asthma and

Allergic Diseases" and "Immune Regulation, Environmental Influences and Immunotherapy" Annual Meeting, Miami, Florida AAAAI

2006 Invited Member, Food Allergy Expert Panel, Bethesda, MD

2006 Invited Participant, Microbial Host Interactions Workshop, St. Petersburg, Florida

2006 Plenary Lecture Presentation and Chair, Major Symposium "Gut reaction to symbiosis – how bugs shape the immune response" American Association of Immunologists Annual Meeting, Boston, MA

2006 Lecturer, "Mucosal Immunology" American Association of Immunologist's Introductory Course in Immunology, Philadelphia, PA

2006 Invited Participant, "IBD Summit Meeting" Cleveland Clinic, Cleveland, OH

Part III: Bibliography

Original Articles

1. Hayama, T., **Nagler, C.**, Umetsu, D.T., Chapman-Alexander, J. and Thorbecke, G.J. Ia restricted interaction between normal lymphoid cells and SJL lymphoma (RCS) leading to lymphokine production. I. Cell Immunol. 1983; 79: 134-149.
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3. Hayama, T., Ponzio, N.M., **Nagler, C.**, Vilcek, J., Coico, R.F. and Thorbecke, G.J. Ia- restricted interaction of normal lymphoid cells and SJL lymphoma leading to lymphokine production III. J. Natl. Cancer Inst 1984; 72: 321-331.
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8. **Nagler-Anderson, C.**, Bober, L.A., Robinson, M.E., Siskind, G.W. and Thorbecke, G.J. Suppression of type II collagen induced arthritis by intragastric administration of soluble type II collagen. Proc. Natl. Acad. Sci. 1986; 83: 7443-7446.
9. **Nagler-Anderson, C.**, Van Vollenhoven, R.F., Gurish, M.F., Bober, L.A., Siskind, G.W. and Thorbecke, G.J. A cross-reactive idiotype on anti-collagen antibodies in collagen induced arthritis - identification and relevance to disease. Cell Immunol. 1988; 113: 447-461.
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11. Van Vollenhoven, R.F., **Nagler-Anderson, C.**, Stecher, V.J., Soriano, A., Connoly, K.M., Nguyen, H.T., Siskind, G.W. and Thorbecke, G.J. Collagen induced arthritis and aging: Influence on arthritis susceptibility and acute phase responses. Aging; Immunology and Infectious Disease 1988; 3:159-176.
12. **Nagler-Anderson, C.**, Verret, C.R., Firmenich, A. and Eisen, H.N. Resistance of primary $CD8^+$ cytotoxic T lymphocytes to lysis by cytotoxic granules from cloned T cell lines. J. Immunol. 1988; 141: 3299- 3305.
13. Allbritton, N.L., **Nagler-Anderson, C.**, Elliott, T.J., Verret, C.R. and Eisen, H.N. Target cell lysis by cytotoxic T lymphocytes that lack detectable hemolytic activity. J. Immunol. 1988 141: 3243-3248.
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Reviews, Chapters, Editorials

1. Scheid, M.P., Chapman-Alexander, J., Hayama, T., Katz, I.R., Lerman, S.P., **Nagler, C.**, Ponzio, N.M. and Thorbecke, G.J. Identification of transplantable reticulum cell sarcomas in SJL/J mice as belonging to the B cell lineage. In *B-lymphocytes in the Immune Response: Functional, Developmental and Interactive Properties*. Klinman, N., Mosier, D.E., Scher, P. and Vitetta, E., Eds. Elsevier, North Holland, 1981: 475-482.
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Thesis

1986 Immunoregulation of an experimental model of autoimmunity: Collagen-induced arthritis

Abstracts

1. Nagler-Anderson, C., Gurish, M.F., Robinson, M.E. and G.J. Thorbecke. Suppression of collagen-induced arthritis by Id coupled lymphoid cells. Fed. Proc. 1986
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21. Smith, D., O. Iweala, H.N. Shi and C. Nagler-Anderson. Presentation of model immunogenic and tolerogenic antigens by the gut associated lymphoid tissue. Keystone Symposium – Microbial-Epithelial-Lymphocyte interactions in mucosal immunity. 2002
22. Bashir, M.E.H., Andersen, P., Shi, H.N. and C. Nagler-Anderson. Enteric helminth infection protects against an allergic response to a dietary antigen. 11th International Congress of Mucosal Immunology. Mucosal Immunology Update 10, Nos. 2 and 3, 2002.
23. Bashir, M.E.H. and C. Nagler-Anderson. Influence of luminal flora on allergic responses to food. Keystone Symposium – Hygiene, Allergy and Asthma, 2003
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26. Iweala, O.I., M.E. McBee, D.B. Schauer and C. Nagler-Anderson. The influence of a Th1 polarizing bacterial infection on the response to dietary antigen. American Association of Immunologists Annual Meeting 2005, San Diego, CA

27. Iweala, O.I., M.E. McBee, D.B. Schauer and C. Nagler-Anderson. The influence of a Th1 polarizing bacterial infection on the response to dietary antigen, 12th International Congress of Mucosal Immunology, 2005, Boston, MA

Curriculum vitae

Employee name	:	Penninks, André Hendrikus
Title	:	Ph.D.
Date of birth	:	12 December 1947 Gender: Male
Signature	:	Initials :
Position	:	Product manager Experimental Immunology Product manager TNO Pharma Study director
Business Unit	:	Toxicology and Applied Pharmacology
Date of entrance	:	1 April 1990
Education (including branch)	:	Biology (Experimental Immunology, Biochemistry, Biological Toxicology) at the University of Utrecht, the Netherlands
Degrees attained	:	1976, Bachelor of Science (Biology) 1979, Doctoral degree in Biology, with majors in Experimental Immunology and Biochemistry (at least equivalent to M.Sc.) 1985, Ph.D. on an Immunotoxicological subject 1988, Board certified Toxicologist (SMBWO) 1990, Board Certified Experimental Pathobiologist (SMBWO) 1997, Registered as certified Toxicologist (NVT) 1998, Eurotox registered certified Toxicologist (Eurotox)
Specialization	:	Immunotoxicology, Pathology
Career	:	1976-1998, Employed at the University of Utrecht, from 1990 for 0.1 fte 1976-1986, Faculty of Veterinary Medicine, dept. Veterinary Pathology, section General Pathology, Working Group Pathology, -Toxicology, University teacher and scientist 1986-1989, Faculty of Veterinary Medicine, dept. Veterinary Pharmacology, Pharmacy and Toxicology, section Immunotoxicology, Assistent Professor : 1989-1990, Faculty of Veterinary Medicine, Research Institute of Toxicology, Associate Professor, Head section Immunotoxicology

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Curriculum vitae

Employee name : Penninks, André Hendrikus

1990-1994,	Head General Toxicology Section at TNO Toxicology & Nutrition Institute; Department of Biological Toxicology
1994-1996,	Head Department of General Toxicology at TNO Nutrition & Food Research Institute; Division of Toxicology
1996-1998,	Head Department of Immuno-, Inhalation- and In Vitro Toxicology at TNO Nutrition & Food Research Institute; Toxicology Div.
1998-present,	Business Unit Toxicology and Applied Pharmacology Product Manager Experimental Immunology Product manager TNO Pharma Senior (Immuno) Toxicologist Projectleader all TNO Food Allergy projects Chair "Knowledge Centre of Food Allergy TNO-University of Utrecht"

International contacts : Many University and Industrial laboratories in the USA, Canada, Japan, Korea, Great-Britain, Denmark, Finland, Norway, Sweden, Germany, Belgium, Switzerland, Italy, Ireland, France, Poland, Hungary, Czech Republic etc.
(see also training record with vists, lectures etc)

Editorial Board : From January 1996-2000 member of the Editorial Board of the Journal Toxicology (Immunotoxicology section)
On a regular basis requested to evaluate manuscripts for publication in various scientific journals (double refereed journals)

Memberships :
- International Society of Immunopharmacology
- Netherlands Society of Toxicology
- Netherlands Society of Immunology
- Working Group: In vitro Toxicology (Member of the Board until '93)
- Working Group: Experimental Pathology
- Working Group: Toxicology (Chairman from '88 - '94)
- Working Group: Toxicology and Risk Evaluation (Member of the Board until '97)
- Industrial Immunotoxicology Discussion Group (group stopped in 2000)
- Dutch Vaccine Group, Vereniging voor de Nederlandse Vaccin Industrie (2003-heden)
- Immunotoxicology and Chemical Allergy Specialty Section of Eurotox (ITCASS 2002-heden)
- Immunotoxicity Inter Laboratory Project, started 2004. Collaboration with Johnson & Johnson PRD, Organon BV, Schering AG, Astra Zeneca R&D, Novartis Pharma AG, Pfizer PGRD, MDS Pharma Services, HSL-UK, Fraunhofer Institute and TNO Pharma

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Curriculum vitae

Employee name : Penninks, André Hendrikus

Co-promotor of PhD thesis: N.J. Snoeij; Triorganotin compounds in Immunotoxicology and Biochemistry, 1987
R.H.H. Pieters; Cellular molecular aspects of organotin-induced thymus atrophy, 1992
G.F. Houben; Vitamin B6 status-dependent immunomodulation by Caramel Colour III, 1992
E.de Jong; Food allergy, human lymphocyte responses to peanut proteins, 1996
L.M.J.Knippels; Oral sensitisation to food proteins and immune mediated effects; a Brown Norway rat food allergy model, May, 1998

Guest teacher : University of Wageningen, immunotoxicology lectures (until 2003)
University of Wageningen, postdoctoral education in Toxicology (POT), cell pathology lectures (until 2001), Food allergy lectures, from 2005.
University of Utrecht, lectures in cell pathology, lectures in immunotoxicology, PhD-training course in immunotoxicity, training course on cytopathology (all ongoing)

Publications : see next pages

Initials: _____ Date: September 2006

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Employee name : Penninks, André Hendrikus

Publications

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The thymus gland in ammonia caramel colour immunotoxicology.

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Employee name : Penninks, André Hendrikus

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Houben, G.F., Kuypers, M.H.M., van Loveren, H., Vos, J.G., Seinen, W. and Penninks, A.H.
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Houben, G.F., Dokkum, W. van, Loveren, H. van, Penninks, A.H., Seinen, W., Spanhaak, S. and Ockhuizen, Th.
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Tox. Appl. Pharmacol., 113, 43-54, 1992

Pieters, R.H.H., Bol, M., Lam, B., Seinen, W. and Penninks A.H.
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Houben, G.F., Van den Berg, H., Van Dokkum, W., Van Loveren, H., Penninks, A.H., Seinen, W., Spanhaak, S., Vos, J.G. en Ockhuizen, T.
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J. Sci. Food Agric., 59, 123-126, 1992

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Curriculum vitae

Employee name : Penninks, André Hendrikus

Jonker, D. and Penninks, A.H.
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Fd. Chem. Tox., 30, 12, 1057-1060, 1992

Houben, G.F., Penninks, A.H., Seinen, W., Vos, J.G. and Van Loveren, H.
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Employee name : Penninks, André Hendrikus

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Knippels L.M.J., Penninks, A.H. and Bannon G.A. Sensitizing Potential of a Peanut protein Extract in Four Different Mouse Strains, Submitted for publication, 2006.

In addition, approximately 195 abstracts are published in abstract books or proceedings of symposia, congresses or in scientific journals.

Curriculum vitae Huub Savelkoul

1. Personal data

Name: Hubertus Fransciscus Jozef Savelkoul
Date of birth: July 11, 1956
Work adress: Celbiology and Immunology, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands; tel: +31.317.483509/483925
E-mail: huub.savelkoul@wur.nl

2. Scientific Career

MSc
Biology, specialisations Zoology and Cell Biology; Wageningen University (1974-1981)
Majors: Biochemistry, Cell Biology, Genetics.
Foreign experience: Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel (1981). Advisor: prof.dr. P. Lonai

PhD
Erasmus University Rotterdam (September 2, 1988, *cum laude*)
Thesis: "Induction and measurement of IgE. A study in mice with emphasis on the regulatory role of lymphokines". Promotor: prof.dr. R. Benner, co-promotor: dr. W. van Ewijk

Research price Erasmus University Rotterdam (1989)

Postdoc: DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California, USA (1988-1990). Advisor: dr. R.L. Coffman

Courses.
Radiation safety C-level: J.A. Cohen Inter-Universitary Institute for Radiation Pathology and Radiation Protection (IRS), Leiden) (1984)
Biostatistics: Post-Academic Course Medicine Rotterdam (1986)
Teaching: Didactic Capacity, Bureau Smidts-Wijnen, Rotterdam (1996)

Registration as Immunologist (SMBWO, 1991)

Lecturer
Assistent Professor (1990-1996) and Associate Professor (1996-2000):
Erasmus University Rotterdam, department of Immunology (chairman: prof.dr. R. Benner)
Unit: Immunoregulation and the development of allergy at childhood age

Professor
Van der Leeuw chair (NWO) in Cell Biology and Immunology, Wageningen University (September 2000-September 2003).
Full professor and head of department as of September 1, 2003

3. Research

PhD Advisor:
Co-promotor: R. Van Ommen (1994), A.C.T.M. Vossen (1995), S.S. Pathak (1995), T. Ten Hagen (1996), H. Koning (1996), M.P. Laan (1999).
Promotor: A. Boonstra (2000), M. Nawijn (2001), C. Kruiswijk (2002), H. Huttenhuis (2005), M.O. Huising (2006), M. Joerink (2006), J.A.J. Arts (2006)

References (1982-2005)
Research papers in peer-reviewed journals: 255; citations: 2350
Others: book chapters, reviews, etc: 80

Approved Research projects: 42 (1994-2006).total 25 M€

Patents: approved 8

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Current group:
4 senior staff, 4 postdoctoral fellows, 12 graduate students, 20 undergraduate students, 5 technical support staff, 1 secretary

4. Memberships
Editorial board member:
Mediators of Inflammation, Science and Technology Intensive Courses, Molecular Immunology

Member Scientific organizations:
Member Scientific advisory board Netherlands Asthma Foundation (1996-2000)
Netherlands Society for Allergology, Netherlands Society for Immunology, European Academy of Allergology and Clinical Immunology, European Society of Paediatric Allergology and Clinical Immunology
International Society for Developmental and Comparative Immunology (ISDCI) (2004-current)
Chairman: Educational Committee Netherlands Society for Immunology (1999-2005)
Chairman: Users group Animal Experiments Wageningen University (2003-current)
Board Member: Netherlands Medical-biological research council (SMBWO) (2000-current)
Board Member: Foundation for Protection against Asthma (SAB) (1999-current)
Program leader in Poverty Related Infection Oriented Research (PRIOR) network (NWO-WOTRO) (2004-current)
Executive board member Allergy Consortium Wageningen (ACW) (2002-current)

Member Frofessional Oragnizations:
Member Scientific advisory board: Adviescommissie Landelijk Overleg Onderwijs-Arbeid, HBO-opleiding Biologie en Medisch Laboratoriumonderzoek, HBO-raad (1999-2004)
Chairman Curriculum board Biology, Wageningen University (2002-2006)
Executive board member Cereales Foundation Wageningen (2005-current)

5. Specializations
basic and applied immunology
antibody formation (isotype switching)
immunoregulation: regulatory T cells, T cell polarization, cytokines,
comparative immunology (aquatic organisms, chicken, bovine, horse)
allergy: food allergens, life-style factors
natural disease resistance and robustness

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: March 9, 2007

TIME: 2:30 PM.

NUMBER: 415-957-0101

PARTICIPANTS:

FDA
Jeremiah Fasano HFS-255
Antonia Mattia HFS-255

External
Charles Morin Morin & Associates

SUBJECT: Discussion of public hearing on human lactoferrin

Dr. Mattia and I contacted Mr. Morin to alert him to our interest in fostering public discussion of issues related to the use of human lactoferrin in foods. Mr. Morin is the agent for GRN 189, submitted by Pharming, Inc. (Pharming), which describes certain food ingredient uses of human lactoferrin from bovine milk. We noted that in FDA's response to a previous notice from a different firm that also involved human lactoferrin (GRN 162), the agency had stated its intent to engage the wider scientific community in consideration of the complex scientific issues raised by the notice. Though Pharming's notice was distinct from GRN 189, in our view some of the issues raised by each notice were related. For that reason, we thought it appropriate to keep Pharming informed about our ongoing efforts to engage the wider scientific community. We explained that we were currently considering a proposal for a Part 15 hearing as a vehicle for this engagement.

Mr. Morin requested an opportunity for a broader selection of Pharming representatives to hear and discuss FDA's plans at a future date. We agreed to this request.

Jeremiah Fasano

R/D:HFS-255:JMFasano:03/09/2007
Init:HFS-255:AMattia:06/01/2010
F/T:HFS-255:JMFasano:06/01/2010

FILE COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

Fasano, Jeremiah

From: Fasano, Jeremiah
Sent: Wednesday, April 04, 2007 10:46 AM
To: 'charleslmorin@earthlink.net'
Subject: RE: April 16th teleconference

Mr. Morin-

Thank you for arranging the line. I've reserved the time slot for the CFSAN participants. Dr. Mattia and Dr. Merker are well-briefed and can handle it without me if necessary, but I will participate if I can.

Regards-

-Jeremiah Fasano

_____
Jeremiah Fasano, Ph.D.
Consumer Safety Officer
DBGNR/OFAS/CFSAN/FDA

jfasano@cfsan.fda.gov
Phone: 301-436-1173
Fax: 301-436-2964

HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

From: Charles L. Morin [mailto:charleslmorin@earthlink.net]
Sent: Tuesday, April 03, 2007 5:55 PM
To: Fasano, Jeremiah
Subject: April 16th teleconference

Dear Dr. Fasano,

This will memorialize the information I left on your voice mail concerning the future teleconference between CFSAN and Pharming. Pharming has now confirmed the availability of all its participants and, thus, can now represent that all can participate on Monday, April 16th starting at 10:30 a.m. (EST). Each such participant as well as those participating on behalf of CFSAN can connect to the teleconference on Monday by dialing 1 (866) 448-6761 and then, when asked to do so, by dialing the dial-in code, i.e., 940357.

Please let me know if there is anything else we need to do prior to our teleconference on April 16th.

Best regards.

Charles L. Morin
Morin & Associates
388 Market Street, Suite 1460
San Francisco, CA 94111
US

Phone: (415) 957.0101
Fax: (415) 957.5905

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: April 16, 2007

TIME: 10:30 AM

PARTICIPANTS:

FDA

Antonia Mattia	HFS-255
Robert Merker	HFS-255
Jeremiah Fasano	HFS-255

External

Charles L. Morin	Morin and Associates
Frans de Loos	Pharming
Bertjan Ziere	Pharming
Sanda van Wetering	Pharming
Harrie van Veen	Pharming
Anurag Relan	Pharming
Charles Elson	University of Alabama at Birmingham

SUBJECT: Discussion of Lactoferrin Public Hearing Proposal

Members of the Division of Biotechnology and GRAS Notice Review met with representatives of Pharming, including the firm's agent (Mr. Morin) and a member of the GRN 189 GRAS panel (Dr. Elson). We wished to discuss our proposal for a public hearing on the use of recombinant human lactoferrin (rhLF) in food.

We began by explaining that although the proposed Part 15 public hearing was prompted in part by the submission of GRN 189, the evaluation of GRN 189 and the public meeting were distinct processes that were not dependent on each other. Nevertheless, we considered that both Pharming and the agency would benefit if Pharming had the opportunity to learn about the kinds of questions we were proposing to ask at the hearing.

The proposed hearing would focus entirely on scientific issues rather than the regulatory status of rhLF. The issues are currently as follows:

- Evaluation of rhLF idenitity with respect to
  - bLF in terms of bioactivities (in various species, including humans)
  - hLF in terms of antigenicity (perception of rhLF as a nonself protein potentially leading to concerns about autoimmunity)

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OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

- The significance of existing exposures to hLF and their utility in a food safety assessment of rhLF
  - infant oral exposure to hLF in human milk
  - adult oral exposure to hLF from exocrine secretions such as saliva
- The consequences of long-term exposure to an exogenous immunomodulator such as rhLF

With respect to the last issue, we explained that we were interested in what sorts of studies, species, and endpoints might or might not be appropriate for this kind of substance and exposure scenario, and the scientific reasoning for any given position.

Pharming noted that the issues we had described were complex and relatively specific and that a third party would require substantial background information to understand them clearly. Pharming then asked about the scope of the hearing and whether their ingredient would be conflated with other ingredients. We explained that the hearing was intended to explore general issues raised by rhLFs as a class, but that any related GRAS determination would continue to be on a case-by-case basis. Pharming would have the opportunity to clearly identify any relevant distinctions between its own product and other rhLFs if the firm chose to give a presentation. Finally, we agreed that a public hearing notice on this topic would require clarity and specificity. We invited Pharming to submit sample text. At a minimum, this would help identify gaps in our draft text.

We then stated that while we intended to hold the meeting in the summer of 2007, at this point the public meeting was still a proposal. As such, it would require clearance by the Center and FDA and we could make no guarantees about the timing or exact content of the meeting notice.

Finally, we explained that the GRAS notification process is not intended to be lengthy or iterative, but that we had offered Pharming the opportunity to provide additional information because of the novelty of the subject matter from a scientific and regulatory perspective. We considered that Pharming had provided a substantive amendment to the original notification, and that we would not be requesting any additional information from the firm. We reminded Pharming that we had not yet arrived at a conclusion of ‘no questions’ for GRN 189 and that all potential outcomes (including ‘no questions,’ ‘no basis,’ or ‘withdrawal without prejudice’ responses) were still possible. We also reiterated that the outcome of our evaluation was not formally connected to the results of the proposed public meeting and that our evaluation could be completed prior to or following the public meeting. However, we would contact Pharming prior to issuing any letter as a courtesy.

Pharming stated that they appreciated the opportunity for dialogue and would pass suggested language to us soon.

Jeremiah Fasano

R/D:HFS-255:JMFasano:04/17/2007
Init:HFS-255:RIMerker:04/18/2007
Comment/Init:HFS-255:AMattia:04/18/2007
Edit:HFS-255:JMFasano:05/24/2010
F/T:HFS-255:JMFasano:05/24/2010

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: May 29, 2007

TIME: 12:40 P.M.

NUMBER: 415-957-0101

PARTICIPANTS:
FDA
Jeremiah Fasano HFS-255

External
Charles Morin Morin & Associates

SUBJECT: Discussion of information relating to recombinant human lactoferrin

Mr. Morin called me regarding the participation of Pharming, whom he represents, in a planned public hearing on the safety of recombinant human lactoferrin.

Mr. Morin first stated that Pharming wished it to be clear that when the 'biological activities' of lactoferrin were referred to as a subject of inquiry at the hearing, in this context this phrase meant immunological activities rather than generic toxicity.

Mr. Morin also stated that Pharming was investigating, and planning to introduce, two additional kinds of evidence at the hearing. The first was the significance of the long-established practice of "wet-nursing" in which infants consumed nonparental hLF. Second, there is limited experience with adult consumption of nonparental human milk and thus hLF. In light of this evidence, Mr. Morin suggested that parts of the public hearing notice referring to consumption of nonparental hLF be carefully phrased.

I thanked Mr. Morin for his suggestions and information and we concluded the call.

Jeremiah Fasano

R/D:HFS-255:JMFasano:05/29/2007
F/T:HFS-255:JMFasano:05/26/2010

FILE COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

AM

Law Offices Of
Morin & Associates

Suite 1460
388 Market Street
San Francisco, California 94111
Telephone: (415) 957-0101 e-mail: charleslmorin@earthlink.net Facsimile (415) 957-5905

July 26, 2007

Antonia Mattia, PhD (HFS-255)
Director
Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied
Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re Pharming Group NV
Notice of GRAS exemption for human
lactoferrin derived from the milk of
transgenic cows expressing a human gene
encoding human lactoferrin
GRN No 000189
Additional information

Dear Dr. Mattia

As both CFSAN and Pharming personnel await the Commissioner's decision concerning whether, when, and specifically how to conduct the CFSAN-requested hearing pertinent to transgenically-produced human lactoferrin, please appreciate that Pharming has expended significant and precious resources for months in preparation for such hearing. Among the many things Pharming has done as a part of such preparation is to rechallenge the qualified expert views that were set forth in its prior

"Response" document (dated December 22, 2006) to ensure that such views are, in fact, representative of the consensus of the qualified expert community, To this end, Pharming has added two, additional, very qualified, very broadly-experienced immunologists to its expert panel – two experts who have no prior consulting or (prior or current) financial ties to Pharming and who – we have discovered – are fiercely independent They are.

1. Bana Jabri, MD, PhD
   Associate Professor
   Departments of Medicine, Pathology, and Pediatrics
   Committee on Immunology
   and
   Co-Director
   University of Chicago Digestive Disease Research Core Center
   University of Chicago, and
2. Martin F. Kagnoff, MD
   Professor of Medicine and Pediatrics
   School of Medicine
   and
   Director
   Laboratory of Mucosal Immunology and
   The Wm K. Warren Medical Research Center for Celiac Disease
   University of California (San Diego)

(For additional information pertinent to the two experts' qualifications, please see their respective CVs, attached as Attachments 1 and 2)

As their affidavits indicate (see Attachments 3 and 4), both were asked to review Pharming's "GRAS Notification", Pharming's "Response" and the "References" cited in those two documents and to indicate their evaluations of the substance set forth in the Response. Importantly, both – "after reviewing and analyzing all of the documents and literature" – indicated that they agreed with the substantive content of the Response and the conclusions reached therein by the expert panel of immunologists. Both of these qualified experts – along with the others – are also helping Pharming prepare its Hearing Presentation to assure that it reflects only the consensus view of the qualified expert community.

I thought you would be interested in this information since importantly it only confirms that information which has already been conveyed to you as being the consensus view of the pertinent, qualified expert community here in question. Please review and consider this information as we move forward with evaluation of Pharming's GRAS request.

Hope you and your colleagues are having a pleasant summer

Sincerely,

Charles L. Morin

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Table Of Contents

Attachment 1

CURRICULUM VITAE
Bana Jabri, M.D., Ph.D.

GENERAL INFORMATION

Address (b) (6)

Professional Address University of Chicago
Department of Pathology
5841 South Maryland Avenue - MC 1089
Chicago. Illinois 60637
Tel. (773) 834 8670
Fax (773) 834-5251
Email. bjabri@bsd uchicago.edu

Citizenship France

Education

ECFMG (Clinical part) 1986
BA in Biochemistry, Université Paris-VII (1988)
M.D Paris, France, Subspecialties in Pediatrics and Gastroenterology (1991)
PH D in Biochemistry, Université Paris-VII (1996)

Certification

Board certified, Paris, France (1991)
Specialty board. Pediatrics Subspecialty: Gastroenterology. Paris, France (1991)

Postdoctoral training

1985-1991	Medical Residency, Assistance Publique Hôpitaux de Paris, Paris, France
1989-1990	Fellow of the "Fondation pour la Recherche Médicale", Clinical Immunology and Immunodeficiency Department, Hôpital Necker, Paris, France (A Fischer and D Guy-Grand)
1991-1994	Fogarty Visiting Fellow, Laboratory of Molecular Biology and Allergology, National Institutes of Health, Bethesda, MD, USA (J.P Kinet)

Academic appointment

1994-1998	Assistant Professor, Universite Paris V, Hôpital Necker, Paris, France
02/1999- 2002	Research Scientist, Princeton University, Princeton NJ, USA
1999-present	Lecturer, Center for Immunobiology,Mount Sinai School of Medicine, New York, NY, USA
2002-2005	Assistant Professor, University of Chicago Department of Pathology, Chicago, USA Secondary appointment in Medicine and Pediatrics.
2005-present	Associate Professor, The University of Chicago, Departments of Medicine, Pathology and Pediatrics. Committee on Immunology

2006-present Co-Director of the University of Chicago Digestive Disease Research Core Center

Hospital appointment

1994-1996 Assistant Professor, Department of Pediatric Gastroenterology. Hôpital Necker Enfants-Malades, Paris, France

Awards and Honors

1991 Prize of Excellence in Pediatric Gastroenterology
1989-1990 Fellowship of the "Fondation pour la Recherche Medicale"
1991-1992 INSERM fellowship for post Doctoral studies
1992-1994 Fogarty Visiting Fellowship (NIH)

Membership

American Gastroenterology Association
American Immunology Association
International Mucosal Immunology

ADVISORY FUNCTIONS

Study Sections

1994-1998 INSERM Gastroenterology/Nutrition study section INSERM Pediatric study section
2000 Ad-Hoc reviewer for the Celiac Program Project (NIH)
2000-2002 Ad-Hoc reviewer for the Crohn's and Colitis foundation
2005 NIH/NIAID Special Emphasis Panel on "HLA Region Genetics In Immune-Mediated Diseases"
2006-2009 CCFA review study section
2006 Ad-Hoc reviewer for NIH/NIDDK Gastrointestinal Mucosal Pathobiology (GMPB) Study Section
2007 Ad-Hoc reviewer for NIH/NIDDK Gastrointestinal Mucosal Pathobiology (GMPB) Study Section

Foundations

2003-present Advisory Board for The University of Chicago Celiac Disease Program
2004- Medical Advisory Board of the National Foundation For Celiac Disease Awareness
2005- Scientific Advisory Board UCSD Celiac Center

Ad-Hoc Reviewer

Immunity, Journal of Clinical Investigation, Journal of Experimental Medicine, Blood, Gastroenterology, Journal of Immunology, European Journal of Immunology

UNIVERSITY OF CHICAGO COMMITTEE ASSIGNMENTS

College

2003-present BSCD Governing Committee
2006-present College Council

Committee on Immunology

2002-present Graduate Recruitment Committee
2002-present Curriculum Committee
2002-present Seminar Committee

2002-2006	Flow Cytometry Facility Committee
2003-present	Retreat Committee

Digestive Disease Research Center

2003-present	Executive Board
2004-2006	Director of the Pilot and Feasibility Project

TEACHING

Teaching

2000-2001	Princeton University Undergraduate Immunology Course 426 (20%)
2003-present	Chair of the Specialization in Immunology (College)
2003-present	Immunopathology Course BIOS 25528 (Instructor, 80%) (College)
2003-present	Advanced Immunology Course BIOS 25257 (20%) (College)

Supervision/Training Responsibilities

Post Doctoral Research Supervisor

Leanne Lee (Medical student)	1999-2001	(NK receptor in CTL)
Bertrand Mersesse	2001-2005	(NK receptor in celiac CTL)
Zarahui Hovhannisyan	2002-2005	(Transglutaminase in celiac disease)
Gerasim Orbelyan	2002-2006	(NK receptor in CTL)
Zhangguo Chen	2003-2005	(NK receptor signaling in CTL)
Sophie de Saint-Mezard	2004-2005	(Intestinal dendritic cells)
William de Paolo	2004-	(Host pathogen interactions)
Bofeng Li	2006-	(Role of IL-15 in autoimmunity)

Graduate and Medical Student Research Supervisor

Rebecca Liu	2003-	(NK receptors in tumor CTL)
Setty Mala	2004-	(Early presentation of celiac disease)
In Young Kim	2006-	(Hsp70· role in Immune regulation)

Undergraduate Research Supervisor

Lisa Bell (Medical student)	2000	(Transglutaminase in celiac disease)
Alexandra Martin (MSTP)	2001-2002	(Transglutaminase in celiac disease)
Nadine Levin	2005-	(Immune modulation by Yersinia)
Jason Solus	2005-	(Regulation of NKR in CTL)

BIBLIOGRAPHY

Peer reviewed research articles

Cuenod, B., Brousse, N., Goulet, O., De Potter, S., Mougenot, J.F., Ricour, C., Guy-Grand, D., Cerf-Bensussan, N. 1992. Classification of intractable diarrhea in infancy using clinical and immunohistological criteria. **Gastroenterology**; 99: 1037-43.

Goulet, O., Kedinger, M., Brousse, N., Cuenod B., Colomb V., Patey, N., De Potter, S., Mougenot, J.F., Canioni, D., Cerf-Bensussan, N., Ricour, C. 1995. Intractable diarrhea of infancy with epithelial and basement membrane abnormalities. **J Pediatr**;127: 212-219.

Scharenberg, A., Lin, S., Cuenod-Jabri, B., Yamamura ,H., Kinet, J.P. 1995. Reconstitution of interactions between tyrosine kinases and the high affinity IgE receptor which are controlled by receptor clustering **EMBO J**; 14: 3385-3394

Donadieu, J., Canioni, D., Cuenod, B., Fraitag, S., Bodemer, C., Stephan, J.L., Signaux, F., Deist, F., Schraub, S., Ranfraing, LE., Griscelli,C., Brousse, N. 1996. A familial T-cell Lymphoma with γδ Phenotype and an original location. **Cancer**, 77: 1571-1574.

Guy-Grand, D., Cuenod-Jabri, B., Malassis-Seris, M., Selz, F., Vassalli, P 1996. Complexity of the mouse Gut cell-immune system: identification of two distinct natural Killer-T cell intraepithelial lineages. **Eur J Immunol**; 26: 2248-2256.

Cuenod-Jabri, B., Zang, C., Scharenberg, A., Paolini, R., Numerof, R., Beaven, M., Kinet, J.P. 1996. Syk dependent phosphorylation of shc: a link between the high affinity IgE receptor and the Ras-MAP kinase signaling pathway. **J Biol Chem**; 27:16268-16272.

Patey, N., Scoazec, J.Y., Cuenod-Jabri, B., Canioni, D., Kedinger, M., Goulet, O., Brousse, N. 1997. Distribution of cell adhesion molecules in infants with intestinal dysplasia (Tufting enteropathy). **Gastroenterology**, 113:833-843.

Lacaille, F, Cuenod, B , Colomb, V., Jan, D., Canioni, D., Revillon, Y., Ricour, C., Goulet, O. 1998. Combined liver and small bowel transplantation in a child with epithelial dysplasia. J. **Pediatr Gastroenterol Nutr**; 27: 230-3.

Bonnerot, C., Briken, V., Brachet, V., Lankar, D., Cassard, S., Jabri, B., Amigorena, S. 1998. Syk protein kinase regulates Fc receptor gamma-chain-mediated transport to lysosomes. **Embo J**; 17:4606-4616.

Cellier, C., Patey, N., Mauvieux, L., Jabri, B., Delabesse, E., Cervoni, J-P., Burtin, M-L., Guy-GranD, D., Bouhnik, Y., Modiglian, R., Barbier J-P., Macintyre, E., Brousse, N., Cerf-Bensussan, N 1998. Abnormal intestinal intraepithelial lymphocytes in refractory sprue. **Gastroenterology**; 114: 471-481.

Park,, SH., Guy-Grand, D., Lemonnier, FA., Wang, CR, Bendelac, A., Jabri, B. 1999. Selection and expansion of CD8αα+TCRαβ+ intestinal intraepithelial lymphocytes in the absence of both classical MHC class I and non classical CD1 molecules. **J Exp Med**; 190:885-890.

Patey, N., Cellier, C., Jabri, B., Delabesse, E., Verkarre, V., Roche, B., Lavergne, A., Briere, J., Mauvieux, L., Leborgne, M., Barbier, JP., Modigliani, R., Matuchansky, C., Macintyre E., Cerf-Bensussan, N., Brousse, N. 2000. Distinction between coeliac disease and refractory sprue: a simple immunohistochemical method. **Histopathology**, 37·70-77

Cellier, C., Delabesse, E., Helmer, C., Patey, N., Matuchansky, C., Jabri, B., Macintyre, E., Cerf-Bensussan, N., Brousse, N. 2000. Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. **Lancet**; 356:203-208.

Jabri, B., Patey, N., Cellier, C , Gache, C., Carvalho, C., Mougenot, JF., Allez, M., Jian, R., Desreumaux, P., Colombel, JF., Matuchansky, C., Cugnenc, H., Lopez-Botet, M., Vivier, E., Moretta, A., Guy-Grand, D., Brousse, N , Schmitz, J , Cerf-Bensussan, N. 2000. Selective expansion of intraepithelial lymphocytes expressing the HLA-E specific NK receptor CD94 in Celiac Disease. **Gastroenterology**; 118:867-879.

Roberts, AI., Lee, L , Schwarz, E., Groh,V., Spies, T., Ebert, CE., Jabri, B. 2001. Cutting Edge: NKG2D receptors induced by IL15 costimulate CD28-negative effector CTL in the tissue microenvironment. **J Immunol**. 167:5527-5530.

Jabri, B, Selby, J., Negulescu, H., Lee, L., Roberts, A.I., Beavis, A., Lopez-Botet, M., Ebert, EC., Winchester, RJ. 2002. TCR specificity dictates CD94/NKG2A expression by human CTL. **Immunity**; 17:487-499.

Green, PH., Fleischauer AT, Baghat G, Goyal, R, Jabri, B, Neugul, AI. 2003. Risk of Malignancy in patients with celiac disease. **Am J Med**, 115:191-195.

Johnson, TC., Negulescu, H., Winchester, RJ., Rotterdam, H., Memeo, L., Diamond, B., Caillat Zucman, S , Grosdidier, E., Cellier, C., Jabri, B., Verkarre, V., Green, PH. The Presence of HLA-DQ8 Does Not Correlate With Severity Of Celiac disease, Comparison of New York and Parisian Cohorts. 2004. **Clinical Gastroenterology and Hepatology,** 2:888-94.

Meresse, B., Chen, Z, Ciszewski, C., Tretiakova, M., Bhagat, G., Krausz, T.N., Raulet, D.H., Lanier, L.L., Groh, V., Spies, T., Ebert, E.C., Green, P.H., Jabri, B. Coordinated inducation by IL-15 of a TCR-independent, NKG2D signaling pathway converts CTLs into natural killer-like, lymphokine activated killer (LAK) cells in celiac disease. 2004 **Immunity**, 21:357-366.

Overhem, KA, Depaolo, R.W., Debord, K.L., Morrin, E.M., Green, N.M., Anderson, D., Brubaker, R.R , Jabri, B., Schneewind, O.. Plague vaccines lacking the immune modulatory properties of rLcrV. 2005. **Infect and Immun**, 73:5125-9

Marketon, M.M., Depaolo, R W., Debord, K.L., Jabri, B. Type III injection of select immune cells during plague infections. 2005. **Science**, 309:1739-41

DeBorad, K.L , Anderson, D.M., Marketon, M M., Overheim, K.A., DePaolo, R.W., Ciletti, N.A., Jabri. B., Schneewind, O. Immunogenicity and protective immunity against bubonic plague and pneumonic plague by immunization of mice with the recombinant V10 antigen, a variant of LcrV. 2006. **Infect Immun**, 74:4910-4.

Meresse, B. Curran, S.A., Ciszewski, C., Orbelyan, G., Setty, M., Bhagat, G., Lee, L., Tretiakova, M., Semrad, C , Kistner, E., Winchester, R.J., Braud, V., Lanier, L.L., Geraghty, D., Green, P.H., Guandalini, S and Jabri, B. Reprogramming of CTLs into natural killer–like cells in celiac disease. 2006. **Journal of Experimental Medicine**, 203:1345-55

Perera L., Shao, L., Patel, A., Evans, K., Meresse, B., Blumberg, R., Geraghty, D., Groh, V., Spies, T., Jabri, B., Mayer, L. Expression of nonclassical class I molecules by intestinal epithelial cells. 2007. **Inflamm Bowel Dis** 19:298-307

Invited reviews

Peer reviewed:

Green, P.H.R. and Jabri, B. 2003. Seminar: Coeliac disease. **Lancet**, 362: 383-391

Sollid, L.M. and Jabri, B. 2005. Is celiac disease and autoimmune disease? **Curr Opin Immunol**, 17:1-6

Jabri, B. and Sollid, L.M. 2006. Mechanisms of disease. Immunopathogenesis of Celiac Disease. **Nature Clinical Practice Gastroenterology & Hepatology**, 3:516-25

Non Peer invited reviewed

Cuenod, B , Cerf-Bensussan, N. 1992. Immunology of intractable diarrhoea. Immunology of Gastrointestinal Diseases, Eds: Mc Donald TT, **Kluwer Academic Publishers**, pp: 75-85.

Jabri, B, Kasarda, DD., Green, P H.R 2005 Celiac Disease **Immunol Rev**, 206:219-31

Jabri, B. and Meresse, B. 2006. NKG2 receptor-mediated regulation of effector CTL functions in the human tissue microenvironment. **Curr Top Microbiol Immunol**, 298:139-56. Review.

Green PH, Jabri B. 2006. Celiac Disease. **Ann Rev Med**, 57:207-21.

Jabri B. and E.C. Ebert. Human CD8 intraepithelial lymphocytes. a unique model to study the regulation of effector cytotoxic T lymphocytes in tissue **Immunol Rev**. 215:202-14

Scientific meetings

Organization

AGA DDW Abstract review (2000-present)
International Congress of Mucosal Immunity Abstract review (2002)
12th International Congress of Mucosal Immunity Steering Committee (2004)
Co-organizer of 12th International Celiac Symposium (2006)

Invited Plenary Talk
- Conference on Mucosal Immunity. Bruxelles, Belgium, 1993
Workshop on Intestinal Inflammatory disease (GETAID). Paris, France, 1996
Workshop on classifications of food allergies Washington DC, USA, 1999
- Workshop on Intestinal Immunity. Pontoise, France, 2000
Third International Workshop on Autoimmunity and Lymphoma. Baltimore, MD, USA, 2001
- Tenth International Symposium on Coeliac Disease Paris, France, 2002
Autoimmune diseases of the digestive tract (organized by the Spanish Society of Immunology), Cordoba, Spain, 2004
Spanish meeting of Immunology. Cordoba, Spain, 2005
- Workshop on 'Genetic control of T cell activation', Lofoten, Norway, 2005
- Keystone Symposium on Innate Immunity to Pathogens, Colorado, USA, 2005
- Dageraad Symposium : "MHC class I (like) molecules: Effects and Defects ", Leiden, Netherlands, 2005
- 9th Meeting of the Society for Natural Immunity. Hawaii. 2005
- Keystone Symposium on Innate Immune recognition. 2006
- DDW, Los Angeles, CA, 2006
- AAI meeting, Boston, 2006
- IL-15: Basic research and clinical applications. NIH, Bethesda, 2006.
- XII International Celiac symposium, New York, 2006
- ESPGHAN, Barcelona, Spain, 2007

Chair
- Tenth International Symposium on Coeliac Disease. Paris, France, 2002
- AGA DDW, State of the Art lecture on the pathogenesis of celiac Disease. Orlando, FL, 2003
- International mucosal Immunity meeting, Boston, USA, 2005
- AGA DDW, Pathogenesis of Celiac disease, Los Angeles, 2007
- AAI, Boston, 2006
- AGA DDW, Pathogenesis of Celiac disease, Washington DC, 2007

Oral Presentations of Abstract
- Keystone Symposium, Mucosal Immunity, USA, 1999
- Keystone Symposium, Interface between Innate and Adaptive Immunity, USA, 2001
- Keystone Symposium, Lymphocyte Activation, USA, 2002
- 7th Annual Meeting of the society for Natural Immunity, Puerto Rico, USA, 2002 (plenary talk)
- AGA, DDW, San Franciso, USA, 2002
- AGA, DDW, New Orleans, USA, 2004
- Eleventh International Symposium on Coeliac Disease, Belfast, Ireland, 2004 (plenary talks)

Extramural Seminar Speaker
- Institut Pasteur, Paris, 1999

- Hôpital Necker-Enfants-Malades, Paris, 2000
- Columbia University, NY, 2000
- Johns Hopkins, Baltimore, 2001
- Harvard University, Boston, 2002
- Columbia University, NY, 2003
- Mayo Clinic, Rochester, 2003
- Columbia University, NY, 2004
- Institut de Pharmacologie Moléculaire et Cellulaire, Nice, 2004
- Celiac Disease Foundation, Stanford, 2004
- Scripps, San Diego, 2005
- University of Oslo, Norway, 2005
- University of Naples, Italy, 2005
- University of Lausanne, Lausanne, 2005
- Institut Curie, Paris, 2005
- UCSD Celiac Center, San Diego, 2006
- Mount Sinai Immunobiology Center Seminar Series. 2007
- NIH Twinbrook seminar series 2007
- UCSD Celiac Center, San Diego, 2007
- Institute of Immunology, University of Oslo, Oslo, 2007
- Institut Pasteur, Paris, 2007

Research Funding

Past funding.
1. Investigator Award in France (1996-1998)
2. DDRC P&F study (P30 DK42086): 'NKG2 Receptors and IEL in celiac disease' (2003-2004)
3 NIH/ NIDDK 058727-06 (PI)
Project period: 9/1/01-6/30/06
Title: Regulation of Human IELs by CD94 and HLA-E

Present funding:

Principal Investigator.
1 Granting agency or source: NIH/ NIDDK 058727-07A1
Project period: 7/1/06 – 6/30/11
Title: Regulation of normal human IEL by NKG2D and IL-15

2. Granting agency or source: NIH/ NIDDK 067180-01A1
Project period: 12/01/04-11/30/09
Title: IEL and NKG2 receptors in celiac disease

Co-Investigator
1. Granting agency or source: NIH/NIAID U54 A1057153-01 (PI Schneewind)
Project period: 09/01/03-8/31/08
Title: Great Lakes regional center for excellence on Bioterrorism: Molecular Analysis and Intervention

2. Granting agency or source: NIH/ NIDDK P30 DK42086 (PI E. Chang)

Project period: 12/1/05-11/30/10
Title: IBD and Mucosal Inflammation, Immunology and Microbiology of the GI Tract

Attachment 2

Curriculum Vitae

Martin F. Kagnoff, M.D.

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University of California, San Diego
Department of Medicine, 0623D
9500 Gilman Drive
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(858) 534-4622 (telephone)
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mkagnoff@ucsd.edu

Laboratory URL Web sites
http://medicine.ucsd.edu/mucosalimmunology
http://celiaccenter.ucsd.edu

Place of Birth
Vancouver, British Columbia, Canada (U.S. Citizen)

Education

M.D.	1965	Harvard Medical School, Boston, MA

Professional Experience

2005-pres	Director, Wm. K. Warren Medical Research Center for Celiac Disease	Univ. of California, San Diego, CA
2003 - present	Professor of Pediatrics	Univ. of California, San Diego, CA
1983 - present	Professor of Medicine	Univ. of California, San Diego, CA
1976 - 1983	Assoc. Professor of Medicine	Univ. of California, San Diego, CA
1972 – 1976	Assist. Professor of Medicine	Univ. of California, San Diego, CA
1972 – 1974	Visiting Scientist	Salk Institute, La Jolla, CA
1970 – 1972	NIH Trainee in Gastroenterology	Boston Univ. School of Medicine, MA
1969 – 1970	Senior Resident in Medicine	New York Hospital, Cornell University
1967 – 1969	Lt. Commander, U.S.Navy, Principal Investigator, Dept. of Experimental Pathology	Armed Forces Radiobiology Research Institute, National Naval Medical Center, Bethesda, MD
1965 - 1967	Intern and Junior Resident in Medicine	Peter Bent Brigham Hospital (currently, Brigham&Women's Hospital), Boston, MA

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Board Certification

- American Board of Internal Medicine
- American Board of Gastroenterology

Professional Memberships

- American Gastroenterological Association
- American Association of Immunologists
- American Association of Physicians
- American Society for Clinical Investigation
- American Physiological Society
- Fellow, American College of Physicians
- Western Association of Physicians
- Society for Mucosal Immunology
- Gastroenterology Research Group

Career Awards

1972-1975	Clinical Investigator Award, NIH
1975-1980	Research Career Development Award, NIH
1985	Western Gastroenterology Research Prize
1994	Fiterman Senior Research Award, American Gastroenterology Association
2001	Rotschild Mayent Award, Institut Curie, Paris France
2004-2005	UCSD Academic Senate Distinguished Faculty Research Lecture in the Sciences

Current Research Support

- National Institute of Diabetes, Digestive and Kidney Diseases (5 P01 DK35108)
  Principal Investigator: Martin F. Kagnoff, M.D.
  Title: "*Intestinal Immune System in Host-Environment Interaction*"

- National Institute of Diabetes, Digestive and Kidney Diseases (5 R01 DK58960)
  Principal Investigator: Martin F. Kagnoff, M.D.
  Title: "*Intestinal Epithelial Response to Foodborne Pathogens*"

- The William K. Warren Foundation
  Principal Investigator and Program Director: Martin F. Kagnoff, M.D.
  Title: *Center for Celiac Disease Research*

Selected University of California Committees and Activities (1984-present)

1984-1985	Chair, School of Medicine Core Curriculum Committee
1984-1985	Member, School of Medicine Committee on Educational Policy
1985-1987	Chair, Faculty Senate Committee on Academic Freedom, UCSD
1987-1989	Chair, University of California Systemwide Academic Senate Committee on Academic Freedom
1987-1989	Chair, Pathophysiology Course Committee, SOM 215
1987-1995	Member, School of Medicine, SBH Prize Committee
1987-1990	Member and Chair (1989-1990), Nominating Committee, UCSD School of Medicine
1989-1990	Chair, Nominating Committee, UCSD School of Medicine
1989-1992	Member, University-wide Task Force on Mandatory Retirement
1990-1992	Member, Faculty Council, School of Medicine
1990-1992	Chair, Committee on Educational Policy (CEP), School of Medicine
1991-1992	Member, Faculty Council Subcommittee on Eastern European Medical School Exchange Programs
1991-1995	Member, Dept. of Medicine Committee on Academic Personnel (Promotions)
1991-pres	Member, School of Medicine Graduate Program in Biomedical Sciences
1991-pres	Member, School of Medicine Graduate Program in Molecular Pathology
1992-1994	Chair, Executive Committee, UCSD Division of Gastroenterology
1997-pres	Director, NIH Institutional Research Service Award in Digestive Diseases and Director, Research Training Program, Division of Gastroenterology
1999-2001	Member, Research Residency Committee, Dept. of Medicine
1999-2000	UCSD Committee on Conflict of Interest, Ad hoc member
2001-pres	Member, Minor Proposition Committee, Biomedical Sciences Graduate Program
2002-pres	Ad hoc Review Committees, UCSD Committee on Academic Personnel, and School of Medicine Committee on Academic Personnel
2003-pres	Member, UCSD Cancer Center
2003-2004	Member, Dean's review committee, Department of Ophthalmology, School of Medicine
2003	Member, DOM Search Committee for Chief, Division of Gastroenterology
2004-2007	Chair, Department of Medicine/Division of Gastroenterology Search Committee for two tenure-track faculty members.

Selected Outside Activities (1991-present)

1991-pres	Member, Scientific Advisory Board, Celiac Sprue Assoc., USA
1991-2001	Member, Advisory Board, Digestive Diseases Center, Harvard Medical School
1991-1993	Member, American Gastroenterological Association Research Committee
1991-1996	Member, Editorial Board, Gastroenterology
1992-1997	Member, Biomedical Research Review Panel, Alberta Heritage Foundation
1992-1994	Member, Basic Sciences Study Section, State of California, AIDS Research Program
1992-1993	American Gastroenterological Association Research Committee; Chair, Senior Fellowship Awards Subcommittee
1992-1999	Chair, NIH, Special Study Section on Mucosal Vaccines Chair, NIH, Special Study Section on AIDS Vaccines Member, NIH, Special Study Section on Sexually Transmitted Diseases Member, NIH, Special Study Section on HIV and Wasting

Selected Outside Activities (1991-present), continued

	Member, NIH, Special Study Section on *H. pylori* Infection
1995	Organizer and Conference Director, 8th International Congress of Mucosal Immunology, San Diego
1996	Co-Director, Keystone Symposia on Molecular and Cellular Biology, "*Mucosal Immunity: Cellular and Molecular Cross-Talk at Mucosal Surfaces*".
1996-pres	Member, Medical Advisory Board, Celiac Disease Foundation
1997-2003	Member, Steering Committee, Gastrointestinal Diseases Section, American Physiological Society.
1999-2004	Student Group Advisor, Medical Scientist Training Program
1999-2000	Member, Conflict of Interest (IRC) Ad Hoc Committee
1999	Member, Scientific Organizing Committee, 10th International Congress of Mucosal Immunology
2000-2004	Vice Chair, Immunology, Microbiology and Inflammatory Bowel Disease Section, Council of the American Gastroenterological Association.
2000	Organizer and Conference Director, Keystone Symposium on Molecular and Cellular Biology, "*Innate and Acquired Immunity at Mucosal Surfaces*".
2001-2004	Member, Research Committee, American Gastroenterological Association
2001-2004	Member, External Advisory Committee, Mt. Sinai School of Medicine Research Program in Immunobiology.
2001-2003	Scientific Advisory Council & Organizing Committee for the 11th International Congress of Mucosal Immunology
2002	Organizer and Conference Director, Keystone Symposium on Molecular and Cellular Biology, "*Epithelial-Microbial-Lymphocyte Interactions*"
2002	Session Chair, Microbial-Mucosal Interactions Workshop, Harvard Medical School
2002-2005	Senior Faculty Advisor, Annual "IBD Research: Junior Faculty Symposium", Northwestern Univ Medical School, Chicago, IL and Johns Hopkins Univ., Baltimore, MD
2002-2005	Member, Scientific Advisory Board, Celiac Sprue Research Foundation
2003	Chair, Immunology and Microbiology Symposium, "Bacteria Meet the Intestinal Epithelium: Strategic Encounters," Annual American Gastroenterology Association Meeting, Orlando, FL.
2003-2004	Organizing Committee for NIH Consensus Conference on Celiac Disease
2003-2005	Member, Steering Committee, 12th International Conference of Mucosal Immunology
2003-pres	Member, Editorial Board, American Journal of Physiology; Gastrointestinal & Liver Physiology
2003-2005	Scientific Organizer, New York Academy of Sciences International Symposium on Inflammatory Bowel Disease, Germany.
2004-pres	American Physiological Society, Publications Committee
2004-pres	Member, External Advisory Board, Digestive Diseases Research Center, Univ. of Virginia
2004-2006	Chair, Immunology, Microbiology, & Inflammatory Bowel Disease Section, Council of the American Gastroenterology Association.
2004-pres	Member, Advisory Board, National Foundation for Celiac Awareness
2004	Associate Editor, Encyclopedia of Gastroenterology, Elsevier Academic Press.

Selected Outside Activities (1991-present), continued

2004	Coordinator & Session Chair, Symposium on "Intestinal Parasites: Friends and Foes," Chair, AGA Distinguished Abstract Plenary Session on Immunology, Microbiology, and Inflammatory Bowel Disorders, annual meeting of American Gastroenterological Assoc., New Orleans, LA
2005	Section Co-Editor, "Host responses to *E coli* and *S. typhimurium*, Eco-Sal Handbook, ASM Press.
2007	External Reviewer NICDR intramural NIH program in Mucosal Immunology and Inflammation
2007-pres	Councillor, Society for Mucosal Immunology
2007	Senior Mentor, AGA Symposium for Junior Faculty in Inflammatory Bowel Diseases

Reviewer for Editorial Boards (2002-present)

- Science
- Cell
- Nature Medicine
- Nature Immunology
- Gastroenterology
- New England Journal of Medicine
- Journal of Immunology
- Journal of Experimental Medicine
- Infection and Immunity
- American Journal of Physiology
- American Journal of Pathology
- Journal of Clinical Investigation

Journal Editorships and Editorial Boards

1992-1996	Associate Editor, Journal of Clinical Investigation
1994-1999	Associate Editor, the Journal of Immunology
1995-2005	Member, Editorial Board, Scandinavian Journal of Immunology
1996-1997	Editor, Journal of Clinical Investigation
1997-2003	Editor in Chief, American Journal of Physiology: Gastrointestinal & Liver Physiology.
2003-pres	Member, Editorial Board, American Journal of Physiology: Gastrointestinal & Liver Physiology
2005	Guest Editor, Seminars in Immunopathology volume, "Immunopathology of the Gastrointestinal Tract."

Grant Reviews

1992-1999	Chair, NIH, Special Study Section on Mucosal and Synovial Gene Transfer
1999	Chair, NIH Special Study Section on Celiac Disease
2000-pres	Ad Hoc Grant and Program reviews for National Institutes of Health and the Medical Research Council of Canada,.
2003-2005	Member, Gastrointestinal Mucosal Pathobiology Study Section, (GMPB), National Institutes of Health
2006	Invited International Reviewer, Qanu Celiac Disease Consortium, The Netherlands

Selected Seminars and Lectures (1995 - present)

01/95	Speaker, Keystone Symposium on "Mucosal Immunity: New Strategies for Protection Against Viral and Bacterial Pathogens", Keystone, Colorado
03/95	Session Chair, New York Academy of Sciences meeting on "Oral Tolerance: Mechanisms and Applications", New York
04/95	Speaker, Symposium on "Neuroimmunology of the Gut: Methodological Advances & Therapeutic Implications", Experimental Biology Mtg, 1995, (FASEB), Atlanta, GA
05/95	Speaker, "The Year in Medicine-Gastroenterology/Hepatology", AFCR/ ASCI/AAP Annual Meeting, San Diego, CA
05/95	Session Co-Chair, Digestive Disease Week, American Gastroenterological Assoc. Meeting, San Diego, CA; Speaker at "Meet the Investigator" session, DDW, San Diego, CA
07/95	Plenary Speaker , 8th International Congress of Mucosal Immunology, San Diego, CA
09/95	Speaker, Celiac Sprue Association (CSA/USA) Regional Meeting, San Diego, CA
09/95	Seminar, Czech Academy of Sciences, Institute of Microbiology, Prague, Czech Republic
09/95	Speaker, 4th United European Gastroenterology Week, Berlin
10/95	Plenary Speaker, Celiac Sprue Association (CSA/USA) annual meeting, San Francisco, CA
11/95	Plenary Speaker, 3rd Seoul International Digestive Disease Symposium, Seoul, Korea
11/95	Speaker, U.S./Japan Cholera and Related Diarrheal Disease Panel, Kiawah, South Carolina
01/96	Speaker, Alberta Gastroenterology Society, Edmonton, Alberta, Canada
02/96	External Examiner and Official Opponent for Ph.D. Thesis of William W. Agace, Department of Microbiology, University of Lund, Lund, Sweden
03/96	Visiting Professor, Division of Gastroenterology, Stanford University, Stanford, C
03/96	Speaker, Oral Tolerance Workshop, American Association of Allergy/ Immunology, New Orleans, LA
04/96	Visiting Professor, Division of Gastroenterology, University of Illinois, Chicago, IL
05/96	Chair and Invited Speaker, Celiac Disease Symposium, American Gastroenterological Association annual meeting, Digestive Disease Week, San Francisco, CA
08/96	Speaker, Argentine Society of Gastroenterology, Immunological Disorders of the Intestine Symposia, Buenos Aires, Argentina
08/96	Speaker, Boehringer Ingelheim, Ridgefield, Connecticut
09/96	Speaker, Seventh International Symposium on Coeliac Disease, Tampere, Finland
10/96	Speaker, American College of Gastroenterology Postgraduate Course, Seattle, WA
11/96	Session Chair and Invited Speaker, Third European Science Foundation Conference on Microbial Pathogenesis, Obernai, France
01/97	Speaker, NIH, Mucosal Think tank on HIV vaccines, Washington, DC
01/97	Member, Scientific Organizing Committee & Session Chair, 9th International Congress of Mucosal Immunology, Sydney, Australia
02/97	Speaker, Canadian Association of Gastroenterology Symposium, "Role of Bacteria in Gastrointestinal Disease", Quebec City, Canada
03/97	Conference Organizer and Speaker, Inflammatory Bowel Disease (IBD) Symposium, Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT.
03/97	Conference Organizer, Keystone Symposia on Molecular and Cellular Biology, "Mucosal Immunity: Cellular and Molecular Cross-Talk at Mucosal Surfaces;" Session chair and Speaker "*Cross-Talk Between Bacterial Pathogens and Epithelial, Lymphoid and Antigen-Presenting Cells,"* Santa Fe, New Mexico

Selected Seminars and Lectures (1995 - present), continued

05/97	Speaker and Session Chair, American Gastroenterological Association Digestive Disease Week, May 10-16, 1997, Washington, DC
10/97	Speaker, Annual Meeting Celiac Sprue Association/United States of America, Inc., Seattle, WA.
10/97	Speaker, Visiting Professor UCLA Dental Research Institute, Los Angeles, CA
11/97	Speaker, 1997 American College of Gastroenterology Postgraduate Course, Chicago, IL. Speaker, Annual Symposium of Harvard Center for the Study of Inflammatory Bowel Disease, Boston, MA
02/98	Visiting Professor, Immunology Graduate Program and GI Division, University of Virginia, Research Seminar and Clinical talks, Charlottesville, VA
03/98	Speaker, Falk Symposium, *"Induction and Modulation of Gastrointestinal Inflammation,"* Saarbrüken, Germany
03/98	Speaker, NIDDK Interagency Coordinating Committee, *"Celiac Disease,"* Bethesda, MD
05/98	Speaker, 1998 Pediatric Academic Society Annual Meeting, New Orleans, LA.
5/98	Session Chair, American Gastroenterological Assoc. Research Forum "Mucosal Immunology," 1998 American Gastroenterology Association Annual Meeting, New Orleans, LA
09/98	Invited Participant, NIH Workshop on "Mycobacterium Avium Complex (MAC) Immunopathogenesis," Rockville, MD
09/98	Keynote Speaker, 1st International Congress on Spondyloarthropathies, Gent Belgium
10/98	Speaker, Washington University Symposium "Gut mucosal-microbial interactions," St. Louis, MO
10/98	Co-Organizer, Session Chair and Speaker, Falk Symposium "Intestinal Mucosa and its Diseases – Pathophysiology and Clinics," Titisee, Germany
11/98	Speaker, 8th Annual Symposium, Harvard Center for the Study of Inflammatory Bowel Disease, "Lymphocytes and IBD: Current Paradigms of Disease Mechanisms and Treatment," Boston, MA
12/98	Speaker, Course: Gastroenterology and Hepatology for Primary Care Physicians, La Jolla, CA
01/99	Keystone Symposium: Chemokines & Chemokine Receptors, Presentation on *"Human Intestinal Epithelial Cells Express an Array of CC and CXC Chemokine Receptors,"* Keystone, CO.
02/99	Speaker, 3rd Annual Winter H. pylori Workshop: *Developments and New Directions in Helicobacter Research· From the Basic Laboratory to the Patient*, "Microbial/Mucosal Interactions: Lessons from Other Systems, Orlando, FL.
04/99	Speaker, Session and Chairman, 8th International Symposium on Coeliac Disease, *"HLA genes in coeliac disease,"* Naples, Italy
05/99	"State of the Art" Speaker on *"Celiac Disease,"* Invited Session Chair, Research Forum *"Mucosal Immunology-Immune Regulation,"* Host: Meet the Professor Lunch – *"Microbial Epithelial Cell Interactions,"* Digestive Disease Week/American Gastroenterological Assoc. Annual Meeting, Orlando, FL
07/99	Invited Speaker, Symposium on Celiac Disease in Memory of Prof. Margot Shiner, Tel-Aviv University Medical School, Israel
09/99	Keynote Speaker, Swedish Medical Research Planning Group for Intestinal and Gastric Diseases, Söderköping, Sweden
09/99	Research Seminar, Pasteur Institute, Paris, France

Selected Seminars and Lectures (1995 - present), continued

09/99	Visiting Professorship and IZKF lecturer, University of Muenster, Germany
12/99	Invited Speaker, Center for the Study of Inflammatory Bowel Disease Workshop on "*Paradigms of Microbial-Mucosal Interaction*", Massachusetts General Hospital, Boston, MA
01/00	Conference Organizer, Session Chair and Invited Speaker Keystone Symposium "Innate and Acquired Immunity at Mucosal Surfaces," Taos, NM
02/00	Invited Speaker, Cystic Fibrosis Foundation Conference on Infection and Inflammation, Chantilly, VA
03/00	Invited Speaker, Immune Deficiency Foundation Sponsored Symposium, American Academy of Allergy & Asthma & Immunology Annual Meeting, San Diego, CA.
04/00	Visiting Professorship, University of North Carolina, Chapel Hill, NC
04/00	Invited Speaker and Symposium Organizer "*Epithelial-Microbial Interactions: Lessons in Communication*," Experimental Biology 2000, San Diego, CA
04/00	Invited Speaker and Participant, NIH Think Tank "*The Biology of HIV Transmission Think Tank*," Warrenton, VA
05/00	Invited Speaker: "*How to Get Published in GI Literature*," Invited Session Moderator, "*Bacterial-Immune Interactions at the Mucosal Interface," Invited Speaker: "What's New in Celiac Sprue?"* American Gastroenterological Assoc. Annual Meeting, Orlando, FL
08/00	Symposium Co-Organizer and Invited Speaker, "*9th International Symposium on Celiac Disease*," Baltimore, MD
09/00	Visiting Professor, Mayo Clinic, Rochester, MN
09/00	Invited Speaker, NIAID "*Developing Immune System Frontiers in Knowledge*," Arlington, VA.
12/00	Invited Discussant, "*Mucosal Signaling Pathways"* Meeting of the Crohn's & Colitis Foundation of America, Amelia Island, FL
03/01	Invited Speaker, 1st Annual Workshop on Immunogenetic Mechanisms of Intestinal Inflammation: Role of Cytokines and Chemokines, Univ. of Virginia, Charlottesvile, VA
03/01	Speaker and Participant, NIH Human Immunology Think Tank, Chantilly, VA
04/01	Speaker and Session Chair, Gut Ecology Workshop, Las Vegas, NV
07/01	Symposium Speaker, 11th International Congress of Immunology, Stockholm, Sweden
09/01	Keynote Address, 56th Annual German Society of Gastroenterology Meeting, Muenster, Germany
09/01	Invited participant, EMBO Conference on "*Microfilament Function and Regulation in Cell Polarity*," Gien, France
09/01-11/01	Invited Speaker for a series of 5 biweekly honorary Mayent-Rothschild research seminars on the theme: "Epithelial Cells: Lessons in Communication", Institut Curie, Paris France
10/01	Invited Faculty Member and Lecturer, course on "Advances in Mucosal Immunity", Naples, Italy
10/01	Invited Research Seminar, Institut Pasteur, Paris France
11/01	Invited Research Seminar, University of Auvergne, Clermont-Ferrand, France
11/01	Invited Research Seminar, University of Tours, Tours France
01/02	Invited Speaker, NIH Workshop: *Animal Models of Autoimmunity*, Bethesda, MD
04/02	Keystone Conference Organizer, Speaker and Session Chair "*Microbial-Epithelial-Lymphocyte Interactions in Mucosal Immunity*," April 5-10, 2002, Breckenridge, Colorado
05/02	Invited Speaker, ULCA Dept of Pathology, Grand Rounds, Los Angeles CA

Selected Seminars and Lectures (1995 - present)

05/02	Invited Chair, Symposium on Food Poisoning:Spectrum 2002 and Immunology, Microbiology and Inflammatory Bowel Disease Plenary Session, Annual Scientific Meeting, American Gastroenterological Association meeting, San Francisco, California
06/02	Invited Speaker "Distinguished Research Faculty Lecture", Hospital Necker, Paris France
06/02	Invited Keynote speaker, International Symposium on Celiac Disease, Paris, France
02/03	Invited Speaker, Symposium on "Innate Immunity and the Gut," Canadian Digestive Disease Week, Banff, Canada
03/03	Invited Speaker: Immunogenetic Mechanisms of Intestinal Inflammation, Role of the Epithelium, University of Virginia, Charlottesville, VA
03/03	Invited Seminar, University of Milan, Dept. of Biotechnology, Milan, Italy
04/03	Invited Speaker, Conference on "Translational Research in Autoimmunity," Portofino, Italy
06/03	Invited Speaker, Falk Symposium No. 133 "Mechanisms of Intestinal Inflammation: Implications for Therapeutic Intervention in IBD," Berlin, Germany
07/03	Invited Seminar, Celiac Disease, Genentech, South San Francisco
10/03	Visiting Professor and Seminar Speaker, "Program in Microbiology/Immunology," Tulane University, New Orleans
10/03	Invited Speaker, 26th Annual Celiac Sprue Association Annual Meeting, Buffalo, N.Y.
3/04	Invited Speaker, Dept of Pediatrics Postgraduate Course, Update on Celiac Disease, Children's Hospital, San Diego, CA.
04/04	GI Grand Rounds on Celiac Disease, Department of Medicine, Columbia University.
04/04	Invited Symposium Speaker, Experimental Biology, Amer. Assoc. of Immunologist Meeting, Washington, DC
5/04	Invited Senior Advisor and Reviewer, CCF sponsored Inflammatory Bowel Disease Junior Investigator Symposium, Northwestern University, Chicago, IL
5/04	Organizer, Research Symposium on Intestinal Parasites, American Gastroenterology Assoc. annual meeting, New Orleans
06/04	Invited Speaker, NIAID Biodefense Workshop, Animal Models for Radiation Injury, Protection, and Therapy, Washington, DC.
6/04	Conference Organizer and Invited Speaker: NIH Concensus Conference on Celiac Disease, Bethesda, MD
7/04	Invited Speaker, International Conference on Microbial-Epithelial Interactions, Newcastle upon Tyne, United Kingdom
10/04	Invited Advisor/Speaker, University of British Columbia, Canada-wide Project on Functional Pathogenomics of Mucosal Immunity, Vancouver, Canada
10/04	Invited Keynote Speaker, Celiac Sprue Assoc. Annual Meeting, Oklahoma City, OK.
11/04	Invited Speaker, PRISM Lecture, UCSD School of Medicine.
11/04	Invited Discussant, Center for the Study of Inflammatory Bowel Disease Symposium on "Stem Cells, Development, and Differentiation," Harvard Medical School, Boston, MA.
03/05	Invited Speaker on "Role of Intestinal Epithelium in Initiating and Regulating Mucosal Inflammation," Berlex Biosciences Meeting on "Recombinant Human GM-CSF in Crohn's Disease," Berkeley, CA.
04/05	Visiting Professor, Grand Rounds Speaker & Seminar Speaker, Dept of Medicine and Division of Gastroenterology, Rush School of Medicine, Chicago, IL
04/05	Invited Speaker, National Cancer Institute Workshop on "Mucosal Immunosurvelliance, Inflammation, and Cancer," Bethesda, MD

Selected Seminars and Lectures (1995 - present), continued

05/05	Speaker on "Pathogenesis of Celiac Disease," & Co-chair, Symposium on "Celiac Disease: A significantly underdiagnosed multi-system disorder," & Chair, "Immunology, Microbiology, & Inflammatory Bowel Disorders" Distinguished Abstract Plenary Session, American Gastroenterological Assoc. Annual Meeting, Chicago, IL
05/05	Academic Senate Distinguished Faculty Research Lecture in the Sciences, "Epithelial Cells: Lessons in Communication and Host Defense," UCSD.
02/06	Guest Speaker, International Symposium on Recent Advances in Inflammatory Bowel Disease, Tokyo, Japan.
03/06	Co-Organizer, AGA Host-Microbial Interactions in Digestive Health & Disease, Marina del Rey, CA
03/06	Visiting Professor, Swedish Medical Center, Seattle, WA "What's New in Celiac Disease: The Scientific Basis of Gluten Intolerance and Evaluation of the Patient in 2006."
05/06	Chair, "Immunology, Microbiology, & Inflammatory Bowel Disease" Session, Chair, "Molecular Basis of Innate Defense in the Intestine" Session, Annual Scientific Meeting, May 20-25, 2006, Los Angeles, CA
05/06	Visiting Professor, Cornell Medical Center, "*What's New in Celiac Disease*?", New York.
06/06	Speaker, Annual Digestive Disease Center Symposium "Autoimmunity in Digestive Health and Disease," June 23, 2006, Stanford, CA
11/06	Invited Keynote Speaker, XII International Celiac Diseases Symposium, "*Summary of Scientific Sessions*."
11/06	Invited Keynote Address, Korean Annual Gastroenterology Society Meeting, Seoul Korea
05/07	Visiting Professor, Univ of Michigan Digestive Diseases Research Center
05/07	Univ of Virginia Medical School Wide Lecture on Intestinal Inflammation

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3. **Kagnoff, M.F.** Reduced platelet adhesiveness following whole-body X-irradiation. Int. J. Radiat. Biol. 15:587-590, 1969.

4. **Kagnoff, M.F.** Motor activity *in vitro* of rat small intestine following whole-body X-irradiation. Radiation Res. 42:565-576, 1970.

5. Armstrong, D., Yu, B.H., Yagoda, A. and **Kagnoff, M.F.** Colonization of humans by *Mycoplasma canis*. J. Infect. Dis. 124:607-609, 1971.

6. **Kagnoff, M.F.**, Armstrong, D. and Blevins, A. Bacteroides bacteremia. Cancer Res. 29:245-251, 1972.

7. **Kagnoff, M.F.**, Donaldson, R.M., Jr. and Trier, J.S. Organ culture of rabbit small intestine: Prolonged *in vitro* steady-state protein synthesis and secretion and secretory IgA secretion. Gastroenterology 63:541-551, 1972.

8. **Kagnoff, M.F.**, Serfilippi, D. and Donaldson, R.M., Jr. *In vitro* kinetics of intestinal secretory IgA secretion. J. Immunol. 110:297-300, 1973.

9. **Kagnoff, M.F.** and Campbell, S. Functional characteristics of Peyer's patch lymphoid cells. I. Induction of humoral antibody and cell-mediated allograft reactions. J. Exp. Med. 139:398-406, 1974.

10. **Kagnoff, M.F.**, Billings, P. and Cohn, M. Functional characteristics of Peyer's patch lymphoid cells. II. Lipopolysaccharide is thymus-dependent. J. Exp. Med. 139:407-413, 1974.

11. **Kagnoff, M.F.** Induction and paralysis: A conceptual framework from which to examine the intestinal immune system. Gastroenterology 66:1250-1256, 1974.

12. Campbell, S.M., **Kagnoff, M.F.** and Watson, J. Demonstration of organ differences in peripheral B cell populations through the use of deficient fetal bovine serum. J. Immunol. 114:992-996, 1975.

13. **Kagnoff, M.F.** Functional characteristics of Peyer's patch cells. III. Carrier priming of T cells by antigen feedings. J. Exp. Med. 142:1425-1435, 1975.

14. **Kagnoff, M.F.** and Campbell, S. Antibody-dependent cell-mediated cytotoxicity; comparative ability of murine Peyer's patch and spleen cells to lyse lipopolysaccharide-coated and uncoated erythrocytes. Gastroenterology 70:341-346, 1976.

15. **Kagnoff, M.F.** Functional characteristics of Peyer's patch cells. IV. Effect of antigen feeding on the frequency of antigen-specific B cells. J. Immunol. 118:992-997, 1977.

16. **Kagnoff, M.F.** Effects of antigen-feeding on intestinal and systemic immune responses. I. Priming of precursor cytotoxic T cells by antigen feeding. J. Immunol. 120:395-399, 1978.

17. **Kagnoff, M.F.** Effects of antigen-feeding on intestinal and systemic immune responses: II. Suppression of delayed-type hypersensitivity responses. J. Immunol. 1209:1509-1513, 1978.

18. **Kagnoff, M.F.** On the etiology of Crohn's disease. Gastroenterology 75:526-527, 1978.

19. Hahn, W.V., **Kagnoff, M.F.** and Hatlen, L.E. Immune responses in human colon cancer: II. Cytotoxic antibody detected in patients' sera. J. Natl. Cancer Inst. 60:779-784, 1978.

20. Hahn, W.V., **Kagnoff, M.F.**, Hatlen, L.E. and Austin, R.K. Immune responses in human colon cancer: I. A microcytotoxicity assay for measuring killing of adherent human colon cancer cell lines. Gastroenterology 75:800-805, 1978.

21. **Kagnoff, M.F.** Effects of antigen-feeding on intestinal and systemic immune responses: III. Antigen-specific serum-mediated suppression of humoral antibody responses after antigen feeding. Cell. Immunol. 40:186-203, 1978.

22. **Kagnoff, M.F.** IgA anti-dextran B1355 responses. J. Immunol. 122:866-870, 1979.

23. **Kagnoff, M.F.** Effects of antigen-feeding on intestinal and systemic immune responses: IV. Similarity between the suppressor factor in mice after erythrocyte-lysate injection and erythrocyte-feeding. Gastroenterology 79:54-61, 1980.

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26. **Kagnoff, M.F.** Two genetic loci control the murine immune response to A-gliadin, a wheat protein that activates coeliac sprue. Nature 296:158-160, 1982.

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Attachment 3

Affidavit Of

Bana Jabri, MD, PhD

Bana Jabri, being first duly sworn, state as follows.

1. Currently, I am employed by the University of Chicago. I have been at the U of C since 2002. My current title is: "Associate Professor, The University of Chicago, Departments of Medicine, Pathology and Pediatrics. Committee on Immunology". I am also Co-Director of the University of Chicago Digestive Disease Research Core Center.

2. Prior to my employment at the U of C, I was employed – in either academic or research positions – at the University of Paris (1994-1998), Princeton University (1999-2002), and Mount Sinai School of Medicine (1999-present).

3. My training consists of a BA and PhD in biochemistry from the University of Paris, a MD (with subspecialties in pediatrics and gastroenterology) from the University of Paris, a medical residency iat the Public Assistance Hospitals of Paris, a fellowship in clinical immunology and immunodeficiency at the Necker Hospital (U of P) in Paris, and a Fogarty visiting fellowship in molecular biology and allergology at the NIH. For further details, please see the attached copy of my C. V.

4. With regard to my experience in gastroenterology- and immunology-related academic and research activities, please see the attached copy of my C.V. for specific details Please note that I am Chair of the "Specialization in Immunology" and that I teach "Immunopathology" and "Advanced Immunology" In addition, I am directly (and indirectly as supervisor of related undergraduate research, graduate and medical school research, and post doctoral research) and significantly involved in immunology-related research

5. I also participate in outside activities related to immunology, including maintaining pertinent memberships (e g , in the American Gastroenterology Association, the American Immunology Association, and International Mucosal

Immunology), functioning as an ad-hoc reviewer (e g., for the Journals of Immunity, Gastroenterology, Immunology, and the European Journal of Immunology), and serving as an ad-hoc reviewer for the "NIH/NIDOK Gastrointestinal Mucosal Pathobiology (GMPS) Study Section""

6 In my capacity as a recognized, qualified expert in gastroenterology and immunology, I was asked by Pharming Group NV – a biotechnology company producing drugs and food-related products from transgenic animals – to participate on an expert panel whose function it is to evaluate the safety of Pharming's rhLF when used as an ingredient in sports and functional foods at a level of 100 mg per product serving – especially as it relates to rhLF's ability, if any, to induce any adverse immunological effect(s). More specifically, I was asked to review Pharming's GRAS Notification (dated December 29, 2005) and Pharming's subsequent Response to CFSAN document (dated December 22, 2006), was supplied with a copy of all references referred to in both the GN and Response, and was asked to indicate 1. whether I agreed with the substance and conclusions set forth in the latter Response document, and 2. whether I had any comments to make which were intended to make that document an even better science-based response. On June 19, 2007 I provided Pharming with a written response to its two requests. Such response was based, in part, on my own, independent research into the pertinent scientific literature (in addition to all of the scientific articles provided by Pharming).

7. With regard to an overall evaluation of the Response, I indicated that after reviewing and analyzing all of the documents and literature, I agree with the overall conclusions of the Expert Panel Statement.

8. With regard to additional comments, I indicated as follows:

A. Differences between endogenous and exogenous human lactoferrin (hLF).

Based on the information provided, the only potentially significant difference between endogenous and exogenous lactoferrin appears to be differences in the type of glycosylation pattern. However, the same type of glycosylation is found in

the exogenous hLF and bovine LF. Even if differences in glycosylation pattern can be associated with differences in immunogenicity, there is no scientific evidence that the type of glycosylation pattern found here in the exogenous hLF would induce adverse immunological effects

B. Determinant spreading, oral tolerance and increased up-take by antigen presenting cells via the mannose receptor.

There is no scientific evidence that differences in glycosylation pattern would affect oral tolerance or epitope spreading. Concerning the role of the mannose receptors in the uptake of antigens by antigen presenting cells, the work by the group headed up by the world expert Dr. Siamon Gordon (who is employed by the Sir William Dunn School of Pathology) suggests that mannose receptors elicit enhanced humoral responses *in vivo*, only when administered in combination with endotoxin (McKenzie, 2007). Furthermore, differences in glycosylation pattern have **not** been reported to play a role in maturation of antigen presenting cells.

C. Immunomodulatory effects of hLF, and impact on inflammatory/autoimmune diseases.

Importantly, the literature on the effects of hLF on the immune system is extremely difficult to analyze, because everything and its opposite have been reported Some studies suggest that hLF has anti-inflammatory properties, other studies suggest that it promotes proinflammatory responses, others that it has no effect A major issue related to all of these studies is the potential contamination not only by TLR ligands, such as LPS, but also by contaminants activating the inflammasome. Examples of contamination of ovalbumine by LPS, promoting the development of asthma in mouse models (see, e g , Bottomly, 2002) and of peptidoglycan (a TLR ligand) by MDP (a NOD2 ligand) have been reported It is hence very difficult to evaluate the significance of the findings on hLF if extensive controls to eliminate potential contaminant(s) have not been realized The only tests available currently, are those looking for LPS contamination. Interestingly,

the sources of lactoferrin used for the reported studies are various (e.g , from Sigma Co (from different countries), from Morinaga Milk Industry Co, Tokyo, and from Dainippon Sumitomo Pharma Co., Ltd.). Analysis for LPS contamination have been performed in a minority of studies and never, to my knowledge, for potential contaminants of the inflammasome (in particular not in the articles looking at induction of active form of IL-18 and IL-1). Different sources of hLF associated with differences in potential contaminants, may explain why conflicting data have been reported on the immunomodulatory effects of hLF *in vitro* and *in vivo*. Altogether, there is **no** compelling and reproducible evidence that hLF has immunomodulatory effects that would promote inflammatory/autoimmune diseases.

This ends Affiant's statement.

Bana Jabri, MD, PhD

STATE OF ILLINOIS)
) SS
COUNTY OF COOK)

SUBSCRIBED and SWORN to before me this 16th day of July, 2007

OFFICIAL SEAL
KAREN GORDON
NOTARY PUBLIC - STATE OF ILLINOIS
My commission expires April 2, 2011

NOTARY PUBLIC

Mannose Receptor Expression and Function Define a New Population of Murine Dendritic Cells[1]

Emma J. McKenzie,[2] Philip R. Taylor, Richard J. Stillion, Andrew D. Lucas,[3] James Harris,[4] Siamon Gordon,[5] and Luisa Martinez-Pomares[5]

***In vitro* the mannose receptor (MR) mediates Ag internalization by dendritic cells (DC) and favors the presentation of mannosylated ligands to T cells. However, in vivo MR seems to play a role not in Ag presentation but in the homeostatic clearance of endogenous ligands, which could have the secondary benefit of reducing the levels of endogenous Ag available for presentation to the adaptive immune system. We have now observed that while MR^+ cells are consistently absent from T cell areas of spleen and mesenteric lymph nodes (LN), peripheral LN of untreated adult mice contain a minor population of MR^+MHCII^+ in the paracortex. This novel MR^+ cell population can be readily identified by flow cytometry and express markers characteristic of DC. Furthermore, these MR^+ DC-like cells located in T cell areas can be targeted with MR ligands (anti-MR mAb). Numbers of MR^+MHCII^+ cells in the paracortex are increased upon stimulation of the innate immune system and, accordingly, the amount of anti-MR mAb reaching MR^+MHCII^+ cells in T cell areas is dramatically enhanced under these conditions. Our results indicate that the MR can act as an Ag-acquisition system in a DC subpopulation restricted to lymphoid organs draining the periphery. Moreover, the effect of TLR agonists on the numbers of these MR^+ DC suggests that the immunogenicity of MR ligands could be under the control of innate stimulation. In accordance with these observations, ligands highly specific for the MR elicit enhanced humoral responses in vivo only when administered in combination with endotoxin.** ***The Journal of Immunology*, 2007, 178: 4975–4983.**

Dendritic cells (DCs)[6] are professional APCs with crucial roles in the induction and control of tolerance to self-Ags and immunity to pathogen-derived Ags These unique cells sample Ag constitutively and migrate from the periphery to secondary lymphoid organs (1, 2) where they present processed Ags to T cells In the absence of infection Ag presentation will result in tolerance, whereas in the presence of microbial signals DC maturation will occur, facilitating the induction of effector responses The induction of tolerance to self and innocuous foreign Ags may also be influenced by the efficient clearance of self-Ags by macrophages (Mϕs), restricting exposure of DCs to Ags

In vivo DCs are a rare but heterogeneous collection of cells expressing a wide range of germline-encoded pattern recognition receptors These receptors encompass several families of molecules including TLRs, C-type lectins, and C-type lectin-like receptors expressed both at the cell surface and intracellularly DCs are able to sense the presence of foreign microbes via these receptors Multiple signals provided by a pathogen are transduced upon ligand recognition and ultimately govern the course of the effector response toward the invader

The mannose receptor (MR) is a C-type lectin that provides an efficient cellular internalization system for both endogenous and microbe-derived molecules and has a well-established role in the maintenance of tissue homeostasis as exemplified in studies of MR-deficient mice generated by Lee et al (3) These mice exhibited defective clearance of neoglycoconjugates and elevated serum levels of multiple lysosomal hydrolases, indicating impaired clearance (3) The MR recognizes sulfated carbohydrates through its cysteine-rich (CR) domain (4, 5), native and denatured collagens through its fibronectin type II domain (6), and oligosaccharides terminating in mannose, fucose, or *N*-acetyl glucosamine through its C-type lectin-like carbohydrate recognition domains (recently reviewed in Ref 7)

No expression of the MR has been documented on murine DC populations in vivo, in agreement with its major role in clearance, the MR is present in most tissue Mϕs and in hepatic and lymphatic endothelia (8) In humans, the MR has been detected in cells located within the dermis, lamina propria, and T cell areas of the tonsil (9), in inflammatory epidermal DCs from patients with atopic dermatitis (10), and in cells lining venous sinuses in the spleen (11) Evidence for the involvement of the MR in Ag presentation to the acquired immune system is limited and in some

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[6] Abbreviations used in this paper DC dendritic cell AP alkaline phosphatase CR cysteine-rich LN lymph node Mϕ, macrophage, mLN mesenteric LN, moDC monocyte-derived DC, MR mannose receptor Pam_3Cys_4 (*S*)-[2,3-bis(palmitoyloxy)-(2-*RS*)-propyl]-*N*-palmitoyl-(*R*)-Cys-(*S*)-Ser-(*S*)-Lys^4-OH ^{1}HCl pLN peripheral LN poly(I C) polyinosinic-polycytidylic acid WT wild type

cases contradictory. The MR is expressed by human and murine DCs generated in vitro: human monocyte-derived DCs (moDCs) and mouse bone marrow-derived DCs. Uptake of mannosylated ligands by moDCs leads to the delivery of Ag to MHCII$^+$ (12) and CD1b$^+$ (13) compartments and enhanced presentation to T cells (14–16). Delivery of the melanoma Ag pmel17 through the MR in human moDCs using an anti-MrmAb-pmel17 fusion protein led to Ag presentation via both HLA I and HLA II molecules (17), indicating that in human DCs the MR could provide an efficient mechanism for Ag acquisition and delivery into Ag processing pathways. In mice, bone marrow-derived DCs were shown to internalize Ag through the MR for presentation to T cells, although MR ligands were not presented as efficiently as ligands for DEC-205, another member of the MR family of proteins (18), and MR expression is required for cross-presentation of the soluble model Ag OVA (19). In contrast, Napper and Taylor recently reported that fibroblasts cotransfected with the MR and MHCII were not able to enhance the presentation of glycosylated Ag to T cells (20), and Ags engineered in fungi to enhance mannosylation elicit T cell responses independently of the MR (21).

Several of the endogenous molecules recognized by the MR are targeted by the immune system in autoimmune diseases such as thyroiditis (thyroglobulin) (22, 23), antineutrophil cytoplasm Ab-associated vasculitis (myeloperoxidase) (Ref. 24 and our own unpublished data), rheumatoid arthritis (collagen II, a major component of cartilage) (25), and Goodpasture's disease (collagen IV) (26). This correlation led us to consider that if the MR contributed to Ag presentation in vivo, it could mediate the inappropriate presentation of its endogenous ligands to the acquired immune system. The aim of this work was to investigate whether the MR could mediate Ag acquisition for presentation to the adaptive immune system under any circumstance in vivo. For this purpose, we have analyzed MR expression in DC, determined the fate of MR ligands upon in vivo administration, and quantified the humoral responses against MR ligands in naive and stimulated animals.

Our results demonstrate that stimulation of the innate immune response has a profound effect on the involvement of MR in the induction of adaptive immune responses. We have identified a novel DC population expressing a functional MR. These MR$^+$ DCs are restricted to peripheral (p) lymph nodes (LNs), and their numbers are controlled by the presence of selected TLR agonists. In agreement with these data, the induction of humoral responses against MR ligands in vivo takes place only in the presence of endotoxin. The relevance of these results in regard to DC heterogeneity and autoimmunity will be discussed.

Materials and Methods

Animals

Mice used in this study (BALB/c, C57BL/6, and MR$^{-/-}$, which were on the C57BL/6 genetic background) were bred within our own institutional colonies, sex matched, and between the ages of 7 and 16 wk at the time of study. Animals were kept and handled in accordance with institutional guidelines. MR$^{-/-}$ mice were provided by Prof. M. Nussenzweig (Rockefeller University, New York).

Reagents

The TLR agonists used in these studies are LPS purified from the *Haemophilus influenzae* type b strain Eagen (a gift from S. Zamze, Edward Jenner Institute for Vaccine Research, Compton, U.K.), flagellin purified from *Salmonella typhimurium* (InvivoGen), polyinosinic-polycytydylic acid (poly(I:C), Amersham Biosciences), and (*S*)-[2,3-bis(palmitoyloxy)-(2-*RS*)-propyl]-*N*-palmitoyl-(*R*)-Cys-(*S*)-Ser-(*S*)-Lys^4-OH ^{3}HCl (Pam$_3$Cys$_4$, Calbiochem).

Tissue digestion

Peripheral (cervical, brachial, axillary, inguinal, and popliteal) and mesenteric LNs were digested with .5 mg/ml collagenase D (Roche) and 1 mg/ml DNase I (Roche) in RPMI 1640 for 25 min at 37°C with gentle shaking. Tissues were further broken down with gentle pipetting. Cell suspensions were washed twice in PBS containing 0.5% BSA and 5 mM EDTA. In some experiments, cell suspensions were enriched in CD11c$^+$ cells using anti-CD11c MACS beads (Miltenyi Biotec) following the manufacturer's instructions, and in other experiments, cells were used directly for FACS staining.

Flow cytometry

Single cell suspensions were blocked for 45 min at 4°C in 5% (v/v) heat-inactivated rabbit serum, 0.5% BSA, 5 mM EDTA, 2 mM NaN_3, and 4 μg/ml FcγRII/III blocking mAb (clone 2.4G2) to reduce nonspecific Fc receptor binding. Blocked cells were incubated with primary mAbs diluted in the above-described blocking solution for 60 min in the dark at 4°C, washed three times with washing buffer containing 0.5% BSA, 5 mM EDTA, and 2 mM NaN_3, and fixed with 1% formaldehyde. If biotinylated mAbs were used, cells were incubated for a further 30 min in the dark at 4°C with streptavidin-allophycocyanin (BD Pharmingen), washed three times in washing buffer, and fixed as above. Ag-specific staining obtained with mAbs was compared with that obtained with isotype-matched control Abs. The primary mAbs used in this study were MR5D3- and MR6C3-Alexa Fluor 488/biotin (MR, rat IgG2a, produced in house), HL3-PE (CD11c, hamster IgG, BD Pharmingen), M5114-biotin (MHCII, rat IgG2b, produced in house), 5C6-biotin (CD11b, rat IgG2b, produced in house), KT15-PE (CD8α, ratIgG2a, Serotec), PO3-biotin (CD86, rat IgG2b, BD Pharmingen), and NLDC-145-biotin (DEC-205, rat IgG2a, Cedarlane Laboratories). Analysis was conducted using a FACSCalibur flow cytometer and CellQuest 3.1 software (both BD Biosciences).

Immunohistochemistry

Slides were fixed for 10 min on ice with 2% paraformaldehyde, permeabilized with 0.1% Triton X-100 in PBS, and then blocked with 5% (v/v) normal goat serum (Invitrogen Life Technologies) in PBS for 30 min to block irrelevant binding sites. Further blocking of endogenous biotin was achieved using an avidin/biotin blocking kit (Vector Laboratories) as per the manufacturer's recommendations. Staining Abs were prepared to appropriate concentrations in 5% (v/v) normal goat serum in PBS and incubated with slides for 60 min. Goat anti-rat IgG-Alexa Fluor 488 secondary Ab (Molecular Probes) diluted in PBS was applied for a further 30 min. In the case of double labeling with another Ab also raised in rat, an additional 30-min blocking step was conducted with 100 μg/ml rat IgG (Sigma-Aldrich) before incubation with the second biotinylated primary reagent for 60 min. This was followed by 30 min of incubation with a streptavidin-Cy3/-Cy5 (Jackson ImmunoResearch) or Alexa Fluor 488 (Molecular Probes) secondary reagent. Slides were counterstained with 400 ng/ml 4′,6′-diamidino-2-phenylindole (Sigma-Aldrich) before mounting. Slides were washed between each step with PBS.

Ear skin explant

Ears from BALB/c mice were removed at the base and split into dorsal and ventral sides. Each half was placed dermal side down into a well of a 24-well tissue culture plate containing 2 ml of medium and incubated for 24 h in a 5% CO_2, 95% humidity incubator at 37°C. Migrated cells were then collected and washed in medium, and cytospins were prepared.

In vivo targeting of MR ligands

Anti-MR mAbs or isotype controls were injected s.c. into the forelimb just above the wrist or, in some experiments, in the leg just above the ankle under anesthesia with 3% isoflurane in air. At various times after injection, the spleen and the peripheral and mesenteric LNs were collected and fresh frozen for immunohistochemical analysis. Injected mAb could be detected in tissue sections by fluorescently labeled goat anti-rat IgG reagents (Molecular Probes/Invitrogen Life Technologies).

Immunizations

Animals were anesthetized as previously indicated before the s.c. injection of Ag into forelimbs just above the wrist. MR6C3$^+$, MR5D3$^+$, and control IgG2a (clone GL113/10, provided by Dr. R. Hodes, National Institutes of Health, Bethesda, MD) were used in these studies in the presence or absence of LPS. After 7–14 days, animals were sacrificed and bled, and sera were prepared and stored at −20°C. The mAbs were purified from hybridoma supernatants prepared in Iscove's medium containing low endotoxin

and IgG-depleted FCS (Invitrogen Life Technologies) using a GammaBind Plus Sepharose column (Invitrogen Life Technologies). All preparations were quantified using a BCA assay (Perbio), analyzed for purity by Coomassie staining, and tested for endotoxin contamination using the *Limulus* amebocyte Lysate assay (Cambrex/BioWhittaker). All proteins were aliquoted and stored at −20°C until required.

ELISA

Total mouse anti-rat IgG produced by each animal was determined by ELISA. Flat-bottom 96-well high-binding enzyme immunoassay/radioimmunoassay (EIA/RIA) plates (Corning) were coated with 10 μg/ml rat IgG (Sigma-Aldrich) at 50 μl/well in PBS overnight at 4°C. Plates were blocked with 3% BSA (w/v) in PBS for 60 min at 37°C before the addition of appropriate dilutions of serum in duplicate for 1 h at room temperature. Wells were then incubated for 1 h with 50 μl/well anti-mouse IgG-alkaline phosphatase (AP) (Sigma-Aldrich) to detect mouse IgG bound to anti-rat Abs or with anti-mouse IgG1-AP or anti-mouse IgG2a-AP (both BD Pharmingen) to detect specific subclasses. All AP conjugates were used at a 1/1000 dilution in PBS. Ab levels were visualized using *p*-nitrophenol phosphate (Sigma-Aldrich). Absorbance was measured at a wavelength of 405 nm using a microplate reader. Plates were washed three times between incubations in PBS supplemented with 0.1% Tween 20 (Sigma-Aldrich). IgG titers were ascertained by calculating the dilution of serum required to achieve an absorbance value of 0.2. Animals that did not make a detectable anti-rat IgG response were assigned an arbitrary value, the minimum dilution level of serum used and, thus, the level of detection.

Statistical analysis

Statistical analysis was performed using ANOVA and the Bonferroni test with GraphPad Prism software version 3.02. Where appropriate, *p* values are indicated within the figures.

Results

MR⁺ cells are found in the outer paracortical areas of selected secondary lymphoid tissues

The expression of the MR in secondary lymphoid tissues was investigated by immunofluorescent staining. In line with our previous studies, MR is abundantly expressed in the medullary regions of LN and the red pulp of the spleen, and small numbers of MR⁺ cells were observed in the outer paracortex of LNs close to B cell areas (8). When different LNs were compared we found that the paracortical MR⁺ cells were restricted to pLNs (Fig. 1*A*, *left panel*) which drain the skin, and absent from mesenteric (m)LNs (Fig. 1*A*, *right panel*) which drain the gut. As reported, MR is absent from the white pulp of the spleen (data not shown and Fig. 4*A*). Further analysis confirmed that paracortical MR⁺ cells also express MHCII (Fig. 1*B*), indicating that the MR could be expressed by a subset of DCs.

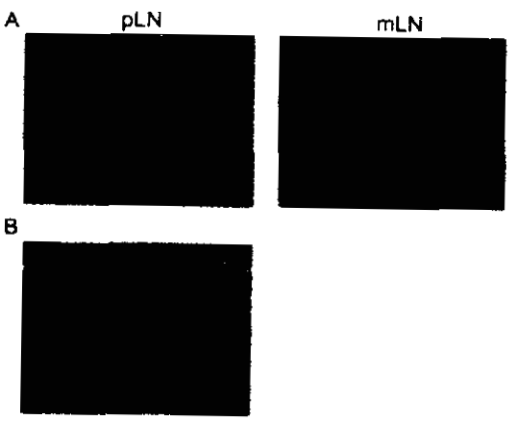

FIGURE 1. The presence of MR⁺ cells in paracortical areas is restricted to selected secondary lymphoid tissues. *A*, Paracortical MR⁺ cells are only present in pLNs. Tissue sections from LNs draining either cutaneous sites (pLN) or gut (mLN) were stained for MR (green) and CD3 (red) as described in *Materials and Methods*. *B*, MR⁺ cells in paracortical areas (green) coexpress MHCII (red). T, T cell area; B, B cell follicle. Original magnification was ×20 in *A* and ×63 in *B*.

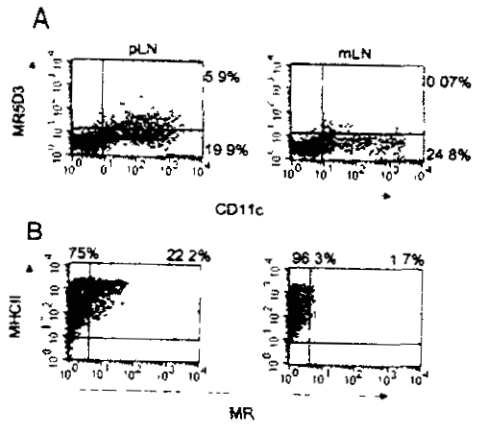

FIGURE 2. The restricted presence of MR⁺ DC to pLN is confirmed by flow cytometry. *A*, Analysis of MR⁺CD11c⁺ cells in total cell suspensions from pLNs and mLNs. Total cell suspensions from pLNs contain a population of MR⁺CD11c⁺ cells that is absent in mLNs. *B*, Analysis of MR and MHCII expression in gated CD11c⁺ cells from cell suspensions from pLNs and mLNs enriched for CD11c⁺ cells. Single cell suspensions from pLN and mLN were enriched for CD11c⁺ cells and the analysis of MR and MHCII expression performed on gated CD11c⁺ cells.

Characterization of MR⁺ cells in lymph nodes by flow cytometry

To characterize the MR⁺MHCII⁺ cells detected in pLNs, we performed flow cytometric analysis of single cell suspensions prepared from pLNs and mLNs using collagenase digestion as described in *Materials and Methods*. Cells with high forward and side scatter were gated (these parameters encompass CD11c⁺ cells) and subsequent analysis was performed. A population of CD11c⁺ MR⁺ cells was identified in pLN that was absent in mLN (Fig. 2*A*). To gain a clearer picture of the phenotype of this population, cells were enriched for CD11c⁺ and then labeled for CD11c, MR, and MHCII as described in *Materials and Methods*. An analysis of MR and MHCII expression was performed on the gated CD11c⁺ cell population. All MR⁺ CD11c⁺ cells expressed

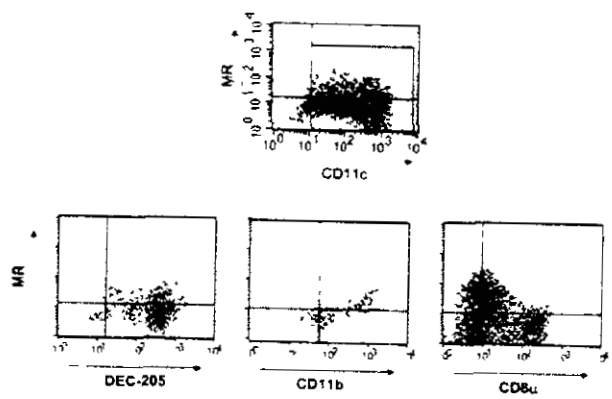

FIGURE 3. Comparison of surface markers expressed by MR⁺ CD11c⁺ and MR⁻ CD11c⁺ cells. CD11c⁺ enriched cells from pLN were gated and analyzed for expression of MR and DEC205, CD11b, or CD8α. MR⁺CD11c⁺ cells were found to be DEC-205$^{int/high}$CD11b^{high}CD8α^{low} whereas MR⁻ CD11c⁺ cells were DEC-205^{high}CD11b^{int}CD8α$^{low/high}$ (where int = intermediate). Different cell preparations were used for each labeling. Quadrants were set according to isotype control labeling.

MHCII, with the majority of the cells expressing high levels of MHCII (Fig. 2*B*). In accordance with previous results, CD11c$^+$MR$^-$MHCII$^+$ cells were absent in mLNs. MR$^-$CD11c$^+$ and MR$^+$CD11c$^+$ cells from pLN were compared for the expression of several DC-associated Ags (Fig. 3). MR$^+$CD11c$^+$ cells were found to be DEC-205$^{inter/high}$CD11b^{high}CD8α^{low} whereas MR$^-$CD11c$^+$ cells were DEC-205^{high}CD11b^{inter}CD8$\alpha^{low/high}$ (where "inter" is intermediate). Based on their levels of DEC-205 and CD11b expression, MR$^+$MHCII$^+$CD11c$^+$ cells seemed to correspond to the interstitial DC population described by Henri et al. (27). Further studies showed that MR$^+$CD11c$^+$ cells expressed the costimulatory molecules CD40 (data not shown) and CD86 (see Fig. 4*B*).

Numbers of MR$^+$ DC are under the control of innate stimulation

To address the possibility of MR$^+$ DC being influenced by innate stimulation in a similar way to that described for cells expressing ligands for the CR domain of the MR (28–30), we analyzed the effect of systemic and local stimulation with microbial products such as LPS and flagellin on MR$^+$ DC numbers by fluorescence microscopy. A prominent increase in MR$^+$ cells in the T cell areas of pLNs was observed after systemic stimulation with LPS and flagellin (Fig. 4*A*). Under these conditions, MR$^+$ cells in T cell areas were also MHCII$^+$ (data not shown). Interestingly, these strong innate stimuli administered through the i.v. route did not alter the absence of MR$^+$ DCs in the T cell areas of mLNs or the spleen, further highlighting the restricted anatomical location of MR$^+$ DCs. We also observed decreased MR expression in splenic red pulp Mϕ after LPS or flagellin treatment. This has previously been shown to occur in response to LPS in vitro (31). Local s.c. administration of LPS (1 μg/site) also induced an increase in paracortical MR$^+$ cell numbers in draining LNs (data not shown). The effect of the systemic administration of two other TLR agonists, Pam_3CSK_4 and poly(I:C), on MR$^+$ DC numbers was also assessed during this study. A variable increase in MR$^+$ DC numbers was observed in pLNs 24 h after the i.v. administration of Pam_3CSK_4 (5–20 μg). Interestingly, the i.v. administration of poly(I:C) (5–10 μg) did not induce any increase in MR$^+$ DC numbers (data not shown). These data indicate that the number of MR$^+$ DCs is

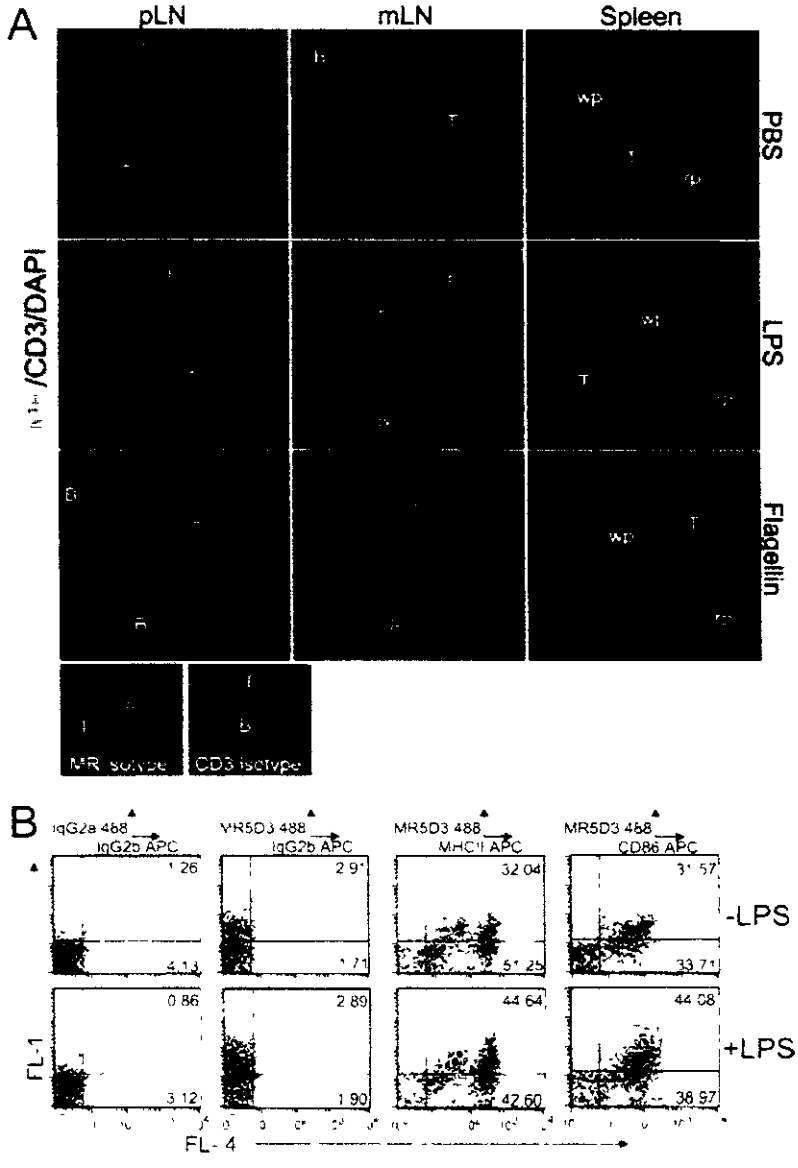

FIGURE 4. Effect of stimulation with microbial mimics on the numbers of paracortical MR$^+$ cells in secondary lymphoid organs. *A*, Analysis of the effect of LPS and flagellin in the numbers of paracortical MR$^+$ cells by immunofluorescence. BALB/c mice were stimulated i.v. with PBS, 5 μg of LPS, or 10 μg of flagellin. Tissues were collected 24 h later, processed for immunofluorescence, and stained for MR (green) and CD3 (red) as described in *Materials and Methods*. Numbers of MR$^+$ paracortical DCs increased after stimulation with microbial mimics in the pLN but not in the mLN or the spleen. Control staining is shown in the *bottom panels*. T, T cell area; B, B cell follicle; wp, white pulp; rp, red pulp. *B*, Analysis of the effect of LPS on the numbers of paracortical MR$^+$ cells by flow cytometry. BALB/c mice were stimulated i.v. with PBS or 5 μg of LPS. pLNs were collected 24 h later, processed for flow cytometry, and stained for CD11c (FL-2, using anti-CD11c-PE), MR (FL-1, using MR5D3 directly conjugated to Alexa Fluor 488 (MR5D3-488)), and MHCII (FL-4, using anti-MHCII-biotin and streptavidin-allophycocyanin (MHC-APC)) or CD11c (FL-2, using anti-CD11c-PE), MR (FL-1, using MR5D3 directly conjugated to Alexa Fluor 488), and CD86 (FL4, using anti-CD86-biotin and streptavidin-allophycocyanin (CD86-APC)), as described in *Materials and Methods*. Rat IgG2a directly conjugated to Alexa Fluor 488 (IgG2a-488) and rat IgG2b biotin and streptavidin-allophycocyanin (IgG2b-APC) were used as controls. In both cases, CD11c$^+$ cells were gated and analyzed for the presence of additional markers. Numbers indicate cell percentages in corresponding quadrant.

regulated by selective TLR agonists These results were supported by the flow cytometric analysis of cells suspensions from the pLNs of LPS-treated or untreated animals which demonstrated that LPS treatment led to the presence of an increased percentage of $CD11c^+$ cells in pLNs (from 2.15 to 3.3% and from 2.69 to 3.55% in two separate experiments), a higher proportion of MR$^+$ MHCII$^+$ and MR$^+$CD86$^+$ cells were detected within the $CD11c^+$ cell population from treated animals (Fig. 4*B*)

MR$^+$ cells in the skin as potential precursors of MR$^+$ DC in LNs

As shown in Fig. 3, MR$^+$ DCs display the characteristics of interstitial tissue DCs with respect to the expression of DC-associated molecules. The restricted presence of MR$^+$MHCII$^-$ cells in pLNs is suggestive of a peripheral tissue origin for the MR$^+$ DCs and because the traffic of DC into lymphoid tissues is known to increase after stimulation with microbes or their products (32–38) we considered the possibility that MR$^+$ DCs are derived from MR$^+$ cells present in the periphery (i.e. skin). To assess this possibility we investigated the phenotype and behavior of MR$^+$ cells in skin. Abundant MR$^+$ cells were observed throughout the dermis of mouse ear skin, while cells expressing MHCII were restricted to the outer dermis and epidermis (Fig. 5*A*). Double immunofluorescence confirmed that dermal MR$^+$ cells lack MHCII expression in situ, as no colocalization of MHCII and MR was observed (Fig. 5*B*). These results suggest that dermal MR$^+$ cells are not phenotypically DCs in situ. Accordingly, when sections were double labeled for MR and CD68, a classical Mϕ marker, the majority of MR$^+$ cells coexpressed CD68 (Fig. 5*B*). Together, these data indicate that the MR is expressed by Mϕ-like cells in the dermis of mouse skin. Explant studies were performed to determine whether dermal MR$^+$ Mϕs were capable of migration out of skin, an inherent property of DCs. Ears were mechanically split into dorsal and ventral sides, transferred into wells containing medium, and incubated at 37°C with 5% CO_2 for 24 h. Migrated cells were collected and cytospins were prepared and labeled for MR and MHCII. We observed a notable heterogeneity in the expression of these two markers and found cells with high levels of MHCII and comparatively lower levels of MR (Fig. 6, *top panels*) as well as the opposite scenario (*middle panels*) and cells with intermediate levels of both markers (*bottom panels*). These differing phenotypes may represent cells at different stages of maturation. In some instances, MR$^+$ cells displayed a dendritic morphology. These results indicate that MR$^+$ Mϕ-like cells can mobilize and acquire DC-like characteristics.

Targeting MR$^+$ DCs in vivo

To investigate the function of the MR in MR$^+$ DCs and the accessibility of these cells to Ag delivered in the periphery, we used purified rat anti-mouse MR mAbs as surrogate MR ligands to target MR$^+$ cells in vivo (39). Preliminary targeting studies were conducted in naive BALB/c mice where 15 μg of mAb (MR6F3, rat IgG2b anti-mouse MR) were injected s.c. in the upper forelimb close to the wrist area. The cervical, brachial, axillary, inguinal, popliteal, and mesenteric LNs and the spleen were collected and processed for immunohistochemistry at various time points thereafter, from 30 min to 24 h postinjection. Injected mAbs were detected by incubating tissue sections with Alexa Fluor 488-labeled goat anti-rat IgG. These experiments indicated that the medullary cells in brachial, axillary, and cervical LN were effectively targeted within 30 min postinjection. Conversely, targeting to paracortical MR$^+$ DCs was poor and was only observed in the brachial LNs (the main draining LNs of this injection site), with only a few targeted cells being clearly visible at 24 h postinjection.

Additional experiments using anti-MR mAb clone 6C3 (MR6C3, rat IgG2a anti-mouse MR) or IgG2a control Ig (clone GLIII/10) demonstrated that targeting to the paracortical region was dose dependent because no MR$^+$ cells in T cell areas were targeted in any LN when 5 μg of mAb were used, even though targeting to medullary cells still occurred (data not shown).

In view of the major effect that LPS had on the numbers of MR$^+$ cells present in T cell areas, we injected BALB/c mice s.c. with 15 μg of purified anti-MR mAb clone 6C3 (MR6C3, rat anti-mouse

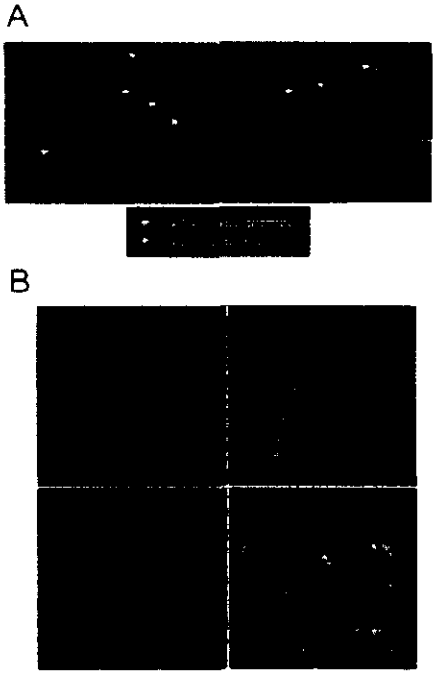

FIGURE 5. MR$^+$ cells in the dermis display a Mϕ-like phenotype under steady-state conditions. *A*, Single labeling analysis of MR and MHCII expression in mouse skin. MR$^+$ cells (green) reside throughout the dermis (yellow arrow), while MHCII$^+$ cells occupy the epidermis (white arrow) and the outer dermal layers (yellow arrow). *Insets* depict isotype controls. *B*, Double labeling analysis of MR$^+$ cells in mouse skin. *Upper panels* show double labeling for MR (green) and MHCII (red) and demonstrate that both markers are not expressed by the same cells. *Bottom panels* show double labeling for MR (red) and CD68 (green), demonstrating that dermal MR$^+$ cells are CD68$^+$. Squares in the *left panels* indicate the areas shown at higher magnification in the *right panels*.

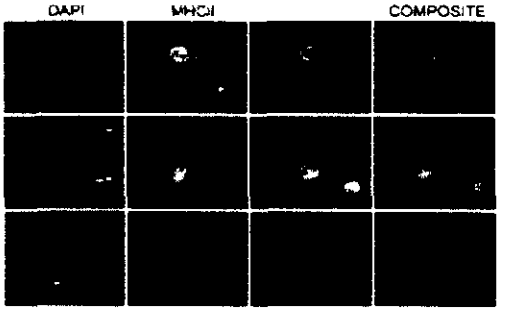

FIGURE 6. MR$^+$ cells are capable of mobilization from skin and acquire MHCII expression. Cells that had migrated out of skin explants were stained for MR (green) and MHCII (red). Skin-derived MR$^+$ cells expressed MHCII heterogeneously; compare *top*, *middle*, and *bottom panels*. Isotype controls for MR are depicted in the *insets* within the *top panels* and those for MHCII are shown in the *insets* within the *middle panels*.

IgG2a), or IgG2a control IgG (clone GLIII/10) in the upper and lower forelimbs of mice treated i.v. with of 5 μg of LPS or PBS 10 min earlier. We detected MR6C3 in the medullary regions of the cervical, brachial, axillary, and inguinal LNs of LPS- and PBS-treated mice. In PBS-treated animals, few targeted cells were detected in the T cell areas of brachial or inguinal LNs (Fig. 7, *A* and *B* show representative inguinal LN). In the presence of LPS, the anti-MR mAb targeted numerous cells within the paracortical areas of brachial, axillary, and inguinal LN (Fig. 7, *C* and *D*, a representative inguinal LN is shown). In all cases, targeted paracortical cells were MR$^+$ and MHCII$^-$, indicating specific delivery to MR$^+$ DCs (Fig. 7, *E–G*). Delivery of anti-MR mAb was exquisitely restricted to local draining LNs because no Ag could be detected in other nondraining lymphoid tissues such as, for example, the spleen (Fig. 7, *H* and *I*). No targeting of the rat IgG2a control Ab to LNs (shown in Fig. 7, *A* and *C*, *insets*) or spleen (Fig. 7, *H* and *I*, *insets*) was observed, indicating that no targeting system selective for rat IgG2a is present in secondary lymphoid organs. Similar results were obtained when the anti-MR mAb was injected s.c. in combination with LPS (1 μg/site), data not shown. Thus, MR$^+$ DCs can acquire MR ligands delivered in the periphery with numbers of MR$^+$ DCs in T cell areas containing MR ligands being increased in the presence of LPS.

Generation of an anti-rat IgG Ab response after immunization with anti-MR mAbs

Because we were able to target MR$^+$ DCs specifically in vivo using rat anti-mouse MR mAbs, we sought to determine whether the delivery of Ag via the MR results in presentation to the adaptive immune system in an immunogenic fashion by assessing the generation of anti-rat IgG Abs in sera from s.c. immunized animals. Preliminary studies using 15 μg of MR6C3, MR5D3, and IgG2a indicated that no detectable response could be obtained in the absence of LPS and that 1 μg was better than 0.1 μg of LPS in promoting a humoral response (data not shown). These experiments also indicated that the mAb clone MR5D3 could elicit a more robust response than MR6C3.

To determine the optimal dose of mAb for the immunization studies, we immunized BALB/c mice s.c. in both forelimbs with varying doses of MR5D3 or control IgG2a in the presence of 1 μg of LPS. After 7 days the animals were sacrificed and serum was analyzed for the presence of anti-rat IgG by using ELISA. Animals immunized with MR5D3 consistently generated higher titers of anti-rat IgG than those immunized with the control protein. Significant differences in the anti-rat response were found between animals immunized with MR5D3 and control IgG2a at doses of 3.75 μg ($p < 0.001$) and 15 μg ($p < 0.05$). Based on these results, a dose of 3.75 μg of immunogen was chosen for use in future experiments (data not shown).

To confirm that the clone used for the immunization had an effect on the level of response obtained, animals were injected in both forelimbs with 3.75 μg of MR5D3, MR6C3, or isotype control in the presence of 1 μg of LPS s.c., and the presence of anti-rat IgG in the serum on day 7 was measured by ELISA (Fig. 8*A*).

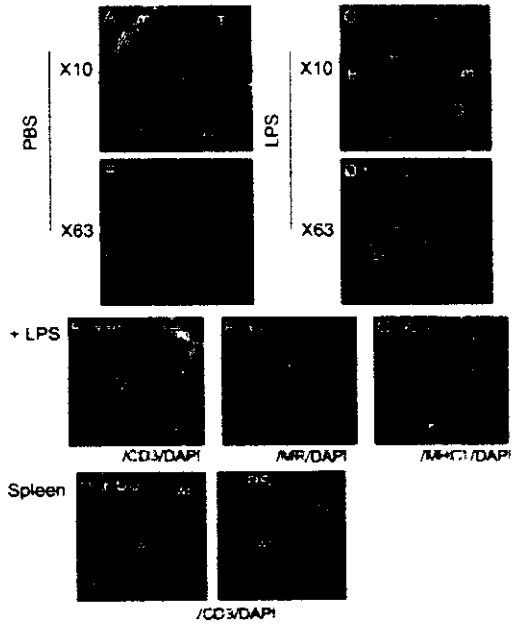

FIGURE 7. Paracortical MR$^+$ cells can be efficiently targeted in vivo using specific MR reagents in the presence of LPS. BALB/c animals were injected s.c. in the forelimb with rat anti-mouse MR6C3 or isotype control mAbs in the presence (*C–G* and *I*) or absence of LPS (*A*, *B*, and *H*). Secondary lymphoid tissues were collected 24 h later and processed for immunofluorescence. Injected mAbs were detected in tissue sections using a goat anti-rat IgG reagent (green). Tissue sections were also labeled for CD3 (red, *A–E*, *H*, and *I*), MR (red, *F*), or MHCII (red, *G*) to analyze the phenotype of targeted cells. T, T cell area; B, B cell follicle; rp, red pulp; wp, red pulp; DAPI, 4′,6-diamidino-2-phenylindole.

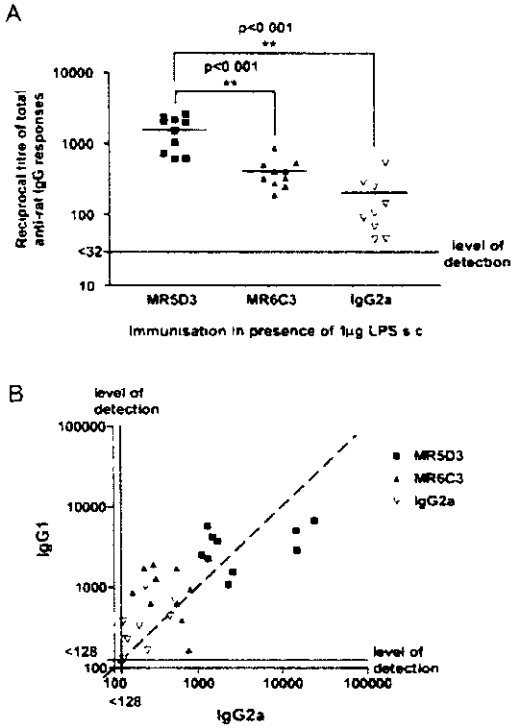

FIGURE 8. Delivery of rat IgG to the MR using MR5D3 induces a more robust humoral response. *A*, Animals were immunized with either MR5D3 (■), MR6C3 (▲), or control IgG2a (▽) mAb in the presence of 1 μg of LPS. After 7 days, the serum was collected and the presence of an anti-rat IgG response was determined by ELISA. Immunization with MR5D3 generated significantly higher titers of anti-rat IgG compared with mice immunized with MR6C3 or IgG2a. Each symbol represents an individual animal. *B*, The anti-rat response was predominantly composed of IgG2a and IgG1 subclasses and immunization with MR5D3 induced a mixed Th1/Th2 response. Each symbol the same as that used in *A* represents an individual animal. An asterisk (*) indicates significant differences.

Differences between the responses induced by MR5D3 and IgG2a and by MR5D3 and MR6C3 were found to be highly significant ($p < 0.001$). These data indicate that Ag delivery through the MR achieved by immunization with MR5D3 in the presence of LPS induced improved Ab production compared with the control. Interestingly, the efficiency of anti-rat responses also appears to be dependent on the clone of mAb used.

Analysis of the presence of the IgG1 and IgG2a subclasses generated in immunized mice (Fig. 8*B*) showed that the animals immunized with MR5D3 generated stronger IgG2a and IgG1 responses compared with animals immunized with MR6C3 or the IgG2a control. The production of both IgG2a and IgG1 indicated that a mixed Th1/Th2 response was generated.

Humoral responses to anti-MR mAbs are abrogated in MR$^-$ animals

The specificity of B cell responses was confirmed by using MR$^-$ mice and wild-type (WT) C57BL/6 control animals. Animals were injected with 3.75 μg of either MR5D3 or control IgG2a in the presence (or absence in the case of WT animals) of 1 μg of LPS in both forelimbs, and sera were analyzed for total anti-rat IgG content by ELISA after 7 days (Fig. 9). In agreement with previous data, the induction of anti-rat IgG responses was dependent upon the presence of a microbial mimic. The results also show that the enhanced anti-rat IgG Ab production obtained in response to anti-MR mAb in WT animals (both BALB/c (Fig. 8) and C57BL/6 (Fig. 9)) was completely abrogated in MR$^-$ animals, indicating that these responses were MR-mediated and specific.

Discussion

In this study, we demonstrate that the delivery of soluble Ag in vivo through the MR leads to enhanced immunogenicity in the presence of innate stimulation. The mechanism behind this effect appears to involve the efficient uptake of MR ligands by a novel population of MR$^+$ DCs restricted to pLNs whose frequency is increased following treatment with TLR agonists. These cells are located in the paracortical areas of pLN and, based on phenotypical analysis by flow cytometry, correspond to a known DC subset that was thought to constitute dermal interstitial DCs (27). We propose that MR$^+$ DCs in pLNs are derived from MR$^+$ cells in the periphery. Results from the skin explant studies are consistent with this hypothesis because a migratory population of MR$^+$ MHCII$^+$ cells with dendritic morphology is obtained even though the MR$^+$ cells located in the dermis have a Mϕ-like phenotype (MHCII$^-$ CD68$^+$). No further stimulus apart from the physical dissociation of the dorsal and ventral sides of the ear was required to induce this migration. Presumably, increased migration would occur if an additional danger signal such as the presence of LPS or cytokines was also provided. Interestingly, tissues drained by mLNs also contain a large population of MR$^-$ Mϕs. MR$^+$ DCs in pLNs appear to be CD68$^-$ (data not shown), indicating that CD68 expression might be lost upon migration. The lack of MR-expressing cells in the paracortical region of mLN indicates that lamina propria Mϕs do not migrate to draining mLN or lose MR expression upon migration under the conditions tested.

Rat anti-mouse MR mAbs were used as specific surrogate ligands to probe the function of the MRs on DC in vivo. This approach has been previously used to deliver Ag *via* CD11c (40), MHCII (41, 42), 33D1 (40), DEC-205 (43, 44), DC-SIGN (45), and FIRE and CIRE (46). Within 30 min postinjection, anti-MR mAbs could be detected in the medulla of LNs draining the site of injection, and by 24 h postinjection MR$^+$ MHCII$^+$ cells containing anti-MR Abs were detected within the paracortex.

The time lag that occurred before the targeted MR$^+$ DCs were detected in draining LNs suggests that these cells may have encountered anti-MR mAbs in the periphery before arrival into the LNs. Others have shown that the time taken for s.c. administered Ag to arrive in the LN is ~18 h, a similar time frame to that observed in our study (47). Moreover, unlike small molecules, the size of mAbs prevents their diffusion through the conduit system into the paracortical area (48), excluding the possibility of free mAbs draining directly into the paracortex and binding MR$^+$ DCs there. The targeting of MR ligands within the paracortex was dramatically improved if LPS was coadministered s.c. or if mice received LPS i.v. before the s.c. injection of mAb. These results are consistent with the increased egress of MR$^+$ cells from the dermis upon stimulation; these cells would internalize anti-MR mAbs in the periphery and transport them to the draining LNs. In contrast, MR$^+$ medullary cells were targeted in the presence or absence of LPS. It is likely that free anti-MR mAbs drained from the site of injection via the lymph into the subcapsular sinus of the local LN before entering the conduit system to the medulla. The targeting properties of the anti-MR mAb in vivo differ from those of anti-DEC-205 Ab, which diffuses throughout the secondary lymphoid tissues after s.c. injection (49). Ag delivery to MR$^+$ DCs was exquisitely restricted to lymphoid tissue draining the site of injection and did not extend to either the spleen, the mLNs, or the pLN located on the contralateral side of the animal, even in the presence of LPS. This is likely to be due to Ab clearance by MR$^+$ medullary cells, which would remove the majority of the injected mAbs reaching the LN in free form. Clearance of anti-MR mAbs by medullary cells would not only prevent Ag access to other lymphoid tissue but, more importantly, to other APCs. In this way, this removal system would limit the acquisition and presentation of MR ligands to the adaptive immune system. These results are consistent with previous studies describing the lack of MR expression in DC in vivo under steady-state conditions (8) and a major defect in homeostatic clearance (3, 50) but normal immunity against *Candida albicans* or *Pneumocystis carinii*, both MR ligands, in MR-deficient mice (50, 51).

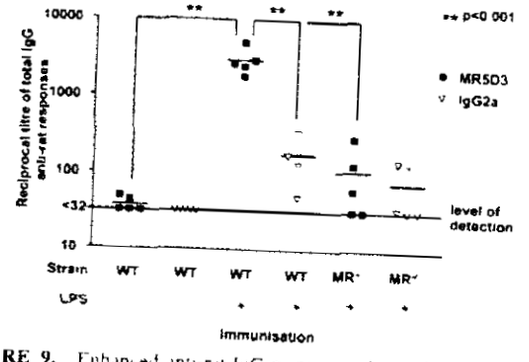

FIGURE 9. Enhanced anti-rat IgG responses obtained against anti-MR mAb are abrogated in MR$^-$ animals. MR deficient mice or C57BL/6 WT control mice were immunized with MR5D3 (■) or control IgG2a (▽) mAb in the presence of LPS. Groups of C57BL/6 were also immunized with MR5D3 or control IgG2a alone. Serum was collected after 7 days and the presence of the anti-rat IgG was determined by ELISA. No anti-rat response was detected in mice receiving mAb alone, whereas significant responses were found in those animals that received MR5D3 and LPS compared with control protein. The enhanced anti-rat IgG response obtained in response to MR5D3 was abrogated in MR$^-$ mice as MR5D3 and control IgG2a elicited similar responses in these animals. Each symbol represents an individual animal. An asterisk (*) indicates significant differences.

After identifying the conditions under which MR could be exploited by professional APCs to internalize Ag for presentation to the adaptive immune system in vivo, anti-MR mAb were used to determine whether the enhanced targeting of MR ligands to DCs in T cell areas correlated with the generation of enhanced humoral responses. This strategy involved the immunization of animals with purified rat IgG preparations and analysis of anti-rat IgG responses in the serum from injected animals.

In the presence of LPS, a single s.c. dose of rat anti-mouse MR mAb induced significant anti-rat IgG production compared with the isotype control mAb. The enhanced anti-rat response was completely abrogated in MR$^{-/-}$ mice, illustrating the capacity of MR^{+} cells to promote B cell responses and the specificity of the system. These results provide the first conclusive in vivo evidence of a role for the MR in the induction of adaptive immune responses. Interestingly, differential humoral responses were induced by immunization with the MR5D3 and MR6C3 clones. Both of these mAbs recognize the C-type lectin-like carbohydrate recognition domain 4-7 region of the MR, but it is not known whether the binding affinities of these clones differ and/or whether their intracellular handling is different. This requires further investigation but may reflect results in the human system the where differential engagement of the MR on moDCs by anti-human MR mAbs induces differential programs of activation. This was also shown to occur for some natural ligands (52).

It will also be important to determine the exact contribution of MR^{+} DCs in the generation of humoral responses, given that Mϕs bearing ligands for the CR domain have been shown to bind injected CR domain-bearing fusion proteins in the subcapsular sinus, potentially mimicking the delivery of Ags by soluble MRs (53). In addition to direct targeting to MR^{+} cells in the draining LNs, it is possible that the mAbs injected in this study also bound to free soluble MR and were delivered to CR domain-ligand^{+} cells located in the subcapsular sinus. In the presence of stimulation, targeted CR domain-ligand^{+} cells would migrate into B cell follicles (28, 30) and present native Ags in complex with soluble MR to differentiating B cells in the germinal center. However, the pattern of anti-MR mAb targeting in vivo is not consistent with the delivery of mAbs to CR domain-ligand^{+} cells via soluble MR. Furthermore, the soluble MR Ag delivery pathway would not be favored under the conditions tested here because CR domain multimerization is required for optimal targeting to CR domain-ligand^{+} cells in vivo (53) and, given that the anti-MR mAb is probably in a monomeric form, it would be an unsuitable ligand for inducing CR domain multimerization.

The induction of CD4^{+} or CD8^{+} T cell responses via Ag delivery through the MR was not addressed in this study and is the focus of future work. However, indirect evidence from the data presented here suggests that T cells can become activated and assist in the process of Ig isotype switching in response to MR ligands. During the course of these studies a role for MR in the cross-presentation of soluble OVA in vitro and in vivo has been suggested by others (19). Intriguingly, in this work the authors demonstrate a defect in the uptake by CD11c^{+} cells and the cross-presentation of soluble OVA in the spleen and bone marrow of MR-deficient animals. These results are not in agreement with our studies demonstrating the lack of MR expression in splenic DC by both immunofluorescence and flow cytometric analysis of splenic CD11c^{+} cells (data not shown). Future studies using chimeric anti-MR mAbs bearing CD4 and CD8 epitopes will clarify this issue because the contribution of other putative receptors or a defect in DC function in the absence of MR can be ruled out by using this system.

In this study, we demonstrate the presence of a previously unknown murine MR^{+} DC subpopulation whose numbers are controlled by innate stimulation. These cells are most likely derived from myeloid skin leukocytes that can mobilize and acquire a DC phenotype under appropriate stimulation. Efficient targeting of MR ligands to MR^{+} DC takes place when LPS is present, and this correlates with an enhanced induction of humoral responses against these ligands. These data provide the first in vivo evidence of a role for MR in Ag presentation to the acquired immune system and reveal potential pathways available for endogenous molecules recognized by the MR to be presented in an immunogenic form to the acquired immune system. Moreover, the correlation between the immunogenicity of MR ligands and the presence of surrogate signs of infection (e.g., endotoxin) observed in our studies parallels the triggering effect that infection can have in the induction of autoimmune diseases and thus place the MR in a pivotal position in the induction of autoimmunity.

The benefits obtained from exploiting a homeostatic receptor as an Ag acquisition system by immunogenic DC would be derived from its usefulness in increasing the sampling ability of APCs. Because the MR has a well established ability to bind pathogen-derived products and, when expressed on DCs, is able to target Ag for presentation (see Ref. 54 for review), the existence of a highly regulated and restricted MR-mediated Ag presentation pathway in the context of infection would ensure the recognition of microbial products that could otherwise escape presentation due to efficient clearance. In this way, the presentation of endogenous molecules would be minimized and this, together with effective induction of central and peripheral tolerance, will limit the generation of pathological immune responses.

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Disclosures

The authors have no financial conflict of interest.

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Brief Definitive Report

Lipopolysaccharide-enhanced, Toll-like Receptor 4–dependent T Helper Cell Type 2 Responses to Inhaled Antigen

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Abstract

Allergic asthma is an inflammatory lung disease initiated and directed by T helper cells type 2 (Th2) The mechanism involved in generation of Th2 responses to inert inhaled antigens, however, is unknown Epidemiological evidence suggests that exposure to lipopolysaccharide (LPS) or other microbial products can influence the development and severity of asthma However, the mechanism by which LPS influences asthma pathogenesis remains undefined Although it is known that signaling through Toll-like receptors (TLR) is required for adaptive T helper cell type 1 (Th1) responses, it is unclear if TLRs are needed for Th2 priming. Here, we report that low level inhaled LPS signaling through TLR4 is necessary to induce Th2 responses to inhaled antigens in a mouse model of allergic sensitization The mechanism by which LPS signaling results in Th2 sensitization involves the activation of antigen-containing dendritic cells In contrast to low levels, inhalation of high levels of LPS with antigen results in Th1 responses These studies suggest that the level of LPS exposure can determine the type of inflammatory response generated and provide a potential mechanistic explanation of epidemiological data on endotoxin exposure and asthma prevalence

Key words asthma • Toll-like receptor • T cell • dendritic cell • lung

Introduction

Asthma is a pulmonary inflammatory disease believed to be due to aberrant Th2 immune responses to commonly inhaled antigens (1) Only a subset of people exposed to these aeroallergens, however, develop pathological Th2 responses, and this process is not well understood In particular, the role of adjuvants and the innate immune system in the induction of Th2 responses is unclear

Respiratory infections have been linked to asthma in both a preventative and facilitating role, implicating Toll-like receptor (TLR) signaling in regulation of Th2-driven airway disease (2) Of particular interest is LPS, a cell wall component of Gram-negative bacteria that is ubiquitous in the environment, including household dusts LPS activates cells through TLR4 with the accessory proteins CD14 and LPS binding protein (3), signaling through a common adaptor protein MyD88 This results in the transcription of several activation markers including MHC II and B7 molecules and the production of IL-1, IL-12, and TNF-α (3)

The role of endotoxin exposure in asthma development in children has been controversial, with studies indicating either a protective role through Th1 induction or an exacerbating effect on asthma severity (1, 4, 5) It has been speculated that the opposing roles of LPS might be explained by differences in exposure levels (6) However, these studies did not address whether the association of household LPS levels with asthma severity is a result of enhanced allergen sensitization or direct irritant effects of LPS on previously sensitized individuals (4, 6) Our objective was to assess if LPS affects Th2 sensitization to aeroallergens and if the amount of LPS exposure affects the disease phenotype

It is now clear that Th1 adaptive immune responses require TLR signals (7) However, Th2 priming is thought to occur either as a default pathway in the absence of TLR signaling or by a currently unidentified Th2-type activating receptor(s) (3) Therefore, the role a microbial adjuvant such as LPS plays in Th2 aeroallergen sensitization at the site of natural exposure, namely the lung, is unknown

To directly address the role of LPS as an adjuvant for Th2 sensitization in the induction of allergic airway responses, we used a murine model of Th2 pulmonary in-

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flammation in which priming occurs after antigen inhalation without the use of alum We show that Th2 sensitization occurs only if inhaled allergens are encountered with LPS, signaling through TLR4 Furthermore, different doses of LPS induce distinct subsets of Th cells and therefore distinct types of inflammatory responses

Materials and Methods

Animals BALB/cJ (WT) and C C3H-*Tlr4*$^{Lps\text{-}d}$ (TLR4d) mice were purchased from The Jackson Laboratory BALB/cAnNCr mice were purchased from the National Cancer Institute 6–10-wk-old female mice were used in all experiments with three or four mice per group

Sensitization Protocols Mice were anesthetized with methoxyflurane (Metofane) and then sensitized intranasally with 100 μg OVA (Grade V, Sigma-Aldrich) in 50 μl PBS on days 0, 1, and 2 as previously described (8) For Fig 3, we sensitized WT or TLR4d mice intrapentoneally with 100 μg OVA in 2 mg aluminum hydroxide (Pierce Chemical Co) in a total volume of 0 25 ml

Airway Challenge Mice were challenged on days 14, 15, 18, and 19 intranasally with 25 μg OVA and killed on day 21 We confirmed that TLR4d and WT mice inhaled the antigen solution equally by administering Evan's Blue (Sigma-Aldrich) intranasally (9)

LPS Depletion and Measurement Endotoxin Detoxi-Gel™ (Pierce Chemical Co) was used according to the manufacturer's instructions to remove >99% of the contaminating LPS in the administered OVA solution (resulting in a total dose <0 001 μg LPS during priming), which was measured by limulus amebocyte assay (BioWhittaker)

Analysis of Bronchoalveolar Lavage (BAL) Mice were killed and BAL inflammatory cells were obtained as previously described (10) We determined statistical significance using an unpaired Student's *t* test

Lung Histology Paraffin-embedded coronal lung sections were prepared as previously described (8) and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) All images are at 100×

Determination of Serum Antibody Concentration Serum was obtained on day 21 for measurement of OVA-specific IgE (11), IgG1, and IgG2a (8) antibodies by ELISA as previously described Hyperimmune serum from OVA/alum immunized BALB/c mice was used for IgE standard and set at 500 U/ml Levels of detection were 125 ng/ml (IgG1), 16 U/ml (IgE), and 81 U/ml (IgG2a)

Lymph Node Cytokine Production Mice were sensitized and challenged with either OVA (WT or TLR4d) or PBS (WT) and on day 21, mediastinal LN cells were isolated and stimulated in vitro with 200 μg/ml OVA and syngeneic T cell–depleted splenocytes Cytokines in culture supernatants were measured using commercially available ELISA kits (R&D Systems) Levels of detection were 25 0 pg/ml (IL-4), 125 0 pg/ml (IL-5), and 1 9 ng/ml (IFN-γ)

Serum and Bone Marrow–derived Dendritic Cell (BMDC) IL-12 Detection and BMDC Activation Markers Serum from mice was obtained 4 h after the third inhalation of OVA with high or low dose LPS and measured p70 levels using commercially available ELISA kits (R&D Systems) For in vitro studies, BMDCs were cultured as previously described (12) from TLR4d and WT mice On day 9 of culture, we added 100 μg/ml OVA, 100 ng/ml TNF-α, or 50 ng/ml LPS and harvested cells and supernatant at 12 h p70 level of detection was 7 8 pg/ml After Fc receptor blocking with 24G2, CDllc^{hi} (HL3) cells were evaluated by FACS® for MHC II (2G9) and B7 2 (GL1, BD Biosciences)

Dendritic Cell (DC) Migration Studies 0 5 mg FITC-OVA (Molecular Probes) was administered intranasally with low dose (0 1 μg) LPS on days 0, 1, and 2 with or without 2 μg TNF-α on day 1 On day 3, we harvested and pooled the draining LNs in each group and blocked Fc receptors and then stained them with anti-CD11c fluorochrome

Results

Dose of LPS Determines Type of Immune Response Generated to Inhaled Antigen We have previously shown that sensitization of mice by exposure to inhaled OVA leads to robust pulmonary Th2 responses (8) To test the role of LPS in these responses, we sensitized mice by intranasal exposure to OVA depleted of contaminating LPS (<0 001 μg) or OVA with a high (100 μg) or low (0 1 μg) dose of LPS These low and high doses of LPS are analogous to reported endotoxin levels of samples from homes of atopic versus nonatopic children, respectively (5) Mice exposed to LPS-depleted OVA showed no airway inflammatory responses after challenge with inhaled antigen (Fig 1 A) and had total BAL cell numbers equivalent to PBS controls In contrast, mice sensitized with OVA containing low dose LPS demonstrated significant increases in total BAL cell numbers as well as lung tissue infiltrates and airway mucus secretion (Fig 1, A and B) Both airway and tissue infiltrates were dominated by eosinophils, consistent with Th2-mediated inflammation Draining lymph node (DLN) IL-5 and IL-13 production confirmed the Th2 nature of the inflammatory response (Fig 1 C) Mice exposed to PBS or low dose LPS alone did not generate pulmonary inflammation after OVA challenge (Fig 2 A)

As LPS is known to be a potent inducer of IL-12 production from APCs in vitro, it might be expected to preferentially stimulate Th1 responses Therefore, we tested whether the surprising induction of Th2 responses was a result of the low dose of LPS exposure Use of a high dose of LPS during intranasal OVA priming resulted in a Th1-associated response dominated by neutrophils and an absence of airway mucus production in the lung (Fig 1, A and B, reference 10) IFN-γ production from DLNs confirmed the induction of a Th1 response in high dose LPS-exposed mice (Fig 1 C) Serum antibody isotype patterns in groups sensitized with OVA containing low versus high dose LPS were also consistent with the generation of Th2 (high IgE and IgG1) versus Th1 (high IgG2a) immunity, respectively (Fig 1 D) Thus, no airway inflammatory response was generated in mice that had been sensitized with LPS-depleted OVA, whereas antigen-specific immune responses were induced in the presence of LPS with low and high doses inducing Th2 or Th1 responses, respectively

TLR4 Signaling Is Required for Th2 Priming to Inhaled Antigens The requirement for LPS in the generation of Th2 responses to inhaled antigen was confirmed in C C3H-*Tlr4*$^{Lps\text{-}d}$ mice (13) expressing a nonfunctional TLR4 (TLR4d) When compared with WT, TLR4d mice ex-

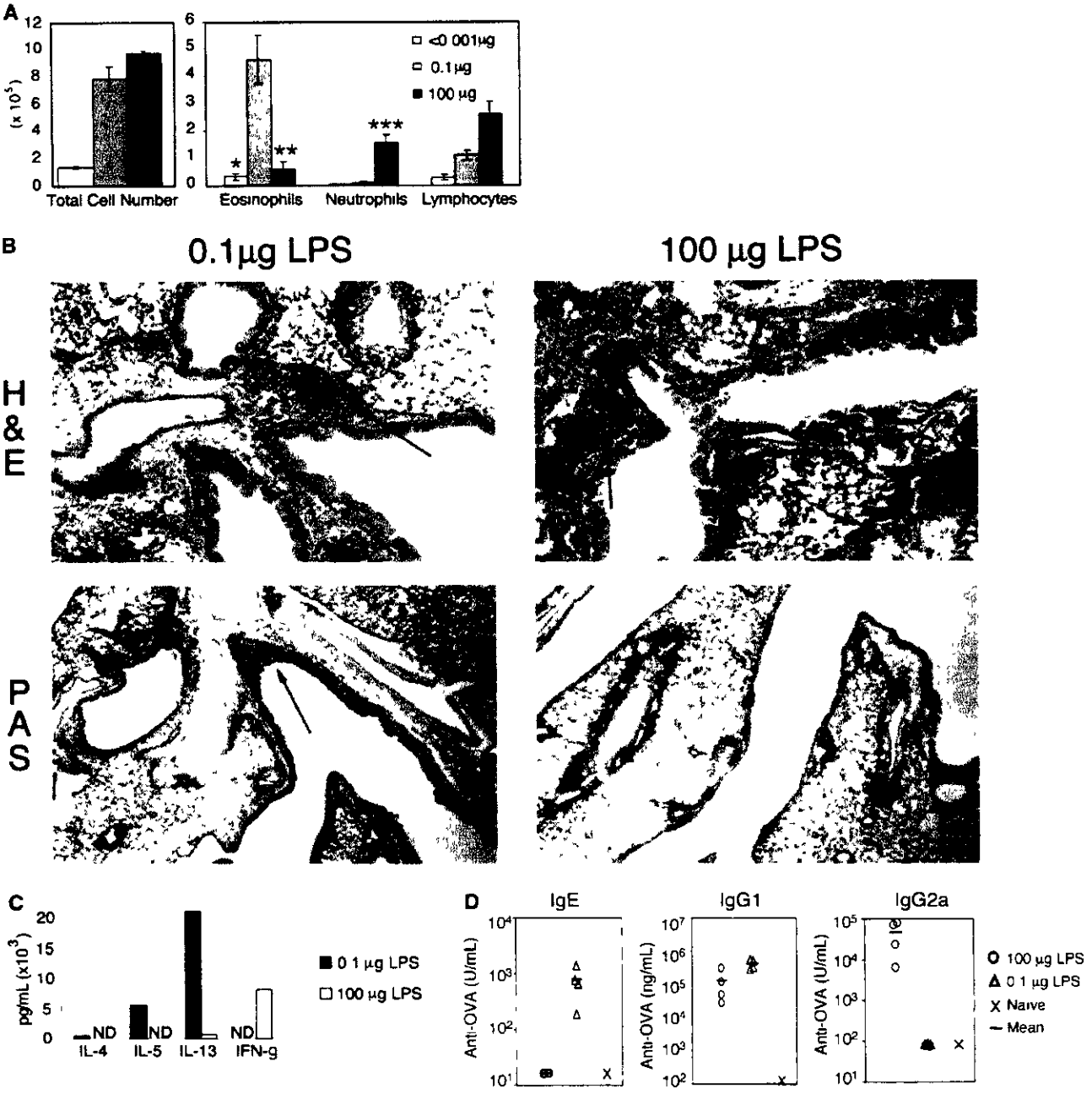

Figure 1. The dose of LPS inhaled with antigen determines the nature of the immune response generated (A) BAL inflammatory cells of BALB/c mice exposed to LPS-depleted OVA (open bars), OVA with low dose LPS (gray bars), or OVA with high dose *Escherichia coli* LPS (solid bars, Sigma-Aldrich) after challenge Monocytes constitute the remainder of BAL cells (not depicted) Bars depict the mean ± standard deviation *, $P < 0.01$ (eosinophils in depleted vs low LPS groups), **, $P < 0.01$ (eosinophils in high vs low LPS groups) ***, $P < 0.01$ (number of neutrophils in high vs low LPS groups) One representative experiment of six is shown (B) Representative lung sections stained with H&E or PAS at 100× Arrows indicate areas or peribronchiolar cellular infiltrate (H&E) or positive mucus staining (PAS) (C) Cytokine production from lung draining LNs in low (solid bars) and high (open bars) dose LPS groups One representative experiment of four is shown ND not detectable (D) Serum antibodies of low (△) and high (○) dose LPS groups are compared with pooled sera from naive BALB/c mice (×) Line depicts the mean $P < 0.05$ (LPS high vs low dose) for IgG1, IgE, and IgG2a responses

posed to OVA in the presence of low dose LPS showed marked reduction in airway inflammation (Fig 2 A) and DLN Th2 cytokine production (Fig 3 B, I N) We obtained similar results using C3H/HeJ mice Th1 responses initiated with high dose LPS were similarly abrogated in TLR4d mice (not depicted)

These data support the observation that LPS is required for the development of Th2 (and Th1) responses to inhaled

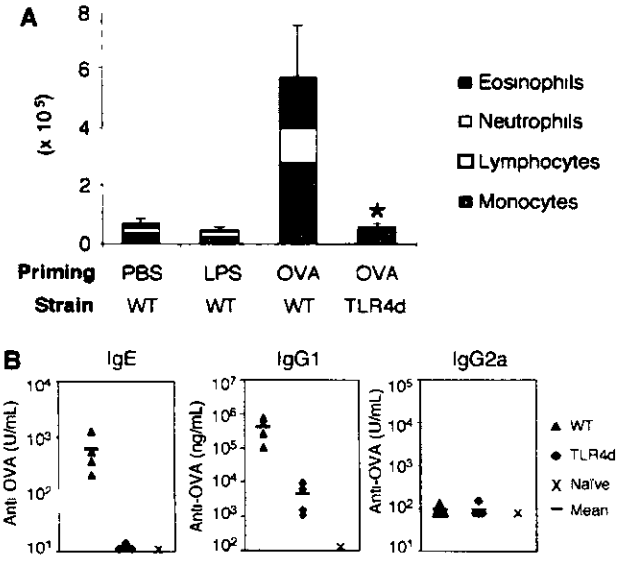

Figure 2. TLR4 signaling is required for Th2 sensitization to inhaled OVA (A) BAL inflammatory cells of WT or TLR4d mice sensitized intranasally with OVA with low dose LPS (0 1 μg), or WT primed with LPS alone, or PBS on day 21 Total bar height represents total cell number in BAL and error bars are based on total cell numbers *, $P < 0.04$ (total BAL cell number from TLR4d vs WT) One representative experiment of six is shown (B) Serum antibody responses by ELISA on day 21 in WT (▲) and TLR4d (◆) mice compared with pooled naive serum (×) $P < 0.05$ (WT vs TRL4d) for IgG1 and IgE responses

antigen However, because LPS signaling is absent during both sensitization and challenge in TLR4d mice, we next asked at what stage LPS was required (6) To address this question, Th2 cell–dependent OVA-specific antibody secretion was measured TLR4d mice demonstrated significantly reduced OVA-specific IgG1 and no IgE or IgG2a antibody responses (Fig 2 B) In addition, there was evidence of a reduced proliferative response in the lung DLN of TLR4d mice as the cellularity after intranasal priming was substantially diminished ($5.9 \pm 1.4 \times 10^6$ in WT vs $2.3 \pm 0.3 \times 10^6$ cells in TLR4d) Thus, there was evidence of abrogated Th2 priming in TLR4d mice by systemic antibody responses, DLN cellularity, lung inflammation, and cytokine responses, consistent with defective T cell priming in the absence of LPS signaling

TLR4d Mice Are Capable of Mounting Th2 Responses Using the Adjuvant Aluminum Hydroxide To confirm that recruitment pathways were intact in the lungs of TLR4d mice, a TLR4 independent mechanism of Th2 priming was used Alum is a potent Th2 adjuvant that does not contain microbial products and therefore should not involve TLR4 signaling to initiate immune responses Therefore, TLR4d and WT mice were immunized intraperitoneally with OVA/alum or intranasally with OVA/LPS 2 wk later, both groups were challenged with inhaled antigen TLR4d mice were fully capable of initiating Th2 immunity in the presence of a non-TLR4 adjuvant as evidenced by eosinophilic BAL inflammation and Th2 cytokine responses in the lung DLNs (Fig 3, A and B) Thus, circumventing deficient Th2 priming with the adjuvant alum results in equivalent pulmonary inflammation in TLR4d and WT mice, indicating that lung recruitment of eosinophils and lymphocytes is not impaired in TLR4d mice

TNF-α Restores Pulmonary Inflammation in TLR4d Mice Adjuvants initiate adaptive immune responses by activating DCs to present antigen in the context of MHC and costimulatory molecules in the DLN (3) We hypothesized that if we could induce DC maturation and migration in the absence of LPS adjuvant signals in TLR4d mice, we could restore T cell priming to inhaled antigen TNF-α is both a product of LPS-stimulated DCs and is known to activate DCs Using this cytokine to circumvent deficient maturation signals by LPS, Th2 responses were completely restored in TLR4d mice with administered TNF-α during sensitization to inhaled antigen This included airway inflammatory responses (Fig 4 A) and antibody responses (not depicted) In addition, TNF-α administration restored DLN cytokine production in TLR4d mice (116 ± 22 vs 1516 ± 590 pg/ml IL-5 and 524 ± 130 vs 2225 ± 1186 pg/ml IL-13 in TLR4d vs TLR4d with TNF-α, respectively) These data indicate that defective T cell priming can be overcome using the LPS/TLR-induced cytokine TNF-α, implicating a role for DC maturation and migration in the LPS adjuvant effect

DC Maturation and Migration to the DLN Are Diminished in TLR4d To test whether the role of low dose LPS with OVA inhalation is to induce DC maturation and migration

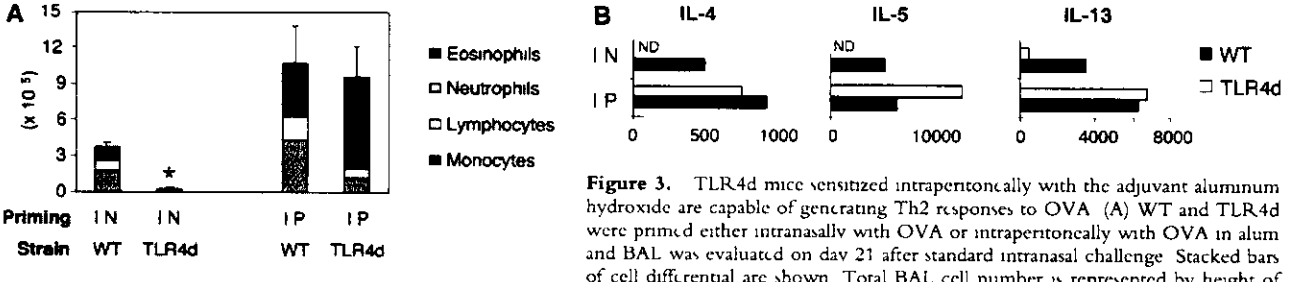

Figure 3. TLR4d mice sensitized intraperitoneally with the adjuvant aluminum hydroxide are capable of generating Th2 responses to OVA (A) WT and TLR4d were primed either intranasally with OVA or intraperitoneally with OVA in alum and BAL was evaluated on day 21 after standard intranasal challenge Stacked bars of cell differential are shown Total BAL cell number is represented by height of stacked bars and standard error is based on total BAL number *, $P < 0.005$ (intranasally primed TLR4d vs WT mice) Mice immunized intraperitoneally with alum alone did not respond (B) Cytokine production in pg/ml from DLN of intranasally or intraperitoneally primed WT (solid bars) or TLR4d (open bars) mice ND, not detectable IFN-γ was not detectable from cultures of WT or TLR4d mice primed intranasally or intraperitoneally with OVA containing a low dose of LPS One representative experiment of two is shown

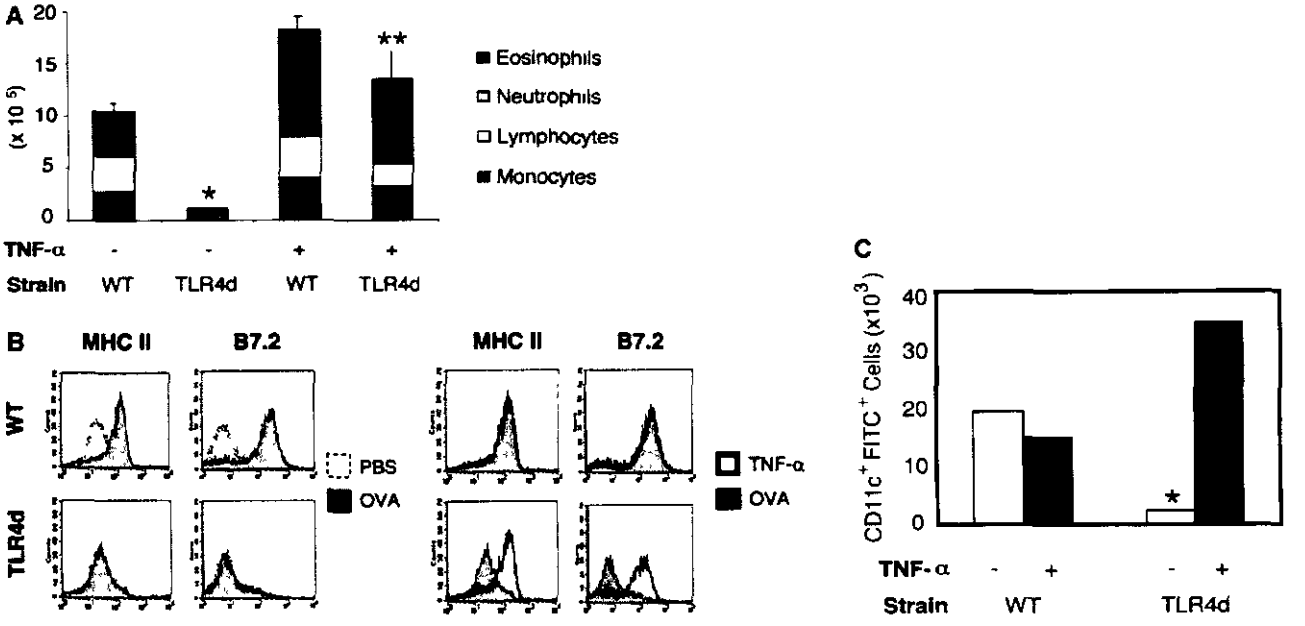

Figure 4. Th2 pulmonary responses and DC activation in response to OVA with LPS are abrogated in TLR4d mice but can be restored with TNF-α (A) We sensitized mice as before with half of groups receiving 2 μg recombinant murine TNF-α (R&D Systems) intranasally on day 1 The number of inflammatory cells recovered by BAL on day 21 is represented by the height of the stacked bars with error bars *, $P < 0.001$ (WT vs TLR4d), **, $P = 0.001$ (TLR4d vs TLR4d with TNF-α) (B) MHC II and B7 2 FACS* analysis of $CD11c^{hi}$ BMDCs from WT or TLR4d stimulated for 12 h with PBS, 100 μg/ml OVA/LPS, or 100 ng/ml TNF-α (C) Number of $FITC^+$ $CD11c^+$ cells in mediastinal LNs on day 3 after intranasal administration of FITC-OVA with low dose (0 1 μg) LPS (gray bars) with (+) or without (−) 2 μg intranasal TNF-α (solid bars) on day 1 One representative experiment of three is shown *, $P = 0.01$ (TLR4d + vs − TNF-α)

resulting in Th2 priming, we examined BMDCs for up-regulation of MHC II and B7 2 in the presence or absence of OVA/LPS or TNF-α Although both activation markers were up-regulated on DCs from WT mice in response to either OVA/LPS or TNF-α, only TNF-α activated TLR4d DCs *in vitro* (Fig 4 B) We then used inhaled FITC-OVA with low dose LPS to track migration in vivo of antigen-containing DCs from the lung to the DLNs in WT versus TLR4d mice Although migration of $CD11c^+$ $FITC^-$ DCs to DLNs was seen in WT mice, no significant antigen-loaded DC migration occurred in TLR4d mice (Fig 4 C) Migration was restored in TLR4d mice upon the administration of TNF-α with FITC-OVA Thus, Th2 sensitization is abrogated in the absence of TLR4-associated DC migration When migration to the DLN is restored using TNF-α in TLR4d mice, Th2 responses are also restored

DC IL-12 Production Differs after Exposure to Low and High Doses of LPS LPS is known to induce both cell surface DC maturation and the production of TNF-α, IL-1, and IL-12 (3) As IL-12 is a potent Th1 skewing cytokine, we hypothesized that differences in IL-12 production following high versus low dose LPS inhalation with OVA might explain the induction of Th1 versus Th2 responses, respectively To test this, serum IL-12 levels were analyzed In contrast to mice immunized with low dose LPS OVA, WT mice immunized with high dose LPS OVA had significantly higher levels of serum IL-12 (Fig 5 A) In vitro evaluation of WT BMDC confirmed that only high dose LPS

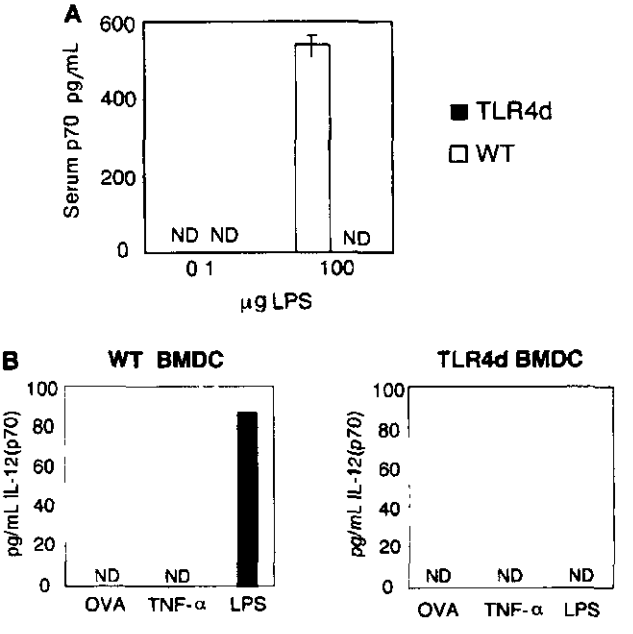

Figure 5 Differential IL-12 production with high and low dose LPS (A) Serum IL-12 (p70) levels on day 2 of priming with inhaled OVA containing either high (100 μg) or low (0 1 μg) levels of LPS (B) IL-12 (p70) production from WT or TLR4d BMDCs after stimulation with 100 μg/ml OVA with low dose LPS, 100 ng/ml TNF-α, or high dose (50 ng/ml) LPS for 12 h *ND*, not detectable

was capable of inducing IL-12 production, whereas OVA (containing low dose LPS) did not (Fig 5 B) These data are consistent with the differential inflammatory response observed in vivo (Th1 vs Th2) and implicate an LPS threshold requirement for IL-12 secretion Interestingly, TNF-α, a cytokine capable of inducing DC maturation and Th2 sensitization, was unable to induce IL-12 in WT BMDCs This is consistent with our observations that TNF-α administration during priming was capable of rescuing Th2 responses in TLR4d mice without the induction of Th1 immunity (Fig 4 A) As expected, no IL-12 was detected from TLR4d serum or BMDCs stimulated with OVA, TNF-α, or LPS

Discussion

The results presented here support a model of sensitization to inhaled inert proteins that requires LPS and the TLR4 signaling pathway In addition, the amount of LPS present during sensitization determines whether Th1 or Th2 immunity is observed Although recent studies in MyD88-deficient mice support a role for TLRs in the generation of Th1 responses to proteins, Th2 responses were shown to be MyD88 independent, suggesting TLR signaling is not important for the induction of Th2 cells (7) However, recent work with MyD88-deficient DCs showed that LPS stimulation induced IL-4 production with normal up-regulation of costimulatory molecules resulting in a Th2 skewing bias (14), suggesting that a MyD88-independent pathway, TIRAP/MAL, is responsible for the observed response We might speculate that the threshold of induction for these two signaling pathways of TLR4 requires distinct levels of signaling intensities, resulting in differential effects on the adaptive immune response The results from this study demonstrate the importance of TLR-dependent adjuvants in the induction of Th2 responses and the LPS dose differential of Th1/Th2 activation

Another study using crystalline OVA in alum intraperitoneally suggested that TLR4-defective mice could not recall Th2-type inflammation to the lung (15) However, the results presented here demonstrate that T cell priming using the adjuvant alum and cell recruitment to the lung are intact in TLR4d mice, as would be expected from an LPS-free, non-TLR–dependent adjuvant such as aluminum hydroxide (Fig 3 A) This discrepancy may lie in the genetic variation that could occur between the substrains of mice used in their study

The data reported here may help explain previously observed differences in the response to inhaled protein, where both tolerance and Th2 immunity have been seen (8, 9) It is plausible that these differences are a result of varying levels of LPS contamination and that one reason this protein has been an effective antigen in many asthma models relates to its inherent LPS contamination (16)

Various animal models indicate that exposure to microbial sequences such as LPS can down-regulate Th2 pulmonary responses (17) Epidemiological data in humans support a differential dose model with endotoxin exposure correlated with both increased and decreased incidence of lung disease and severity (1) Our data provide a model to explain these conflicting findings in that OVA exposure in the presence of high dose LPS fails to induce Th2 cells, but instead induces both IL-12 production and a Th1 response By contrast, low dose LPS is not sufficient to induce Th1 cells but is required to induce Th2 inflammation In the absence of LPS there is no significant lung response Thus, different levels of LPS exposure resulting in different Th cell inflammatory responses might explain the discrepancies in human studies Recently discovered missense mutations in human TLR4 could likewise provide an explanation for the variability in human sensitization to ubiquitous aeroallergens (18)

Respiratory syncytial virus (RSV) infections during childhood have also been identified as a major risk factor for the development of asthma (2) Although RSV is likely to have multiple pathways of influencing asthma, it was recently found that the innate immune response to RSV is mediated by CD14 and TLR4 (19) This raises the question of whether LPS has a unique role in asthma or if other TLR ligands could induce Th2 sensitization

We thank R Flavell and R Medzhitov for critical review of the manuscript and discussion, and P Ranney and L Xu for technical assistance

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Attachment 4

Affidavit Of

Martin F. Kagnoff, MD

Martin F. Kagnoff, being first duly sworn, states as follows:

1. I have been employed by the University of California (San Diego) School of Medicine since 1972. My title until July 1, 2007 was Professor of Medicine and Pediatrics, UCSD and my new title beginning August 1, 2007 is Research Professor of Medicine and Pediatrics, UCSD. I also currently serve as Director, Laboratory of Mucosal Immunology and The Wm. K. Warren Medical Research Center for Celiac Disease at UCSD.

2. Prior to my employment by UCSD School of Medicine (in various positions) I was a "Visiting Scientist" at the Salk Institute.

3. My professional educational training consists of an MD from Harvard Medical School; an internship and residency at Peter Bent Brigham Hospital (currently Brigham & Women's Hospital) in Boston; senior residency in medicine at New York Hospital (Cornell University); and an NIH trainee in gastroenterology at Boston University School of Medicine.

4. With regard to my experience in gastroenterology- and immunology-related academic and research activities, please note that for decades I have been significantly involved in gastroenterology/immunology-related research and currently I am focusing on research areas involving the role of the intestinal immune system in host-environment interactions, the intestinal response to foodborne pathogens, and celiac disease. In these capacities, I have published numerous, peer-reviewed scientific articles. I also teach as a member of graduate programs in the biomedical sciences and molecular pathology. Finally,

I have directed a NIH funded Research Training Program at UCSD for the past decade.

5. I also participate in various, related, outside activities. These include memberships in nine professional organizations (including the American Association of Immunologists and the American Gastroenterological Association), participation on various advisory boards and NIH-related review sections, as a reviewer for twelve editorial boards (including Science, Cell, Nature Immunology, Gastroenterology, Journal of Immunology, and Infection and Immunity), and have served as a past Editor in Chief of the American Journal of Gastroenterology: Gastrointestinal and Liver Physiology, as a past Editor and Associate Editor of the Journal of Clinical Investigation, and on the editorial boards of several journals.

6 In my capacity as a widely-recognized, world authority in gastroenterology and immunology (especially in mucosal immunology), I was asked by Pharming Group NV – a biotechnology company producing drugs and food-related products from transgenic animals – to participate on an expert panel whose function it is to evaluate the safety of Pharming's rhLF when used as an ingredient in sports and functional foods at a level of 100 mg per product serving – especially as it relates to rhLF's ability, if any, to induce any adverse immunological effect(s). More specifically, I was asked to review Pharming's GRAS Notification (dated December 29, 2005) and Pharming's subsequent Response to CFSAN document (dated December 22, 2006), was supplied with a copy of all references referred to in both the GN and Response, and was asked to indicate 1. whether I agreed with the substance and conclusions set forth in the latter Response document, and 2. whether I had any comments to make which were intended to make that document an even better science-based response. On July 11, 2007 I provided Pharming with a written response to its two requests. Such response was based, in part, on my own, independent research into the pertinent scientific literature (in addition to all of the scientific articles provided by Pharming).

7. With regard to an overall evaluation of the Response document, I indicated that after reviewing and analyzing all of the documents and literature, I generally agree with and support the expert panel's responses and conclusions (as set forth in the response document).

This ends affiant's statement.

Martin F. Kagnoff, MD

STATE OF HAWAII)
) SS
COUNTY OF KAUAI)

SUBSCRIBED and SWORN to before me this 23rd day of July, 2007

NOTARY PUBLIC

TANYA L CHYTKA
Expiration Date May 23, 2011

TANYA L. CHYTKA NOTARY PUBLIC STATE OF HAWAII

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11-05-07P05:12 RCVD

Re: Request for FDA to Notify Ventria Bioscience and Pharming Group N.V. of the Impact of New Legislation on GRAS Notifications for Recombinant Human Lactoferrin

Dear Dr. Tarantino:

On behalf of our client, Agennix, Inc., we respectfully request that the Food and Drug Administration (FDA) notify Ventria Bioscience (Ventria) and Pharming Group N.V. (Pharming) that, because of recently enacted legislation, the FDA will no longer review pending or future GRAS Notifications for recombinant human lactoferrin (rhLF). 1/ This action is necessary to implement Section 912 of H.R. 3580, the Food and Drug Administration Amendments Act of 2007 (FDAAA), which President George W. Bush signed into law on September 27, 2007. Section 912 specifically prohibits the sale of foods containing pharmaceutical components such as rhLF.

I. STATUTORY BACKGROUND

In general, Title IX of the FDAAA is devoted to enhancing FDA's authority with regard to the postmarket safety of drugs. More specifically, Section 912 entitled, "Prohibition against food to which drugs or biological products have been added," makes it a prohibited act under the Federal Food, Drug and Cosmetic Act (FFDCA) to introduce into interstate commerce any food to which "has been added a drug approved under section 505, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which

1/ Pharming's GRAS Notice No. GRN 000189 was submitted to FDA on December 29, 2005 and is still pending. Ventria's GRAS Notice No. GRN 000162 was submitted to FDA on December 16, 2004 and withdrawn on November 6, 2006, but without prejudice to being resubmitted in the future.

substantial clinical investigations have been instituted and for which the existence of such investigations has been made public."

Section 912 also contains the following exceptions to this prohibition, such that foods containing such components may be sold in interstate commerce if:

(1) such drug or such biological product was marketed in food before any approval of the drug under section 505, before licensure of the biological product under such section 351, and before any substantial clinical investigations involving the drug or the biological product have been instituted;

(2) the Secretary, in the Secretary's discretion, has issued a regulation, after notice and comment, approving the use of such drug or such biological product in the food; or

(3) the use of the drug or the biological product in the food is to enhance the safety of the food to which the drug or the biological product is added or applied and not to have independent biological or therapeutic effects on humans, and the use is in conformity with—

(A) a regulation issued under section 409 prescribing conditions of safe use in food;

(B) a regulation listing or affirming conditions under which the use of the drug or the biological product in food is generally recognized as safe;

(C) the conditions of use identified in a GRAS notification to the Secretary, provided the Secretary has not questioned the general recognition of safety determination in a letter to the notifier;

(D) a food contact substance notification that is effective under section 409(h); or

(E) such drug or biological product had been marketed for smoking cessation prior to the date of the enactment of the Food and Drug Administration Amendments Act of 2007. 2/

2/ Section 912 contains a fourth exception – the drug is a new animal drug whose use is not unsafe under section 512, which is not relevant to our discussion of recombinant human lactoferrin and will not be addressed in this letter. Nor is the subparagraph dealing with smoking cessation relevant to this letter.

II. VENTRIA'S AND PHARMING'S PROPOSED DISTRIBUTION OF RECOMBINANT HUMAN LACTOFERRIN WOULD BE PROHIBITED UNDER SECTION 912 OF THE FDAAA

Ventria and Pharming are each attempting, through the GRAS notification process, to gain FDA authorization for a drug to be used in a food product that would, due to passage of Section 912 of the FDAAA, result in that food being prohibited from distribution under the FFDCA. Section 912 of the FDAAA specifically prohibits such distribution, and Ventria's and Pharming's proposed uses for recombinant human lactoferrin do not fall within any of the statutory exceptions to this prohibition. Accordingly, FDA should take the necessary action to ensure that Section 912 is fully implemented and applied with respect to recombinant human lactoferrin products.

A. Recombinant human lactoferrin is a drug for which substantial clinical investigations have been instituted and the existence of such investigations have been made public—and is therefore prohibited from being added to food

Recombinant human lactoferrin meets the threshold test under Section 912 because it is a drug for which substantial clinical investigations have been instituted and the existence of such investigations have been made public. In fact, Agennix has been developing recombinant human lactoferrin (rhLF) as a pharmaceutical drug under the authority of the FDA since 1996 (see, e.g., IND No. 6799, IND No. 11728 and IND No. 8546). Agennix is currently preparing to enter Phase III clinical trials with rhLF in advanced non-small cell lung cancer, for which Agennix received Orphan Drug designation from the FDA, and has been granted Fast Track designation by the FDA for both first-line combination therapy and third-line monotherapy. Agennix is also preparing for a Phase IIb trial in advanced renal cancer, and has been granted Orphan Drug designation by the FDA for this indication. Moreover, the existence of Agennix's clinical investigation of recombinant human lactoferrin has long been open and within the public domain. For example, an Agennix press release of May 10, 2001 explicitly stated "Agennix has completed numerous pre-clinical and clinical trials with rhLF demonstrating the enormous potential of lactoferrin in a wide range of clinical conditions." See also Agennix press release of May 22, 2003 "VAMC and Agennix Successfully Complete Safety Phase of Lactoferrin Cancer Trial." In addition to these press releases, Agennix has made other public statements detailing clinical investigations with recombinant human lactoferrin that pre-date both GRN No. 000162 and GRN No. 000189. 3/ Section 912 of the FDAAA clearly prohibits a drug compound, such as recombinant human lactoferrin that is actively being studied as a pharmaceutical product, to enter the marketplace in a food product.

3/ For the complete text of these press releases and a partial list of relevant publications, please see Attachment A.

B. Ventria's and Pharming's recombinant human lactoferrin products do not meet any of the exceptions found in Section 912 of the FDAAA

Ventria and Pharming's use of recombinant human lactoferrin would not be allowed under any of the exceptions found in Section 912 of the FDAAA. These are described further, as follows:

1. Recombinant human lactoferrin has never been marketed in food.

Recombinant human lactoferrin does not qualify for the first exception to the prohibition because the compound has not been marketed in human food products prior to the initiation of clinical investigations by Agennix in 1996. In fact, rhLF has **never** been marketed in human food products. Therefore, by the plain language of the statute, rhLF does not qualify for the first exception for food products that were marketed first.

2. Ventria's and Pharming's proposed uses of recombinant human lactoferrin have not been the subject of FDA rulemaking to allow such uses in human food, and FDA should not initiate such rulemaking

Recombinant human lactoferrin similarly does not qualify for the second exception in Section 912, which allows the Secretary, at the Secretary's discretion, to issue a regulation after notice and comment, approving the use of the drug in a food. FDA has issued no such regulation. Again, therefore, under the plain language of the statute, rhLF does not qualify for the rulemaking exception in Section 912.

Nor should the agency initiate such rulemaking. The use of recombinant human lactoferrin in conventional foods does not present any compelling reason that would justify the expenditure of FDA's limited resources for this purpose. Indeed, the initiation of rulemaking is no routine matter. Congress gave the Secretary complete discretion on whether to initiate rulemaking, and such discretion should only be used in matters that have a significant public health benefit. Before FDA should even consider the question of initiating a rulemaking proceeding, Ventria and Pharming should be required to show — in addition to its safety – that the use of rhLF in conventional food products is necessary to benefit public health.

FDA has not even been satisfied of the safety of rhLF, and has notified Ventria that the agency plans to conduct a public scientific inquiry into the complex scientific issues presented. FDA's response letter acknowledging Ventria's withdrawal, dated November 20, 2006, informed Ventria that the agency had ceased to evaluate their notice but "plans to engage the wider scientific community for further consideration of the complex scientific issues in the notice." 4/ Moreover, the only potential benefit of Ventria's and Pharming's proposed uses of rhLF would be therapeutic, which would render the product a drug--exactly the type of component the FDAAA was enacted to keep out of conventional foods. Accordingly, rhLF would not be a proper subject of such rulemaking.

4/ *See* Agency Response Letter GRAS Notice No. GRN 000162 *at* http://www.cfsan.fda.gov/~rdb/opa-g162.html.

3. Ventria's and Pharming's proposed uses of human lactoferrin are to have a biological or therapeutic effect on humans

Ventria's and Pharming's proposed use of recombinant human lactoferrin similarly does not fall under Section 912's third exception, which allows for the use of the drug in a food if it is being used to enhance the safety of the food and not to have independent biological or therapeutic effects on humans (and it meets one of the other conditions, such as receiving a food additive approval or receiving agency authorization under the GRAS notification process). The inclusion of rhLF in food is clearly not to enhance the safety of the food, but to promote precisely what Section 912 prohibits – an independent biological or therapeutic effect on humans. Indeed, as will be highlighted below, both Ventria and Pharming have publicized recombinant human lactoferrin's pharmaceutical-like properties while making no mention of any increased safety of the food product itself.

i. Published statements attributable to Ventria regarding its proposed uses of recombinant human lactoferrin

The following are published statements attributable to Ventria regarding its proposed uses of rhLF. These statements speak for themselves. Additional statements are also contained in Attachment B.

Prepared Remarks of Mr. Scott Deeter, Ventria President and CEO before the Subcommittee on Rural Enterprises, Agriculture, and Technology (entitled "Different Applications for Genetically Modified Crops") June 29, 2005 5/

- "Ventria Bioscience is a plant-made pharmaceutical company that utilizes rice and barley as a factor to produce biologic products. Ventria's initial products provide human health benefits...."
- "Ventria believes this technology will lead to more affordable medicines for a much broader patient population than what is possible with conventional biopharmaceutical production technology today."
- "These advantages pave the way for a paradigm shift in biopharmaceutical production for the benefit of patients worldwide." (citing reasons for Ventria's economic advantage)
- "As an illustration of the strength of Ventria's technology, I would like to describe some of the human health products in development. Ventria's first two human health products are proteins called Lactiva™ and Lysomin™." [Note: Lactiva™ is the proposed trade name for recombinant human lactoferrin.]

5/ *See* Attachment C.

- "There are several products being developed by Ventria that will incorporate Lactiva™ and Lysomin™. One product has been developed for children suffering from acute diarrhea. The World Health Organization estimates that 1.9 million children under the age of 5 die annually due to diarrhea. To address this crisis, Ventria added Lactiva™ and Lysomin™ to an oral rehydration solution, which is a common first line therapy given to children suffering from diarrhea. By adding Lactiva™ and Lysomin™, Ventria believes it can improve the recovery rate and reduce the severity or duration of diarrhea in these children."

- "Ventria is also exploring the use of Lactiva™ and Lysomin™ for the prevention of diarrhea in the military.... This is a silent enemy attacking American troops. Ventria has set its goal to reduce the diarrheal attack rate by 50% with the preventive administration of Lactiva™ and Lysomin™."

- "Another use of Lactiva™ that is being developed is for the management of inflammatory bowel disease, or IBD. IBD afflicts over one million Americans and over four million people worldwide. IBD is an extremely debilitating disease that causes severe abdominal pain, weight loss, poor absorption of nutrients and chronic gastrointestinal ulcers."

- "Ventria is also working with University of Cincinnati to develop a treatment for chronic lung infections caused by Pseudomonas, which is the leading cause of death for patients suffering from Cystic Fibrosis."

ii. Published statements attributable to Pharming regarding its proposed use of recombinant human lactoferrin

Pharming made similar statements with respect to its rhLF products having biological or therapeutic effect in humans: 6/

Excerpts from Pharming's website, available at http://www.pharming.com 7/

- "Human lactoferrin (hlf) is a natural protein that helps to fight and prevent infections and excessive inflammations and strengthens the defense system of the human body."

- "Lactoferrin is a multi-functional protein with many beneficial properties, which makes it a good candidate for a number of product applications. Since the protein has the ability to bind iron, is a natural anti-bacterial, anti-fungal and anti-viral, is an antioxidant and also has immunomodulatory properties, large groups of people might benefit from orally administered lactoferrin."

6/ *See* Attachment D for corroborating press articles indicating that Pharming's rhLF has independent biological or therapeutic effects in humans.

7/ *See* Attachment E.

Pharming Press Release, Jan 31, 2007 available at http://www.pharming.com 8/

- "In addition, a "Feasibility" subsidy was granted that will allow the Company, in collaboration with the Erasmus Medical Center in Rotterdam, to establish the role of lactoferrin in bone formation and explore a business and clinical development strategy to develop lactoferrin as a new product in the field of bone diseases such as osteoporosis. Lactoferrin is one of Pharming's current products under development."

Pharming Press Release, Nov. 24, 2004 available at http://www.pharming.com 9/

- "Human lactoferrin is a natural protein that helps to fight and prevent infections and strengthens the defense system of the human body. The protein is present in substantial quantities in mother's milk and plays an important role in the defense system of infants. The protein is also present in various body fluids and continues to play an important role against a wide range of bacterial, fungal and viral pathogens in adults."

- "Pharming is developing recombinant human lactoferrin (rhLF) as a nutraceutical and intermediate while evaluating applications of the product for the pharmaceutical market."

4. The addition of human lactoferrin to food products does not enhance food safety

Given the extensive statements above, any claims at this point in time by either company that rhLF is intended to be used to enhance the safety of food products would be inconsistent with their repeated, prior public statements. On the contrary, it is clear that the benefits rhLF is intended to have are precisely the independent biological or therapeutic effect on humans prohibited by Section 912. This becomes even clearer when considering the foods to which Ventria and Pharming have indicated they would add rhLF. Specifically, Ventria has indicated that it intends to add rhLF to functional foods and drinks such as frozen yogurt, popsicles, meal replacements, performance beverages and bars (including granola and "Ensure"-type drinks), medical foods, and oral rehydration solutions. 10/ Similarly, Pharming has indicated rhLF would be added to meal replacements, sports beverages, frozen yoghurt, yoghurt, ice cream and other frozen deserts, cereal, energy and health bars, and milk-based meal replacements. 11/ Given the highly processed nature of these foods and the lack of any food safety hazard that requires the addition of an agent intended to enhance the safety of the food, the addition of rhLF would not be for any increase in food safety, but rather expressly for its biological or therapeutic effect.

8/ *See* Attachment F.
9/ *See* Attachment G.
10/ Ventria GRAS Notice No. GRN 000162 pages 5 and 28.
11/ Pharming GRAS Notice No. GRN 000189 page 53.

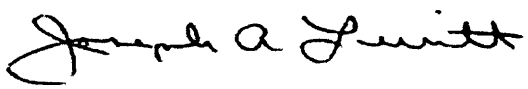
Moreover, to qualify for this statutory exception, the compound must meet BOTH criteria—i.e., enhance the safety of the food AND not have a biological or therapeutic effect on humans—and rhLF fails on both counts. Ventria and Pharming have each clearly marketed the use of rhLF for its biological and therapeutic uses, which renders it a drug. Each company's public statements focus specifically on rhLF's biological or therapeutic effect. Neither company can credibly claim at this point that the intended use of the rhLF is to enhance food safety. Indeed, consumers would purchase products containing rhLF for the alleged biological or therapeutic effects and not due to any increase in the safety of these foods. Therefore, Ventria's and Pharming's proposed use of recombinant human lactoferrin does not fall within Section 912's third and final relevant exception. Consequently, the sale of products containing such components is prohibited.

III. ACTION REQUESTED

Section 912 of the FDAAA makes clear that the addition of drug compounds, such as recombinant human lactoferrin, to conventional foods is strictly prohibited, unless one of the carefully defined exceptions is met. The proposed uses of recombinant human lactoferrin in food do not fall within any of the exceptions delineated in Section 912. Because it is now unlawful to use recombinant human lactoferrin in foods, it would be incongruous (and an unjustified use of scarce FDA resources) for the agency to still consider any review of GRAS notifications for its use in foods. In light of the above, we hereby request that the FDA notify Ventria and Pharming that the use of rhLF in foods is now prohibited by law, and that the agency will no longer review such submissions.

Thank you for your consideration of the information provided in this letter. Please let us know if you have any questions.

Sincerely,



Joseph A. Levitt
Counsel to Agennix, Inc.

Enclosures

cc: Gerald Masoudi, Chief Counsel
Food and Drug Division

Michael Landa, Deputy Director for Regulatory Affairs
Center for Food Safety and Applied Nutrition

ATTACHMENT A

EXAMPLES OF DISCLOSURES OF AGENNIX CLINICAL INVESTIGATIONS WITHIN THE PUBLIC DOMAIN PRIOR TO THE SUBMISSION OF GRAS Notice NO. GRN 000162 (DEC. 16, 2004) AND GRAS Notice NO. GRN 000189 (DEC. 29, 2005)

Agennix Receives U.S. Patent On Production of Recombinant Lactoferrin

HOUSTON--(BW HealthWire)--July 13, 1998--Agennix Incorporated today announced that it has been granted a patent from the United States Patent and Trademark Office (no. 5,766,939) for the production of recombinant lactoferrin. The patent specifically covers production of recombinant human lactoferrin and lactoferrin polypeptides including functional polypeptides in various organisms. Lactoferrin is a natural antimicrobial protein. Agennix is in early phase clinical development for lactoferrin-based products for a variety of inflammatory indications, according to Denis R. Headon, Ph.D., President and CEO.

"This patent, in addition to the three which were issued in November 1996, represent a strong patent portfolio for our core technology," Headon said. "Our patented technology allows us to advance the research and development of a new class of anti-inflammatory drugs, which are currently in various clinical development studies for the treatment of inflammatory conditions. We have a manufacturing agreement with Royal Gist-Brocades N.V. of the Netherlands."

Agennix's patent estate now includes U.S. patents covering cDNA sequences encoding human lactoferrin, methods for producing recombinant human lactoferrin from a variety of host cell systems, and the production of lactoferrin polypeptides, in addition to numerous patents covering applications of lactoferrin.

Agennix Receives Broad Patent Covering Production of Human Lactoferrin in Eukaryotic Cells

Houston, TX -- May 10, 2001 -- Agennix, Incorporated, a developer of protein and peptide-based drugs targeting oncology, infectious disease and dermatology, today announced the issuance of a patent broadly covering the method for expressing human lactoferrin in eukaryotic cells. The patent, U.S. Patent No. 6,228,614, broadens the Company's previous patent coverage to include a greater variety of potential production systems for this protein. The patent is assigned to the Baylor College of Medicine and is licensed exclusively to Agennix. The issuance of this patent brings to Agennix 13 U.S. patents in its growing portfolio. Corresponding patents have now been granted in many countries throughout the world..

"This patent significantly expands our patent coverage of recombinant human lactoferrin," said Richard Barsky, CEO of Agennix. "The issuance of this patent confirms our view that Baylor scientists have made substantial contributions in the field of lactoferrin. These contributions are now being recognized by patent offices worldwide."

The inventors on the patent are Drs. Orla Conneely, Bert O'Malley, both on Agennix's Scientific Advisory Board, and Denis Headon, President and Chief Scientific Officer of Agennix.

Agennix is a privately-owned biopharmaceutical company focused on research and development of recombinant human lactoferrin (rhLF), a natural and safe anti-infective and anti-inflammatory protein, and a variety of related peptides. Holding 40 issued patents and 84 pending patents, the

Company is engaged in programs that address large market opportunities and unmet medical needs in the areas of oncology, infectious disease and dermatology. Agennix has completed numerous pre-clinical and clinical trials with rhLF demonstrating the enormous potential of lactoferrin in a wide range of clinical conditions. More information about Agennix is available on the Company's web site at: www.agennix.com.

VAMC and Agennix Successfully Complete Safety Phase of Lactoferrin Cancer Trial

HOUSTON, May 22, 2003 - The Department of Hematology/Oncology at the Veterans Affairs Medical Center, Houston (VAMC) and Agennix Inc., announced the completion of the safety portions of two Phase 1/2 cancer trials with oral recombinant human Lactoferrin (rhLF). The trials are taking place in the United States and in South America. Teresa G. Hayes, M.D., Ph.D., the Principal Investigator at the VAMC, is conducting the U.S. trial. The South American trial is being conducted at six centers in Argentina, Brazil and Chile.

Lactoferrin, a protein found naturally in milk and other exocrine secretions, plays an essential role in stimulating the body's immune system. The clinical trials are evaluating rhLF as a single agent for the treatment of solid tumor cancer patients, who had progressed on standard chemotherapy and whose tumors were not resectable. The trial design was based on results from over twenty-five different animal experiments showing that lactoferrin significantly inhibits tumor growth and metastases in a variety of solid tumors.

Thirty patients were enrolled in the safety phase of the trials, and administered one of four different doses of rhLF, ranging from 1.5 g/day to 9 g/day, for either 14 days (South America) or in cycles of 14 days with a 14 day gap (VAMC). Patients were observed for adverse events, and tumor size was measured radiologically at baseline and 8 weeks following start of therapy. Of the 30 patients enrolled, 29 completed dosing, with one patient withdrawing prior to completion due to progressive disease. The drug, which is administered orally, was well tolerated without a single drug related serious adverse event or a drug-related laboratory abnormality greater than Grade 2. Safety results are presented in an abstract submitted to the upcoming annual meeting of the American Society of Clinical Oncology.

To date, nineteen patients are evaluable for tumor response (those with a baseline and an 8-week post rhLF treatment CT scan). Of these, 53% (10 of 19 patients) had stable disease by RECIST criteria, three of whom showed some tumor shrinkage. Patients with tumor shrinkage had ovarian cancer, metastatic melanoma or gastric cancer.

In the ongoing efficacy phase of the trials, an additional 38 patients will be treated with the highest rhLF doses. Complete results are expected later in the year.

About VAMC:

The Houston Veterans Administration Medical Center is the largest facility in the nationwide VA system. Patients from a large catchment area, including Texas, Louisiana and Mississippi, come to the Houston VA for cancer treatment. The modern facility, certified as a Cancer Center by the American College of Surgeons, is staffed with Board-Certified Hematologists and Oncologists. All modalities of cancer care are available at the VAMC, including chemotherapy, radiation therapy, and surgery.

About Agennix:

Agennix, a Houston-based biopharmaceutical company, is the world leader in the development of recombinant human lactoferrin (rhLF), a natural immuno-stimulatory and anti-inflammatory protein, and a variety of patented peptides. RhLF has been administered to over 300 people without a single drug-related serious adverse event, and has demonstrated pre-clinical efficacy in treating cancer and asthma, and accelerating wound healing. Phase II clinical trials are underway in all three areas. Agennix is the first and only company to manufacture commercial quantities of human lactoferrin and holds global patents on its technology, with 71 issued patents and more than 30 patents pending. More information about Agennix is available on the Company's web site at: www.agennix.com.

Agennix Inc. Presents Positive Results Showing Accelerated Wound Healing and Wound Closure with Topical Recombinant Human Lactoferrin

HOUSTON, June 18, 2003 - Agennix, Inc. announced results from animal experiments showing that topical recombinant human lactoferrin (rhLF) gel increased the rate and incidence of wound closure, relative to placebo and to the approved drug therapy. These results were presented by Dr. Jose Engelmayer and Dr. Atul Varadhachary in Seattle, at the 13th Annual Meeting of the Wound Healing Society. An abstract of the results was published in the March-April issue of the journal Wound Repair and Regeneration (volume 11, No. 2, A25).

There are 12.5 million people worldwide with chronic wounds (diabetic ulcers, venous ulcers, and pressure ulcers). Many of these patients suffer from severe complications such as leg amputations. Current therapy is only partially effective. RegranexT (recombinant-human platelet-derived growth factor-BB, becaplermin) is the only approved pharmaceutical product and shows 9-23% improvement over placebo and 4-22% improvement over good ulcer care alone in published studies of diabetic foot ulcers. Thus, there is an unmet clinical need for more effective drugs.

RhLF accelerates wound healing in animal models, with a novel mechanism of action. Agennix tested the efficacy of rhLF in mice with open, full thickness wounds, using both healthy mice and diabetic mice with impaired healing. RhLF consistently increased the rate of wound healing and the incidence of closure relative to mice treated with placebo as well as those treated with the approved drug.

Healthy mice treated with 1% topical rhLF had a 38% increase in the incidence of wound closure over placebo, and a 36% increase over the approved drug, which was highly statistically significant ($p<0.01$). In diabetic mice, rhLF treated animals had an 83% increase in incidence over placebo treatment ($p<0.01$). Healing was also evaluated in mice with infected wounds, a

clinically relevant condition that contributes to the blocking of healing. In these animals, rhLF treated animals had an 86% increase in incidence over placebo and a 71% increase over the approved drug, both of which were statistically significant. RhLF treatment also significantly increased the percent of wound closure on each of the measurement days, compared to both placebo and the approved drug.

RhLF treatment appears to be safe and well tolerated. RhLF has been administered to over 300 people (149 topically and 200 orally) without a single serious drug related adverse event. Agennix has a blinded, multi-center Phase 1/2 clinical trial currently underway at Joslin-Beth Israel Hospital (Harvard University) and New York University School of Medicine, to evaluate the safety and efficacy of topical rhLF in patients with diabetic foot ulcers. After the initial dose escalation phase, which has completed enrollment, patients will be randomized between placebo and two doses of rhLF. Results from the efficacy phase of the trial are expected in early 2004.

About Agennix:

Agennix, a privately owned Houston-based biopharmaceutical company, is the world leader in the development of recombinant human lactoferrin (rhLF), a natural immunomodulatory, anti-infective and anti-inflammatory protein. RhLF has been administered to over 300 people without a single drug-related serious adverse event. Oral rhLF has demonstrated preclinical efficacy in treating cancer and asthma, in addition to the accelerated wound healing observed with topical rhLF. Agennix is the first and only company to manufacture substantial quantities of human lactoferrin and holds global patents on its technology, with over 71 issued patents, and more than 50 pending patents. Agennix has five human clinical trials currently underway in the treatment of cancer, asthma, and diabetic foot ulcers.

VAMC and Agennix Release Lactoferrin Cancer Trial Results at ASCO

HOUSTON -June 9, 2004 - The Department of Hematology/Oncology at the Veterans Affairs Medical Center (VAMC), Houston and Agennix Inc., disclosed results from two Phase I/II cancer trials with oral recombinant human lactoferrin (rhLF). The trials are taking place in the United States and in South America. Teresa G. Hayes, M.D., Ph.D., Principal Investigator at the VAMC, is conducting the U.S. trial. The South American trial is being conducted at six centers in Argentina, Brazil and Chile. The data was published at the 40th Annual Meeting of the American Society of Clinical Oncology (ASCO) in New Orleans.

Patients with advanced or metastatic cancers that had failed standard chemotherapies were dosed with oral rhLF administered as a single agent. RhLF appeared to be safe and well tolerated without a single drug related SAE. Among the 45 evaluable patients with a variety of tumor types, rhLF showed a 69% reduction in the average tumor growth rate ($p<0.001$). The effect in the major tumor types was even more dramatic - 89% and 105% reductions (both $p<0.05$) in average tumor growth rates in non-small cell lung cancer (NSCLC) and renal cell cancer (RCC), respectively.

In the nine NSCLC patients, there was an apparent survival benefit. The median survival already substantially exceeds the published median survival in this patient population, and the 12-month survival rate of 57% with rhLF is far higher than the 19% rate expected from the literature ($p<0.05$). Among the six RCC patients, there was one (17%) confirmed durable partial response (currently 53% tumor shrinkage by RECIST compared to tumor size at the start of rhLF therapy 10 months ago). The other five RCC patients all showed shrinkage or a reduction in the rate of

growth of their target lesions. The median PFS in the RCC patients of 6.1 months compares favorably to the expected rate from the literature of 2.5 months, and the 4-month PFS of 83% is much higher than the expected rate of 20% ($p<0.01$). Results in both NSCLC and RCC, though with small numbers of patients, also compare favorably with the published literature on approved drugs. Larger Phase II trials are underway in both NSCLC and RCC.

Rick Barsky, CEO of Agennix, said, "We are pleased with the results of these initial trials. An orally administered, non-toxic, safe and well tolerated drug that is effective against a wide range of common cancers would be tremendously exciting to patients and physicians."

About VAMC:

The Houston Veterans Administration Medical Center is the largest facility in the nationwide VA system. Patients from a large catchment area, including Texas, Louisiana and Mississippi, come to the Houston VA for cancer treatment. The modern facility, certified as a Cancer Center by the American College of Surgeons, is staffed with Board-Certified Hematologists and Oncologists. All modalities of cancer care are available at the VAMC, including chemotherapy, radiation therapy, and surgery.

About Agennix:

Agennix, a privately owned Houston-based biopharmaceutical company, is the world leader in the development of recombinant human lactoferrin (rhLF), a natural immunomodulatory protein. Oral rhLF has been shown to be safe and well tolerated. It has also been shown to be effective in cancer, asthma and wound healing pre-clinical models, and in Phase I/II human clinical trials in cancer. Agennix is the first and only company to manufacture substantial quantities of human lactoferrin and holds global patents on its technology, with 73 issued patents, and more than 50 pending patents. Agennix has six Phase II human clinical trials currently underway in the treatment of cancer, asthma, and diabetic foot ulcers.

More information about Agennix is available on the Company's web site at http://www.agennix.com.

Agennix Data Published in the International Journal of Cancer; Journal Features Cover Image of Tumor Regression Following rhLF-Treatment

HOUSTON -August 18, 2004- Agennix Inc. today announced that its pre-clinical recombinant human lactoferrin (rhLF) data was published in an article entitled "Oral Lactoferrin Inhibits Growth of Established Tumors and Potentiates Conventional Chemotherapy," in the September 1st issue of the International Journal of Cancer (IJC) (Vol. 111, pp 398-403).

The publication described the anti-cancer activity of rhLF, a natural immunomodulatory protein, in pre-clinical experiments. Oral rhLF demonstrated anti-cancer activity when administered alone and in combination with chemotherapy in tumor-bearing mice. Monotherapy of a squamous cell carcinoma in a syngeneic murine model caused a 66% tumor growth inhibition that was statistically significant over placebo ($p<0.01$) and comparable to that obtained by therapy with Cis-platinum, docetaxel or radiotherapy. Mice receiving both rhLF and Cis-platinum showed a statistically significant improvement over either drug alone. Tumor growth inhibition with oral rhLF was also observed in a murine renal cell carcinoma model ($p<0.01$). In a mammary adenocarcinoma model, oral rhLF also induced tumor shrinkage including complete rejection of established tumors.

Separately, the IJC featured a cover image showing the regression of lung metastases in a patient with renal cell carcinoma (RCC) following treatment with rhLF. The patient had a durable partial response following monotherapy with rhLF after having previously progressed on a four-drug regimen with capecitabine, interferon, gemcitabine and thalidomide. Agennix is currently conducting Phase II trials in RCC and non-small cell lung cancer.

About Agennix:

Agennix, a privately owned Houston-based biopharmaceutical company, is the world leader in the development of recombinant human lactoferrin (rhLF), a natural immunomodulatory protein. Oral rhLF has been shown to be safe and well tolerated. It has also been shown to be effective in cancer, asthma and wound healing pre-clinical models, and in Phase I/II human clinical trials in cancer. Agennix is the first and only company to manufacture substantial quantities of human lactoferrin and holds global patents on its technology, with 73 issued patents, and more than 50 pending patents. Agennix has six Phase II human clinical trials currently underway in the treatment of cancer, asthma, and diabetic foot ulcers, with company sponsored U.S. INDs in each area.

More information about Agennix is available on the Company's web site at http://www.agennix.com.

Additional Published References Disclosing Agennix Clinical Investigations

Guttner, Y.; Windsor, H. M.; Viiala, C. H.; Marshall, B. J., Human recombinant lactoferrin is ineffective in the treatment of human Helicobacter pylori infection. Alimentary Pharmacology & Therapeutics. 17(1):125-129, January 2003.

Annual Meeting of the American Society of Clinical Oncology (ASCO). Abstract No. 947, "Phase I/II Clinical Trial of Oral Recombinant Human Lactoferrin in the Treatment of Chemo Resistant Solid Tumors" June 2003

TG Hayes, GR Varadhachary, G Falchook, D Smith, HM Dhingra, and A Varadhachary. Oral recombinant human Lactoferrin (rhLF) slows tumor growth in metastatic NSCLC and other advanced incurable cancers: Results of a Phase II study. Proceedings of the American Society of Clinical Oncology, 2004.

ATTACHMENT B

ADDITIONAL STATEMENTS BY VENTRIA CLAIMING THAT ITS RECOMBINANT HUMAN LACTOFERRIN HAS INDEPENDENT BIOLOGICAL OR THERAPEUTIC EFFECTS IN HUMANS

Comments Submitted by Mr. Scott Deeter, Ventria President and CEO to USDA, APHIS Docket No. APHIS – 2007-0006 – Regarding Ventria's Permit Application, March 30, 2007

- "Ventria Bioscience has developed affordable products that have been shown to help children recover from diarrhea faster."

- "Benefits of Ventria's Products -- There are several potential applications for Ventria's products and technology which offer numerous benefits to society on a global basis. For Ventria's first products, the economic and societal benefits are estimated to be significant as follows: Reduce duration of childhood diarrhea by 4 million days annually in the US and help these children get back to school sooner; Potentially save hundreds of thousands of lives globally; Help parents return to work sooner with an economic impact of $1.6 billion over five years in the US alone..."

Excerpts from Ventria's Website, available at http://www.ventria.com

Ventria's own website indicates that the Company's intended use of human lactoferrin is to treat diseases including acute diarrhea, fungal infections and topical infections, as well as inflammation. The company's website further defines its own terms for its products – which are referred to as Biopharmaceuticals:

- Biopharmaceutical: The application of biological technology research to the development of pharmaceutical products that improve human health, animal health, and agriculture

- Output Traits: In agricultural biotechnology, input traits are traits that improve the agronomic performance of the plant (I.e. RoundUp Ready(R) Corn). Output traits are traits that change the way the plant is used. In this case, Ventria's output traits are biopharmaceuticals.

- Pharmaceutical: Of or pertaining to the knowledge or art of pharmacy, or to the art of preparing medicines according to the rules or formulas of pharmacy; as, pharmaceutical preparations.

- Plant-Made Pharmaceuticals: The art of preparing medicines according to the rules or formulas of pharmacy through the use of plants.

- Therapeutic Proteins: A protein, of or pertaining to the healing art; concerned in discovering and applying remedies for diseases; curative.

Presentation to the USDA's Advisory Committee on Biotechnology & 21st Century Agriculture, Scott Deeter, Ventria's CEO, June 17, 2003.

- Described Company's vision of using plants as the "host for the manufacture of the active ingredient for a drug." The presentation also highlighted Ventria's focus on treating acute respiratory infections and diarrheal diseases.

CORROBORATING PRESS ARTICLES INDICATINIG THAT VENTRIA'S RECOMBINANT HUMAN LACTOFERRIN HAS INDEPENDENT BIOLOGICAL OR THERAPEUTIC EFFECTS IN HUMANS

"Bioengineered rice takes center of debate over using food crops to grow drugs." ***San Jose Mercury News, April 16, 2004***

Ventria is represented as a company that uses rice as a "factory for producing human medicine." Scott Deeter, Ventria's CEO, asserts that lactoferrin and lysozyme are intended to treat diseases such as anemia and diarrhea and states, "Ventria sees itself -- as a biotechnology company hard at work on medical products that could save lives."

"Biotech company cultivates new field." ***Sacremento Bee, January 25, 2004***

Ventria CEO Scott Deeter asserts that his lactoferrin and lysozyme "would be the first genetically engineered plant-produced pharmaceuticals to reach the market." He further states that Ventria's rice is intended to "treat severe diarrhea" and is "not intended as food".

"California OKs GM Pharm Crops." ***The Scientist, April 8, 2004***

In a scientific periodical, Ventria's lactoferrin and lysozyme are described as "drugs" with Ventria's CEO Scott Deeter making the claim that "Ventria's products have the potential to save the lives of 2 million children a year."

"Biotech firm to make drugs in GM rice." ***The Independent, February 1, 2004***

According to The Independent, Ventria says that its plants "will become 'factories' that manufacture therapeutic proteins to combat life-threatening illnesses". It adds that "plants improved through the use of biotechnology" can produce them "for innovative treatments for diseases such as cancer, HIV, heart disease, diabetes, Alzheimer's disease, kidney disease, Crohn's disease, cystic fibrosis and many others."

March 30, 2007

United States Department of Agriculture
Docket No. 05-006-1 and Docket No. 05-007-1
Regulatory Analysis and Development, PPD
APHIS, Station 3C71
4700 River Road Unit 118
Riverdale, MD 20737-1238

Re: Docket No. APHIS – 2007 - 0006

Dear Secretary of Agriculture Johanns:

Ventria Bioscience has reviewed all public comments posted as of 5:00 pm EST for Docket No. APHIS – 2007-0006 and would like to add the following comments to its application so that the public has a full understanding of Ventria's plans and products.

Ventria Bioscience has developed affordable products that have been shown to help children recover from diarrhea faster[1]. To achieve this, Ventria produces its products in rice seed, utilizing soil, sun, water and nutrients as raw materials and the rice plant as the biological factory. Once the products are manufactured in the rice seed under a closed and dedicated supply system, the products are extracted from the rice seed and formulated into medical foods, such as oral electrolyte solutions. Ventria's seed is NEVER sold and the seed is destroyed in the extraction process.

We request that you consider the product benefits, the safety profile of the products and Ventria's use of a closed and dedicated system of production as you consider Ventria's permit application.

Benefits of Ventria's Products

There are several potential applications for Ventria's products and technology which offer numerous benefits to society on a global basis. For Ventria's first products, the economic and societal benefits are estimated to be significant as follows:

- Reduce duration of childhood diarrhea by 4 million days annually in the US and help these children get back to school sooner;
- Potentially save hundreds of thousands of lives globally;
- Help parents return to work sooner with an economic impact of $1.6 billion over five years in the US alone;
- A $228 million positive economic impact over five years to farmers and rural communities from Ventria's field production activities in Kansas;
- A $50 million positive economic impact over five years from direct employment in Ventria's bioprocessing operations in Junction City, Kansas;

[1] Zavaleta, N, etal. Efficacy of a Rice-based Oral Rehydration Solution Containing Recombinant Human Lactoferrin and Lysozyme in Peruvian Children with Acute Diarrhea. Journal of Pediatric Gastroenterology and Nutrion. Volume 44, No 2:258-264 , February 2007

- $37.5 million in savings to the US Government and American taxpayers when compared to government subsidized rice production; and
- Successful introduction of these first products may lead to additional products being developed using plants as a biological factory. This multiplies the benefits to society and the US economy.

Below is a more detailed review of the major benefits of Ventria's products and technology:

Ventria's Products Help to Improve Child Health and Save Lives

A double-blind and controlled study published in the Journal of Pediatric Gastroenterology and Nutrition found that Ventria's products helped to reduce the duration of acute diarrhea by 30%, or a day and a half. (Average duration: 5.21 days for control vs. 3.67 days for Ventria's products). In addition children receiving Ventria's product were more likely to recover from their diarrhea and were less likely to relapse into another episode of diarrhea[1].

In the US there is an average of 2.2 episodes of diarrhea per child per year with 10% requiring a hospital visit[2]. According to the US Census Bureau, there are 20.3 million children under the age of 5 suggesting 45 million episodes of diarrhea per year among American children and approximately 4.5 million hospital visits for childhood diarrhea annually[3]. If one-third of these children consumed Ventria's product during their diarrhea we estimate that it may reduce duration of childhood diarrhea in the US by approximately 4 million days on an annual basis[4].

Globally, childhood diarrhea is the second leading killer of children under the age of 5, claiming 2 million lives annually[5]. It is our hope that Ventria's products, if distributed broadly with the same global reach as current oral electrolyte solutions today, would save hundreds of thousands of children from this scourge. That is the objective we are striving for in our product development and clinical program.

Ventria's Products May Reduce the burden on Families and Caregivers

Ventria's ambition is to make a significant impact by helping reduce the duration of diarrhea by at least one day and this is supported by studies showing a significant reduction in duration of diarrhea as mentioned above. According to Pediatrician Dr. Robert Wittler, Professor, Pediatric Infectious Diseases, University of Kansas School of Medicine, "Oral rehydration is a safe and effective way to treat most diarrheal illnesses and this study confirms the advantages of adding proteins contained in breast milk to oral

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[2] Vernacchio, L etal. Diarrhea in American Infants and Young Children in the Community Setting. The Pediatric Infectious Disease Journal. Volume 25, Number 1, January 2006.

[3] US Department of Commerce, US Census Bureau. www.Census.Gov; May 10, 2006.

[4] Ventria Bioscience estimates.

[5] United Nations Environment Programme, United Nations Children's Fund and World Health Organization, Children in the New Millenium: Environmental Impact on Health, 2002 www.unep.org/ceh/children.pdf

rehydration solution. This is an important study as decreasing the duration of diarrhea lessens the likelihood of children becoming significantly dehydrated and allows children to return to daycare and school quicker and their parents who work outside the home can return to work sooner."

Thus, in addition to saving lives and helping children recover faster, Ventria believes its products will lead to an improvement in family life as parents and caregivers who work outside the home can return to work sooner. What is the benefit of this to the US economy? Assuming there are 4 million days of reduced childhood diarrhea, as estimated above, and assuming 59% of children live in homes where the caregiving parent or parents work[6], we estimate that the parent could return to work an aggregate of 2.4 million days sooner. Assuming a daily wage of $136 represents $326 million in economic benefit in the US alone[7]. Over a five year period, this represents $1.6 billion in positive economic impact.

Ventria's Production has a Significant Benefit for Farmers and Rural Communities

Farmers stand to profit from this technology for many reasons. First, they earn approximately $150 in additional profit per acre plus additional economic impact from more intensive management required of Ventria's production, requiring an additional $300 per acre. For example, a corn farmer that is currently generating $587 per acre from corn production would generate an economic impact of $1,037 per acre, or an increase of $450 per acre if they switch to Ventria's production[8]. Second, they are able to receive a more consistent revenue stream versus their alternatives because they do not shoulder losses caused by poor yields, weather damage, disease or insect damage, or other negative impacts typically faced by farmers today. Third, the farmers are trained in new value-added farming practices, quality control, and regulatory requirements. Finally, farmers are able to enter multi-year agreements which provide more certainty about future cash flow, thereby improving their financial outlook.

Based on the above, we estimate an economic benefit to farmers of $600 per acre in positive economic impact compared to their alternative with corn. With a projected 30,000 acres of production per year upon full scale commercialization of Ventria's products, we estimate the resulting economic benefit to be $18 million in direct economic benefit per year for farmers and the rural community of Junction City, Kansas. If we assume a full economic benefit with a multiplier of 2.54, then the estimated economic benefit for farmers and rural communities from Ventria's products in the first five years of full-scale production is $228 million[9].

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[6] http://www.tlc.state.tx.us/redist/pdf/e1018/education.pdf;

[7] US Department of Labor, Bureau of Labor Statistics. www.data.bls.gov

[8] Ventria Estimates and Daniel O'Brien, Associate Professor and Extension Agricultural Economist, Kansas State University. Decision Tools for Corn Production. www.agmanager.info

[9] Junction City/Geary County Economic Development Commission. 2.54x is the economic impact multiplier used by Junction City/Geary County Kansas.

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Ventria's Bioprocessing Facility will Create Jobs for Kansans

In order to extract the products from the rice grain, Ventria designed and is constructing a bioprocessing facility in Junction City, KS. This is a $6 million capital improvement project and is expected to employ 10 people within the first year of operation. Employment will expand as the demand for Ventria's products grows. It is estimated that an employment of 50 people in Junction City, Kansas will be required for full-scale production. We estimate this to be a total economic benefit to the region of $50 million over the first five years using an economic impact multiplier of 2.54[9].

Value-added Agriculture Reduces Dependence on Farm Subsidies

Rice is the most subsidized of any of the food crops per pound produced, receiving $5.2 billion in government subsidies from 2001 to 2005[10]. During this time, 108 billion pounds of rice have been produced in the US[11]. This represents a subsidy of 5 cents per pound, or approximately 50% of the current market price for rough rice on the Chicago Board of Trade[12]. These subsidies are not available to Ventria's products. Thus, Ventria's production saves taxpayer money, while simultaneously improving the livelihood of American farmers. This is the promise of value-added agriculture which most farmers enthusiastically support. When Ventria produces at full-scale, the taxpayer savings over a similar five year period amount to approximately $37.5 million in savings to the US Government and American Taxpayers when compared to government subsidized rice production.

Future Opportunities for Kansas and American Agriculture

The use of plants as a biological factory is a technology with significant future product opportunity to improve human health, agriculture, animal health, bioenergy and the environment. In Kansas, plant biotechnology is an engine for future economic growth with the State's enactment of the Kansas Economic Growth Act ("KEGA"), which included a commitment to invest $580 million in the biosciences in the next 10 to 15 years. According to Kansas Technology Enterprise Corporation, this will "build off the state's homegrown strengths in the biosciences and ensure the growth of bioscience-related jobs and economic prosperity."[13]

Several market research analysts have predicted that using plants to produce new products for pharmaceutical, nutritional and industrial applications will become a multi-billion dollar market[14]. Frost & Sullivan, a leading market research firm estimated that plant-made pharmaceuticals alone would be a $2.2 billion market by 2011[15]. Of course, we must begin to commercialize these products to convert this vision into reality.

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[10] Environmental Working Group; Farm Subsidy Database. www.ewg.org

[11] United States Department of Agriculture, Economic Research Service. http://usda.mannlib.cornell.edu

[12] Chicago Board of Trade, Rough Rice closing price on March 30, 2007; www.cbot.com

[13] Kansas Technology Enterprise Corporation, Kansas Bioscience Initiative: Economic Growth Through Discovery and Innovation. www.ktec.biz

[14] Theta Reports; Biopharming: The Emerging World Market of Plant-Based Therapeutics, November 2002.

[15] "Biopharming" in Plants – a Future Method of Biopharmaceutical Production? Frost & Sullivan, Phil Webster, November 2004.

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Safety of Ventria's Products

Ventria is developing lactoferrin, lysozyme and human serum albumin as products for use in medical foods and other applications. All three products are naturally occurring human proteins found in saliva, tears, and Mother's milk.[16]

Ventria's recombinant human lactoferrin and lysozyme have been extensively reviewed by scientific experts as part of the GRAS (Generally Recognized As Safe) process. These products have undergone extensive safety testing and have been used in clinical studies in adults and children with no safety concerns. The scientists represented on Ventria's GRAS panel are recognized qualified experts in fields of food allergy, mucosal immunology, pediatric nutrition, and carbohydrate allergies. These panels have UNANIMOUSLY concluded that Ventria's products are substantially equivalent to the native human proteins and are SAFE FOR THE INTENDED USES, such as medical foods.

Human serum albumin (HSA) is a safe protein with a well established safety profile in many applications. It is not toxic or allergenic in humans. Biochemical and biophysical analysis has shown that Ventria's HSA is equivalent to native HSA. Both Ventria's HSA and native HSA are completely degraded by stomach digestive conditions within 30 seconds or less and both are completely denatured with cooking.

HSA plays an important role in human health by helping regulate blood pressure and sequestering toxins from the bloodstream. HSA often provides life-saving therapy for trauma patients, burn victims, and individuals undergoing surgery[17]. There is currently a shortage of HSA available from native sources. Producing HSA in rice provides a safe and affordable source of HSA for the biomedical community.

Containment and Production Practices

As mentioned, Ventria utilizes a closed system of production that is significantly different from traditional seed practices followed by the conventional rice industry. Although, rice produced by conventional breeding is no safer for humans than Ventria's products, the regulatory requirements are much different. Ventria must employ a dedicated and closed system of production in order to maintain compliance with USDA regulations.

One very important distinction is that Ventria does not sell seed and has not requested approval from USDA to sell its seed. Because Ventria does not have this approval from USDA, it would be illegal for Ventria to sell seed. Ventria maintains ownership of the seed, rice plants, harvested seed, and stored seed throughout its supply chain. Another

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[16] Lönnerdal, B and Atkinson S, 1995 Nitrogenous components of milk, A. Human milk proteins. In Handbook of Milk Composition. Editor: Jensen R G, Academic Press pp 351-368.

[17] Peters, T, 1996, All about albumin, Biochemistry, Genetics, and Medical Applications. Academic Press, pp 231.

important distinction with the conventional rice industry is that Ventria's final product is not rice seed, rather it is the extracted protein. During the extraction process the rice seed is destroyed.

Ventria maintains a separate supply chain for its products. Ventria's supply chain consists of dedicated facilities and equipment for planting, harvesting, transportation, storage and processing of its rice. This is part of our permit requirements and incorporated into our Standard Operating Procedures (SOPs). Ventria's supply chain is completely separate from commercial rice production with no shared equipment or facilities.

Another aspect of Ventria's closed production system is that Ventria's rice is self-pollinating and the life of its pollen is only a few minutes. Because it is self-pollinating, Ventria's rice does not require insects or wind to carry pollen for reproduction. This significantly reduces any risk of inadvertent contamination. Many peer-reviewed and published research studies as well as the Association of Official Seed Certifying Agencies (AOSCA) have determined that 10 feet was an adequate distance between rice seed fields to maintain purity of Foundation Seed, the highest purity standards for seed[18]. More recent studies have shown that outcrossing in even adjacent plants is unlikely. No studies have shown outcrossing beyond 30 feet. Ventria grows its rice in areas that do not have production of commercial rice within hundreds of miles. In fact, there is no commercial rice being grown in Kansas or North Carolina, the locations for Ventria's field production for 2007.

Finally, movement of seed by Ventria prior to processing requires a movement permit approval from USDA. Movement permits include detailed SOPs and incorporate shipping and receiving location information and have specific requirements pertaining to the movement.

In closing, Ventria Bioscience has an outstanding track record and has responsibly assumed its duty to properly steward this technology toward commercialization over nine years of successful field production in a closed and dedicated system. We have met all regulatory requirements and have adjusted our production practices as the regulatory requirements have evolved to the current set of regulations. We are committed to continuing this successful track record.

We respectfully request that you utilize a scientifically supportable decision framework and approve the permit application so that we can begin to deliver the promises of these products. The benefits to society are great and the product safety is self evident.

Respectfully submitted,

/sig/
Scott E. Deeter
President & CEO
Ventria Bioscience

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[18] The Rockerfeller Foundation, The World Bank, The USDA, APHIS. 1993. Rice Biosafety. 37 pp.

Lactoferrin
Lysozyme

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Products

Lactoferrin

Lactoferrin is a glycoprotein that belongs to the transferrin family of iron binding proteins. It is found in human breast milk as well as most epithelial surface secretions including tears, nasogastric, saliva, and bronchial. Lactoferrin is a multifunctional protein that has the following properties:

- Binds two molecules of iron with very high affinity
- Anti-bacterial
  - Inhibits bacterial growth by withholding iron
  - N-terminal region is an antimicrobial peptide
- Anti-viral
- Anti-fungal
- Antioxidant
- Immunomodulatory
- Acts synergistically with lysozyme to potentiate the activity of both proteins

Because of the numerous important roles lactoferrin plays in the human body, a wide variety of potential products could be pursued. The following are some examples of how lactoferrin could be used to enhance human health:

- Gastrointestinal health
  - Dietary management of acute diarrhea
- Treatment of topical infections and inflammations
  - Alleviation of fungal infections

For more information on lactoferrin, please see our lactoferrin **references page.**

VENTRIA BIOSCIENCE

Glossary of Terms

Term	Definition
AFLP	Amplified Fragment Length Polymorphism (AFLP). Selected markers are amplified in a PCR, which makes amplified fragment length polymorphism an easy and fast tool for strain identification in agriculture, botany, microbiology and animal breeding.
Agronomic Evaluation	Evaluation of field-crop production characteristics.
Biochemistry	The chemical characteristics and reactions of a particular living system or biological substance.
Biopharmaceutical	The application of biological technology research to the development of pharmaceutical products that improve human health, animal health, and agriculture.
Biosynthesis	The production of a chemical compound by a living organism.
Biotech	Biological science when applied especially in genetic engineering and recombinant DNA technology.
Biotechnology	Biological science when applied especially in genetic engineering and recombinant DNA technology.
Cereal Genetics	A branch of biology that deals with the heredity and variation of grains.
Cereal Grain Gene Expression	The unique set of genes involved in the development and maturation of cereal grains
Cereal-Transformation	Transformation is the process of stably incorporating new DNA into an organism. Cereal transformation refers to the the process applied specifically to the cereal plants: rice, wheat, barley, corn, sorghum, etc.
cGMP Facility	cGMP refers to Good Manufacturing Practices, a

	rigerous set of manufacturing guidelines that the FDA uses to document and ensure that the products it regulates are produced safely, and consistently.
Chromosome	Linear, or sometimes circular, DNA-containing bodies of viruses, prokaryotic organisms, and the cell nucleus of eukaryotic organisms that contain most or all of the genes for that particular organism.
CJD	Creutzfeld Jacob Disease, the human variant of mad cow disease.
Commercialization	The act of managing something on a business basis for profit.
Crop Biology	The particular area of biology related to crop plants.
Cultivar	A race or variety of a plant that has been created or selected intentionally and maintained through cultivation.
Cultivation	The art or act of cultivating; improvement for agricultural purposes or by agricultural processes; tillage; production by tillage.
Cyanide	A compound formed by the union of cyanogen with an element or radical.
Delivery System	A method of introducing a product (usually a pharmaceutical product) into an individual. Examples are: pills (for dietary delivery), liquids (for injectable delivery), mists (for inhaled delivery), etc.
Dimeric Molecules	A molecule consisting of two identical simpler molecules. After the ary made, many proteins must assemble in this fashion before they become biologically active.
DNA	A nucleic acid that carries the genetic information in the cell and is capable of self-replication and synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or cytosine and guanine. The sequence of nucleotides determines individual hereditary characteristics.
Expression Host	The environment which provides the necessary tools for production of proteins.

Expression Vector	Typically a small, circular piece of DNA that is transformed (inserted) into a particular expression host for the purpose of producing the protein coded for by the DNA.
Extraction	The act of extracting, or drawing out; most pharmaceuticals must be extracted and purified away from their production host.
FDA	Food and Drug Administration (FDA). The U.S. Agency responsible for regulation of foods and drugs in the United States.
Field Breeding	The propagation of animals or plants within a portion of land or a geologic formation containing a specified natural resource.
Field Trials	The act or process of testing, trying, or putting to the proof within a portion of land. In this case, referring to the growing of transgenic plants in an open field.
Formulation	The act, process, or result of formulating or reducing to a formula.
Gene	A hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism.
Gene Expression	The full use of the information in a gene via transcription and translation leading to production of a protein and hence the appearance of the phenotype determined by that gene. Gene expression is assumed to be controlled at various points in the sequence leading to protein synthesis and this control is thought to be the major determinant of cellular differentiation in eukaryotes.
Gene Pyramiding	The act of breeding together genes, contained in different loci, that
Genomics	The study of all of the nucleotide sequences, including structural genes, regulatory sequences, and noncoding DNA segments, in the chromosomes of an organism.
Germination	The process of germinating; the beginning of vegetation or growth in a seed or plant; the first development of germs, either animal or vegetable.

GI Tract	Gastrointestinal (GI) Tract. Tubular passage of mucous membrane and muscle extending about 8.3 meters from mouth to anus; functions in digestion and elimination.
Glycosylation	The process of adding sugar units such as in the addition of glycan chains to proteins.
Grain Certification	Seeds grown in the United States can be certified by state agencies to be of a particular qualtiy.
Hepatitis C	An infection of the liver that is caused by an RNA virus, is transmitted primarily by blood and blood products, as in blood transfusions or intravenous drug use, and sometimes through sexual contact. Most cases of non-A, non-B hepatitis are of this type.
Host Production System	The organisim that is used to produce the target molecule. In this case, the organism is transformed with a DNA construct, which contains the instructions for producing the target molecule.
Host Tissue	The particular tissue in an organism that is producng the recombinant protein.
Human Health Products	Refers to a broad classification of products that can improve human health.
Human Nutrition	A process or series of processes by which the living organism as a whole (or its component parts or organs) is maintained in its normal condition of life and growth.
Human Pathogens	An agent that causes disease, especially a living microorganism such as a bacterium or fungus.
Human Therapeutics	That part of medical science which treats the discovery and application of remedies for diseases.
Lactoferrin	Iron binding protein of very high affinity (Kd 10exp 19 at pH 6.4, 26 fold greater than that of transferrin) found in milk and in the specific granules of neutrophil leucocytes.
Lysozyme	Glycosidase that hydrolyses the bond between N acetyl muramic acid and N acetyl glucosamine, thus cleaving an important polymer of the cell wall of many bacteria. Present in tears, saliva and in the lysomes of phagocytic cells, it is an important antibacterial defence, particularly against gram-positive bacteria.

Molecular Biology	The study of the biochemistry of cells, it is closely linked to cell biology, in particular the biochemistry of DNA and cogeners.
Molecular Breeding	The act or process of generating or bearing of molecules.
Molecular Screening	To detect unsuspected disease of two or more atoms combining by chemical bonding.
Molecule(s)	The result of two or more atoms combining by chemical bonding.
Monocots	Any of a class or subclass (Liliopsida or Monocotyledoneae) of chiefly herbaceous seedplants having an embryo with a single cotyledon, usually parallel-veined leaves, and floral organs arranged in cycles of three.
Nutraceuticals	A food or naturally occurring food supplement thought to have a beneficial effect on human health.
Nutrition	A process or series of processes by which the living organism as a whole (or its component parts or organs) is maintained in its normal condition of life and growth.
Output Traits	In agricultural biotechnology, input traits are traits that improve the agronomic performance of the plant (I.e. RoundUp Ready(R) Corn). Output traits are traits that change the way the plant is used. In this case, Ventria's output traits are biopharmaceuticals.
Pathogenesis	The origin and development of disease.
Pharmaceutical	Of or pertaining to the knowledge or art of pharmacy, or to the art of preparing medicines according to the rules or formulas of pharmacy; as, pharmaceutical preparations.
Phenylalanine Ammonia-Lyase	An enzyme that catalyzes the deamination of l-phenylalanine to form trans-cinnamate and ammonia. It may also act on l-tyrosine. Since the enzyme deprives neoplastic tissue of phenylalanine, it has been used experimentally in the treatment of acute lymphoblastic leukemia. The enzyme is obtained from many plants and is used as an enzymic marker for lignification and other developmental processes in plant cells.
Photosynthesis	Process by which green plants, algae and some

	bacteria absorb light energy and use it to synthesize organic compounds (initially carbohydrates). In green plants, occurs in chloroplasts, that contain the photosynthetic pigments.
Photosynthetic	Relating to or using or formed by photosynthesis.
Physiology	The biological study of the functions of living organisms and their parts.
Plant Biotechnology	A set of biological techniques developed through basic research and now applied to research and product development through the use of plants.
Plant-Made Pharmaceuticals	The art of preparing medicines according to the rules or formulas of pharmacy through the use of plants.
Post-Translational Modification	The enzymatic processing of a polypeptide chain after translation from messenger RNA and after peptide bond formation has occurred.
Production Host	The organism used to produce or make a particular protein.
Production Vector	A small circular piece of DNA transformed into a host organism for the purpose of producing a particular protein.
Promoters	A region of DNA to which RNA polymerase binds before initiating the transcription of DNA into RNA.
Proteomics	The study of how the entire set of proteins produced by a particular organism interact
Purification	The act of purifying; the act or operation of separating and removing from anything that which is impure or noxious, or heterogeneous or foreign to it; as, the purification of liquors, or of metals.
Recombinant Human Blood Proteins	Proteins normally found in human blood that are produced in a different system using recombinant DNA technology.
Recombinant Molecules	Molecules prepared by recombinant DNA technology.
Recombinant Proteins	Proteins prepared by recombinant DNA technology.

Self-Pollinating	Self-pollination in plants means that the female part of the plant is fertilized by pollen from the male part of the same plant. This explains why self-pollinating crops do not require wind or insect pollination to reproduce, thus reducing the risk of outcrossing.
Therapeutic Proteins	A protein, of or pertaining to the healing art; concerned in discovering and applying remedies for diseases; curative.
Transgene	DNA integrated into the germ line of transgenic organisms.
Transgenic	This term describes an organism that has had genes from another organism put into its genome through recombinant DNA techniques.
Transgenic Cereals	Cereal plants (ie rice, wheat, corn, barley, etc.) containing foreign DNA. Usually inserted through the transformation process.
Transgenic Grains	Grains containing foreign DNA. Usually inserted into the plant through the transformation process.
Trimeric Molecules	A molecule formed by combining three identical smaller molecules.
USDA	United States Department of Agriculture (USDA).
In Vitro	In vitro refers to an experiment done in "glass" or within the confines of a laboratory and NOT within a host.
In Vivo	In vivo refers to an experiment done in "living" tissue. In the living body of a plant or animal.

VENTRIA BIOSCIENCE

Plant-made Pharmaceuticals & Industrials:

Expectations & Realities

Scott Deeter

Ventria Bioscience

June 17, 2003

What are PMPs and PMIPs?

- Plant-made Pharmaceuticals (PMPs)
  - New category of products
  - Plant is the host for the manufacture of the active ingredient for a drug.
  - Following harvest, plant material is processed to recover and purify the active ingredient.
  - Active ingredient manufacture and marketing is regulated by FDA
- Plant-made Industrial Products (PMIPs)
  - New category of products
  - Plant is the host for the manufacture of an industrial product, such as an industrial enzyme.
  - Following harvest, plant material is processed to recover and purify the industrial compound.

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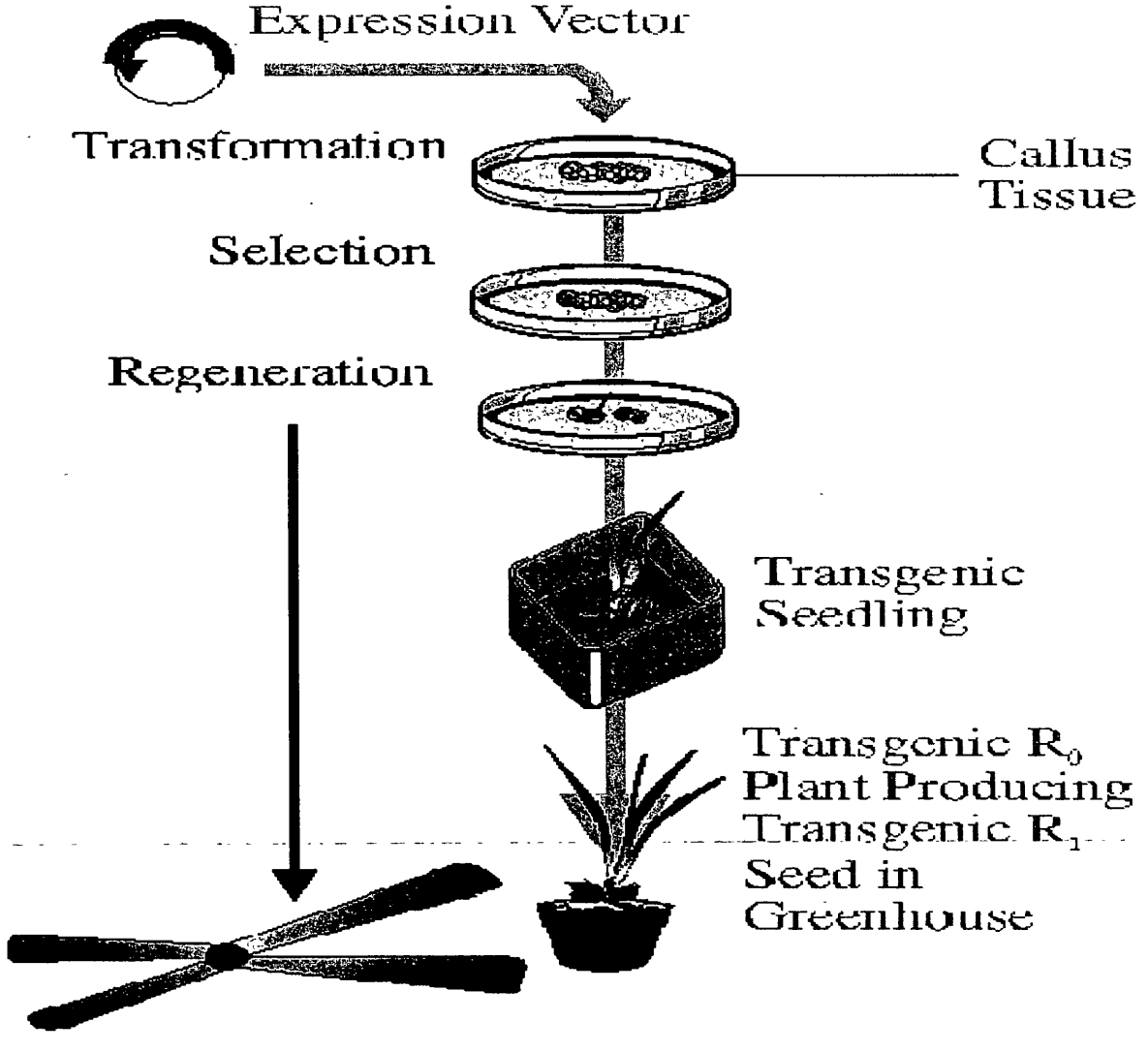

Expression Vector
Transformation
Callus Tissue
Selection
Regeneration
Transgenic Seedling
Transgenic R_0 Plant Producing Transgenic R_1 Seed in Greenhouse

Why Plant-made Pharmaceuticals?

- Plants can produce complex biologics:
  Plants such as rice and barley are able to produce complex biologics such as monoclonal antibodies and complex proteins.

- Plants are highly scalable:
  Rice and barley can scale up capacity at the rate of 2,500 times annually.

- Plants are economically superior:
  Production economics are very attractive, especially for oral and topical biologicals. Capital cost is a fraction (10%) of the traditional systems of production.

Why Food Crops?

- Grains such as rice and barley are free of infectious contaminants from human or animal origin such as viral (west nile virus) and prion (BSE) contamination. In addition, grains do not have toxic contaminants that are difficult to remove (ie. phenolics).
- These crops provide a host that is generally recognized as safe ("GRAS") and well characterized.
- Rice and barley can store the target biopharmaceutical for more than 2 years, reducing the need to over-produce or maintain idle capacity.
- The seed and target gene are stable and replicable across multiple generations, providing consistent quality, a critical requirement of FDA.
- Using the plants biological process for storing proteins achieves much better economics as a result of the high yields and ability to maintain quality of raw material inventory.

Why Self-Pollinating Crops?

- Male and female reproductive system contained within the same plant, providing self-fertilization.
- Several studies have shown that beyond 30 feet, no outcrossing has been detected.
  - Rice Biosafety: World Bank Technical Paper, Clegg et al. 1993. Result of a 40-member consultation supported by the USDA/APHIS, the World Bank, IRRI and the Rockefeller Foundation. This study summarized several studies showing no outcrossing beyond 30 feet.
  - UC-Davis: Biggs experiment station. 4 replications, with 15 foot Liberty-Link transgenic center circle surrounded by 48 foot radius non-transgenic. Beyond 6 feet, no outcrossing was detected. Between 0 ft and 6 ft from the donor, the outcrossing rate was 0.00001% (16 seeds out of 1,680,000 tested).
- Ventria utilizes a minimum setback distance of 100 feet.

Environmental Stewardship

- Grown under permit issued by USDA.
- Ventria directly manages production using standard operating procedures ("SOPs") with trained field personnel, maintaining ownership of seed and grain. (HACCP). Rice and barley are processed before any product is sold.
- 100% internal audit of our SOPs and regulatory requirements.
- Dedicated field production equipment that comes into contact with plant material.
- Dedicated harvesting, storage and processing infrastructure.
- Double-contained transportation.
- Ventria's production practices are audited by CCIA and inspected by USDA/APHIS. (Multiple inspections in 2003)

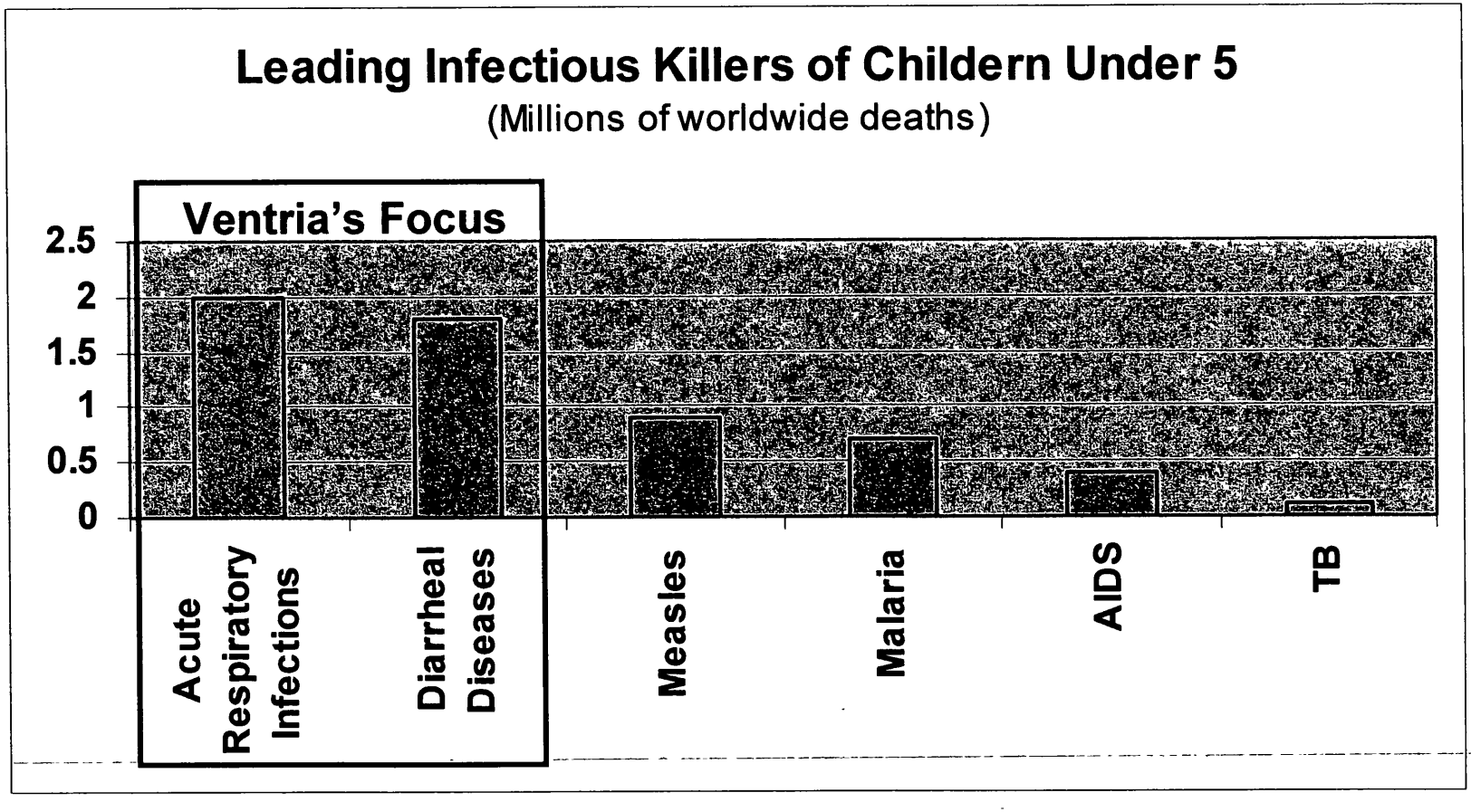
Expectations

- Plant-made Pharmaceuticals:
  - Today: ~ 100 acres; 18 companies
  - 2008: ~ 10,000 acres; 6 companies
- Plant-made Industrial Products:
  - Today: ~ 500 acres; 8 companies
  - 2008: ~ 100,000 acres; 6 companies

Selected PMPs in Development

Company	Targets	Host
Biolex	Multiple	Lemna
Dow	Multiple	Corn
EpiCyte	Herpes	Corn
Large Scale Biology	Surgical Blood Loss Fabry's Disease???	Tobacco (Viral)
Meristem	Cystic Fibrosis	Corn
Monsanto	Cancer	Corn
SemBioSys	Cosmetics	Canola Safflower
Ventria	Infectious Disease Gastrointestinal Disease	Rice

Global Health Problems



Source: *World Health Organization*

Global Health Problems

Why solutions to these problems have not been developed?

Problem

- **Inability to produce therapeutic solutions in sufficient quantities**
- **High cost of products produced in traditional systems**

Global Health Problems

Ventria's Solution

- **Orally delivered Lactoferrin and Lysozyme produced using ExpressTec™, plant-made pharmaceutical production system**
- **Able to produce 1,000's of kilograms with minimal capital investment**
- **Biologic yield is very high, and the processing is efficient, reducing the cost of production.**

Realities

….the Benefits will take time to deliver

- Many products in the pipeline
- New Product Development: 5+ years
- First PMPs: 2006
- Many products by 2008

Bioengineered rice takes center of debate over using food crops to grow drugs

April 16, 2004
San Jose Mercury News

There are two very different views of Ventria Bioscience, the company that can turn a field of rice into a factory for producing human medicine.

There's the way Ventria sees itself -- as a biotechnology company hard at work on medical products that could save lives.

"Without our technology those products could never end up in use for human health, not in our lifetime," says Ventria Chief Executive Scott Deeter.

And then there's the view of opponents, who see the firm as a harbinger of a new biotech age that threatens the purity of the food supply and puts growers of conventional crops at risk.

"As a California rice farmer, I say: Don't grow drugs in my food crop," says Greg Massa, who grows rice in Glenn and Colusa counties.

Whichever view is correct, this tiny, 11-year-old Sacramento company is now front and center in a national debate over using food crops to grow drugs, as well as industrial and nutritional products.

Scores of companies and academic labs have been attempting this for years, confining their crops to greenhouses or to small field parcels.

Meanwhile, Ventria has been moving beyond the more typical small patches.

This year, Ventria hoped to grow up to 120 acres of its genetically engineered rice. But it was willing to limit production to one out of 10 selected California counties -- far enough south of the Central Valley rice belt to allay the concerns of growers who feared field contamination. But the California Secretary of Food and Agriculture blocked its permit, saying that he wants more time to hear from the public.

Deeter now says Ventria will likely plant only a small test field in California this year and may leave the state to plant a larger field in 2005.

Still, the mood remains decidedly upbeat at Ventria's headquarters in Sacramento.

Deeter says the privately held company, which has 20 employees, remains a few years away from commercializing its first products. It has spent more than $20 million on two human proteins that are usually found in mother's milk, tears and saliva but, through genetic manipulation, can also be produced in rice.

Sponsoring research The company is sponsoring research to show that in the right doses, the two proteins -- lactoferrin and lysozyme -- can be helpful in treating diarrhea.

Worldwide, as many as 3 million children under 5 die each year from the dehydration that accompanies severe diarrhea. Even in the United States, hundreds of youngsters and thousands of elderly patients die from it each year.

For decades, standard treatment has been a solution of table salt and carbohydrates. This summer, a clinic in Lima, Peru, will begin a Ventria-sponsored study to see if adding the lactoferrin and lysozyme, extracted from a flour made from the modified rice, gets even better results.

"The benefits of this approach are enormous," said Dr. William B. Greenough III, a professor of medicine at Johns Hopkins University.

Greenough was part of a team in Bangladesh that developed the standard treatment that is credited with saving the lives of 3 million infants each year.

Maryland company Greenough is also co-founder of Cera Products, a Maryland company that has been talking about adding Ventria's lactoferrin and lysozyme to its product for treating diarrhea.

Ventria is conducting studies in Los Angeles to see if lactoferrin, which plays a role in absorption of iron in the intestines, can help women suffering from anemia.

The company's critics don't dispute the potential benefits.

Instead, they see Ventria as opening a door to an industry that is still not adequately regulated.

For example, because genetically engineered lactoferrin and lysozyme are virtually the same as natural human proteins, the company can market them as "medical foods," a designation that does not require a detailed Food and Drug Administration review. But for a variety of reasons, Ventria is taking a more cautious approach and will ask the FDA to review the proteins for safety.

Environmentalists, rice growers and some experts worry about inadvertent mixing of Ventria's rice with the non-engineered food crop.

Voicing concerns "I have concerns that any pharmaceutical product grown in a food crop could end up in food," said Steve L. Taylor, professor of food science at University of Nebraska.

Even if the products prove safe, he said, "a lot of people have trouble thinking of Rice Krispies with human genes in them." Says Bill Reese, a research analyst with Friends of the Earth: "First of all, these are not human proteins." Small differences might trigger allergies and other unexpected responses, he said.

Even if there should be a mix-up between Ventria's rice and non-engineered food crops, the two human proteins pose no hazard to consumers, says Delia Bethell, Ventria's vice president

of clinical development. "If you breast-feed a baby for a year, that child consumes 277 grams of lactoferrin," she said. A person who ate Ventria's genetically modified rice over an entire year, she said, would consume only 60 grams.

VENTRIA BIOSCIENCE

The privately held company is genetically modifying rice plants to produce potentially useful proteins, turning fields of rice into factories for drugs, food supplements and other products.

Headquarters: Sacramento

Number of Employees: 20

Primary Products: Genetically engineered human lactoferrin and lysozyme for treating diarrhea and iron deficiency

Chief Executive Officer: Scott E. Deeter

Board Members: William J. Rutter and Pablo Valenzuela, co-founders of Chiron

The Benefits: Five thousand acres of rice can produce enough genetically engineered proteins to treat millions of diarrhea cases -- at less than 1 percent of the cost of making them in standard biotechnology factories.

Critics Say: Company's plans may threaten purity of the food supply and could put conventional growers at risk.

SEEDS OF DOUBT *BY THE SACRAMENTO BEE*

Biotech company cultivates new field

By Mike Lee and Edie Lau -- Bee Staff Writers

Published Sunday, January 25, 2004

A Sacramento biotechnology company is pushing the $500 million California rice industry to a new frontier with a proposal to grow commercial rice engineered to make drug compounds.

The controversial plan is ambitious and somewhat mysterious. The company, Ventria Bioscience, will not reveal where it hopes to cultivate what would be America's first genetically engineered plant-produced pharmaceuticals to reach the market.

Citing fear of vandalism by militant environmentalists, Ventria's chief executive officer, Scott Deeter, will say only that somewhere in California the company hopes to grow 130 acres of rice that produce two anti-microbial proteins.

A California Rice Commission committee struggling to write rules for the pharmaceutical rice will review Ven-tria's plans at a public meeting Thursday.

It seems likely that Ventria will continue to farm where it has grown engineered rice in experimental plots since 1997: in the northern Central Valley, the heart of California rice country.

And that has local rice farmers' anxiety levels soaring.

"I feel very vulnerable that genetically modified rice could come into the state ... and cause significant disruption to our ability to market our rice to our customers," said Bryce Lundberg, director of organic certification for Lundberg Family Farms, a 67-year-old Richvale business that is the nation's largest organic rice processor.

Lundberg -- who is leading a campaign to bar biotech rice from California -- and others in the rice industry worry about scaring off Japanese buyers, who are wary of genetic engineering.

Ken Chinen, a Japan-born professor of international business at California State University, Sacramento, said that with the recent discovery of mad cow disease in this country and the Asian chicken flu epidemic, the timing is terrible for introducing anything that raises doubts about food safety.

"Japanese consumers are becoming very sensitive about the safety of food, especially from foreign countries," Chinen said.

Deeter said his company's rice, while not intended as food, is safe for human consumption. And Ventria will work hard to keep its rice isolated, Deeter said, though he thinks it's unnecessary to plant the rice far from food rice fields.

"Rice grows where it grows," he said. "There's no risk here."

This spring -- perhaps in March, if weather cooperates -- the company would like to plant 65 acres each of two biotech rice varieties.

In a few years, Deeter said, Ventria hopes to expand to as many as 1,000 acres.

Under state law, Ventria's plan must be reviewed by a 12-member committee of scientists, growers and business representatives operating under the state Rice Commission. The law, the California Rice Certification Act of 2000, reflects the state's interest in protecting its rice markets. It gives California's agricultural secretary final say on growing restrictions and sets fines of up to $5,000 per violation.

Ventria submitted a sample protocol to the Rice Commission last March and has met with the review committee three times to hash out details of a more specific containment plan.

"We still have some significant work to do," said Tim Johnson, president of the California Rice Commission. "Our future depends on doing it right."

Last week, the U.S. Department of Agriculture announced its plans to consider tightening its regulation of pharmaceutical compounds grown in food, in part because of rapid advances in development of the technology.

But the Ventria proposal will not be affected because it already has been approved by the USDA as a field test, said Jim Rogers, a spokesman for the agency's Animal Plant Health Inspection Service.

Rogers said Ventria must comply with its existing USDA permit, which requires special precautions to prevent the escape of gene-carrying pollen to nearby crops, including an unplanted buffer zone around the field.

"We want to make sure these plants don't affect other plants," he said.

Rice farmers have long known that scientists were moving genes around in ways not possible through traditional breeding, with a goal of inventing new crop types. Still, they thought pharmaceutical rice was a ways off.

"We have jumped all the way to the most sensitive topic," said Kent S. McKenzie, director of the grower-funded California Cooperative Rice Research Foundation, who serves on the committee reviewing the Ventria plan.

The advent of pharmaceutical rice is not entirely unexpected, though. Ventria has been in Sacramento since 1993, a startup founded by a University of California, Davis, biologist.

Originally named Applied Phytologics, it hatched from the idea that plants could serve as biological factories that cheaply produce proteins with medicinal and nutritional benefits.

The company planted its first engineered rice outdoors in 1997. After exploring several possibilities, including baby formula made with plant-engineered ingredients, it settled on two products for its market debut: human lysozyme (LY so zime) and human lactoferrin (lak toe FAIR in).

Both are proteins found in mother's milk, thought to reduce infections in nursing infants.

Deeter said the company intends to sell the rice-derived lysozyme and lactoferrin for use in oral rehydration products to treat severe diarrhea.

He said 65 acres of Ventria rice could generate 1,400 pounds of lactoferrin, enough to treat at

least 650,000 sick children. The same acreage of lysozyme rice would yield enough protein to treat 6.5 million patients.

Dr. William Greenough III, a professor of medicine at Johns Hopkins University, said oral rehydration solutions, a mixture of sugar and electrolytes, save the lives of more than 3 million people a year worldwide.

Greenough said adding anti-microbial proteins is appealing because existing products don't tackle causes of diarrhea; they merely prevent dehydration.

Despite the potential health benefits, the notion that a genetically engineered crop would have absolutely no hazard may be a hard sell for the public.

"There's no such thing as 100 percent certainty when you're talking about living organisms," said Doreen Stabinsky, a former CSUS environmental studies professor with a doctorate in genetics from UC Davis.

Now a scientific adviser for Greenpeace International, Stabinsky helped coordinate a Greenpeace "action" in 2001 that publicly pinpointed Ventria's rice in a Sutter County field.

Food industry trade groups also have expressed reservations about plant pharmaceuticals.

"This is a technology that deserves to blossom," said Stephanie Childs, spokeswoman for Grocery Manufacturers of America, which represents the nation's name-brand foods. "However, we are concerned that ... regulations are not in place to ensure the safety of the food supply. ... It would only take one accident to destroy an entire industry sector."

Mainstream scientists are similarly wary. Last week, a National Research Council committee examining biological methods for containing genetically engineered organisms recommended using non-food "host organisms" for products that should be kept out of the food supply.

Such concerns are based on the difficulty of corralling biotech genes. In November 2002, for instance, USDA inspectors discovered experimental pharmaceutical corn growing in Nebraska amid soybeans.

The biotech industry, once bullish on the prospect of growing drugs in plants, is pulling back. Nationwide, the number of field experiments on plant-made pharmaceuticals is down from a peak of 19 in 2001, to four in 2003.

Deeter said Ventria is sensitive to concerns about the escape of biotech genes, which is why the company engineers crops such as rice and barley that are self-pollinating, thus less likely to breed with crops in nearby fields.

The company's processing facility is within 50 miles of where the rice is grown, Deeter said. Ventria leases the fields but owns all the equipment, used solely on its own rice.

Ventria's proposal under review by the Rice Commission committee involves about 50 procedures the company will use to keep its rice out of the food chain.

Among them: sealing truck containers that carry Ventria rice, keeping 100-foot buffers between the company's fields and conventional varieties, and providing a test kit so inspectors can monitor for escaped genes.

The draft proposal is light on some details, including how Ventria will prevent birds from spreading its rice; what constitutes "proper" disposal of rice plants; and whether the company will notify nearby growers.

Deeter said he worries that if the location becomes public, anti-biotech activists will destroy Ventria's crops, as they did in 1999 at UC Davis and elsewhere.

Besides state and USDA hurdles, pharmaceuticals also are overseen by the U.S. Food and Drug Administration. But Ventria is categorizing its rice as "medical food" -- which does not require FDA review.

Ventria does plan to voluntarily submit documents to FDA, Deeter said, demonstrating that its proteins are safe enough to be consumed in ordinary food.

About the Writer The Bee's Mike Lee can be reached at (916) 321-1102 or mflee@sacbee.com.

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Rules for rice [108k JPG]

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NEWS ANALYSIS

California OKs GM pharm crops

by Charles Q Choi

Email: Charles Q Choi - cqchoi@nasw.org
News from The Scientist 2004, 5(1):20040408-04

Published 8 April 2004

International groups of scientists, consumers, and environmental activists are urging the California Department of Food and Agriculture (CDFA) to reject an emergency proposal to grow pharmaceutical rice. The rice would be the first time a genetically modified (GM) food crop in the United States was planted for commercial-scale drug production.

"The implication is there could be a precedent set here on biopharmaceutical crops on which we don't have a full national policy in place yet, and there are clearly questions here about human safety," Michael Hansen, an ecologist and senior research associate at Consumers Union's Consumer Policy Institute in Yonkers, NY, told The Scientist.

On March 29, the California Rice Commission, which makes recommendations to CDFA, approved by a 6 to 5 vote the Sacramento, Calif.–based biotech firm Ventria Bioscience's proposal to grow rice genetically engineered with human genes to produce lactoferrin and lysozyme.

Both proteins are found in bodily secretions such as milk, tears, and saliva, and possess antibiotic, antifungal, and antiviral properties. Among other ailments, the company hopes these drugs can kill bacteria that cause severe diarrhea, such as

Escherichia coli, Pseudomonas spp., or Vibrio cholera. "Ventria's products have the potential to save the lives of 2 million children a year," said Ventria president and chief executive officer Scott Deeter.

Ventria sought approval via an emergency proposal to grow up to 120 acres of the crops in 10 Californian counties away from the state's primary rice fields. The planting season lasts from April to July, and since the standard review process can take months, Ventria went on a fast-track process to avoid delaying plans until next year. CDFA secretary A.G. Kawamura must decide whether to approve, deny, or modify the rice commission's recommendation by April 12.

The Union of Concerned Scientists (UCS) urged Kawamura to deny the recommendation. "I don't know of any emergency involved here to deny the public any right to participate in these deliberations and accomplish any approval in only 10 days. Not getting into the

field as soon as possible is not really an emergency," said Margaret Mellon, director of the UCS food and environment program.

Deeter objected, saying Ventria's proposal had been discussed in public meetings since March 2003, with attendees including the UCS and Consumers Union. "We've by no means been trying to sneak this through. We're absolutely committed to listening to any data and scientific evidence that's new," Deeter said.

Consumer and environmental groups fired off letters urging Kawamura to deny the recommendation and hold public hearings on the application instead. The Consumers Union, along with four other groups, noted that pharmaceutical crops might trigger food allergies, kill off wildlife or beneficial microbes, or transfer disease-resistant traits to related weeds.

The Center for Food Safety also noted that groups in Japan, the largest foreign market for Californian rice, have said they might reject GM rice or even rice grown near GM crops.

"Ventria says they can limit contamination, but cannot offer a 100% guarantee. For us, even the smallest chance of contamination is too much for us to risk," Yoko Tomiyama, chairperson of Consumers Union Japan, wrote in a March letter to the California Rice Commission.

As of now, it's unclear whether Ventria can begin planting this year even with CDFA approval, because further US Department of Agriculture (USDA) consent is also needed.

"From discussions with USDA officials, we understand that Ventria Bioscience does not yet have a Plant Pest Act permit to grow pharmaceutical rice in California counties south of the major rice-growing area of the state," Mellon and UCS senior staff scientist Jane Rissler wrote in a letter to Kawamura on March 31. "Since the USDA permit may take up to 120 days to obtain and is required before planting can begin, the company may not be able to plant this spring even with CDFA approval."

Deeter agreed. "Submitting a permit is a fairly exhaustive process that doesn't happen quickly, from past experience," he said.

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Return to citation in text: [1]

Biotech firm to make drugs in GM rice

Independent on Sunday, The, Feb 1, 2004 by Geoffrey Lean Environment Editor

GM crops specially engineered to produce drugs are to be grown commercially for the first time, The Independent on Sunday can reveal.

An American biotech company plans, in spring, to start growing medicines in rice to treat diarrhoea. Its proposals were examined last week by regulators in California, who have no power to stop it.

The rice will usher in a second generation of GM crops, which are bound to further polarise opinion around the world. They could offer real benefits to millions - but they also pose far greater health risks.

Top officials at the Department for Environment, Food and Rural Affairs believe that the danger is so great that the new crops should never be grown in Britain. But Downing Street has cautiously endorsed them.

The possibilities for growing drugs in plants - "pharming" - have been researched for years, with scientists developing a range of vaccines and other medicines in several common foods. But now Ventria Bioscience, in Sacramento, is to plant 130 acres with two new varieties of GM rice that will produce lactoferrin and lysozyme, infection-fighting chemicals that it will market for use in oral rehydration products to treat diarrhoea.

It says this could generate enough lactoferrin to treat at least 650,000 sick children, and sufficient lysozyme for 6.5 million patients. It hopes to expand production to 1,000 acres within a few years. The company will not disclose the location of the site for fear of sabotage. Its plans have caused alarm in California. Organic farmers, in particular, fear that the GM rice will contaminate their crops; the company says there is "no risk".

On Thursday, the arguments were thrashed out before a meeting of the California Rice Commission, which is drawing up protocols under which the rice can be grown. But Tim Johnson, the commission's president, told The Independent on Sunday that neither it nor the state's agriculture secretary, to whom it reports, has the power to stop the rice being cultivated.

He said that the commission was instead working out precautions - such as the distance the GM rice must be from conventional crops - to try to minimise risks.

The chemicals in the rice are relatively mild - they are found in mother's milk - but they could pave the way for stronger ones. Scientists have developed vaccines to treat measles, antibodies to treat cancer, provide contraceptives and prevent genital herpes - in potatoes, maize, wheat, rice, alfalfa, carrots and tomatoes.

The company says that its plants "will become `factories' that manufacture therapeutic proteins to combat life-threatening illnesses". It adds that "plants improved through the use of biotechnology" can produce "innovative treatments for diseases such as cancer, HIV, heart disease, diabetes, Alzheimer's disease, kidney disease, Crohn's disease, and many others".

Ventria Testimony Before The Subcommittee on Rural Enterprises, Agriculture, and Technology, United States House of Representatives, June 29, 2005

Hearing Name:	Different Applications for Genetically Modified Crops
Committee:	Subcommittee on Rural Enterprises, Agriculture, and Technology
Date:	Wednesday, June 29, 2005

Prepared Remarks of Mr. Scott Deeter

President and CEO Ventria

Good afternoon Chairman Graves (R-MO), Members of the Committee, Ladies and Gentlemen. My name is Scott Deeter and I am President & CEO of Ventria Bioscience. I appreciate the opportunity to address the Committee on behalf of Ventria Bioscience. I will briefly describe the company, our technology and our products and would be happy to answer any questions.

First, let me provide an introduction to Ventria Bioscience. Ventria Bioscience is a plant-made pharmaceutical company that utilizes rice and barley as a factory to produce biologic products. Ventria's initial products provide human health benefits, however the Company's technology has the potential to address many challenges faced by other sectors of the economy including animal health, energy and industrial processing.

Ventria was founded with the support and guidance of several leaders in biotechnology and agribusiness who form the Company's Board of Directors. Ventria's Chairman is Thomas N. Urban, Jr. former Chairman and CEO of

Pioneer Hi-Bred International. Other Board members include William J. Rutter, Ph.D. and Pablo Valenzuela, Ph.D., who were Co-Founders of Chiron; William H. Rutter, an attorney by training and an entrepreneur; William W. Crouse, a limited partner of Healthcare Ventures; Dean Hubbard, Ph.D. President of Northwest Missouri State University and Melvin D. Booth, former President of MedImmune, Inc. and Human Genome Sciences, Inc. These industry leaders have committed their resources, their time and their talents to realize the vision of improving healthcare on a global basis utilizing the tools of modern biotechnology combined with the industrial might of American agriculture.

The company's core technology is a highly efficient and unsurpassed method of producing biological products in the seed of self-pollinating rice and barley. This technology was discovered in collaboration with University of California as well as other leading research institutions in the United States.

Ventria believes this technology will lead to more affordable medicines for a much broader patient population than what is possible with conventional biopharmaceutical production technology today. Ventria's technological innovation results in a substantial improvement in the economics of biopharmaceutical production. For instance, the capital investment required for Ventria to produce 500 kilograms is estimated to be $4 million. As a comparison, to produce the same amount using conventional technology, such as mammalian cell culture, would require capital investment exceeding $125 million, a more than 30 fold increase. In addition, the operating costs of Ventria's technology are less than 10% of the conventional technology.

There are several reasons for this economic advantage. First, Ventria has been able to achieve extraordinarily high yields of the product in the seed of rice and barley. Second, barley and rice are self-pollinating crops that can easily achieve the necessary geographic isolation from their food crop counterparts to eliminate concerns of cross contamination with the food supply. Third, because these

crops can be stored in ambient conditions for up to two years without degradation, they allow for continuous operation of a processing facility, thereby increasing capacity utilization and reducing cost. Fourth, because rice and barley are safe for human consumption, they are ideal for products that can be delivered orally, thereby eliminating the need for expensive separation technology that is required by conventional systems to remove infectious or toxic contaminants. These advantages pave the way for a paradigm shift in biopharmaceutical production for the benefit of patients worldwide.

As an illustration of the strength of Ventria's technology, I would like to describe some of the human health products in development. Ventria's first two human health products are proteins called Lactiva™ and Lysomin™. These two proteins are found naturally in mother's milk, saliva and tears and they have been suggested to contribute to the improved health status that has been widely reported for breast fed children when compared to their bottle fed counterparts. These proteins are part of the reason why breast feeding is the best form of nutrition for infants and is highly recommended by most pediatricians.

Ventria currently produces Lactiva™ and Lysomin™ in the seed of rice through contract relationships with selected and well trained growers. Ventria's field production is regulated under a permit that is issued by the United States Department of Agriculture's Animal and Plant Health Inspection Service ("APHIS"). In fact, last year alone, Ventria's field location was inspected eight times by APHIS inspectors. Once harvested the seed is pulverized into a powder and transported to the processing facility where the final product is isolated into either a concentrate or isolate

The United States Food and Drug Administration ("FDA") has regulatory authority over Ventria's products for human health. As part of Ventria's pre-market activities, we reviewed the safety of Lactiva™ and Lysomin™ with a panel of scientific and medical experts that have unanimously concluded that these

products are Generally Recognized as Safe ("GRAS") for human consumption. The results of the panel review were summarized and submitted to FDA where they are awaiting clearance prior to commercial sales for human health.

There are several products being developed by Ventria that will incorporate Lactiva™ and Lysomin™. One product has been developed for children suffering from acute diarrhea. The World Health Organization estimates that 1.9 million children under the age of 5 die annually due to diarrhea. To address this crisis, Ventria added Lactiva™ and Lysomin™ to an oral rehydration solution, which is a common first line therapy given to children suffering from diarrhea. By adding Lactiva™ and Lysomin™, Ventria believes it can improve the recovery rate and reduce the severity or duration of diarrhea in these children. This hypothesis is the basis of a recently completed study in Peru with 150 children suffering from acute diarrhea. Ventria expects the results of this study to be published shortly. Ventria's production technology enables the cost effective addition of Lactiva™ and Lysomin™ to oral rehydration solution for the benefit of millions of children globally.

Ventria is also exploring the use of Lactiva™ and Lysomin™ for the prevention of diarrhea in the military. During Operation Iraqi Freedom, 70% of deployed troops suffered a diarrheal attack and 43% reported decreased job performance as a result of this attack. During the Viet Nam War, it has been reported that hospitalizations due to diarrhea were four times more prevalent than malaria. This is a silent enemy attacking American troops. Ventria has set its goal to reduce the diarrheal attack rate by 50% with the preventive administration of Lactiva™ and Lysomin™. If we achieve our objective, it would improve military morale, efficiency, and manpower. In terms of manpower productivity alone, this may pay for itself due to the cost effectiveness of Ventria's technology. Incidentally, this is a similar problem to that experienced by the millions of Americans who travel overseas.

Another use of Lactiva™ that is being developed is for the management of inflammatory bowel disease, or IBD. IBD afflicts over one million Americans and over four million people worldwide. IBD is an extremely debilitating disease that causes severe abdominal pain, weight loss, poor absorption of nutrients and chronic gastrointestinal ulcers. Ventria is testing the potential for Lactiva™ to improve the quality of life for the millions with this disease.

Ventria is also working with University of Cincinnati to develop a treatment for chronic lung infections caused by Pseudomonas, which is the leading cause of death for patients suffering from Cystic Fibrosis. Ventria and our collaborators have shown successful inhibition of this infection and we are jointly planning a pre-clinical program to further develop this product.

Recently, Ventria was the recipient of an SBIR grant from National Institutes of Health, National Institute on Aging relating to the use of one of Ventria's products to inhibit biofilms constructed by pathogenic bacteria. These types of infections affect more than 10 million Americans annually. Infections that are protected by biofilms are 100 to 1,000 times more resistant to antibiotics, so it is important to inhibit the formation of these biofilms before they can establish themselves at the wound site. Ventria has worked with scientists from University of Iowa and Howard Hughes Medical Institute to develop a natural human protein that has been shown to inhibit the ability of pathogens to construct these biofilms. Using its plant -made pharmaceutical technology Ventria produced and purified this protein and has shown the effective inhibition of biofilm formation. With the SBIR grant, Ventria will further develop this product with the goal of improving patient recovery by reducing the establishment of biofilms that lead to antibiotic resistant pathogens.

This concludes my testimony on behalf of Ventria Bioscience. I would like to thank Chairman Graves and the Committee members for your kind attention and would be happy to answer any questions you may have.

ATTACHMENT D

CORROBORATING PRESS ARTICLES INDICATINIG THAT PHARMING'S RECOMBINANT HUMAN LACTOFERRIN HAS INDEPENDENT BIOLOGICAL OR THERAPEUTIC EFFECTS IN HUMANS

"Pharming licenses lactoferrin production tech to NZ group" available at http://www.in-pharmatechnologist.com (June 6, 2005)

- "The Dutch biotechnology firm said its partnership with AgResearch covers the production of recombinant human lactoferrin (rhlf), made in a herd of transgenic cattle. Pharming is developing rhlf both for pharmaceutical and nutraceutical applications."

"Drug from GM Animal Gets Thumbs Down," BioED Online available at https://BioEdOnline.org (February 24, 2006)

- "Pharming, a biotech company based in Leiden, the Netherlands, is awaiting approval from the US Food and Drug Administration for an antibacterial agent called lactoferrin, which they produce in the milk of GM cows. Samir Singh, chief business office with Pharming, believes the company will get a positive response by the end of this year. As human lactoferrin would be a "nutriceutical" – a food additive intended to boost health – it has fewer hurdles to clear than a drug."

Breaking News on Pharmaceutical Technology - Europe

Print

Previous page : Pharming licenses lactoferrin production tech to NZ group

Pharming licenses lactoferrin production tech to NZ group

30/06/2005- **Pharming has licensed rights to its protein production technology, based on the use of transgenic animals, to AgResearch, the largest government owned research organisation in New Zealand, *reports Phil Taylor.***

The Dutch biotechnology firm said its partnership with AgResearch covers the production of recombinant human lactoferrin (rhLF), made in a herd of transgenic cattle. Pharming is developing rhLF both for pharmaceutical and nutraceuticals applicatons.

The two companies have now pooled their resources for the manufacturing of rhLF, with the NZ company taking responsibility for the production of rhLF and purification, as well as providing research capabilities for product development. AgResearch will also fund the initial production of rhLF and support the commercialisation of the ingredient in the South Pacific and Asia.

Pharming has granted AgResearch a research license to its proprietary technology for the production of recombinant proteins, and in return the Dutch firm will have the first right to review new products arising out of AgResearch's protein discovery and R&D projects. The commercial rights of Pharming will cover recombinant bovine and human proteins produced using its proprietary technology.

Pharming is currently preparing a filing on rhLF for Generally Recognized as Safe (GRAS) registration with the US Food and Drug Administration (FDA), which would clear the way for its use as an ingredient in foods.

Meanwhile, the company is also seeking partners to help it advance the pharmaceutical applications of the ingredient. These could include its use in treatments for dry eye or eye infections.

contact the editor

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February 24, 2006

Drug from GM animal gets thumbs down

Protein made in goatsâ€™ milk doesnâ€™t make it to market.

by Mark Peplow
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An application to market a drug made in the milk of genetically modified (GM) goats was turned down this week. The decision means that, despite more than a decade of work using GM animals to produce drugs, no products have yet been approved for use.

GTC Biotherapeutics of Framingham, Massachusetts, has spent almost 15 years developing a herd of genetically modified goats whose milk contains a human anticoagulant called anti-thrombin. The company planned to market the drug under the name ATryn.

But the London-based European Medicines Agency (EMEA) turned down their request on 23 February, saying the product hadnâ€™t been tested enough.

"It's important to stress that the grounds for refusal have nothing to do with the use of a transgenic animal," says Martin Harvey Allchurch, spokesman for the EMEA.

Easy breeding

ATryn was designed for people lacking a working anti-thrombin gene, who can have an increased risk of blood clots. At the moment they are given blood-thinning drugs such as Warfarin, but this can raise the risk of bleeding to death during childbirth or surgery. At such times anti-thrombin itself is used, the only present source of which is human blood.

GTC spokesman Tom Newberry says that goats' milk is an ideal place to make these proteins, because it can deliver large quantities relatively cheaply and reliably. Some therapeutic proteins are currently produced in bioreactors, huge brewing vats that typically contain cultured Chinese hamster ovary cells. But large, complex proteins such as anti-thrombin are difficult to make this way. And breeding goats is easier than building reactors.

GTC added a copy of the human anti-thrombin gene to a goat gene that makes milk. The engineered DNA was injected into an embryo, and a goat herd built up by conventional breeding. "Getting the protein into the milk is the easiest part," says Newberry. The difficult part is purifying the proteins and doing enough clinical trials, he adds.

Trial, trial again

The difficult part is purifying the proteins and completing sufficient clinical trials, Newberry says.

The EMEA recommended that GTC test their drug on 12 patients undergoing surgery. But the company only presented evidence from five cases, which the EMEA says is too few. Newberry says that the drug also tested positively during nine childbirths, but that the EMEA excluded these from the surgical tally.

The agency also pointed out that the marketed product would have an extra filtration step that was not included in the trials. Finally, they said that GTC had done too few studies to assess whether patients developed antibodies in response to ATryn.

The company plans to appeal against the decision.

Moo milk

Despite the setback, the next such application is just around the corner. Pharming, a biotech company based in Leiden, the Netherlands, is awaiting approval from the US Food and Drug Administration for an antibacterial agent called lactoferrin, which they produce in the milk of GM cows.

Samir Singh, chief business officer with Pharming, believes the company will get a positive response by the end of this year. As human lactoferrin would be a 'nutriceutical' â€" a food additive intended to boost health â€" it has fewer hurdles to clear than a drug.

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- Lactoferrin -

Human lactoferrin

Human lactoferrin (hLF) is a natural protein that helps to fight and prevent infections and excessive inflammations and strengthens the defense system of the human body. The protein is present in significant amounts in numerous human biological fluids and mucus secretions, including tears and lung secretions, and has been shown to fight bacteria that cause infections of the eye and lungs. In addition, hLF is present in substantial quantities in mother's milk and plays an important role in the defense system of infants, as well as adults. Lactoferrin promotes the health of the gastro-intestinal system by improving the intestinal microbial balance.

Market opportunity

Lactoferrin is a multi-functional protein with many beneficial properties, which makes it a good candidate for a number of product applications. Since the protein has the ability to bind iron, is a natural anti-bacterial, anti-fungal and anti-viral, is an antioxidant and also has immunomodulatory properties, large groups of people might benefit from orally administered lactoferrin.

Pharming has a patent on human lactoferrin from the Japanese Patent Office, which covers the production and purification of hLF with Pharming's technology as well as its use in sports and food formulations. In Japan, bovine lactoferrin is currently used as an additive in food products and as a nutritional supplement. Japan represents a significant market for recombinant human lactoferrin.

Pharming's hLF approach

Because of its unique biological activities, Pharming is developing its human lactoferrin as a food supplement using its protein production technology. Pharming's human lactoferrin is produced from the milk of transgenic cows, a method that fits functional food development very well as cow's milk is a common food source worldwide. Pharming has filed a GRAS (Generally Recognized As Safe) notification for its hLF with the US FDA .

The company has medium-size production facilities to supply its hLF for further research and development purposes. In addition, the company has a partnership with the New Zealand based research institute AgResearch for development of its human lactoferrin. Pharming and AgResearch invite investors, companies and institutes to partner for further development of human lactoferrin for oral applications.

Pharming Receives Subsidies For Research In Osteoporosis
Identification of new therapeutic approaches and diagnostic tools

Leiden, The Netherlands, January 31, 2007. Biotech company Pharming Group NV ("Pharming" or "the Company") (Euronext: PHARM) announced today that its wholly owned subsidiary DNage has been granted two SenterNovem subsidies totalling just over €1 million, over a period of three years, to develop products in the field of osteoporosis.

The granting of these subsidies exemplifies Pharming's strategy to expand its research engine and to strengthen its product pipeline. Several early stage programs have already been initiated at Pharming/DNage and partnered with academic institutions and biotech companies. The Company has started to finance these programs through national and European subsidy programs.

Under a so-called International Innovation Subsidy, DNage will use its unique animal models (for aging diseases) and work with an international consortium of academic institutions and biotech companies to identify new targets (to which therapeutic products can be targeted) and therapies in the field of osteoporosis. Moreover, the study will focus on the identification of new diagnostic tools that can identify the disease at an early stage (biomarkers) to facilitate early diagnosis.

In addition, a "Feasibility" subsidy was granted that will allow the Company, in collaboration with the Erasmus Medical Center in Rotterdam, to establish the role of lactoferrin in bone formation and explore a business and clinical development strategy to develop lactoferrin as a new product in the field of bone diseases such as osteoporosis. Lactoferrin is one of Pharming's current products under development.

Osteoporosis is a skeletal disorder characterized by weakened bones leading to increased risk of fractures and disability. It is estimated that every one in three women and one in eight men over the age of fifty will develop osteoporosis and that more than 75% of osteoporosis patients are diagnosed very late or not at all. There is currently no cure for osteoporosis, although several treatments may slow down its progress. Bisphosphonates are the most commonly prescribed class of drugs for the treatment of osteoporosis with a market size of € 7 billion and growing rapidly. However, poor adherence to current therapy and the lack of early diagnosis present key market opportunities. There is a strong medical need for new products (using new therapeutic targets) and new biomarkers.

About Pharming Group NV
Pharming Group NV is developing innovative products for the treatment of genetic disorders, ageing diseases, specialty products for surgical indications, intermediates for various applications and nutritional products. Pharming has two products in late stage development - Rhucin® (recombinant human C1 inhibitor) for hereditary angioedema (MAA under review by EMEA) and human lactoferrin for use in food products (GRAS notification under review by US FDA). The advanced technologies of the Company include innovative platforms for the production of protein therapeutics and technology and processes for the purification and formulation of these products, as well as technologies in the field of tissue repair (via its collaboration with NovaThera) and DNA repair (via its acquisition of DNage BV). Additional information is available on the Pharming website, http://www.pharming.com and on http://www.dnage.nl

About SenterNovem
SenterNovem, an agency of the Dutch Ministry of Economic Affairs, promotes sustainable development and innovation and aims to achieve tangible results that have a positive effect on the economy and on society as a

whole. International projects are coordinated by SenterNovem in collaboration with Eureka, a pan-European network for market-oriented, industrial research and development partnerships. Founded in 1985, Eureka currently counts 38 full member countries including the European Union. It initiates about 180 projects each year including Eureka clusters, Umbrella networks, and Eurostar projects with an estimated yearly industrial investment of €1 billion. Additional information is available on the SenterNovem website, http://www.senternovem.nl

This press release contains forward looking statements that involve known and unknown risks, uncertainties and other factors, which may cause the actual results, performance or achievements of the Company to be materially different from the results, performance or achievements expressed or implied by these forward looking statements. The press release also appears in Dutch. In the event of any inconsistency, the English version will prevail over the Dutch version.

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Pharming Announces Positive Results Of Study With Human Lactoferrin

Leiden, November 24, 2004. Pharming Group N.V. ("Pharming" or "the Company") (Euronext: PHARM) announced today the positive results from a key study with recombinant human lactoferrin (rhLF). The Company will use these results for Generally Regarded as Safe (GRAS) registration of rhLF for nutritional applications.

The results demonstrate that rhLF can be consumed orally at high amounts with no adverse effect. Pharming has conducted the extensive animal toxicology study in cooperation with the TNO Institute for Nutrition and Food Research to observe the effect of the oral intake of rhLF. After publication of the study results, the Company will prepare its GRAS filing along with an expert opinion on use of rhLF for nutritional applications.

'I am very pleased with the positive outcome of this study with recombinant human lactoferrin, as well as the positive results in animal studies with human fibrinogen,' said Dr. Francis J. Pinto, CEO of Pharming. 'Based on these achievements, Pharming will consider making additional investments to accelerate the development of these innovative products.'

Recently, Pharming completed initial studies in animal models with recombinant tissue sealant / fibrinogen (rhTS / rhFIB). The results of these studies indicate that rhTS / rhFIB is effective in stopping bleedings and may provide advantages over commercially available plasma fibrin sealants. The Company has started licensing discussions with several parties to accelerate commercial production of these products.

Recombinant Human Lactoferrin
Human lactoferrin is a natural protein that helps to fight and prevent infections and strengthens the defense system of the human body. The protein is present in substantial quantities in mother's milk and plays an important role in the defense system of infants. The protein is also present in various body fluids and continues to play an important role against a wide range of bacterial, fungal and viral pathogens in adults.

Pharming is developing recombinant human lactoferrin (rhLF) as a nutraceutical and intermediate while evaluating applications of the product for the pharmaceutical market. Pharming has demonstrated that rhLF is safe, effective and comparable to the natural hLF. Pharming plans to file for Generally Regarded as Safe (GRAS) status for rhLF and commercialize the product for nutritional applications.

Background on Pharming Group N.V.
Pharming Group N.V. is developing innovative protein therapeutics for unmet medical needs. The Company's products include potential treatments for genetic disorders and specialty products for surgical indications. Pharming's lead product for hereditary angioedema is in Phase III of clinical development. The advanced technologies of the Company include novel platforms for the production of protein therapeutics, as well as technology and processes for the purification and formulation of these products. Additional information is available on the Pharming website, http://www.pharming.com

This press release contains forward looking statements that involve known and unknown risks, uncertainties and other factors, which may cause the actual results, performance or achievements of the Company to be materially different from the results, performance or achievements expressed or implied by these forward looking statements.

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November 1, 2007

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Re: **Interpretation and Implementation of Section 912 of the Food and Drug Administration Amendments Act of 2007**

Dear Dr. Brackett:

Like many in the food industry – including attorneys who are responsible for advising their pertinent clients about food-related, legal requirements and compliance with same, you and your colleagues are currently faced with understanding, interpreting, and implementing the provisions of new Section 912 of the "Food and Drug Administration Amendments Act of 2007". Depending upon how these provisions are interpreted and implemented, the effects of Section 912 on CFSAN's scope of and day-to-day operations could be anywhere from slight to very significant (indeed, devastating).

It is very unfortunate – indeed, inappropriate – for all concerned, except the real intended beneficiary, that this new Section was sprung upon the public and their representatives without adequate advance notice and opportunity for review and consideration. Indeed, one wonders why those responsible for protecting the interests of CFSAN did not prevent this Section – as currently worded – from being enacted. Nevertheless, until and unless portions of Section

912 are modified (which event is not likely), the Section's provisions are currently the law; thus, they need to be **effectively** dealt with.

To this end, please find below some **comments** which I hope are helpful to you and your colleagues in interpreting and implementing the new Section in a manner that is reasonable and fair. For your convenience, each area of comment and subpart of the new Section is first set forth in highlighted fashion and then immediately followed by my comments (if any).

Background pertinent to Section 912

You and your colleagues may be aware of all that follows in this subpart; however, if you are not, since such background is key – especially to interpreting Section 912 – the pertinent background is summarized here.

In the first six months of 2005, at least four letters were conveyed to CFSAN personnel (copies of which are attached) whose purposes had **nothing** to do with safety and **everything** to do with inappropriately protecting narrow interests. More specifically, the content of the letters indicate that they were intended to persuade FDA **not** to authorize any use of human lactoferrin – especially its use in food – **except** for any FDA authorization(s) for use of human lactoferrin products as approved or licensed drugs. If FDA had acted so as to implement this intended end point (which, thank you very much, it did not), the result would have been to create a monopoly – a situation that would have removed any competitive pressures with regard to pricing. Accordingly, the letters were really all about inappropriate protectionism, i.e., protecting product prices.

In early October of 2005 I learned of the existence of the above-referenced four letters. Since one of them (i.e., the first) specifically referenced

one of my clients, i.e., Pharming Group, N.V., I responded in writing to CFSAN to the comments contained in the four letters. A copy of my response is attached. Rather than summarize its contents and since the letter is fairly short, I recommend that it be read. Please note that, importantly, the letter – in essence – argues for the **neutral** (not selfish) application of the laws in question.

Evidently the writers of the four letters correctly concluded that their letter had not positively (from their point of view) influenced FDA and decided further measures were needed to force the protectionist end in question. Thus, Section 912, whose current language exactly tracks – in some places – the language used in some places in the four letters.

The power of interpretation

Please recall that there is **no** private right of action under the provisions of the FD&C Act. Accordingly, it is up to FDA to interpret the provisions of Section 912 and such interpretation(s) are likely to stand unless overturned by a court of competent jurisdiction in litigation brought by some plaintiff against FDA or via other legal process. Accordingly, FDA should make every effort to interpret and implement in a manner consistent with Congressional intent.

Congressional intent

On its surface, new Section 912 appears to concern itself with drugs, yet – in essence – it is really all about food-related matters. Isn't it curious that the new section was **not** included among those included under "Title X" which deals with "FOOD SAFETY"?

Notwithstanding the foregoing, new Section 912 is included under "Title IX" – whose intent – according to Congress – is to concern itself with provisions pertinent to "SAFETY OF DRUGS". "Subtitle B" of Title IX also makes clear that Congress' intent was to fill the subtitle with sections pertinent to ensuring "Drug Safety". Accordingly, when interpreting and implementing new Section 912, FDA should make every effort to interpret and implement in a manner that ensures safety and **not** any other agenda.

Section 912

New Section 912 is organized into two, distinct subparts. Subpart one deals with a general rule and subpart two deals with four exceptions (or in total eight exceptions, since subpart "(3)" contains five subparts). Obviously, if a given factual scenario satisfies any one of the eight exceptions, then the general rule does not apply to the factual scenario in question. Each of the subparts is discussed below.

The general rule

The general rule states (i.e., prohibits) as follows:

> The introduction or delivery for introduction into interstate commerce of any food to which has been added a drug approved under section 505, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations

have been instituted and for which the existence of such investigations has been made public unless–

This portion of Section 912 prohibits "the introduction or delivery for introduction into interstate commerce of any food to which" one or more of three, qualifying substances have been added. Accordingly, thus far, for the general rule to apply, there must be:

1. a **food** involved;
2. the **addition** of one or more of the three, enumerated, qualifying substances (thus, if one or more of the substances has **not** been "added" to the food but rather naturally appears in the food, then presumably the general rule would **not** apply);
3. an **introduction** or delivery for introduction; and
4. **interstate commerce** (please note that Section 912 does **not** indicate that interstate commerce is presumed).

Section 912 continues by enumerating the above-referenced three prohibited, qualifying substances. The first two are well-known and logically included since they amount to either a drug "approved" pursuant to Section 505 of the FD&C Act, i.e., a "new drug", or a biological product "licensed" under Section 351 of the PHS Act[1]. Thus, if an added substance is neither an approved, new drug nor a licensed biological (pursuant **only** to either of the two, quoted Sections, i.e., Sections 505 and 351), then the general rule would **not** apply. Moreover, if the added substance is either an old drug, or a grandfathered product, or one of many OTC products, then the general rule would also **not** apply. The fact that Section 912 purports to be about "drug safety" and keeping, in general, drugs out of food unless one of the exemptions applies is belied by

[1] Please note that this reference to "biological product" does not incorporate animal biologics.

the fact that not all drugs – indeed, many if not most drugs – are not included in the general rule.

At this point Section 912 finally gives up its subtext when it reveals the third qualifying substance whose presence will activate the general rule, i.e., a "drug" or a "biologic product" for which "substantial clinical investigations have been instituted" (and for which the existence of such investigations have been made public). What, one might ask, does mere institution of "substantial clinical investigations" have to do with drug safety. Of course, unless such investigations have, in fact, lead to either drug approval or biological product licensure, the answer is **nothing** at all, since many substances are investigated but never eventually authorized for use by FDA as either drugs or biologics[2]. Of key relevance is the controlling wording pertinent to this third qualifying substance which comes directly from the first, attached, letter to CFSAN (see page 2, first full paragraph, line 4, first three words) and its inclusion serves only to service the interests of a single company. Worse, its presence in the FD&C Act subverts the stated, legitimate purposes of the FD&C Act – especially as they relate to safety. Accordingly, this third category of substances should be interpreted so as to limit its scope as much as is reasonable. To achieve this, FDA should consider the following:

1. Please note that the first two categories of qualifying substances are approved drugs or licensed biologicals, while the third category of qualifying substances evidently includes only "drugs" or "biological products". Since the purpose of Section 912 is supposed to be about

[2] **Please note** that if the term "substantial clinical investigations" is defined to mean something short of that quantity of investigations needed to demonstrate safety of a substance for its intended use, then institution of such short quantity (which will not lead to approval or licensure) will result, nevertheless, in the general rule applying and such substance being banned from food unless one of the exemptions applies. If neither approval nor licensure occurs or an exception applies, then such substance will be inappropriately forced into a "black hole" – unable to be used in perpetuity for drug and/or food use.

the safety of only certain **approved** drugs and **licensed** biologicals, the scope of the third category of substances should **not** be interpreted to include a broader range of products, i.e., old drugs, grandfathered products or most OTCs. Rather, the third category should be confined to substances either actually or likely (because the substantial clinical investigations in question have proven that the given substance in question is safe for its intended use) to be approved under Section 505 or licensed under Section 351;

2. Also, please note that the term "substantial clinical investigations" is used, but is not defined despite the fact that such term is an important controlling phrase, i.e., its definition determines what is or is not included in the third group of qualifying substances; thus, it needs to be defined by FDA because its meaning is not clear. When defining such phrase, FDA should consider that the term is stated in the plural, thus, one investigation **pertinent to an intended use** is not enough. In addition, since not just any substance amounts to either a "drug" or a "biological" but only those substance that meet the definitions of the two terms – which definitions both **require a specific intended use** (see, 21 USC §§ 321(g) and (p) and 42 USC § 262(i)) – FDA should require substantial clinical investigations for a **particular use** (and not merely a series of investigations – each one investigating a different use);
3. Moreover, since all such investigations are required to have been "instituted", all such qualifying, clinical investigations should be required to have been conducted pursuant to the IND regulations and also to have been well-designed and well-conducted (since to allow just any old "investigation" to qualify would make a mockery of what is supposed to be about safety); and

4. Finally, since the investigations in question are required to have "been made public", FDA should require that each such investigation – to qualify – must have been published in a legitimate, peer-reviewed, scientific journal (since to allow any old publication of information, such as only on a web site, to qualify would make a mockery of what are supposed to be real and substantial clinical investigations).

Critical to the proper functioning of Section 912 is a determination of whether – with regard to any drug or biological (whether approved, licensed, or not) – "substantial clinical investigations have been instituted" in support of the substance being added to food. But who is going to have the burden of demonstrating that such investigations have been instituted concerning a specific, qualifying substance to be added to food? Is CFSAN – with its limited resources already stretched up to, if not beyond, the breaking point – going to be responsible for keeping track of all "substantial clinical investigations" pertinent to all substances which are being added to food which may amount to a qualifying drug or biological product? You know better than I that the answer is, generally speaking, "no". For sure, in the **very limited** instances in which CFSAN (i.e., FDA and the Justice Department) is involved in bringing an enforcement action based in part or whole on a violation of Section 912, then in such instances FDA will have the burden of proof of proving that – with respect to a specific, qualifying substance being added to food – substantial clinical investigations have been instituted. But in all other instances, the party with the vested interest, i.e., the entity that has instituted the substantial clinical investigations in support of some qualifying substance being added to food, should bear the burden of adequately demonstrating that such investigations exist and otherwise meet any and all pertinent requirements that have been made applicable by CFSAN.

The exemptions

Section 912 next sets forth eight exemptions to the above-stated general rule. As indicated above, if any one exemption applies to a given factual scenario, then the general rule is inapplicable.

Exemption one

The first exemption applies to any

> such drug or such biological product which was marketed in food before any approval of the drug under section 505, before licensure of the biological product under such section 351, and before any substantial clinical investigations involving the drug or the biological product have been instituted.

Several comments seem appropriate. First, the substance being marketed in food must be either a "drug" or a "biological product", as those terms are defined by Section 201 of the FD&C Act and by Section 351 of the PHS Act otherwise, such substance is **not** one included in the general rule. Second, a qualifying substance need only to have been "marketed", i.e., as the dictionary indicates, merely **offered** for sale, prior to any of the three dates which control the exemption in order to meet the exemption requirements; importantly, the statute does **not** require that any sale has been consummated. Third, the general rule requires that, at least, more than one clinical investigation have been instituted before the general rule applies; accordingly, the exemption should apply provided the marketing requirement has occurred before **all** of the qualifying substantial clinical investigations **have been completed** with regard to a specific indication

for use and appropriately published. To require otherwise is to go beyond the general rule and expand the original boundaries of the general rule.

Exemption two

The second exemption applies if FDA or

> the Secretary, in the Secretary's discretion, has issued a regulation, after notice and comment, approving the use of such drug or such biological product in the food.

Given the context established by subsequent subsections 3A, 3B, 3C and 3D (which subsections appear to deal with FDA authorizations pertinent to use of substances in food resulting from food additive petitions, GRAS Affirmation petitions, GRAS Notifications, and food contact substance notifications (all Section 409-type activities) – but see clarifying comments appearing after each of those subsections below), subsection 2 – assuming that it is not intended to be duplicative of subsection 3(A) – appears to apply to, at least, a regulation issued under Section 409(d). However, notwithstanding that the express language of subsection 2 expressly requires issuance of a regulation "approving" a certain use, since Section 409(d) does **not** require any "approval" (and does not use any form of the term "approve") but rather only requires the Secretary to issue a regulation "prescribing...the conditions..." of safe use, use of the term "approving" in subsection 2 cannot be taken literally. Rather, such term must be interpreted to mean "authorizing" via an appropriate regulation.

Moreover, notwithstanding that the intention **may** have been to tie the exemption found in subsection 2 to only the conduct prescribed in Section 409(d) of the FD&C Act, no language in subsection 2 actually limits its scope to Section

409(d). Thus, subsection 2 may well apply to any lawful regulation issued by the FDA pursuant to any pertinent authority – such as that which is provided to FDA in other subparts of Section 409 and Section 701 of the FD&C and which was used to promulgate, e.g., 21 CFR Part 182.

Subsection "3" exemptions

The five exemptions set forth in subsections 3A-3E are all qualified by the lead-in language that appears after "(3)" and before subsection "(A)". Such language indicates that the substance in question is exempted from coverage by the general rule if

> the use of the drug or the biological product in the food is to **enhance the safety** of the food to which the drug or the biological product is added or applied and **not to have independent biological or therapeutic effects** on humans, and the use is in conformity with.... (Emphasis added).

This language also requires several comments. First, unless the substance in question is a "drug" or a "biological" as those terms are defined under Section 201 of the FD&C Act and Section 351 of the PHS Act, Section 912 does **not** apply. Second, the phrase "enhance the safety" is not defined. It should be interpreted to include any of CFSAN's authorization procedures which are expressly referenced in subsections 2 and 3A-3D because such substances are universally deemed safer than any substance which has not been prior reviewed and authorized by CFSAN; thus, such former (i.e., reviewed and authorized) substances should be deemed to enhance a food's safety. Third, the qualifying substance must have been "added" to the food; any naturally-occurring

substance would not qualify. (Please note use of the phrase "or applied"; the phrase is redundant, since in every case where a qualifying substance has been "applied" to the food it would also have been "added" to the food). Fourth, the phrase "not to have independent biological or therapeutic effects on humans" appears in the subsection but such phrase is not defined or explained. CFSAN should interpret this phrase to mean that the substance in question is not being promoted – independent of the food use in question – for its ability to induce certain biological or therapeutic effect(s) in the humans intended to consume the food to which the substance in question is being added. The terms "biological" and "therapeutic" have a long history of use at FDA; thus, their use should not create confusion – **unless** someone has a private agenda via which one advocates that such terms mean something much more expansive than what for decades the terms have been understood to mean. CFSAN should reject such new, expansive definitions appreciating that if adopted such new definitions would probably potentially include virtually every food additive and GRAS substance currently in 21 CFR Parts 172-186 – since all such substances (including, for example, such commonly-used items as water, hydrochloric acid, sodium chloride, sucrose, and ferric sulfate) can have – as qualified experts indicate – a bioactive and/or therapeutic effect at the molecular level. While such advocacy may appear rather benign at the surface, if adopted it could gut use of numerous currently regulated substances – all of which are otherwise appropriate for use in food and have been safely used for decades. Of course, if any drug claim is ever being made for such substance, the substance's use is illegal unless such substance has been prior authorized for use by FDA for the specific claim being made. Thus, adhering to well- and long-understood definitions will serve to preserve the status quo – which has been very carefully crafted by Congress and has well-served the US public for decades.

Exemption 3A

This exemption exempts from the general rule a qualified substance provided

> a regulation issued under section 409 prescribing conditions of safe use in food

has been promulgated. Such exemption obviously includes any use of a food additive which has been approved by CFSAN via issuance of an appropriate regulation in response to a food additive petition. But – not so obviously – since the authority to regulate GRAS substances also emanates from Section 409, such exemption would also include any substance that has been affirmed or otherwise listed as GRAS by CFSAN via a "regulation" (such as would result via the GRAS affirmation process set forth in 21 CFR § 170.35 – which process is legally still viable despite the **proposed** GRAS Notification process which was proposed on April 17, 1997 (see 62 FR 18938) – but has never been finalized or via use of Sections 201, 409 and 701 of the FD&C Act as was used to list those substances set forth in 21 CFR Part 182).

Exemption 3B

This exemption exempts from the general rule a qualified substance provided

> a regulation listing or affirming conditions under which the use of the drug or the biological product in food is generally recognized as safe

has been promulgated. Since this exemption especially requires a regulation, presumably it includes any GRAS substance listed or affirmed via the filing of a GRAS affirmation petition pursuant to 21 CFR §170.35. (See, e.g., 21 CFR Parts 184 and 186). However, it would also include any substance listed via the regulation-related means (see Sections 201, 409 and 701 of the FD&C Act) used to list those very numerous substances currently set forth in 21 CFR Part 182. Thus, this exemption would apply to any qualified substance listed or affirmed via any legitimate, regulation-resulting means.

Exemption 3C

This exemption exempts from the general rule a qualified substance provided

> the conditions of use identified in a notification to the Secretary of a claim of exemption from the premarket approval requirements for food additives based on the notifier's determination that the use of the drug or the biological product in food is generally recognized as safe, provided that the Secretary has not questioned the general recognition of safety determination in a letter to the notifier.

It appears that this exclusion is limited to the status gained from receiving a "no questions" letter from CFSAN in response to the filing of a GRAS Notification pursuant to the procedure set forth in the rule proposed on April 17, 1997 (62 FR 18938) which has not yet been finalized. (See, proposed 21 CFR § 170.36). This exemption is not in play unless CFSAN has no questions; thus, if questions

are raised, the exemption would not apply until such questions are resolved and a "no questions" letter is issued.

Exemption 3D

This exemption exempts from the general rule a qualified substance provided there exists

> a food contact substance notification that is effective under section 409(h).

This exemption appears to be limited to food contact substance notifications. For the exemption to apply, i.e., for it to be "effective", FDA must **not** have objected to the notification via the procedure set forth in 21 USC § 348(h)(2)(A).

Exemption 3E

This exemption exempts from the general rule a qualified substance provided

> such drug or biological product had been marketed for smoking cessation prior to the date of the enactment of the Food and Drug Administration Amendments Act of 2007.

This exemption appears limited to a qualified substance "marketed" for smoking cessation prior to September 27, 2007; thus, from a practical point of view, it would be expected to have very limited applicability with regard to use of food.

Exemption 4

This exemption exempts from the general rule a qualified substance provided

> the drug is a new animal drug whose use is not unsafe under section 512.

This exemption is limited to new animal drugs found by FDA to be safe pursuant to the requirements set forth in Section 512 of the FD&C Act. Please note that it would **not** be applicable to animal biologics used in human food because the term "biological product" appears to be confined to Section 351-type substances, i.e, those intended for use in humans.

Independent assessment of GRASness

Since Section 201(s) and Section 409 (pertinent to the definition and approval of food additives) were enacted in 1958, the portion of such sections which refers to GRAS substances (i.e., Section 201(s), first paragraph, the "if" clause prior to the exemptions) has been widely interpreted as permitting a determination that a substance and its use is GRAS via either independent assessment or via some process established by CFSAN, i.e., via general regulation or via the GRAS Affirmation process or via the GRAS Notification process. With regard to qualifying substances – which activate the general rule – I believe that independent assessment is no longer permitted. As a result of Section 912, the only way now to GRAS a substance and, thus, obtain a qualifying exemption is to obtain GRAS status via (obviously) subsections 3B or 3C or (not so obviously) via subsections 2 or 3A. Section 912 does **not** provide any exemption for independent assessment.

Grandfather clause

Section 912 does **not** appear to include any general grandfathering (i.e., exempting) of qualified substances whose regulatory status was determined prior to September 27, 2007, unless subsection 1 is applicable. Thus, if a substance is a qualifying substance **and** does not fall into any of the eight exemptions discussed above **and** has been added to food, it would be prohibited from being introduced into commerce.

CFSAN function

CFSAN is in **no** way automatically prohibited from going forward with any function – including GRAS Notifications – set forth in any of the above-discussed eight exemptions. If the substance under consideration is not a qualifying substance and, thus, does not activate the general rule, then it should be business as usual for CFSAN. If, however, the substance in question is a qualifying substance, then CFSAN may still proceed to reach one of many, specifically referenced, final determinations which result in such substance being exempted so long as the specific criteria, if any, associated with the exception scenario being pursued are met.

* * * * *

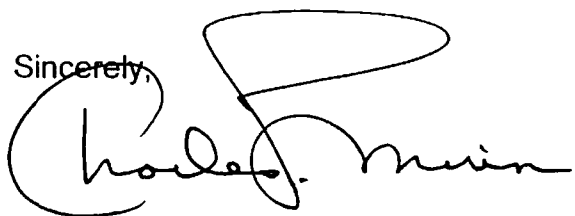
I hope the foregoing information has been helpful to you and your colleagues. It seems to me – and I hope you share this view – that CFSAN should interpret and implement the provisions of Section 912 so as to preserve – when possible – the delicate balance with regard to food safety that has evolved

and prevailed over the last several decades – especially since 1958 (with passage of Section 201(s) and Section 409 of the FD&C Act). In addition, CFSAN should act so as to prevent any protectionism that could significantly *imperil*, if not destroy, the delicate balance so hard won over the last five decades – a balance which has served the public and all other interested parties well.

If I can provide any further input concerning any aspect of Section 912, please let me know.

Thank you in advance for considering my views.

Sincerely,



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Re: Use of Recombinant Human Lactoferrin
In Food and Dietary Supplements

Dear Drs. Tarantino, Schneeman, Weiss, and Walton,

We have become aware that Ventria BioScience (Ventria) is conducting human clinical trials of recombinant human lactoferrin (rhLF) in the United States and that it intends to market its rhLF as an ingredient in medical food or dietary supplements.[1] In addition, Pharming Group

[1] Celia Lamb, *Regulators Block Plans for Genetically Altered Rice*, Sacramento Business Journal, April 9, 2004 (Attachment 1); Celia Lamb, *Altered Rice Still Headed to Market*, Sacramento Business Journal, April 16, 2004 (Attachment 2).

N.V. (Pharming) recently announced its intention to file a notification with FDA that its rhLF product is generally recognized as safe (GRAS) for use in food.[2]

Recombinant human LF is not now, and has not ever been, used as an ingredient in food or dietary supplements, and the safety of such product as a food additive or new dietary ingredient has not been established. Our client, Agennix, Inc. (Agennix), has conducted substantial clinical testing of rhLF as a drug under investigational new drug (IND) applications prior to any entry of rhLF into the marketplace as an ingredient in a dietary supplement. This ingredient is therefore excluded from the definition of a dietary supplement, and any rhLF product marketed as a dietary supplement is an unapproved new drug in violation of section 505 of the Federal Food, Drug, and Cosmetic Act (FD&C Act).[3]

It is important that CFSAN and CDER coordinate regulation of the safety of rhLF in food, dietary supplements, and drugs. Whatever safety requirements are appropriate for drug uses of rhLF should also be applied to its food and dietary supplement uses.

We urge FDA to investigate the attached press reports and to take action to prevent the unlawful marketing of these products. At this time, rhLF can be classified only as an investigational new drug or an unapproved food additive in the United States.

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[2] Press Release, Pharming Announces Positive Results of Study with Human Lactoferrin (November 24, 2004), *available at* http://www.pharming.com/index.php?act=show&pg=90 (Attachment 3).

[3] Section 201(ff)(3) of the FD&C Act.

I. Background

In September 1996, FDA approved the initial IND submitted by Agennix for the study of rhLF in treating gastrointestinal disorders.[4] Since that time, FDA has approved five additional IND applications for the study of rhLF for use in treating dermal concerns,[5] asthma,[6] GVHD,[7] cancer,[8] and most recently, mucositis.[9] Agennix currently is conducting Phase II human clinical trials of rhLF for the treatment of cancer, asthma, and diabetic wounds.

Ventria is now also producing rhLF. According to press coverage, Ventria has been selling its rhLF "for research uses" since the fourth quarter of 2003.[10] Newspapers report that the company has begun clinical trials in Southern California of a product containing purified lactoferrin and iron for the treatment of iron deficiency.[11]

Pharming announced in November 2004 that an animal toxicology study of its rhLF showed positive results and would be published in a scientific journal.[12] Pharming also announced that it would submit the results of this and other studies, as well as expert opinions, to FDA in support of GRAS recognition of rhLF for use in food.

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4 IND No. 6799.

5 IND No. 8546.

6 IND No. 10897.

7 IND No. 11230.

8 IND No. 11728.

9 IND No. 11870.

10 *Altered Rice Still Headed to Market*, *supra* (Attachment 2).

11 *Id.*

12 Pharming Announces Positive Results of Study with Human Lactoferrin, *supra* (Attachment 3).

II. Recombinant hLF Differs From Natural hLF

Although human lactoferrin (hLF) naturally is present in breast milk, recombinant human lactoferrin (rhLF) may differ from natural hLF in significant ways. Ventria acknowledges that its rice-based rhLF differs from natural hLF in that "Three major types of glycans are present in recombinant human lactoferrin, and all of them carry the typical core structure of the plant glycan with both xylose and fucose. None carries sialic acid as in the native form of human lactoferrin."[13] Ventria acknowledges that "certain protein targets require human glycans for optimal efficacy and stability when reintroduced into the human system," and that recent commentary has discussed the importance of correct glycan structure.[14] In response, Ventria offers but does not support the assertion that "There should generally be little effect of plant glycan structures in plant produced proteins."[15]

FDA has long expressed concern regarding the potential differences between natural products and their recombinant counterparts, and required appropriate oversight of such recombinant agents.[16] Although some products derived through recombinant means have been approved for use, including recombinant Factors VIII and Factor VIIa and etanercept (Enbrel), these products have been carefully reviewed by FDA before approval. In requiring the IND applications submitted by Agennix, CDER has recognized the possibility that the recombinant

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13 Ning Huang, *High-Level Protein Expression System Uses Self-Pollinating Crops as Hosts*, BioProcess International 54, 55 (January 2004)(Attachment 4).

14 *Id.*

15 *Id.*

16 51 Fed. Reg. 23309 (June 26, 1986).

nature of the product may result in changes to its safety profile, and that these potential changes must be reviewed by FDA before the product may be tested or marketed.

III. RhLF Is Not GRAS for Use in Food and Would Require a Food Additive Petition and Regulation

Pharming has announced its intention to bring rhLF to the market as an ingredient in a medical food product. Ventria also has suggested that it may make rhLF available as a food ingredient. Neither of these companies legally can market rhLF as a component of food until FDA reviews a food additive petition and promulgates a food additive regulation.

The only pertinent exception to the requirement of a food additive regulation would be a GRAS determination. Absent a food additive regulation, Pharming and Ventria would be required to demonstrate that rhLF is GRAS prior to using it as a food ingredient, whether in traditional food or in medical food.[17] RhLF is not listed as GRAS in any FDA regulation and has not been the subject of a GRAS notification. Moreover, we find no evidence that any other authoritative body has demonstrated that rhLF is GRAS.

The data currently available in the published literature regarding the safety of rhLF are not adequate to meet the regulatory requirements for a GRAS determination. Although several companies are investigating rhLF, the existing published scientific evidence is not of the quantity and quality required by 21 C.F.R. 170.30(b) for a GRAS determination, even if corroborated by unpublished studies and other data and information. Nor can the safety of rhLF be demonstrated through experience when used in food. The recombinant product does not yet have a substantial

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[17] Medical foods are subject to special labeling requirements under 21 C.F.R. 101.9(j)(8), but are not exempt from the requirement that all ingredients be determined to be safe.

history of consumption in food by a significant number of consumers, as required by the regulations.[18]

There are little public data regarding the safety of consuming a larger quantity of human lactoferrin, whether natural or recombinant, than normally is present in the adult diet.[19] Although lactoferrin exists in breast milk, it is not a common component of the diet at any later stage in life. Milk products can contain small quantities of bovine lactoferrin, but this protein differs from human lactoferrin.[20] For instance, the amino acid sequences in bovine lactoferrin differ from the sequences in hLF,[21] and the products can have significantly different biological effects.[22]

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18 21 C.F.R. 170.30(c), (f).

19 On previous occasions, FDA has denied GRAS status for naturally-occurring products due to a lack of information on human use in the United States. For instance, FDA determined that inadequate data supported the safe use of miracle fruit and its extracts and concentrates. 39 Fed. Reg. 34468 (September 25, 1974); 42 Fed. Reg. 26467 (May 24, 1977).

20 FDA has expressed no objection to the marketing of lactoferrin derived from bovine milk (milk-derived LF) as an ingredient in "sports and functional foods." CFSAN Response Letter Re: GRAS Notice No. 000077 (August 14, 2001). FDA also expressed no objection to the marketing of an anti-microbial spray of milk-derived LF to be applied to beef carcasses that aLF Ventures self-determined as GRAS. The agency noted that the level of lactoferrin remaining on the beef was comparable to the amount naturally occurring in the beef. CFSAN Response Letter Re: GRAS Notice No. 000130 (August 21, 2003).

21 F.L. Schanbacher *et al.*, Bovine Mammary Lactoferrin: Implications from Messenger Ribonucleic Acid (mRNA) Sequence and Regulation Contrary to other Milk Proteins, 76 J. Dairy Sci. 3812 (1993); R.E. Goodman & F.L. Schanbacher, Bovine Lactoferrin mRNA: Sequence, Analysis, and Expression in the Mammary Gland, 180 Biochem. Biophys. Res. Commun. 75 (October 15, 1991).

22 For example, one researcher found that bovine lactoferrin helps halt the process of blood vessel development while human lactoferrin facilitates the growth of new blood vessels. K. Norrby, Human Apo-lactoferrin Enhances Angiogenesis Mediated by Vascular Endothelial Growth Factor A In Vivo, 41J Vasc Res. 293 (July - August 2004).

Accordingly, rhLF is not lawfully marketed as an ingredient in food until it is subjected to the premarket review and approval process for food additives under Section 409 of the FD&C Act. The product only can be marketed pursuant to a food additive regulation establishing the specific conditions under which the additive can be used in the food supply. Until that time, any food product containing rhLF as an additive is adulterated, in violation of Section 402(a)(2)(C) of the FD&C Act.

This result is consistent with the view expressed by FDA in a recent proposed rulemaking that a food additive regulation may be required for products created through recombinant technology:

> FDA recognizes that because breeders utilizing rDNA technology can introduce genetic material from a much wider range of sources than previously possible, there is a greater likelihood that the modified food will contain substances that are significantly different from, or are present in food at a significantly higher level than, counterpart substances historically consumed in food. In such circumstances, the new substances may not be GRAS and may require regulation as food additives.[23]

Finally, CFSAN must take into account the impact that any GRAS determination for rhLF would have upon the INDs for this substance. CFSAN therefore must coordinate with CDER on the consideration of any GRAS premarket notification of rhLF, in order to make certain that the safety requirements of the two Centers are consistent.

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[23] 66 Fed. Reg. 4706, 4711 (January 18, 2001)(internal citations omitted). Although some recombinant products have been determined to be GRAS, these products are distinguishable from rhLF in that the individual ingredients had long marketing histories demonstrating safety, and a substantial amount of data supported the safety of the recombinant form specifically. 55 Fed. Reg. 10932 (March 23, 1990); 58 Fed. Reg. 27197 (May 7, 1993)

IV. Dietary Supplement

Ventria also has suggested that it would promote rhLF for use in dietary supplements and that it currently is conducting clinical trials of this use. RhLF is excluded from the statutory definition of "dietary supplement," however, because it was not marketed prior to FDA acceptance of an IND to research the product as a drug.

The statutory definition of "dietary supplement" excludes articles:

> authorized for investigation as a new drug . . . for which substantial clinical investigations have been instituted and for which the existence of such investigations has been made public, which was not before such approval . . . or authorization marketed as a dietary supplement or as a food.[24]

Agennix has been conducting clinical trials on the use of rhLF as a drug for several years, and has made public these investigations. Results of a study conducted under an Agennix IND were published in 1999 in the journal Alimentary Pharmacology and Therapeutics,[25] and the company has published and presented other clinical trial findings on numerous subsequent occasions.[26] Two ongoing clinical trials sponsored by Agennix currently are listed on the NIH

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[24] Section 201(ff)(3)(A) of the FD&C Act; Pharmanex v. Shalala, 221 F.3d 1151, 1154 (10th Cir. 2000).

[25] A.R. Opekun *et al.*, Novel Therapies for Helicobacter Pylori Infection, 13 Aliment. Pharmacol. Ther. 35 (1999)(Attachment 5).

[26] The company has made presentations at, for instance, the Annual Meeting of the American Association for Cancer Research (AACR)(March 2004), the Wound Healing Society meeting (June 2003), and the American Society of Clinical Oncology (ASCO) meeting (2003), and has published results in the International Journal of Cancer, among others. A. Varadhachary *et al.*, Oral Lactoferrin Inhibits Growth of Established Tumors and Potentiates Conventional Chemotherapy, 111 Int'l J. Cancer 398 (2004).

clinicaltrials.gov website. Agennix also frequently issues press releases describing the company's activities, and stated as early as 2001 that:

> Agennix is a privately-owned biopharmaceutical company focused on research and development of recombinant human lactoferrin (rhLF) . . . and a variety of related peptides. . . . Agennix has completed numerous pre-clinical and clinical trials with rhLF demonstrating the enormous potential of lactoferrin in a wide range of clinical conditions.[27]

We are not aware of any evidence suggesting that human lactoferrin, whether from a natural source or derived through recombinant technology, was marketed as a dietary supplement or food prior to the initial IND submitted by Agennix in September 1996.[28] The cost of producing natural lactoferrin, at $3,600 per gram at 90 percent purity, has been prohibitive.

V. Conclusion

FDA should take appropriate action to ensure that Ventria and Pharming do not attempt to avoid the regulatory requirements applicable to rhLF for use in food or dietary supplements. The activities being conducted by Ventria and Pharming are cause for significant concern because the safety of their products has not been established. The testing or marketing of these products without appropriate oversight may put patients at risk.

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[27] Press Release, Agennix Receives Broad Patent Covering Production of Human Lactoferrin in Eukaryotic Cells (May 10, 2001)(Attachment 6).

[28] If rhLF had been marketed as a dietary supplement prior to the Agennix clinical trials, the product still could not be marketed without a 75-day premarket notification to FDA of intent to market a new dietary ingredient. We conclude that rhLF is a new dietary ingredient because we are not aware of any marketing of the ingredient prior to October 15, 1994. Nor is the ingredient exempt from the requirement on the basis of being extracted from human food.

As FDA has previously stated, allowing these articles to be marketed as food or dietary supplements would undermine FDA's regulation of new drugs. FDA has noted that Congress determined, in enacting section 201(ff)(3) of the FD&C Act, that allowing marketing of these types of products "would not be fair to the pharmaceutical company that brought, or intends to bring, the drug to market, and would serve as a disincentive to the often significant investment needed to gain FDA approval of new drugs."[29]

Sincerely yours,

Peter Barton Hutt
Ruth K. Miller

cc: Joseph R. Baca (HFS-600)
Robert E. Brackett, Ph.D. (HFS-1)
Vasilios H. Frankos, Ph.D. (HFS-810)
Steven K. Galson, M.D., M.P.H. (HFD-1)
David J. Horowitz (HFD-300)
Gerald F. Masoudi (GCF-1)
Susan Walker, Ph.D. (HFS-810)

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[29] FDA, Final Decision in Pharmanex, Inc., Administrative Proceeding, Docket No. 97P-0441 at 4-5 (May 20, 1998).

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May 19, 2005

VIA HAND DELIVERY

Robert Martin, Ph.D. (HFS-255)
Office of Food Additive Safety
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Dear Dr. Martin:

We have become aware that a GRAS notice, Number 162, has been submitted for human lactoferrin purified from rice. In assessing the adequacy of this notice, we request that the Division of Biotechnology and GRAS Notice Review consider the comments in the enclosed letter. This letter was sent by us to officials in CDER and CFSAN in anticipation of the submission of a GRAS notice for recombinant human lactoferrin; we were not aware at the time that the notice had already been filed.

Per our telephone conversation this morning, please forward the attached letter to all relevant persons within the Division. Please do not hesitate to call me or Peter Barton Hutt at (202) 662-5522 if we can provide additional assistance.

Regards.

Ruth K. Miller

Enclosure

Agennix Incorporated

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REC'D MAY 23 2005

May 20, 2005

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AGENNIX

Re: **Ventria GRAS Notice No. 162: Use of Recombinant Human Lactoferrin In Food and Dietary Supplements**

Dear Drs. Tarantino, Schneeman, and Martin,

With respect to the petition for GRAS listing of recombinant human lactoferrin (rhLF) by Ventria BioScience (GRAS Notice #162), we respectfully submit the following public documents for consideration:

"Consumers Union's Comments on USDA Animal Plant Health Inspection Service (APHIS) Environmental Assessment for Field Test of Permit of Ventria Bioscience rice genetically engineered to express human lactoferrin USDA/APHIS Docket No. 05-006-1"
Consumers Union – March 2005

Eight Greenway Plaza Suite 910 Houston Texas 77046 Telephone 713/552-1091 Facsimile 73/552-0795

"Comments on APHIS Environmental Assessment for Permit Application No. 04-302-01r for Outdoor Cultivation of Rice Expressing a Novel, Recombinant Human Lactoferrin Submitted to USDA's Animal and Plant Health Inspection Service Docket No. 05-006-1"
Friends of the Earth - March 25, 2005
(Particularly pages 11-12; 18-22; 34-35)

"Comments on Two Environmental Assessments on Permit Application Number 04-302-01r: Ventria Rice Expressing Lactoferrin (Docket 05-006-1), and Permit Application Number 04-309-01r: Ventria Rice Expressing Lysozyme (Docket 05-007-1)"
The Center for Food Safety - March 24, 2005
(Particularly pages 14-16)

These public documents were submitted by respected consumer advocacy groups in response to Ventria's requests for approval to grow GMO Pharma rice containing recombinant human lactoferrin. The documents point out that recombinant human lactoferrin has not been shown to be safe for general human consumption, and in fact, depending on the full recombinant sequence (which Ventria has never determined or disclosed) and the specific glycosylation, it could be hazardous. There clearly is not a consensus within the scientific community that recombinant human lactoferrin is safe for its intended use and thus it fails to meet the requirements for being considered GRAS.

Additionally, as discussed in the previous submission in this matter by our legal counsel (Peter Barton Hutt and Ruth Miller of Covington & Burling), recombinant human lactoferrin is, and has been, in active clinical development as an investigational new drug regulated by the FDA (CDER), with open INDs in the U.S. since 1996. Granting the present request for GRAS listing would directly contradict recombinant human lactoferrin's existing regulatory status with the FDA as an investigational new drug.

We believe the information submitted currently, together with our previous submission, clearly demonstrates that **Ventria's petition for GRAS listing of recombinant human lactoferrin should be DENIED.**

Please do not hesitate to contact us at the address or phone number below if we can provide any additional information that would be helpful.

Sincerely,

Rick Barsky
Chief Executive Officer

Attachments

Agennix Incorporated

OU

RECD JUN 3 2005

June 1, 2005

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Agennix

Re: **Ventria GRAS Notice No. 162: Use of Recombinant Human Lactoferrin In Food and Dietary Supplements**

Dear Drs. Tarantino, Schneeman, and Martin,

With respect to the petition for GRAS listing of recombinant human lactoferrin (rhLF) by Ventria BioScience (GRAS Notice #162), we respectfully submit the following additional public document for consideration:

"Human lysozyme and lactoferrin therapeutic proteins also have been implicated in pathological conditions": Comments submitted to USDA/APHIS Docket Nos. 05-006-1 and 05-007-1"

Professor Joe Cummins, The University of Western Ontario – April 2, 2004

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Re: Pharming Group N. V.
Comments in response to comments filed in opposition to CFSAN "approval" of GRAS Notification number 162

Dear Drs. Tarantino and Mattia:

On December 22, 2004 Ventria BioScience filed a GRAS Notification with CFSAN pertinent to use of its human lactoferrin product in food. CFSAN subsequently entitled such Notification GRN No. 162.

Subsequently, Covington & Burling and one of its clients, i.e., Agennix Incorporated, filed comments pertinent to GRN No. 162 (on February 4 and May 19, 2005 and on May 20 and June 1, 2005 respectively) which – in essence – requests that CFSAN deny Ventria's request for GRAS status of its human lactoferrin product. Since such comments also specifically reference Pharming and its future intent to file a GRAS Notification concerning its human lactoferrin product for use in food **and,** thus, make such comments pertinent also to Pharming's future notice, Pharming has requested that I – as their US regulatory counsel – respond to the filed comments. Thus, what follows are these comments.

First, Pharming has **no** position on whether Ventria's notification should be "approved" or "denied" by CFSAN. Pharming expects CFSAN to review such notification (and all such notifications) consistent with **only** the regulatory requirements pertinent to GRAS filings and to base its final decision **solely** on whether Ventria (or any similarly situated petitioner) has met the legal burden emanating from such requirements.

Second, to the extent that the above-referenced comments argue the obvious – and they, in part, do – that is, that if one seeks to place a product into interstate commerce which product is adequately associated with a claim or claims that legally amount to a claim or claims pertinent to, for example, an infant formula, a health claim, a dietary supplement or a drug, then such entity must comply with all regulatory requirements pertinent to the specific claim or claims in question, Pharming agrees with such statement(s). It's **not** clear from a review of Ventria's GRAS Notification that Ventria is seeking to avoid or ignore such requirements; however, CFSAN can assure that Ventria does not simply by including appropriate language in any final GRAS "approval" letter (if such letter is otherwise to be

issued) similar to the language CFSAN included in its "approval" letters to those seeking GRAS status for their bLF products.

Third, the comments argue that "recombinant human LF is not now, and has not ever been, used as an ingredient in food...". However, to the extent that any hLF product is specifically shown to be identical to or substantially similar to native hLF then such argument is scientifically incorrect since hLF has been safely consumed as a part of food, i.e., mother's milk, for thousands of years – just as bLF has been.

Fourth, the argument that once a substance is associated with an approved IND it cannot be used **in any way** as a component of food is nonsense and **not** supported by any law, including Section 201(ff) (3) of the FD&C Act. As the comments conveniently fail to mention, CFSAN currently has full and express authority to "approve" a substance for food use – even though authorized for investigational use – under Section 201 (ff) (3)(B)(ii) of the FD&C Act.

Fifth, the comment that all safety requirements pertinent to use of a substance as a drug must also be applied to that same substance if used as a component in food is not supported by any current law (and **none** is cited in support of such statement). A substance intended for use as a drug must meet all pertinent regulatory requirements applicable to drugs while the same substance intended for use merely as a component of food must meet the requirements pertinent to food and – to the extent applicable – requirements pertinent to whether a food additive or a GRAS substance.

Sixth, the comments seem to argue that either Ventria or Pharming or both are attempting to commercialize their respective hLF products without first interfacing appropriately with CFSAN. At least with regard to Pharming, such a suggestion is not accurate. Ventria has filed a GRAS Notification pertinent to its product (and had done so prior to the filing of the above-referenced comments) and Pharming intends to file a GRAS Notification pertinent to its.

Seventh, in contradictory fashion, the comments acknowledge that – under current food law – one can have a substance to be used in food either approved by CFSAN as a food additive or determined by CFSAN to be GRAS, **but then** argue that the hLF products must be regulated only via food additive petition and then argue that such products cannot be "approved" at all in any fashion. As CFSAN

knows from decades of applying currently, pertinent, legal requirements to both food additives and GRAS substances, only the first argument is correct. As has been known for decades, if one can show (via the regulatory requirements pertinent to GRAS determinations) either via "experience based on common use in food" or on "scientific procedure" (or on both) that the use(s) of a substance in food is safe under the conditions of its intended use, such use can be determined to be GRAS – if the determination is made by a consensus of qualified experts. (Section 201(s) of the FD&L Act).

Eight, the comments acknowledge that there exists a valid "pertinent exception", i.e., a GRAS determination, to the general food additive rule, but argue that the commenter's review of "the published literature" does not qualitatively or quantitatively support the safety of either Ventria's or Pharming's product. With all due respect to such "review", it is the data and information actually in a GRAS Notification – not otherwise – that legally determines GRASness, and Pharming's GRAS Notification will be filled with as much published, quality information – if not more – than has ever been presented to CFSAN in such a submission. To suggest that there currently exist a paucity of relevant information in the scientific literature is to have missed over 1,000 pertinent published scientific papers.

Ninth, the comments seem to argue that either Ventria's or Pharming's products or both may differ from native hLF and therefore, cannot be shown to be safe. If the information in a GRAS Notification adequately demonstrates safety and general recognition (as these terms have been applied for decades), then the subject of such notification can – indeed should be – deemed GRAS regardless of whether the subject form is exactly like the native form. Of course, to the extent that the subject form is substantially equivalent or identical to the native form will presumably be of interest to all parties concerned – including the qualified experts.

Tenth, the comments indicate that only "some products" (indicating a very small number) derived via recombinant activities have been approved by FDA, and then list only three drug products. Rather than citing irrelevant, approved drug products, perhaps the commenter should have cited the over twenty substances intended to be used in food that have been determined to be GRAS by CFSAN – **all** of which emanated from recombinant technology and **all** of whose safety –

contrary to the commenter's assertion (see footnote 23) – was based solely on "scientific procedures" (not – as asserted – on "long marketing histories").

Eleventh, the comments seem to want to differentiate hLF from bLF probably because the arguments contained in the comments are weakened by the fact that bLF has been determined to be GRAS. And importantly – and regardless of whether bLF and hLF are identical – bLF has been repeatedly determined by CFSAN to be GRAS based solely on scientific procedures (**not** on any experience based on common use in food).

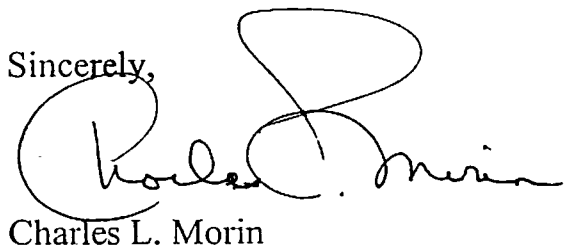
In summary, Pharming has not sought and will not seek to avoid any U.S. regulatory requirements pertinent to lawful use of its hLF product in food in the U.S. Indeed, to that end, it has repeatedly communicated with CFSAN over the years and even met with CFSAN (as long ago as in January of 2001) to make sure that it was pursuing the proper regulatory pathway for use of its product in food. It will continue to do just that.

* * * * *

If after receiving the foregoing information you should have questions or need additional information, please let me know.

Thank you in advance for considering the above-referenced information.

Sincerely,



Charles L. Morin

From: Charles L. Morin [mailto:charleslmorin@earthlink.net]
Sent: Thursday, November 15, 2007 5:58 PM
To: Brackett, Robert
Subject: Request for a meeting

Dear Dr. Brackett,

Please find attached an e-copy of a letter whose formal version is on its way to you via FedEx for delivery Friday, November 16th. I will contact you early next week to try to make arrangements for a meeting date which is convenient for all interested parties.

Best regards.

Charles L. Morin
Morin & Associates
388 Market Street, Suite 1460
San Francisco, CA 94111
US

Phone: (415) 957.0101
Fax: (415) 957.5905

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November 15, 2007

Robert E. Brackett, PhD (HFS-001)
Director (Room 4B-064)
Center for Food Safety
and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Pharming Group N.V.
Notice of GRAS exemption for human lactoferrin derived from the milk of transgenic cows expressing a human gene encoding human lactoferrin
GRN No. 000189
Request for a meeting

Dear Dr. Brackett:

As background, this letter concerns the current regulatory status of Pharming's GRAS Notification (i.e., GRN No. 189) concerning use of human lactoferrin for certain food uses (as specifically set forth in the GN). Such uses are identical to those already authorized by CFSAN in connection with use of bovine lactoferrin. (See GN number 77 and its associated "no questions" letter dated 08/14/01). So far, the regulatory events associated with Pharming's GN are as follows:

Date	Event	Running Clock
12/29/05	Pharming files GN (supported by qualified experts)	NA
12/30/05	CFSAN receives GN	NA

Date	Event	Running Clock
01/03/06	CFSAN acknowledges receipt of GN	NA
01/12/06	CFSAN "files" GN	Day 0
05/17/06	Pharming receives email from CFSAN; CFSAN has no questions about the content of the GN; CFSAN has questions about whether hLF induces any adverse, non-allergic response by the adaptive immune system.	Day 125
09/01/06	CFSAN and Pharming hold teleconference concerning CFSAN's questions and related matters	Day 232
12/22/06	Pharming files a qualified experts' comprehensive response to CFSAN's questions	Day 344
12/26/06	CFSAN receives Pharming's response	Day 348
03/09/07	CFSAN and Pharming hold teleconference concerning whether to hold a Part 15 hearing in the near future	Day 421
07/26/07	Pharming updates its GN file	Day 560
10/05/07	Pharming meets with Dr. Mattia to unstall review of its GN	Day 631
10/12/07	Pharming meets with Dr. Tarantino to unstall review of its GN	Day 638
11/15/07	Pharming requests meeting with Dr. Brackett to unstall review of its GN	Day 671

As you can see, CFSAN's process of reviewing Pharming's GN and reaching a final decision on the merits has become overwhelmingly stalled. At

this point, such stall has consumed over **eight** months and is increasingly functioning to irrevocably and substantially harm Pharming. The Part 15 Hearing that was supposed to have taken place by mid summer has not occurred; indeed, there has not yet even been a notice published in the Federal Register announcing such a Hearing. Neither Drs. Mattia nor Tarrantino (through no fault of theirs) can unstall the ongoing regulatory process. Thus, you need to intercede to unstall the process.

Accordingly, to make this all happen, Pharming respectfully requests a meeting with you as soon as can be arranged. Pharming suggests the following dates for your consideration – an afternoon meeting on November 26, 27, 28 or December 3, 4 or 5.

The suggested agenda for such meeting – in order to unstall this entire matter – would include (subject to your input) a discussion of:

1. whether there needs to be some sort of public involvement in a GRAS Notice review (especially since the pertinent regulation does not call for such involvement);
2. if not, then number 5;
3. if so, whether such involvement must amount to a hearing (Part 15 or otherwise) or whether another, just-as-useful means – such as notice and comment – might suffice;
4. if there must be public involvement when and how such will take place;
5. if no public involvement is necessary, when Pharming can reasonably expect a final decision on the merits of its GN.

As each delay day occurs, Pharming becomes more and more harmed by the ongoing stall. Thus, we hope that you will act quickly to accommodate Pharming's request for a meeting.

If after reviewing the foregoing you should have questions, please let me know.

Thank you in advance for your attention to and consideration of Pharming's request.

Sincerely,

Charles L. Morin

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
College Park, MD 20740

MEMORANDUM OF MEETING

Date: December 11, 2007

Place: Center for Food Safety and Applied Nutrition, FDA, College Park, MD

Participants:

Industry

Frans de Loos, Pharming
Charles L. Morin, Morin and Associates
Anuras Relan, Pharming

FDA

Michael Landa, Deputy Director for Regulatory Affairs, OCD/CFSAN
Laura Tarantino, Director, Office of Food Additive Safety, CFSAN
Jeremiah Fasano, OFAS/CFSAN
Catherine Copp, Office of Regulations Policy and Social Sciences, CFSAN
Louisa Nickerson, Office of Chief Council, Office of the Commissioner, FDA
Anne Crawford, Science Policy Analyst, Executive Operations Staff, OCD/CFSAN

Subject: Pharming GRAS Notification

The meeting was held at the request of Mr. Morin to discuss issues related to Pharming's GRAS submission for human lactoferrin.

Mr. Morin expressed interest in hearing from FDA what is necessary to get to a final determination on his client's (Pharming) GRAS submission which was filed in 2005. He noted that the Office of Food Additive Safety (OFAS) has indicated it might want to have a public meeting to get input before reaching a final decision.

Participants discussed the effect of the provisions of the newly passed Food and Drug Administration Amendments Act (FDAAA) (Section 912) on the Pharming GRAS submission for lactoferrin. Mr. Morin stated he believes that Section 912 becomes relevant only after a decision on GRAS status has been made and only then if a product is introduced into U.S. commerce. Mr. de Loos indicated Pharming is interested in getting a response to their GRAS submission, irrespective of the potential impact of Section 912.

Mr. Morin indicated he/Pharming has updated information on the GRAS submission since March 2007 to address outstanding issues and is willing to share this information with FDA.

At the close of the meeting, FDA participants indicated that CFSAN still does want public input on the scientific issues presented by the GRAS submission, but in light of today's meeting needs to consider how and when to do that. FDA participants agreed to get back to

Mr. Morin/Pharming, shortly after the first of the year, on the timeline for moving forward on a decision on the lactoferrin GRAS submission.

Anne B. Crawford

Docname:H:\MEMORANDUM OF MEETING - Pharming121107.doc
Drafted:ABCrawford:HFS-022:12/18/07
Edit/Clear: MLanda:HFS-002:12/18/07; JFasano:HFS-255:12/18/07; LTarantino:HFS-200:12/18/07; CCopp:HFS-004:12/18/07
Review/clear:LNickerson:GCF-1:12/18/07
f/t:ABCrawford:HFS-022:12/19/07

cc: MLanda: HFS-002
LTarantino:HFS-200
JFasano:HFS-255
CCopp:HFS-004
LNickerson:GCF-1
HHorn:HFS-022
RWheeler HFS-022

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December 12, 2007

MEMORANDUM

Section 912 of The FDA Amendments Act of 2007 Does NOT Apply Retroactively

This memorandum addresses the statutory construction of the newly enacted Section 912 of the Food and Drug Administration Amendments Act of 2007 (FDAAA), specifically whether Section 912 has a retroactive application. 1/ The short answer is no—Section 912 of the FDAAA does not apply retroactively to products that were lawfully marketed prior to enactment on September 27, 2007.

As a general rule, retroactive application is not favored by courts, and statutes are ordinarily given a prospective application barring express intent of retroactivity by Congress. The long-standing presumption against retroactive legislation is deeply routed in our jurisprudence and embodies a legal doctrine "centuries older than our Republic." 2/ This rule favoring prospective application of statutes remains strong in the courts today. Section 912 of the FDAAA clearly does not warrant an exception to the general presumption against retroactivity.

The Supreme Court provides a history of the presumption against retroactivity and expounds upon the standard in *Landgraf v. USI Film Products*. 3/ The Court notes that the presumption against statutory retroactivity is "deeply rooted in this Court's jurisprudence and finds expression in several provisions of our Constitution." 4/ When determining the retroactive

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1/ Section 912 entitled, "Prohibition against food to which drugs or biological products have been added," makes it a prohibited act under the Federal Food, Drug and Cosmetic Act (FFDCA) to introduce into interstate commerce any food to which "has been added a drug approved under section 505, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and for which the existence of such investigations has been made public."

2/ *Landgraf v. USI Film Products*, 511 U.S. 244 (1994) (citing *Kaiser Aluminum & Chemical Corp. V. Bonjorno*, 494 U.S. 827 (1990)).

3/ *Id.*

4/ *Id.* at 265. Citing the *Ex Post Facto* Clause (prohibiting retroactive application of penal legislation), Article I, § 10, cl. 1 (prohibiting States from passing another type of retroactive legislation, laws "impairing the Obligation of Contracts), Fifth Amendment's Takings Clause (preventing the legislature (and other government actors) from depriving private persons of vested property rights except for "public use" and upon payment of "just compensation"), The

or prospective application of a newly enacted statute, courts first determine whether Congress expressly intended retroactive application, and if so, whether retroactive application would have an impermissible retroactive effect -- which is the case if it would impair rights a party possessed when he acted, increase a party's liability for past conduct, or impose new duties with respect to transactions already completed. 5/

Importantly, the Supreme Court in *Bowen v. Georgetown University Hospital* found that "congressional enactments and administrative rules will not be construed to have retroactive effect unless their language requires this result." 6/ The Supreme Court has also found that "requiring clear intent assures that Congress itself has affirmatively considered the potential unfairness of retroactive application and determined that it is an acceptable price to pay for the countervailing benefits. Such a requirement allocates to Congress responsibility for fundamental policy judgments concerning the proper temporal reach of statutes, and has the additional virtue of giving legislators a predictable background rule against which to legislate. " 7/ The Court has gone so far as to say that "even where some substantial justification for retroactive rulemaking is presented, courts should be reluctant to find such authority absent an express statutory grant." 8/

The language of Section 912 of the FDAAA does not warrant retroactive application. Nowhere in the FDAAA did Congress provide a clear statement indicating that it intended to upset the normal presumption of prospective application. Nor is there any legislative history to suggest this. As stated by the Supreme Court, "if the statute would operate retroactively, our traditional presumption teaches that it does not govern absent clear congressional intent favoring such a result." 9/ The result is clear – Section 912 of the FDAAA operates prospectively.

Due Process Clause (protecting the interests in fair notice and repose that may be compromised by retroactive legislation).

5/ *Ojeda-Terrazas v. Ashcroft*, 290 F.3d 292 (5th Cir. 2002). See e.g., *Landgraf v. USI Film Products*, 511 U.S. 244 (1994). Congress clearly did not intend Section 912 of the FDAAA to apply retroactively, therefore, a full discussion of whether retroactive application of the statute would have an impermissible retroactive effect is not necessary. It is clear, however, that retroactive application of Section 912 of FDAAA would unjustly impair the property rights of manufacturers who currently market food products that contain ingredients that would be defined as "drugs" and have done so for years. Any considerations of fair notice, reasonable reliance, and settled expectations would find that, absent clear direction from Congress to the contrary, these manufacturers should not be deprived of their rights.

6/ 488 U.S. 204, 208 (1988). See e.g., *Greene v. United States*, 376 U.S. 149 (1964), *Claridge Apartments Co. v. Commissioner*, 323 U.S. 141 (1944), *Miller v. United States*, 294 U.S. 435 (1935), *United States v. Magnolia Petroleum Co.*, 276 U.S. 160 (1928). Numerous courts have similarly found that express intent is required for retroactivity to be applied, stating that "words in a statute ought not to have a retrospective operation unless they are so clear, strong and imperative that no other meaning can be annexed to them, or unless the intent of the legislature cannot otherwise be satisfied." *Alyeska Pipeline Service Co. V. U.S.*, 624 F.2d 1005 (1980). See e.g., *Union Pacific Railroad Co. v. Laramie Stock Yards Co.*, 231 U.S. 190 (1913), *United States v. Heth,* 7 U.S. (3 Cranch) 399 (1806).

7/ *Landgraf* at 272.

8/ *Bowen* at 208.

9/ *Landgraf* at 280.

-----Original Message-----
From: Levitt, Joseph A. [mailto:JALevitt@HHLAW.com]
Sent: Thursday, December 13, 2007 3:43 PM
To: Tarantino, Laura M
Cc: Landa, Michael; Masoudi, Gerald F
Subject: Memorandum on Why Section 912 is NOT Retroactive

Laura--

This follows up on my earlier letter and our telephone conversation concerning FDA's implementation of Section 912 of the FDA Amendments Act of 2007. One question you said was being discussed was whether Section
912 was retroactive in its application. We have since researched the point, and the case law is very clear: There is a strong presumption that Congress intends all statutes to apply only PROSPECTIVELY. Accordingly, if Congress wants a statute to apply retroactively, it must say so expressly. Because Section 912 has no such express statement, it must be applied prospectively only.

Attached is a memorandum that summarizes the case law on this point, including Supreme Court cases. Please share with the Office of Chief Counsel for use in developing your implementation plan. We hope this assists the agency in dismissing any concern about retroactivity, and instead allows you to apply your attention to Section 912's prospective application, particularly with respect to recombinant human lactoferrin.

Please let me know if you have any additional questions on this subject.

Best regards,

Joe

_____

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December 26, 2007

Laura M. Tarantino, PhD (HFS-200)
Director (Room 3044)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
4300 River Road
College Park, MD 20740

Re: Pharming Group NV
Notice of GRAS exemption for human lactoferrin derived from the milk of transgenic cows expressing a human gene encoding human lactoferrin
GRN No. 000189
Follow up to 12/11/07 meeting

Dear Dr. Tarantino:

This letter is being forwarded to you since during our recent meeting you indicated that future communications should be with you. Please make it available to your colleagues as you deem appropriate.

Thanks very much to you and your FDA colleagues for providing Pharming with an opportunity to discuss matters pertinent and important to obtaining a final determination – on the merits – concerning its GRAS Notification, i.e., GRN No. 000189.

We appreciated the opportunity to discuss the status of CFSAN's review and its progress towards a "no questions" letter.

During the meeting, several important (indeed, critical) matters were discussed. These are summarized and memorialized below in highlighted subparts

Information pertinent to Pharming's GN

Repeatedly over the last almost nine months, Pharming has been informed that CFSAN has **no** further questions for Pharming with regard to the need for any additional information pertinent to Pharming's GN – that is, that Pharming has supplied all that is required to meet Pharming's legal obligation of providing sufficient scientific information to support its GN. During the meeting this position was again affirmed by CFSAN personnel.

Pharming has been repeatedly encouraged by CFSAN to keep its GN file updated, and Pharming has updated from time to time its GN file. To that end, please find attached additional updating information.

The current status of Pharming's GN

Contrary to what Pharming has been **repeatedly** told over the last several months, i e., that nothing was impeding progress on Pharming's GN except for obtaining final, go ahead approval from upper FDA management for conduct of a Part 15 hearing, we were very surprised to learn during the meeting that all progress on Pharming's GN had stopped some time ago because it is believed by, at least, some at CFSAN that Pharming's product cannot successfully make it through and out of the gauntlet posed

by Section 912 and, therefore, there is no point in wasting CFSAN resources on completing the GN when Pharming's product will never be able to be introduced into interstate commerce. This very critical acknowledgement warrants several responses.

First and most importantly, CFSAN has no legal authority to significantly deviate from the express legal duty imposed on it with respect to receiving, reviewing, and making a final determination on the merits of a GRAS Notification. The duties imposed on CFSAN expressly emanate from 21 CFR § 170.36. Failure to act in a reasonable fashion on a GN amounts to unlawful conduct and arbitrary and capricious conduct in violation of the Administrative Procedures Act. Accordingly, Pharming expects that from this time forward, Pharming's GN will receive the attention and action it is legally entitled to.

Second, Section 912 in **no** way prohibits CFSAN from acting in accordance with Section 170 36 or other APA-related legal requirements. Indeed, Section 912 has **nothing** to do with the regulatory process involved when GRASing a substance. Section 912 requirements do **not** even arise unless and until:

1 a **qualifying substance** has been added;

2 to a **food**;

3. which food has been **introduced**,

4 into **interstate commerce**.

Then and only **then** does the regulatory status of the qualifying substance come into question. Of course, to the extent that any such substance – **at that time** – is found to meet any of the eight, express exceptions (to the general prohibition rule of Section 912), then such substance would **not** be prohibited from being introduced into interstate commerce.

Third, any attempt to currently apply Section 912 to Pharming's hLF product would be arbitrarily and capriciously premature for two reasons. First, until and unless the many, key words and/or phrases used in Section 912 and left undefined are defined (perhaps in a guidance document), no reasonable person could apply Section 912 to any actual, factual scenario. Second, unless there exists an actual factual scenario to which Section 912 requirements can be applied, any hypothetical scenario is speculative and not ripe for adjudication Accordingly, CFSAN needs to deal with Section 912 matters **only** after an actual, factual scenario arises which triggers Section 912's provisions.

Finally, as indicated to you during the meeting, receiving CFSAN's no questions letter in response to Pharming's GN is very important to Pharming even if Pharming were never to introduce its hLF product into interstate commerce in the United States. Needless to say, introduction of a new product on a world-wide basis is a very complicated, multi-faceted process. The strategy for doing so should be left to the sponsor, while regulatory bodies should respond as required by law to the regulatory implications actually and when raised by such strategy.

Need for a hearing

CFSAN has indicated – although less vigorously recently – that it may need some sort of public hearing before it can make a final determination on Pharming's GN. Although Pharming does not object per se to meaningful public participation when it can be of real value, in this case Pharming believes **no** public hearing is necessary. To date (and over the last approximately 30 years), more than 200 products – including drugs, devices and food substances to be added to food – emanating from numerous

transgenic platforms (all incorporating use of recombinant technology) have been via various types of submissions reviewed and authorized by FDA for use in interstate commerce. **Not one**, including numerous, injectable, **human proteins** has required any public hearing before being authorized for use by FDA. To date, CFSAN has not cited to Pharming any adequate reason(s) for altering this well-established and long-followed precedent. If CFSAN feels the need for additional, qualified expert advice, it should, for example, bring in for a day a group of qualified experts and let them consider whether the scientific opinions conveyed by Pharming and its independent, qualified experts to CFSAN are indeed correct and representative of the consensus view among qualified experts

With regard to additional qualified expert input, please note the information contained in the attached update document. It includes, among many other pieces of information, additional reviews by qualified expert groups from around the world concerning the impact of consuming hLF As you will see, they all agree with Pharming's experts' assessment that such consumption does not pose any unique risk of untoward event to those doing the consuming. In addition and very importantly, such document establishes that the proposed daily consumption level of hLF pales in comparison to the huge, natural, background of hLF that an individual can be exposed to on a daily level for, perhaps, a lifetime.

For all the foregoing reasons, Pharming believes that no hearing is necessary and that CFSAN currently has before it all the information – including various opinions of numerous, eminently-qualified experts – it needs to make a final decision on Pharming's GN.

Pharming trusts that there will be no more delay in dealing with Pharming's GN. It looks forward to hearing from you by the end of January (or sooner, if possible) as to

exactly what process is now to be applied to Pharming's GN and what timelines will be followed for applying and reaching an end to such process.

Thank you again for your and your colleagues' efforts to resolve the current, rather lengthy stall associated with the GN If at any time I can be of assistance to you with regard to any aspect of this matter, please do not hesitate to contact me.

Sincerely,

Charles L. Morin

Pharming, NV

Updating Information Pertinent To GRN No. 000189 Which Involves Production Of Recombinant Human Lactoferin From Transgenic Cows

December 26, 2007

Table Of Contents

Pharming, NV

Updating Information
Pertinent To
GRN No. 000189
Which Involves
Production Of
Recombinant Human Lactoferrin
From
Transgenic Cows

A. Introduction

From time to time Pharming – via the encouragement of CFSAN – has forwarded additional information pertinent to its GRAS Notification (i.e., GRN No. 000189 which pertains to certain specified food uses of human lactoferrin derived from the milk of transgenic cows expressing a human gene encoding human lactoferrin) to keep such GN updated. What follows is more of such information. For the convenience of the reader, it has been subdivided into those subareas which have been the bases of discussion with CFSAN over the last approximately nine months.

B. Subareas Of Discussion

1. Subarea 1 (concerning the precedential value emanating from the GRASing of bLF)

The scientific literature indicates that bovine lactoferrin and human lactoferrin have very similar biological activities (with regard to their ability to

induce immunological effects). Bovine lactoferrin has been significantly, safely, and long-consumed by both infants and adults either in purified form (as a functional food or as an ingredient added to numerous foods) or via the drinking of cow's milk. To what extent can this long history of safe, human consumption of bovine lactoferrin contribute to assessing the safety of transgenically produced human lactoferrin when added to and consumed in certain foods?

2. Pharming Update

a. Introduction

This subarea raises several subsidiary subareas. Each of these is identified below and then responded to.

b. Identification of bovine lactoferrrin

The term "lactoferrin" refers to a specific glycoprotein found in, among other tissues, the milk of almost all mammals. (Nuijens, 1996; Lonnerdal, 1995). Bovine lactoferrin (i.e., "bLF") is the version of lactoferrrin found in the milk of cows. (Nuijens, 1996). Bovine lactoferrin is – like human lactoferrin (i.e., "hLF) – an iron-binding glycoprotein (of about 80 kDa) which is substantially similar in structure and function compared to its human homologue, i.e., hLF. (Nuijens, 1996). The amino acid sequence of bLF (which contains 689 amino acids) shows 69% homology with hLF. (Pierce, 1991). The sequence of bLF contains five possible N-linked glycosylation sites (as compared to three such sites in the sequence of hLF). Four sites, i.e., Asn 233, 368, 476, and 545, are always utilized (Spik, 1994) while the fifth (Asn 281), located in the N-lobe, is glycosylated in about 30% and 15% of the molecules in bovine colostrums and mature milk, respectively (van Veen, 2002; Wei, 2000; Yoshida, 2000). (For

additional, extensive technical information concerning bLF see GRAS Notices 0042, 0067, 0077 and 0130).

c. bLF's and hLF's ability to induce immunological effects

Bovine lactoferrin and human lactoferrin have both been extensively studied – especially over the last two decades and often in the same study – for their potential to induce immunological effects. Indeed, more than 100 such studies have been published in the scientific literature and are directly referenced in this update document.[1] Pharming generally agrees that such studies indicate that bLF and hLF have very similar biological activities (with regard to their ability to induce immunological effects). However, to be as precise as possible, one should note that a careful reading of all such studies also indicates that:

1. bLF induces – qualitatively or quantitatively – certain immunological effects not induced by hLF; and
2. hLF does **not** induce any immunological effect not also induced by bLF

Thus, bLF is somewhat more biologically active than hLF. (More about such activity in subpart (7)).

d. bLF's safety

(1.) Long term

Milk from cows and other dairy products have been significantly, consistently, and long-consumed by populations all over the earth for well over 2,000 years. (See, e.g., GRN Nos. 42 and 77). As sources became more available and safety became assured, increasing numbers of individuals consumed increasing amounts of milk and dairy products. Today, in the United

[1] In addition, over 200 other such studies are indirectly referenced in the referenced, published articles. Since they are older studies and duplicative of the substance contained in the referenced studies, they are not directly referenced.

States, so long as milk and dairy products are derived from locations following good dairy practices and are properly pasteurized, they are broadly viewed as being generally recognized as safe – based on, at least, long history of common use as food.

Current, daily consumption of milk and dairy products (and, thereby, bLF) in the United States amounts to the following:

Age Groups	MDP (g/d)[2]		bLF (mg/d)[3]	
	Ave.	**90%**	**Ave.**	**90%**
1-12	396	731	40	74
13-19	377	747	38	75
≥ 20	240	500	24	50

(GRAS Notice 0042/0077). Thus, persons consuming milk and dairy products are regularly and for a lifetime exposed to from 24-75 mg bLF/day (with such exposure decreasing by about half, i.e., from a max of 75 to a minimum of 24, over one's life).

This level of long-term exposure to bLF is **not** known to be associated with any adverse, immunologically-related effects. (See, e.g., GRAS Notices 0042, 0067, 0077, amd 0130).

(2.) GRAS status

In addition to the above-described, long history of safe consumption of bLF – via the consumption of cow's milk and other dairy products – bLF (as a stand alone substance derived from milk) has been repeatedly reviewed by FDA as to whether it is generally recognized as safe ("GRAS") (for certain intended use(s)) and repeatedly determined by FDA to be GRAS. (See FDA "no questions" letters – dated 8/14/01, 10/23/01, 8/21/03, and 5/27/04 – in response

[2] MDP = milk and dairy products
[3] One gram or ml of milk contains 0.1 mg bLF. (Barth, 1997).

to GRAS Notice numbers 000067, 000077 and 000130). In each such instance, GRASness was based primarily on "scientific procedures", i.e., on animal toxicity and genotoxicity studies, which demonstrated that bLF exposure levels up to 2,000 mg/k BW/day were neither toxic nor genotoxic. (See, e.g., FDA letter – dated 8/14/01 – in response to GRN 000077 at page 2). In addition, GRASness was based on results from human studies in which (1.) infants were exposed to dose levels ranging from 1.4 mg/d (0.3 mg/kg BW/d) to 2.9 g/d (1.0 g/kg BW/d) and study durations from 11 days to 5 months and (2.) adults were exposed to dose levels ranging from 100 mg/d (1.7 mg/kg BW/d) to 3.6 g/d (60 mg/kg BW/d) and study durations from single dose to 8 weeks. (See, e.g., GRAS Notice 0042/0077). In about half of these studies (i.e., 5 of 12), subjects were exposed to both bLF and hLF (but not at the same time, i.e., in separate arms). Finally, GRASness was also based on an expert panel of immunologists' determination that lactoferrrin is highly unlikely to induce either an allergic response or any autoimmune disease, especially in adults. (Id. at page 3). Accordingly, FDA authorized two uses of bLF as an "antimicrobial" agent and, importantly, for "use as an ingredient in sports and functional foods at a level of 100 milligrams per product serving". (See, GRAS Notices 000067, 000077, and 000130).

e. Safety of hLF

(1.) Introduction

Assessing the safety of hLF (including the safety of long-term consumption) – like assessing the safety of bLF – is dependent on several subassessments. Each of these is identified and discussed below.

(2.) hLF naturally occurs in humans

Notwithstanding that bLF does **not** naturally occur in humans, is approximately 31 percent different than hLF in its amino acid sequence, has

more glycosylation sites (i.e., two more) than does hLF, and induces (at a minimum) the same immunological effects as hLF, bLF has been appropriately deemed by FDA to be GRAS – and at the exact daily exposure level and uses that Pharming is seeking for its hLF product. In contrast, hLF is native to humans and – as discussed in significant detail in subsequent sections – is present both endogenously and exogenously at significant levels far in excess of that being sought (via Pharming's GRAS Notice) as a daily exposure level; thus, one would not expect hLF to induce any safety concerns beyond those induced by bLF. And there is **no** direct, scientific evidence that hLF does.

(3.) Long history of exposure to hLF

As discussed in very significant detail in the updating information pertinent to subareas 3 and 4, virtually all humans have significant and lifelong exposure to both endogenous and exogenous hLF from about week 12 in utero onward. As also discussed, such long-term exposure is at levels significantly higher than those long-term exposure levels to bLF. Accordingly, if lesser levels of exposure to bLF are sufficient to determine that bLF is GRAS (at the same level of daily exposure and use as is being requested by Pharming), then significantly greater levels of long-term exposure to hLF should suffice to determine that hLF (at the same levels GRASed for bLF) is GRAS.

(4.) Regarding host organism

It may be obvious, but since it is of a critical nature it should be noted that both bLF and – in this specific instance – Pharming's hLF are produced by the same host organism, i.e., a cow, and appear in the same, naturally-occurring, bovine product, i.e., cow's milk. As Pharming's GRAS Notice discusses in significant detail, the transgenic cows in question are treated just like dairy cattle pursuant to good dairy practices and are no different than ordinary dairy cattle except that they contain a single, extra gene – in this case responsible for

producing hLF (GRAS Notice 0189). Such cattle do not represent any identified risk beyond those that have been identified over decades and which are currently adequately dealt with via compliance with good dairy practices. In addition, the milk emanating from such transgenic cattle is identical to milk emanating from normal dairy cattle except for the presence of hLF. Such presence has been shown – see next section – to be of no risk to either the dairy cows or to an individual consuming up to 2000 mg hLF/kg BW/day. Thus, the fact that the hLF here in question emanates from a transgenic source presents – **in this specific instance** – no additional, novel risks.

(5.) Regarding "scientific procedures" evidence

Bovine lactoferrin was determined by its sponsors and FDA to be GRAS based on "scientific procedures" (e.g , see GRAS Notice 0042/0077; see also, GRAS Notices 0067 and 0130) More specifically, the safety of bLF was evaluated in a series of published animal toxicity and genotoxicity studies[4] which demonstrated that consumption of bLF at levels up to 2000 mg/kg BW/day given up to 13 weeks produced no adverse effects. (See "no questions" letter from FDA – dated 08/14/01 – to sponsor of GRAS Notice 0077). With one exception (discussed at length in subsection 7 that follows), FDA determined that such testing was adequate since it issued a "no questions" letter.

Pharming also is primarily relying on "scientific procedures" to demonstrate that its hLF is GRAS. It too tested its hLF via published, animal toxicity and genotoxicity studies – although such testing was considerably **more extensive** than that conducted in support of bLF. Such testing demonstrated that hLF was also **not** genotoxic and also did **not** produce any adverse effects when consumed up to 2000 mg/kg BW/day. (GRAS Notice 0189).

Pharming also included other published information emanating from Rhesus monkey studies which indicated that hLF was safe to consume up to 79 mg/kg BW/day. (GRAS Notice 0189, page 31).

[4] Clinical studies are discussed in the next section.

(6.) Regarding clinical evidence

The sponsor of GRAS Notices 0042 and 0077 (pertinent to use of bLF) also provided human clinical information supporting the GRAS status of bLF. (GRAS Notice 0042, pages 85-91). Such studies – which in about half the instances (as indicated above) also included separate evaluation of hLF – exposed subjects as follows:

Infants

Doses – from 1.4 mg/day (0.3 mg/kg/day) to 2.9 g/day (1.0 g/kg/day)

Duration – from 11 days to 5 months

Adults

Doses – from 100 mg/day (1.7 mg/kg/day) to 3.6 g/day (60 mg/kg/day)

Duration – from a single dose to 8 weeks

FDA found these studies to be sufficient, since it issued a "no questions" letter.

Despite the fact that humans are naturally exposed to significant quantities of hLF from in utero to death (i.e., from 50-3100 mg/kg BW/day during the first 12 months of life and from 10-200 mg/kg BW/day during the rest of life (see subparts 6, 8 and 10 infra)),Pharming also included clinical information in its GN in support of the safety of hLF. Such twenty-six studies exposed subjects as follows:

Infants

Doses – from 700 mg/day (150 mg/kg/day) to about 5 g/day (1538 mg/kg/day)

Duration – from 1 day to 21 days

Adults

Doses – from 52 mg/day (0.87 mg/kg/day) to 15 g/day (250 mg/kg/day)

Duration – from 1 day to 42 days

As can be seen by comparing the two sets of doses and durations pertinent to bLF and hLF:

1. the number of clinical studies in support of hLF are significantly more numerous;
2. the hLF doses to which infants and adults were exposed were significantly greater; and
3. the durations were longer for infants for bLF and about the same in adults for hLF and bLF.

Accordingly, if the GN's pertinent to bLF contained enough clinical information, then the GN pertinent to hLF certainly contains enough clinical information.

(7.) Regarding assessment of qualified experts

The GN's pertinent to bLF and hLF both initially included professional assessments by qualified experts. Eventually both **also** included professional assessments by qualified experts with respect to the potential of either substance to induce immunologically-related effects and the meaning of any such effects. The latter assessment pertinent to bLF was one page long and consisted only of conclusions. In contrast, Pharming's comparable assessment was 40 pages long and included both extensive, in-depth, scientific discussion and expert conclusions. In addition, such latter assessment was twice updated. Since the former assessment pertinent to bLF was deemed to be sufficient, presumably Pharming's assessment will also be deemed, at the very least, sufficient.

(8.) Regarding ability to induce adverse immunological effects

With regard to both hLF and bLF (since **both** are known to induce essentially the same immunological[5] effects at the same oral dosages), the

[5] Since the term "immunological" incorporates numerous terms – such as "innate immunity", "adaptive immunity", "T-cells", "cytokines", and many more, an understanding of these terms and the manner in which they are interrelated is critical to Pharming's update document. Thus, it seems appropriate – at this specific point – to provide some helpful background information concerning what such terms and their related activities entail – so as to promote common understanding. Since such information is quite basic and, therefore, not particularly helpful to a "qualified expert", it has been set forth in a stand-alone

question has been raised – primarily by research conducted over ten years ago – as to whether any of the effects induced are adverse effects. Presumably the answer is no since FDA determined that bLF is GRAS. Since hLF is intended for the same uses at the same ingestion level as bLF was GRASed for, presumably it also does not induce any adverse, immunological effects.

Nevertheless, for the sake of thoroughness, each of the immunological-related questions asked about the potential for either bLF or hLF to induce any adverse, immunological effect are set forth below and then answered via reference to current, direct, and consensus, scientific evidence.

(a.) Th1 cell activity

The pertinent, scientific literature inconsistently suggests that lactoferrin has immunoregulatory properties influencing both innate and acquired immunity. (See, e.g., the comprehensive review by Fischer, 2006). In particular, it has been suggested that lactoferrin influences T cell maturation, proliferation and differentiation into T-helper 1 (Th1) or T-helper 2 (Th2) cells. Th1 and Th2 cells are two functional subsets of Th- or CD4-positive T cells, whose function depends upon the specific types of cytokines that are generated. (Rafiq, 2000; Mosmann, 1986, Abbas, 1996). CD4-positive Th1 cells produce IFNγ and IL-2, but not IL-4 or IL-5, and drive cellular immunity to attack viruses and other intracellular pathogens; conversely, CD4-positive Th2 cells produce IL-4, IL-5 and IL-13, but not IFNγ or IL-2, and drive humoral immunity that up-regulates

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attachment. (See, Pharming response, Attachment 1). Notwithstanding its basic nature, however, the information is important and is, therefore, intended as a part of Pharming's update document. Such information – over 6 pages of it – is not set forth with numerous quotes because almost all of it comes from two, authoritative, sources, i.e , two widely-respected and widely-used medical school textbooks by two widely-respected immunologists – specifically, that by Abbas, Abul K. (at UCSF Medical School) and Lichtman, Andrew H (at Harvard Medical School) entitled· Basic Immunology· Functions and Disorders of the Immune System, Second Edition, Saunders Elsevier (Phil, PA) (2006) and that by Abbas, Abul K. (at UCSF Medical School) and Lichtman, Andrew H. (at Harvard Medical School) entitled: Cellular and Molecular Immunology, Fifth Edition, Saunders Elsevier (Phil, PA) (2005) These sources were used because they are widely respected and represent the consensus, established viewpoint of qualified experts. The authors are to be credited for the information presented – including that appearing in many of the footnotes (in particular, the definitions).

antibody production to attack extracellular organisms. Whereas Th1 cells are known as important producers of IFNγ, other cell types are also able to produce IFNγ, including (in particular) NK cells and nonpolarized memory T cells. (Ye, 1995; Biron, 1999). It is important to note that increased IFNγ production does not necessarily reflect increased Th1 cell activity.

The establishment of the Th1/Th2 balance is determined early during immune responses and depends on many factors including antigen structure, the functional status of antigen-presenting cells (APCs), the strength of T cell activation, the presence of cytokines, co-stimulatory signals and the microenvironment. (Rafiq, 2000). Both Th1 and Th2 cells negatively cross-regulate the function of one another through their respective cytokines. (Romagnani S, 1994; Maggi, 1992). Furthermore, it should be noted that IL-18, frequently reported as being upregulated upon lactoferrin oral administration, does not skew Th responses towards a Th1 response. Rather, Th1 responses are highly dependent on and stimulated by IL-12. Once Th1 cells are polarized, then IL-18 can act on them to enhance IFNγ production. IL-18 also enhances IFNγ production of NK cells. Thus, production of IL-18 does not correlate to induction of Th1 responses. (Nakanishi, 2001; Okamura, 1998).

Regarding oral administration of lactoferrin, most of the data comes from orally administrated bovine lactoferrin (bLF) rather than human lactoferrin (hLF). Since there is sufficient evidence indicating that both proteins are comparable in structure and function (Baker, 2000; Nuijens, 1996), the effects observed on the immune system as a result of either bLF or hLF administration have been used as model for oral administration of Pharming's hLF.

Review of the available, scientific literature[6] concerning oral administration of lactoferrin indicates that there are contradictory results with respect to the

[6] These preclinical and clinical studies – involving **oral administration** of lactoferrin – include those by Artym (2003), Haversen (2003), Hayes (2005), Iigo (2004), Ishii (2003), Kruzel (2006), Kuhara (2006), Kuhara (2000), Nakajima (1999), Sfeir (2004), Takakura (2006), Takakura (2004), Tanaka (1999), Togawa (2002), Ueno (2006), Varadhachary (2004), Wakabayashi (2006), Wakabayashi (2004), Wakabayashi (2003), Wakabayshi (2002), Wang (2000), Zimecki (2006), Zimecki (2005), Zimecki (1998) and Zimecki (1995) Other, older studies also have been conducted. Their findings are all referred to in one or more of the studies referred to in the above-referenced list. All of the above-referenced studies are fully cited and summarized in Pharming's response, Attachment 2.

evidence showing that lactoferrin affects proliferation and differentiation of T cells into Th1 and Th2 cells[7]. The induction of either Th1 or Th2 biased immune responses by lactoferrin is complex as the observed effects appear to be, at least in part, dependent on the mode of lactoferrin delivery and on whether any ongoing inflammatory or immune response is occurring. (For a review of all pertinent studies, see Fischer, 2006). Based on the available data, Pharming and its expert panel concluded that the evidence for orally administered lactoferrin eliciting a positive and adverse $CD4^+$ Th1 biased response is not convincing. This is because most studies suggest a change in Th1 cell activity based on alterations in cytokine levels, in particular IFNγ levels, but did not identify the cell-type responsible for the cytokine production. As mentioned above, increased IFNγ production does not specifically indicate increased Th1 cell activity. More likely, it indicates enhanced NK cell activity. In addition, the information is not convincing because some papers show potential Th1 responses (i.e., IFNγ secretion) within a few days. However, there is a critical time element involved in that it takes weeks for Th1 and Th2 cells to become firmly polarized. (Murphy, 1996). Even in culture, where one can create an optimal environment, it takes at least a week – and usually 2-3 weeks – to generate $CD4^+$ Th1 and Th2 cells. (Perez, 1995).

Finally and not least importantly, even if – for sake of argument – oral consumption of human lactoferrin were to enhance Th1 responses, that would not necessarily be deleterious. First of all, there is nothing in the direct evidence that demonstrates that lactoferrin given orally enhances any pathologic Th1 responses[8]. On the contrary, there is evidence from a rat colitis model and other

[7] However, please note that Zimecki, et al. reported that lactoferrin inhibits proliferation and cytokine production by Th1 cells – but not Th2 cells. (Zimecki, 1996).

[8] Guillen (2002) did report increased severity of collagen-induced arthritis in transgenic mice expressing human lactoferrin associated with an apparently enhanced Th1 response. However, this conclusion was based on cytokine levels which, as argued elsewhere, do not automatically imply a Th1 response, and the continuous and chronic systemic exposure in this model is quite different from the oral exposure envisaged in humans. In contrast to these results, the same group earlier demonstrated that periarticular injection of hLF in mouse models of autoimmune arthritis and septic arthritis demonstrated significant treatment benefits. (Guillen, 2000). Also in sharp contrast are the many, preclinical results reported by Zimecki et al. which only demonstrated very beneficial results in immunosuppresssed and autoimmune animals when fed lactoferrin. (Zimecki, 2007)

rat and mouse studies that demonstrate that oral consumption of lactoferrin inhibits a pathologic Th1 response via upregulation of IL-10 and inhibition of IFN-γ. (Zimecki, 2006; Takakura, 2006; Togawa, 2002). In addition, there is considerable evidence that – to the extent lactoferrin influences T-cell maturation, proliferation, and differentiation – it does so only beneficially. (Fischer, 2006).

(b.) Release of specific cytokines

With respect to increased release of specific cytokines in the gut and/or systemically following oral administration of lactoferrin, various animal studies generally reported only local changes in the expression/production of both Th1 (e.g., IFNγ, IL-2) and Th2 (e.g.,IL-4, IL-10) cytokines. (Wang, 2000; Kuhara, 2000; Iigo, 2004; Wakabayashi, 2006; Varadhachary, 2004). In addition, various animal studies indicate that oral lactoferrin administration might increase both local and systemic IL-18 levels. (Iigo, 2004; Wakabayashi, 2004; Kuhara, 2006; Hayes, 2005). Pharming's expert panel concludes, however, that the effect of IL-18 will occur locally and not systemically. Regarding the systemic levels of IL-18, oral administration of lactoferrin at doses up to 9 gram per day in human adults with solid tumors only resulted in a 15% increase of circulating IL-18, which is considered very low. (Hayes, 2005). More importantly, in this study no serious adverse events were reported and lactoferrin was well-tolerated by all subjects at a dosage of 150 mg/kg/day – which is very significantly higher than the level of maximum daily consumption that Pharming proposes in its GRAS Notification. In another study, a transient increase of IL-18 was observed in serum of hepatitis C patients receiving lactoferrin at an oral dosage of 600 milligrams per day for 12 months. (Ishii, 2003). However, the data showed large variation and the observed increase of IL-18 decreased again after 3 months to baseline levels. Taking all such information into account, Pharming and its experts conclude that to the extent cytokines are reported to be released upon oral administration of lactoferrin, such reports do not indicate a consistent pattern of enhancement or any adverse effect.

In conclusion, it is Pharming's and its experts' opinion that, based on the available data, there is not convincing evidence that demonstrates to a reasonable certainty that lactoferrin specifically enhances any adverse Th1 responses or can significantly increase any adverse, systemic cytokine levels over time. In contrast, there is sufficient evidence that lactoferrin enhances innate immune responses in the gut, e.g., by increasing IL-18 production[9] (most likely locally, not systemically) and by increasing NK cell activity, both of which are considered beneficial rather than deleterious. Indeed, there is no direct evidence that increasing innate function is in any way detrimental; rather, such increased function is considered beneficial. (Fischer, 2006).

(c.) Effect of oral administration of hLF on autoimmune or other inflammatory disorders

((a.) Introduction)

Finally, this presentation considers whether oral consumption of human lactoferrin induces any adverse effect(s) with regard to autoimmunity or other inflammatory disorders. Both areas of potential effects are addressed below.

((b.) Autoimmunity)

T cell responses to antigens are classified on the basis of the amount and kind of cytokines produced. Using this classification, T cell responses in MHC-class-II-restricted autoimmune diseases appear to be predominantly of the Th1 type. (Rosloniec, 2002). Thus, Pharming understands the potential autoimmune concern to be about whether oral administration of lactoferrin enhances Th1 responses and, thus, whether same could lead to the onset or enhancement of autoimmune diseases. Although the mechanisms of autoimmunity are not yet sufficiently understood, the concern here is considered highly unlikely by experts

[9] Lactoferrin has been shown to enhance IL-18 production by intestinal epithelial cells, thus enhancing the innate immune response. Human intestinal epithelial cells have been shown to condition human dendritic cells along a non-inflammatory Th2-like pathway, rather than towards Th1 responses. (Rimoldi, 2005).

consulted by Pharming. **First** (and, perhaps, most importantly), there is a growing body of scientific evidence that indicates that orally administered lactoferrin significantly inhibits and/or diminishes and/or improves (rather than initiates or enhances) autoimmune diseases. (See, e.g., Zimecki, 2007 (orally administered lactoferrin only induces numerous, beneficial[10] effects in various studies conducted in immunosuppressed and autoimmune mice and rats); Kruzel, 2006 (orally administered lactoferrin causes reduction of clinical signs of multiple sclerosis in patients – in parallel to normalization of cytokine production by peripheral blood cells); Zimecki, 2006 (orally administered lactoferrin significantly diminished the clinical symptoms of experimental autoimmune encephelomyelitis in Lewis rats); and Togawa, 2002 (oral administration of lactoferrin significantly reduced colitis in rats))[11]. **Second**, it is very possible that Th1 cells are not even involved in the pathogenesis of autoimmune diseases. Rather, such diseases may well be induced by the recently discovered T-helper 17 subset. (Hue, 2006; Yen, 2006). **Third**, as already discussed above, the evidence that orally administered lactoferrin elicits an adverse, Th1-biased response or potentiates a pre-existing Th1-mediated immune response is considered highly unlikely. **Fourth**, hLF is naturally expressed in saliva and the gastro-intestinal tract; thus, humans have a significant daily naturally-occurring exposure to hLF[12]. For instance, the intake of lactoferrin from saliva alone is about 20 mg/day. (Tanida, 2003). Consequently, humans are tolerant to hLF. Once oral tolerance has been established, it is very hard to disrupt, even in patients with chronic stimulation of the immune system. (Zivny, 2001). Moreover, the oral administration of an autoantigen has been shown to be beneficial in the treatment of various experimental, autoimmune diseases and this method of inducing immune non-responsiveness has currently been applied

[10] For a discussion of all such benefits, see pages 18-22 of this update document.

[11] See also, two other studies showing similar results, i.e., Guillen, 2000 (which study demonstrated that periarticular injection of hLF in mouse models of autoimmune arthritis and septic arthritis demonstrated significant treatment benefits) and Zimecki, 1995 (which study demonstrated that intraperitoneal injection of bLF in mice significantly inhibited autoimmune hemolytic anemia).

[12] Such daily, natural exposure also emanates from lactoferrin produced and released by or in, for example, human milk, neutrophils and various mucosa. Indeed, as Pharming's GN indicates (at pages 26 and 28 of Attachment 1), human lactoferrin is virtually ubiquitous throughout the human body.

to the prevention and treatment of human autoimmune diseases. (See reviews by Wardrop, 1999; and Sosroseno, 1995). **Fifth**, although there is extensive reporting on the presence of autoantibodies against lactoferrin, there is no evidence that these antibodies play any role in the pathology of autoimmune diseases. There is a large body of scientific literature on antilactoferrin auto-antibodies as a component of antineutrophil cytoplasmic antibodies (ANCA). (See review by Malenica, 2004). In addition, individuals with a wide range of autoimmune conditions have anti-lactoferrin autoantibodies. Despite this large body of scientific literature on these antibodies, there is no evidence showing them to have any role in the etiology of autoimmune disease, and there is a general consensus among qualified experts that they are an epiphenomenon, i.e., unrelated to treatment, disease activity, duration of disease, or disease extent. (Malenica, 2004; Chikazawa, 2000; Guillen, 1998; Roozendal, 1998; and Audrain, 1996). Furthermore, all individuals possess low but detectable amounts of circulating and mucosal human lactoferrin. Therefore, it is considered highly unlikely that oral administration of human lactoferrin, even to an individual with an ongoing autoimmune disease, would increase autoantibody levels. Even if oral lactoferrin were to increase the level of such antibodies, it would be clinically irrelevant, i.e., unlikely to have any impact on disease pathogenesis or severity.

Anti-lactoferrin autoantibodies have **not** been shown to be involved in the pathogenesis of any disease. In contrast, there is data that autoantibodies in general may help clear and degrade autoantigens, thus reducing T cell sensitization to them. (Mizoguchi, 1997). It should also be pointed out that there have been multiple trials in which autoantigens were fed to patients with autoimmune diseases to see if this might ameliorate the disease. For example, these trials have fed human insulin to autoimmune diabetics, collagen to rheumatoid arthritis patients, and myelin proteins to patients with multiple sclerosis. These trials have not shown any consistent benefit to the patients; however, there were no deleterious effects from autoantigen feeding and this was done in substantial numbers of individuals. (Faria, 2005).

In conclusion, Pharming and its experts conclude that it is highly unlikely that oral consumption of Pharming's lactoferrin at the level here in question would lead to the development or the perpetuation or enhancement of an autoimmune response.

((c.) Inflammatory disorders)

As discussed above, there is sufficient evidence that lactoferrin enhances innate immune responses in the gut Thus, it is a potential concern that this may lead to promotion of inflammatory disorders in the gut. Pharming understands this concern, particularly as it relates to inflammatory bowel disease (IBD), a term which commonly incorporates ulcerative colitis (UC) and Crohn's disease (CD). Both diseases are chronic inflammatory conditions of the gut in which Crohn's disease may affect any part of the gastrointestinal tract, whereas UC mainly affects the colon. In IBD, there appear to be multiple levels of immune responses, including innate, adaptive and regulatory immune responses. There is emerging literature that innate immune defects can contribute to the development of IBD. (See, e g., Beckwith, 2005; Hugot, 2001; Ogura, 2001). However, neither Pharming nor its experts are aware of any scientific evidence that supports the idea that a low-level Th1 response or enhancement of the innate immune response, even on a chronic basis, would be detrimental or trigger IBD. In contrast, lactoferrin has been repeatedly shown to enhance the production of IL-18 by intestinal epithelial cells, thereby increasing innate immunity, which is considered beneficial rather than deleterious for susceptible individuals. This beneficial enhancing of innate immunity has been confirmed in a recent open label trial in patients with Crohn's disease who received granulocyte-macrophage colony-stimulating factor (GM-CSF). (Dieckgraefe, 2002). GM-CSF is a cytokine involved in enhancement of the qualitative function of various immune cells, and stimulates the expansion and differentiation of haemopoetic progenetors. (Armitage, 1998). The results showed an

enhancement of the intestinal innate immune response resulting in an amelioration of the disease. (See also, Fischer, 2006).

Even with regard to individuals who have a "leaky" gut[13], such as can be found in inflammatory bowel disease, orally administered exogenous lactoferrin is simply supplementing large endogenous production of lactoferrin in alimentary secretions. There are low levels of antibodies to various foods in intestinal secretions and serum, but there is no evidence that these have any detrimental effect. There is also no evidence that immunologic reactions to food have any adverse effect in inflammatory bowel disease or that any foods exacerbate inflammatory bowel disease.

In contrast to the concern that orally administered lactoferrin may impact negatively on inflammatory bowel disorders, there is a growing body of scientific evidence – as Zimecki et al. point out – that demonstrates just the opposite, i.e., that orally consumed lactoferrin exhibits "distinct anti-inflammatory properties." (Zimecki, 2006). Such conclusion – the authors indicate – is supported by a growing number of studies incorporating a number of models "including experimentally induced bowel inflammation in rats (Togawa et al., 2002), autoimmune disorders in mice (Zimecki et al., 1995; Guillen et al., 2000), experimental endotoxemia in mice (Kruzel et al., 2002), and inflammatory reactions to Mycobacterium bovis (Zimecki et al., 1994)." (Zimecki, 2006; see also, Zimecki, 2007 which reported on the anti-inflammatory effect of hLF in rats and mice; Fischer, 2006 which reported on the anti-inflammatory effects of hLF in mice; and Haverson, 2003 which reported on the anti-inflammatory effects of hLF in an experimental colitis model in mice). In all such models, lactoferrin exhibited significant anti-inflammatory properties.

Moreover, lactoferrin induces TGF-β production which is widely considered an anti-inflammatory cytokine. (Zimecki, 2005; Ward, 2002). Since TGF-β is an anti-inflammatory cytokine associated with the induction of antigen-specific regulatory T cells and such cells produce TGF-β or IL-10, these cells can

[13] To the extent that the "leaky" gut concept exists – and such concept is **not** generally recognized – it generally refers to the movement of molecules with a molecular weight of less than 1000 daltons.

inhibit the induction of inflammatory responses. In particular, these cytokines suppress IFN-γ production and activity from activated Th1 cells. Lactoferrin can even exhibit strong anti-inflammatory effects in dexamethasone-induced acute colitis in a mouse model. (Haverson, 2003).

In further contrast to suggesting that human lactoferrin – a substance native to humans – might be responsible for either autoimmune or other inflammatory disorders, there is a growing body of scientific evidence showing that defects in innate immunity can lead to an abnormal adaptive immune response, some of which are manifest by autoimmune disease. A good example of this is the non-obese diabetic (NOD) mouse, which has some well-defined defects in innate immune responses. Stimulation of the NOD innate system by a variety of means blocks the development of the autoreactive T cell response to islet cells and, thus, prevents diabetes. In inflammatory bowel disease there is emerging literature that innate immune defects can contribute to the development of IBD. (Korzenik, 2006). For example, a colitis susceptibility gene has been identified which appears to function by regulating innate immunity. (Beckwith, 2005; Hugot, 2001; Ogura, 2001). In addition (and as mentioned above), there is a trial in which GM-CSF has been administered to patients with Crohn's disease to enhance their innate immunity and, thus, ameliorate their disease. (Dickgraefe, 2002). Thus, autoimmune or chronic inflammatory diseases are more likely to result from deficient innate immune cytokine production or function.

Of course, there is no scientific evidence that suggests – let alone demonstrates – that orally consumed human lactoferrin induces any deficiency in any innate immune mechanism. In fact, orally consumed human lactoferrin does just the opposite, i.e., it enhances innate immunity, which is deemed beneficial. (See, e.g., Zimecki, 2007; and Fischer, 2006).

(8.) Conclusion

Fortunately, there are now numerous, published, preclinical and clinical studies and other information – most of which have been reviewed above –

which are pertinent to and evaluate the potential immunomodulatory ability of hLF. Thus, the primary question arising at this time from such studies and information with regard to the safety of hLF is: What do all such studies and information currently indicate about such potential?

Clearly, the growing body of evidence indicates that lactoferrin can induce a broad range of immunological effects with regard to the manner in which a human immunologically reacts given the exposure to an invading pathogen or other substance. Early on, some thought that such evidence might indicate that lactoferrin may have a causal role in human disease (such as Crohn's disease, irritable bowel syndrome or autoimmune disorders); thus, they asked: Are such effects possibly adverse to the individual? More recent evidence increasingly indicates that lactoferrin also has a well-established ability to induce various, clearly beneficial effects. Thus, to some the evidence in the aggregate may seem confusing – perhaps even inconsistent. Fortunately, all of the above-referenced studies and information have been recently and thoroughly reviewed for the purposes of determining what exactly all such evidence currently indicates. Qualified experts now conclude – as discussed in greater detail below – that oral consumption of lactoferrin does **not** induce detrimental effects; rather, its numerous effects function only in beneficial – but differing – manners.

Fischer et al [14] recently reviewed approximately 80 different studies and related publications pertinent to lactoferrin's potential immunoregulatory properties[15] – especially as they relate to lactoferrin's ability to regulate Th1 and Th2 responses. (Fischer, 2006)[16]. After discussing the findings of all such studies and information, Fischer et al concluded that lactoferrin does **not** induce any adverse, non-allergic immune responses – via either the innate or adaptive immune defense mechanisms. More specifically, the authors concluded that:

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[14] Fischer et al. are a group of experts in nutrition and immunology that is – when not involved in a visiting professorship – employed as a group by the French National Institute of Agronomy in Parris to investigate aspects of the physiology of nutrition and the alimentary canal.

[15] The Fischer paper was peer reviewed prior to publication.

[16] There are a few preclinical studies on Pharming's reference list which do not appear on the reference list attached to the Fischer article. Since such studies supply only information like that already reviewed by Fischer, they do not alter the scope of the substance discussed or the conclusions reached by Fischer, et al.

1. under **nonpathogenic conditions**, lactoferrin functions to set up the immune system – by, for example, influencing T-cell maturation, proliferation and differentiation into Th1 and Th2 cells – so as to be able to effectively respond to the conditions described immediately below;
2. under **infectious disease conditions**, lactoferrin affords protection by inducing a Th1 polarization in diseases in which the ability to control infection or tumor relies on a strong Th1 response;
3. under **inflammatory conditions**, lactoferrin functions as an anti-inflammatory by reducing the Th1 component to limit excessive inflammatory response; and
4. under **noninfectious disease conditions**, lactoferrin provides protection against Th1- or Th2-induced diseases, such as autoimmune or allergic diseases, through correction of the cytokine Th1/Th2 imbalance.

Thus, consistent with Pharming's and its experts' assessment, Fischer et al. also concluded that the available information indicates that oral consumption of lactoferrin – even at levels exceeding the level of use here at issue – results in only beneficial immunological effects.

Zimecki et al.[17] also published a review of the implications of the ability of lactoferrin to influence immunoregulatory function – especially as it relates to their preclinical work involving immunosuppressive and autoimmune animals. (Zimecki, 2007; see also, Artym, 2003; Artym, 2003; Zimecki, 1999; and Baveye, 1999). After reviewing the pertinent information, the group concluded that oral administration of lactoferrin.

1. accelerates reconstruction of immune system function (including restoring innate and acquired, antigen-specific cellular, and

[17] Zimecki et al. are a group headed up by Dr. Michael Zimecki, an immunologist (who is Chief, Laboratory of Immunobiology and Chief, Department of Experimental Therapy – both at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences) and which group also includes experts in several other medical-related disciplines and which group has tested, evaluated, and published more articles concerning lactoferrin than, perhaps, any other entity.

humoral immune response) in instances of immuno-suppresssion;

2. accelerates counteraction (i.e , inhibition) of autoimmune disease development;
3. normalizes the ration of major blood cell types;
4. lowers clinical scores of disease and diminished pathohistological changes; and
5. reduces serum levels of both pro- and anti-inflammatory cytokines

Thus, like Fischer et al. and Pharming's experts , Zimecki et al. found no adverse effects induced by the oral consumption of human lactoferrin.

Kruzel et al.[18] also reviewed the information – pertinent to the effects that oral consumption of lactoferrin has on the immune system. (Kruzel, 2007). They reported that – contrary to inducing any adverse effects – lactoferrin functions:

1. as a frontline defense protein involved in protection and prevention;
2. as a mediator that bridges innate and adaptive immune functions to achieve successful outcome;
3. to protect cells from oxidative injury; and
4. most importantly, as an immune sensor directing specific immune responses to achieve immune homeostasis.

Thus, Kruzel et al. – like numerous, other qualified experts (including Pharming's qualified experts) who have carefully and thoroughly evaluated the potential of lactoferrin to induce adverse immunological effects – concluded that lactoferrin does **not** induce adverse immunological effects.

Finally, since it is very difficult – despite numerous attempts – to find a mucosal adjuvant among substances likely to be orally consumed, Pharming and its experts conclude that it is very unlikely that further preclinical or clinical testing

[18] Dr. Marian Kruzel is an immunologist who is currently a visiting professor in the Department of Integrative Biology and Pharmacology at the University of Texas, Health Science Center (Houston, Texas). Her group includes experts in immunology, pathology and microbiology. She is also part of the Zimecki group referenced above.

of Pharming's lactoferrin at the daily level here at issue and even for longer periods of exposure would result in any demonstration that Pharming's lactoferrin is able to induce – via oral consumption – any adverse immunomodulatory effect.

f. Conclusion

All of the scientific information set forth above – including long-term data – indicates that bLF is not only safe for consumption at the levels here in question but also GRAS. Accordingly, FDA has repeatedly determined that it is GRAS. Based upon structural and functional similarities, one would expect that hLF would be as safe as bLF (if not more so since it is native to humans). All of the scientific information provided in support of hLF – which is considerably more than that provided for bLF – indicates that hLF – like bLF – is safe for ingestion at the same levels and is also GRAS. Thus, the history of safe consumption of bLF is – in effect – also the history of the safe consumption of hLF.

3. Subarea 2 (concerning potential antigenicity of hLF)

Human lactoferrin in its native forms and human lactoferrin in its transgenically-produced forms are virtually, but not absolutely, identical. With regard to the differences, since human lactoferrin in the normal population has four polymorphic sites, differences between exogenous and endogenous human lactoferrin can arise because of different polymorphic alleles in the exogenous and endogenous molecules. Additionally, another difference can arise as a characteristic arising from the transgenic organism in which the protein is expressed, i.e., transgenically-produced proteins will be glycosylated in a way characteristic for the expression system and, thus, the resulting glycosylation pattern may differ from its native form. Due to these known differences between native and transgenically-produced human lactoferrin, to what extent would such differences be expected to impact on the potential antigenicity of oral exposure to transgenically-produced human lactoferrin?

4. Pharming Update

a. Introduction

At the outset, it seems important to emphasize that this entire presentation is primarily about the two substances (here in question) about which Pharming has expertise and which are the focus of Pharming's GRAS Notification – that is, Pharming's and native **human lactoferrin**[19]. Thus, much of what is in the GRAS Notification and this presentation is only about human lactoferrin (sometimes referred to in this presentation as hLF). However, Pharming recognizes that human lactoferrin is one in a broad set of mammalian lactoferrins (including, especially, bovine lactoferrin – sometimes referred to in this presentation as bLF)[20]; accordingly, Pharming has also – from time to time – included in its updating documents information about other lactoferrins because such information is helpful in establishing a broader context of safety of human lactoferrin.

b. Comparability of the two lactoferrins

(1.) Introduction

This subarea of discussion considers whether Pharming's exogenous lactoferrin is structurally significantly different from the polymorphic, endogenous lactoferrin produced naturally by the individual consumers comprising the U.S. population and, if so, whether any such structural difference may have a

[19] This information may or may not be applicable to other versions of human lactoferrin. While there are likely to be very significant similarities between versions of hLF produced from transgenic sources, there may also be differences – in some cases, a difference that makes a difference – thus, each version must be safety assessed based on its own particulars.

[20] To the extent hLF is considered to be a "known biological response modifier" (KBRM) of the human immune system, bLF has also been demonstrated to the same extent to be a KBRM. (See, subarea B(2)(e)(8)). bLF has been determined to be GRAS and at a level equivalent to the level being requested in Pharming's GN.

significant, negative impact on the way in which such exogenous lactoferrin is recognized and responded to by the human immune system. Accordingly, what follows is a discussion of the extent to which both lactoferrins are identical, the extent to which both lactoferrins are different, and the importance of any such difference.

(2.) Both lactoferrrins are almost entirely the same

As thoroughly discussed in considerable detail in Pharming's GRAS Notification (please see, Attachment 1, pages 4-5, 12-13, and 32-34), both Pharming's exogenous lactoferrin ("rhLF") and endogenous lactoferrin ("hLF") are overwhelmingly identical. As a reminder, such identicalness extends to the fact that **both** lactoferrins:

1. are the same metal-binding, glycoprotein, i.e., hLF (Thomassen, 2005, van Berkel, 2002; Anderson, 1989);
2. have the same amino acid sequence and composition based on the nucleic acid sequence pertinent to the allelic variation seen in the normal population (see, GRN No. 00189, subsection III(C)(1)(e));
3. have the same N-terminal protein sequence (van Berkel, 2002);
4. have the same three-dimensional, protein structure (Thomassen, 2005);
5. are N-glycosylated (van Berkel, 2002);
6. have the same number and location of glycosylation sites (van Veen, 2004);
7. show the same chromatographic profiles upon analytical Mono S analysis (van Berkel, 2002);
8. have the same core-molecular weight (although overall molecular weight slightly differs – Pharming's hLF is slightly lower – due to the differences in the carbohydrate moieties attached to the lactoferrin core) (van Berkel, 2002);

9. show the same tryptic degradation kinetics, i.e., digestibility (van Veen, 2004);
10 have the same iron-binding and iron-release properties (van Berkel, 2002); and
11. are equally effective against experimental infections with multidrug-resistant S. aureus and K. pneumoniae in mice (van Berkel, 2002).

Thus, from the point of view of considering any difference which actually makes any significant difference, Pharming's lactoferrin is identical to endogenous lactoferrin – except for the difference that is discussed below.

(3.) How the lactoferrins are different

CFSAN initially questioned whether Pharming's exogenous, human lactoferrin differs from endogenous human lactoferrin in two, different ways. Each is discussed below.

((a.) With regard to their respective amino acid sequences)

CFSAN first questioned whether the amino acid sequence of Pharming's exogenous lactoferrin is structurally different from that of endogenous lactoferrin.[21] As Pharming's GN explains (see pages 12-13) and as discussed below, the two lactoferrins are not really different.

Careful comparison of the ten, published, amino acid sequences of endogenous lactoferrin demonstrates that such naturally-occurring sequences may naturally differ from one another in six instances[22], i.e., in amino acid

[21] Specifically, CFSAN questioned whether "Pharming's lactoferrin is distinct from the endogenous lactoferrin of individual consumers with respect to expected differences between the amino acid sequences of the exogenous lactoferrin and the polymorphic endogenous lactoferrin alleles present in the general population."

[22] Only **four** of these instances have been scientifically confirmed, i.e., at amino acid positions 4, 11, 29 and 561. (van Veen, 2004). The other two, i.e., positions 14 and 413, have not yet been confirmed. Pharming's lactoferrin has – with regard to these latter two amino acid positions – the same amino acids as reported in 9 of the 10 above-referenced amino acid sequences. (See pages 12 and 13 of Pharming's GN, Attachment 1) It is possible that the latter two differences may not be real. Thus, FDA's question refers to four.

positions 4, 11, 14, 29, 413, and 561. In each such natural instance, the amino acid present is one of only two possibilities. Thus, there exists a well-known and well-documented naturally-occurring range of amino acid variation in endogenous lactoferrin.

Pharming's lactoferrin does **not** differ from but rather exactly duplicates this range, i.e., it is no more different from such range than any one of the ten, known endogenous lactoferrins In each of the six, above-referenced amino acid positions, Pharming's lactoferrin incorporates exactly the same one of two possible amino acids, as does any one of the ten endogenous lactoferrins. Thus, there exists **no real difference** here (with regard to amino acid sequence) between what occurs endogenously and what occurs exogenously. (Please note that with regard to all other amino acid positions, they are all identical).

There is no scientific evidence that an individual producing any one of the above-referenced, naturally-occurring, endogenous lactoferrins reacts – immunologically speaking – differently when exposed to any one of the other above-referenced endogenous lactoferrins. Indeed, extensive and long-term human experience demonstrates just the opposite. For example, infants when consuming human milk are exposed – in the vast majority of instances – to an exogenous hLF variety that differs from their own endogenous variety and all without adverse, immunological reaction, probably due – assuming, for sake of argument, that the infant's immune system even recognizes the exogenous variety as being different – to oral tolerance[23] and/or anergy[24]. It should be noted that the daily exposure levels to exogenous hLF that an infant naturally experiences (i.e., 48-3077 mg hLF/k BW/d) far exceed the daily exposure level

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[23] The term "**oral tolerance**" is defined as the suppression of systemic humoral and cell-mediated immune responses to an antigen after the oral administration of that antigen, due to anergy of antigen-specific T cells or the production of immunoregulatory mediators such as transforming growth factor-β or interleukin-10. Oral tolerance is a physiological mechanism for preventing immune responses to food antigens. For a thorough discussion of how tolerance is established, etc., see Iweala , 2006 or Faria, 2005.

[24] The term "**anergy**" is defined to mean a state of unresponsiveness to antigenic stimulation. Lymphocytic anergy (also called clonal anergy) is the failure of clones of T or B cells to react to antigen, and this may be a mechanism of maintaining immunologic tolerance to self antigens. In clinical practice, anergy refers to a generalized defect in T cell-dependent cutaneous delayed-type hypersensitivity reactions to common antigens. (Abbas, 2006).

being requested in Pharming's GRAS Notification, i.e., 100 mg/product serving (or 1.91-3.95 mg hLF/k BW/d).[25] In addition, patients – of all varieties and ages – who receive transfusions of blood products, e.g., fresh, frozen plasma, are routinely exposed to an exogenous human lactoferrin that differs from their own. This exogenous lactoferrin – present in the plasma at varying concentrations from 42-202 µg/ml – is predominantly derived from degranulating neutrophils. (Scott, 1989). Importantly, patients who receive such transfusions commonly have ongoing inflammatory reactions, e. g., trauma; even so, such very numerous, systemic exposures to these exogenous lactoferrins in these patients have not been reported to have led to any known, adverse, immunological event. Oral exposure to human lactoferrin should be even less potentially immunogenic than this type of very intimate, blood-to-blood exposure. Since Pharming's exogenous human lactoferrin only duplicates endogenous, human lactoferrin (with regard to amino acid sequence), one would also expect such exogenous lactoferrin **not** to induce any adverse, immunological event (as a result of its amino acid sequence) And there in **no** evidence that it could or does

Finally and not least importantly, in nature, there are numerous, naturally-occurring, mammalian lactoferrins (i.e., iron-binding glycoproteins) all of which are similar in structure and function compared to their human homologue (See, e.g., Nuijens, 1996). Why would one expect that oral consumption of Pharming's exogenous hLF – whose amino acid sequence only duplicates that which occurs naturally in endogenous human lactoferrin – would induce any adverse immunological event when bLF – whose amino acid sequence is 31 percent different that endogenous human lactoferrin – does not and is deemed GRAS, even when consumed long-term at significant levels (i.e., at least at levels ≥ 100 mg/product serving)? Of course, the answer is that one should not have any such expectation because there is **no** direct, scientific evidence to support such expectation.

[25] The issue concerning the impact of the differences between the adult and infant gut and immune system was discussed in an earlier section. (See, subpart IIIB2e4)

((b.) With regard to their respective glycosylation patterns)

As CFSAN correctly notes, Pharming's exogenous lactoferrin does differ from endogenous lactoferrin with regard to the type of carbohydrate structures that are attached at each of the three glycosylation sites. However, that is the extent of their structural differences (as Pharming's GN, Attachment 1, discusses at pages 4 and 33), since both lactoferrins incorporate the same number and location of glycosylation sites and both utilize these glycosylation sites in the same fashion. (van Veen, 2004).

With regard then to the specific glycans attached at each of the glycosylation sites, the only glycans attached to the glycosylation sites of natural hLF (from human milk) are N-linked, complex-type glycans. (van Berkel, 2002; Spik, 1982). In addition to the complex, N-linked glycans that are attached to the endogenous lactoferrin glycosylation sites, Pharming's exogenous lactoferrin also bears oligomannose and/or hybrid-type, N-linked glycans (van Berkel, 2002) – as one would expect, since the distribution and structures of attached glycans is species-, tissue-, cell type- and protein-specific. (James, 1995; Opdenakker, 1993). Furthermore, the complex, N-linked glycans of Pharming's hLF contain N-acetylgalactosamine next to galactose, which is typical for N-linked glycoproteins produced in bovine milk, such as bovine lactoferrin. (Van den Nieuwenhof, 1999; Coddeville, 1992) Finally, the glycans of Pharming's hLF contain less fucose compared to natural hLF. (van Berkel, 2002). However, as a result of crystallography studies, it has been determined that – despite the differences in N-linked glycosylation – the three-dimensional structure of Pharming's hLF and natural hLF are identical. (Thomassen, 2005).

Thus, the attached glycan-related differences then are the only known structural differences that exist between endogenous lactoferrin (from human milk) and Pharming's exogenous lactoferrin.

((c.) The importance of the differences)

At this point, the key focus becomes: Does the above-described difference (with regard to exactly what glycan is attached at each of lactoferrin's three glycosylation sites) make any real difference with regard to the ability of Pharming's *exogenous lactoferrin to disrupt previous tolerance to endogenous* lactoferrin? The direct evidence indicates that it does not.

The mere fact that a difference exists – as here – between two forms of a molecule (one of which naturally occurs endogenously – in this case in human milk – and the other of which (i.e., the exogenous form) differs from that naturally-occuring form only with regard to the kinds of glycans attached at each of the glycosylation sites) does not – by itself – amount to direct evidence that such difference will affect the latter molecule's potential immunogenicity. For example, please note that endogenous hLF (from human milk) and endogenous hLF (from human neutrophils) also differ in their respective glycosylation patterns. (Derisbourg, 1990). The glycan associated with neutrophilic hLF is not fucosylated – thus, it resembles the glycan pattern of human serum transferrin. (Spik, 1994). However, such difference in glycosylation pattern does not affect hLF's function with respect to isoelectric point, stability of the iron-saturated form, rate of clearance, or antigenicity. (Derisbourg, 1990, Moguilevsky, 1985).

And there exists another, just as relevant, well-known example, which demonstrates that consumption of a differently glycosylated lactoferrin does **not** lead to any adverse consequences with regard to immune response or any interruption of tolerance. The example, of course, involves the human *consumption of bLF*[26] *which is long known to be safe* (and at levels far exceeding

[26] Bovine lactoferrin (bLF) is also – like hLF – an iron-binding glycoprotein (of about 80 kDa) which is similar in structure and function compared to its human homologue. (Nuijens, 1996). The amino acid sequence of bLF (which contains 689 amino acids) shows 69% homology with hLF. (Pierce, 1991). The sequence of bLF contains five possible N-linked glycosylation sites, i.e., two more than hLF. Four sites, i.e., Asn 233, 368, 476, and 545, are always utilized (Spik, 1994) while the fifth (Asn 281), located in the N-lobe, is glycosylated in about 30% and 15% of the molecules in bovine colostrums and mature milk, respectively. (van Veen, 2002; Wei, 2000; Yoshida, 2000)

the level here at issue, i.e., 100 mg per product serving) as a result of a long and well-documented history of safe use (and by humans of every variety, including age, race and ethnic background). (See, subarea B(2)). Since Pharming's exogenous lactoferrin and bLF are both produced by the bovine mammary gland which determines the type of glycosylation (in this case, a mammalian type of glycosylation) **and** since, similar to Pharming's hLF, bLF bears oligomannose-type glycans and complex-type glycans with N-acetylgalactosamine next to galactose (Coddeville, 1992) (but more of the same since bLF has 5 attachment sites as compared to hLF's 3) **and** since historical human consumption of bLF at or exceeding the level of consumption of hLF being proposed in Pharming's GN has not resulted in any reported, adverse, immunological events, one would not expect that consumption of Pharming's exogenous lactoferrin would induce any adverse, immunological event. And there is no direct evidence that it does – absolutely none.

Of course, under certain circumstances, it may be possible (and documented) that a specific difference in glycosylation pattern may make a significant difference in the way in which a specific glycosylated protein will be recognized by the human immune system. (Cobb, 2005). But in the specific instance at hand, the single difference that exists between Pharming's human lactoferrin and endogenous human lactoferrin is not recognized (i.e, documented) as a difference which leads to an adverse, immune response. Finally and not least importantly, neither Pharming nor its experts are aware of any direct evidence that indicates that there is any protein to which humans are tolerant – including bLF and hLF – which will induce any adverse, immune response merely as a result of a difference in glycosylation. Therefore, it is extremely unlikely that such difference will alter the normal way in which Pharming's hLF is recognized and processed.

d. Potential for adverse effect(s)

(1.) Introduction

There has been some speculation that Pharming's human lactoferrin might – via the oral route of exposure – possibly induce certain adverse, immunological effects due either to differences in different polymorphic alleles or in glycosylation pattern. These speculations are addressed below.

(2.) Determinant spreading

With regard to the potential for determinant spreading from alloepitopes, Pharming and its experts conclude that such event is very unlikely to occur in the situation involving consumption of Pharming's hLF. An epitope is any molecular structure that can be recognized by the immune system Epitopes, or the antigen from which they are derived, can be composed of protein, carbohydrate, lipid, nucleotide, or a combination thereof. (Abbas, 2006). It is through recognition of foreign, or non-self, epitopes that the immune system can identify and destroy pathogens. T cells are known to respond **only** to linear epitopes, i.e., peptide fragments (usually 8 or 20 amino acids in length) digested from the native protein, that are presented in association with major histocompatibility complex (MHC) molecules [27] (Abbas, 2006). An epitope is considered linear, if the target of the immune response is apparent in the series of adjacent amino acids without any requirement for secondary or tertiary structure (folding) as would occur in a native protein. Although single amino acid substitutions (which result in a different linear epitope) have been reported to alter epitope spreading resulting in increased immune response, the amino-acid substitutions in Pharming's

[27] A **major histocompatibility complex** molecule is defined to mean a heterodimeric membrane protein encoded in the major histocompatibility complex (MHC) locus that serves as a peptide display molecule for recognition by T lymphocytes Two structurally distinct types of MHC molecules exist. Class I MHC molecules are present on nucleated cells, bind peptides derived from cytosolic proteins, and are recognized by $CD8^+$ T cells. Class II MHC molecules are restricted largely to professional antigen-presenting cells, macrophages, and B lymphocytes, and bind peptides derived from endocytosed proteins, and are recognized by $CD4^+$ T cells. (Abbas, 2006).

lactoferrin exactly mirror those in the various forms of endogenous lactoferrin in the general population. Thus, no "recognizable" difference results and no epitope spreading or disruption of oral tolerance would be expected.

Any discussion of glycosylation – with regard to determinant spreading – is irrelevant to linear peptide fragments which are the only entity which determines T cell response and, thus, T cell tolerance. Moreover, neither Pharming nor its experts are aware of any evidence showing that a mere difference in glycosylation would alter epitope spreading or that oral tolerance can be disrupted by the introduction of a differently glycosylated version of the same, native protein.

Therefore, while it is true that polymorphisms present in Pharming's lactoferrin can differ from those in the endogenous lactoferrin for a given individual, such naturally-occurring, amino acid substitutions – which fall within the range of variation that can be found in a normal population – are considered not to be immunogenic and, therefore, of little or any risk. Moreover, since T cells recognize only linear peptide epitopes, the concern about the effect, if any, of glycosylation is likely to be irrelevant to the discussion of T cell tolerance.

(3.) Enhanced pro-inflammatory Th1 response

As indicated in Pharming's GN, there is already a fairly sizeable endogenous lactoferrin production that occurs in humans as a result of human lactoferrin being produced in salivary glands and in intestinal mucosa (and elsewhere). Therefore, ingestion of Pharming's human lactoferrin would simply supplement an already existing endogenous protein. Humans are already tolerant to human lactoferrin and bovine lactoferrin and once mucosal tolerance is established, it is quite difficult to "break" it. For example, a recent study looking at chronic ingestion of foreign proteins by humans (Zivny, 2001) showed that the major response to chronic antigen feeding is T cell anergy (the major mechanism

of tolerance to chronic antigen feeding) even though there are low titers of antibodies to dietary proteins present in secretions and serum, such as ovalbumin, bovine gammaglobulin and soy proteins. These anergic, antigen-specific T cells actively contribute to maintenance of homeostasis in the intestine in the face of massive antigen challenge. (Zivny, 2001). This is why significant consumption of bovine lactoferrin does not result in any breakage of tolerance to bLF and why the same significant consumption of Pharming's lactoferrin will not disrupt any tolerance to endogenous lactoferrin. Indeed, one would expect Pharming's lactoferrin to be even less immunostimulatory and more tolerogenic than bovine lactoferrin.

Finally, Pharming and its experts conclude that it is very unlikely that consumption of Pharming's lactoferrin would result in perturbation of intestinal barrier function. (Dickenson, 1998).

(4.) Increased uptake by antigen-presenting cells via the mannose receptor

Although it may be theoretically possible that the differences in glycosylation between Pharming's lactoferrin and endogenous lactoferrin could result in increased lactoferrin uptake by an antigen presenting cell (APC) via mannose receptors in such a manner that the Th1 response is potentiated, Pharming is not aware of any direct evidence to support this. On the contrary, uptake by a mannose receptor appears to lead to an anti-inflammatory response, rather than a Th1 response. (Chieppa, 2003). Furthermore, the mannose-type glycans attached to the backbone of Pharming's lactoferrin are also attached – but in even greater number – to the backbone of bovine lactoferrin, which is deamed GRAS by CFSAN and is not reported to give rise to harmful, Th1 responses. Finally, Pharming is not aware of any direct evidence demonstrating that differential glycosylation alters antigen uptake and potentiates immune reactivity for native proteins. In conclusion, the risk of disruption of previous

tolerance to endogenous lactoferrin via any increased uptake of Pharming's lactoferrin by APCs via the mannose receptor is considered remote.

(5.) Conclusion

5. Subarea 3 (*concerning exposure to exogenous hLF*)

Describe the daily, long term, i.e., greater than 90 days, oral exposure that a human has to exogenous human lactoferrin.

6. Pharming Update

a. Introduction

Perhaps obvious to anyone reasonably informed is the fact that an infant[28] has considerable, long-term, oral exposure to exogenous[29] human lactoferrin during the approximately 12 months[30] that an infant is consuming – on a daily basis – significant quantities of human milk Perhaps not so obvious is the fact that a human in utero also experiences such exposure over a period of approximately eight months or that significant numbers of children, adolescents, or adults may also have similar exposures. Information pertinent to all of these exogenous hLF exposures is discussed below.

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[28] For purposes of this updating document, the term "*infant*" is used to mean a young child from birth to 12 months of age. (See, e.g., Lane, 2005).

[29] This response deals only with information concerning long term, oral, human exposure to "exogenous" human lactoferrin; it does not address such exposures to "endogenous" human lactoferrin. Such latter exposures are addressed in the next response

[30] The American Academy of Pediatrics – the US entity recognized as the most qualified to establish gold standard medical practices pertinent to infants – recommends that infants be fed human milk for 12 months. (See, e.g., AAP, 2007).

b. Fetal exposure

Fetal exposure to exogenous hLF occurs throughout the entire pregnancy, and results from several different exposure scenarios. The first kind of exposure to exogenous hLF begins very early in utero – via diffusion – and results from embryonic invasion of and contact with the maternal decidua (i.e., the uterine lining). (King, 2003). At this stage of development, there is no alimentary canal or circulatory system, thus what internal exposure to hLF later occurs in the formed alimentary canal cannot occur at this stage; nevertheless, diffusion at this early stage results in the same kind of internal exposure to hLF as later occurs via real oral exposure as a result of the alimentary canal being then in place. During such early exposure via diffusion, human LF ranges from being significantly present early on, to increasing during the first trimester, to upwards of 95ug hLF/g of decidual tissue at term. (King, 2003; Niemela, 1989). The second kind of exposure to exogenous hLF results from the formation of the umbilical cord. Via this feeding (oral-like) mechanism the fetus is exposed – also via diffusion – to a continuous bathing of tissues by a maternal plasma containing approximately 200 ng hLF per ml of plasma. (Pacora, 2000). Finally, the last type of exposure occurs via fetal immersion in and ingestion of hLF in amniotic fluid. (Jauniaux, 2000).

With regard to hLF exposure from amniotic fluid, please recall that during the third week of pregnancy the amniotic cavity forms and surrounds the embryo. (See Fig. 1 for details).

Figure 1

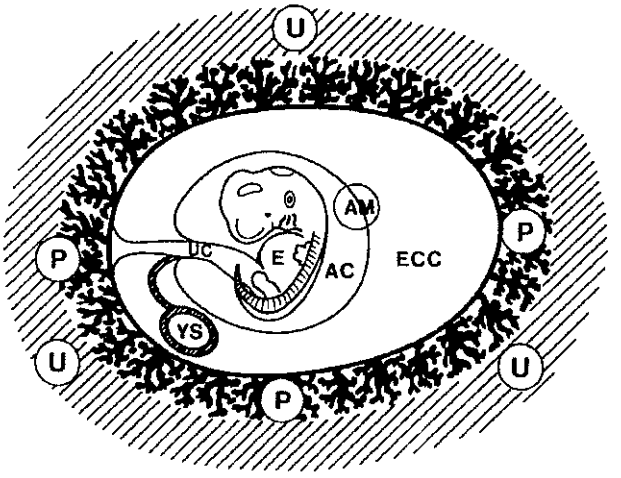
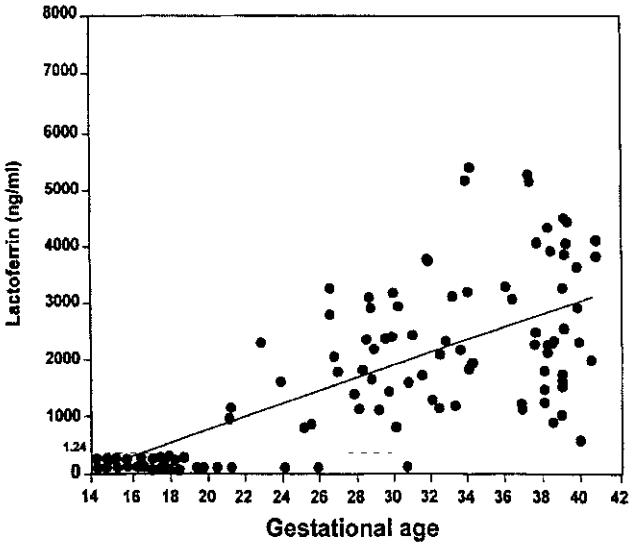


Diagram showing the different anatomical barriers inside the first trimester gestational sacs. U = uterine; P = placenta; UC = umbilical cord; ECC = exocoelomic cavity; SYS = secondary yolk sac; AC = amniotic cavity; AM = amniotic membrane. (As appears in Jauniaux, 2000).

By the 10th week of gestation the amniotic cavity contains approximately 30 ml of amniotic fluid and peaks to as much as 1 liter after 34-37 weeks. (Sadler, 2000). During the first trimester, hLF measures < 2 ng/ml of amniotic fluid. (Jauniaux, 2000). After 20 weeks, the level of hLF significantly rises (to a mean of 3000 ng/ml amniotic fluid) and remains high until term. (Jauniaux, 2000; Pacora, 2000; Niemaela, 1989, Figure 2).

Figure 2



Amniotic fluid lactoferrin concentrations and gestational age (in weeks). Lactoferrin increased with advancing gestational age ($r = 0.68$; $P < .0001$ by Spearman rank correlation) *as appears in* (Pacora, 2000).

Clearly, after 20 weeks gestation until term fetal tissues are being continuously bathed in the hLF contained in the amniotic fluid. (See, e.g., Table 1 below).

Table 1
Fetal Exposure to hLF in Bathing Amniotic Fluid

Age (weeks)	Total AF (ml)	ng hLF/ml AF	Wt (kg)	mg hLF/kg/d[31]
20	350	800	0.45	6.22
38-40	900	3000	3.35	8.96

Amniotic Fluid volumes from (Sadler,2000)
Concentrations of hLF in AF estimated from (Pacora, 2000)
Fetal weights from (p. 492 Appendix 2 and Fig. 8.1 p. 91 in O'Rahilly, 2001)

Continuity of amniotic fluid with the intestinal lumen occurs at three weeks gestation when the buccopharyngeal membranes ruptures forming the future mouth. (Weaver, 1991). By 7 to 8 weeks, the cloacal membrane ruptures, forming the future anus. (Weaver, 1991). From 11 weeks gestation, effective fetal swallowing is developed and is continuously recycling the amniotic fluid through the gastrointestinal tract into the amniotic cavity and back through the primordial mouth. (Weaver, 1991). By 12 weeks, the ability to absorb is present

[31] This figure equals an average case scenario of maximum exposure.

(Milla, 1991), including intact proteins and lipids. (Weaver, 1991). After this time, the amount of hLF ingested in amniotic fluid progressively increases until birth. (See, Table 2 below.)

Table 2
Fetal Ingestion of hLF in Amniotic Fluid ("AF")

	Gestational Age (WKS)	
	20	38-40
AF po ml/day	13-16	450
Ng hLF/ml AF	800	3000
Wt (kg)	0.450	3.350
Tot. hLF ng/day	11,600	1,350,000
Mg hLF/day	.0116	1.35
Mg hLF/k BW/day	0.03	0.40

Amniotic Fluid volumes from (Sadler,2000)
Concentrations of hLF in AF from (Pacora, 2000)
Fetal weights from (p. 492 Appendix 2 and Fig. 8.1 p. 91 in O'Rahilly, 2001)
Amniotic Fluid swallowed from (Pritchard, 1966)

There are no documented adverse consequences of fetal exposure to increasing levels of hLF throughout pregnancy. In fact, necessary benefit is implied by increased hLF when the integrity of natural barriers are threatened or breached (e.g., during villitis, intrauterine infection, invasion of the embryo into the decidua) and via the constant presence of hLF in the external environment (i.e., in the decidua, amniotic fluid, vernix caseosa, and maternal cervical mucus)

and ingested environment (i.e., in the amniotic fluid, vernix caseosa, colostrum, and breast milk) of humans as they transition from fetus to newborn[32,33,34,35].

c. Infant exposure

During the first twelve months of life, an infant encounters significant, long-term, oral exposure to exogenous human lactoferrin via one or more of three different, exposure scenarios. Each is discussed below.

(1.) Via human milk from one's mother.

The typical daily exposure of an infant – over the first 12 months of life – to hLF via the consumption of human milk – especially that of an infant's own mother – is dependent on three factors, i.e., the amount of human milk consumed per day, the amount of hLF in such daily-consumed human milk, and the weight of the infant at the time such milk is being consumed

The concentration of hLF in human milk varies very significantly during the human period of lactation. (Nagasawa, 1972; Lönnerdal, 1976; Mathur, 1990; Nuijens, 1996). During the first few days postpartum – when human colostrum is being expressed – hLF concentration may approach 10 mg/ml by day two and then taper off by day seven to about 5 mg/ml (Nuijens, 1996, Davidson, 1987).

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[32] Please note that amniotic fluid sampled before 20 weeks gestation supports the growth of bacteria (e g., *E coli*) but remains consistently bacteriostatic or bacteriocidal after 36 weeks gestation coinciding with the rise in hLF in the third trimester. (Pacora, 2000; Schlievert, 1975).

[33] There is increased immunohistochemical staining for hLF with chronic inflammation of placental villi (Thaler, 1993).

[34] In a study of lactoferrin in amniotic fluid and suppression of IL-6 production in intrauterine infection, Otsuki, 1999 suggests that lactoferrin could be useful in the treatment of chorioamnionitis.

[35] Vernix caseosa is a complex proteolipid unique to humans. In utero, the vernix caseosa detaches from fetal skin and is ingested with the amniotic fluid At birth, it covers the infant and is similar to colostrum and breast milk in derivation. Akinbi, 2004 demonstrated hLF in vernix caseosa by immunostaining and Western analysis. The authors conclude, "The third trimester fetus subsequently swallows a complex mixture of detached vernix, pulmonary surfactant, and amniotic fluid long before the forgut encounters breast milk. There is, therefore, considerable functional and structural synergism between the prenatal biology of the vernix caseosa and postnatal biology of breast milk."

Thereafter, the concentration continues to decline during mid lactation, eventually persisting at about 2 mg/ml in mature human milk. (Schanbacher, 1993).

For purposes of determining the **maximum**, natural exposure to hLF during infancy (the time during an entire human life of highest, natural exposure to hLF), let one assume the worst (or the highest exposure), i e., that the concentration of hLF reaches 10 mg/ml in colostrum during the first few days of infancy. At this point, an average[36] infant's weight would be approximately seven pounds (or 3.25 kilograms, assuming 1 kilogram per 2.2 pounds). (Lane, 2005; U.S. EPA, 2002). Later on, when hLF concentration drops to 2 mg/ml, the infant's weight would be between approximately 16 pounds at six months and 21 pounds at 12 months (or 7 53-9.84 kilograms, assuming 1 kilogram per 2.2 pounds). (Lane, 2005; U.S. EPA, 2002). At the 90th percentile[37], an infant would consume an average of 1000 ml per day during early infancy (months 1 and 2) and mid infancy (months 3-6). (U.S. EPA, 2002). As Table 3 below shows, this consumption could equate to a typical daily exposure (over the first 6 months of an infant's life) of ≥ 266 mg hLF per kilogram of infant body weight and (over the first year of an infant's life) of from 48-3077 mg hLF per kilogram of infant body weight[38].

Table 3

Daily Infant Exposure to hLF

Age	Weight	Human Milk Consump. (per day)	Maximum Amt. Of hLF In Human Milk	Total hLF Potentially Consumed Per Day	Total hLF Consumed Per Day Per Kilogram Of Body Weight
Newborn	7 15 lbs. (3.25 k)	1000 ml	10 mg/ml	10,000 mg	3077 mg/kg BW
Six months	16.57 lbs. (7.53 k)	1000 ml	2 mg/ml	2,000 mg	266 mg/kg BW

[36] Average weight is used here (instead of the 90th percentile weight) in order to establish **worst case**, i.e , reasonably highest exposure per kilogram of infant body weight.

[37] Consumption at the 90th percentile is used here in order to establish **worst case**, i.e., reasonably highest exposure per kilogram of infant body weight.

[38] Even for infants not consuming human milk, they consume from 105-245 mg bLF/day from infant formula. (GN 000042/77, page 255).

Twelve months	21 lbs. (9.5 k)	500 ml	2 mg/ml	1,000 mg	48 mg/kg BW

Importantly, the gene for human lactoferrin in the normal population incorporates 21 single nucleotide polymorphisms – nine of which generate a variant, but normal, amino acid. (Teng, 2006). The frequency of these normal variants can range from 1-43 percent. (Teng, 2006). Thus, practically speaking, given the large number of infants at any one time in the US population, most infants are exposed long-term to a variety of exogenous human lactoferrin that is different from the endogenous hLF being produced by any one of such infants.

Finally, there are approximately 4.2 million infants born each year in the United States (NCHS, 2004), of which approximately forty percent are still being breast fed at six months. (CDC, 2004). Thus, each year approximately 1.68 million infants are involved in the above-discussed, six-month (long-term) consumption pattern pertinent to exogenous hLF.

(2.) Via human milk from a female other than one's mother

There are numerous instances[39] in which an infant's mother cannot provide – for various potential reasons – an adequate supply of human milk. In such instances, substitute human milk can often be obtained either from a "wet nurse"[40] or from a commercial source (such as the not-for-profit "Human Milk Bank Association of North America" (HMBANA)[41] or the for-profit "Prolacta").

When such alternative sources of human milk are resorted to, infants consume such milk at the same rate and for the same, long-term period as described (in subpart 1) above. Such consumption will often expose an infant to

[39] Such instances are estimated to be ≥ 10 percent. (HMBANA, 2005).
[40] A wet nurse is a woman who breastfeeds a non-related infant or adult. This common practice dates back to, at least, the time of the Code of Hammurabi, i.e., 2250 BC.
[41] HMBANA consists of ten North American sites that collect, process and distribute human milk. It will distribute approximately 900,000 ounces in 2007. (HMBANA, 2007).

an exogenous form of hLF that differs from the form of endogenous hLF being produced by the infant.[42]

(3.) Via clinical applications

The majority of banked human milk is used for clinical applications in infants. (See, e.g , HMBANA, 2007). The most common diagnosed conditions which banked human milk is used to treat include: premature births, allergies, respiratory disorders, GI/bowel problems, and jaundice. (E.g., see HMBANA, 2005) When such milk is used, it is consumed at daily rates and for long periods of time like those set forth in subpart I above. As noted above, such consumption often exposes an infant to several exogenous forms of hLF that differ from the form of endogenous hLF being produced by the infant.

Table 4 below sets forth some of the published studies pertinent to long-term exposure to exogenous hLF by infants.

Table 4

Clinical Long-term Exposure To Exogenous hLF By Infants

Study	Age	Indication	Duration	Total ml DBM	mg hLF	mg hLF/kg BW/d
(Arnold, 1995)	6wk-12mos	GER Seizures Pneumonia In utero drugs	11.5 m.	175-350,000	350-700,000	210-307
(Asquith, 1987)						
Term infants	9-27 wk	Food allergy, FTT	4 5 m.	67,000	134,000	132-306
	3-6 m	Maternal illness	3 m	55,000	110,000	162-376
	3-6 m	ALL	3 m	45,000	90,000	133-308

d. Adult exposure

There is also considerable human experience with regard to long-term consumption of hLF beyond infancy. For example, suckling beyond infancy

[42] Since HMBANA pools its sources of human milk (up to a maximum of 20 donors per 300 ml of human milk), infants receiving human milk from HMBANA would be exposed to multiple versions of hLF. (Asquith, 1987; HMBANA, 2007).

commonly occurs throughout the world, depending on cultural norms and available resources. (Wickles, 1953; Ford, 1945 (as cited in www.geocities.com/HotSprings/Spa/3156/history.htm?200712); Ploss, 1935); thus, for centuries, hLF has been ingested by non-infants via milk from wet nurses (Hill, 1987). Such consumption has only increased with the advent of banked donor milk since about 1910. (Wickles, 1953). In addition, provision of human breast milk to the very old or infirm has been practiced, by Asian cultures, for centuries. (Baumslag, 1987). Total numbers of non-infant individuals ingesting hLF (in human milk) are impossible to ascertain given the universal acceptance of human milk as a safe, familiar food and the informal use of wet nurses or banked milk by families. Indeed, such practices are so commonplace that governmental entities do not keep any count of them.

The significant increase in milk banking (Sakamoto, personal communication, 2007; HMBANA website, 2007) **and** the more recent emergence of recombinant hLF (rhLF) **and** the ever-increasing awareness among experts of the safety and significant nutritional/clinical utility of hLF has significantly extended the nutritional/clinical use of hLF in human milk to treat a wide range of pathology in humans from premature birth through adulthood.[43] Such clinical/nutritional applications include, but are not limited to: feeding problems, food intolerance, food allergies, prematurity, chronic diarrhea, Hirschsprung's

[43] The majority of banked human milk is donated for the feeding of sick premature infants (Sakamoto, personal communication July 3, 2007 who provided a copy of the HMBANA: 2005 Diagnoses Chart prepared by Linda Gonzales San Jose Mothers' Milk Bank). Prioratizing banked milk distribution ranks recipients from most to least critical in the following order; 1) Premature infants, sick; 2) Premature infants, well, ; 3) Infants < 12 months with medical conditions likely to respond to donor milk therapy; 4) Individuals > 12 months old, likely to respond to donor milk therapy; 5) Research contracts for clinical use in well-designed studies; 6) Individuals > 12 months with chronic medical conditions, high normal functioning and low dose need; 7) Individuals > 12 months with chronic medical conditions, high normal functioning and high dose need; 8) Individuals > 12 months with chronic medical conditions, low level functioning , and low dose need; 9) Individuals > 12 months with chronic medical conditions, low level function, and high dose need; 10) Infants for short –term use, no specific medical condition, and 11) Laboratory reserch (milk that cannot be used for human consumption due to drugs used by donor, lack of complete testing of the donor, or age of the milk. (Tully, 2002). Some United States nurseries, including those at the University of Kentucky and at the Wilmington Delaware Medical Center have even used banked human milk as a standard initial feed for premature infants (Arnold, 1990). The practice is more routine in the U.K., Australia and Sweden, but published studies only cite exposures up to six weeks and, therefore, were not included in the discussion of chronic (≥ 90 days exposure). (Boyd, 2007; Arnold, 2002; Lucas,1990).

disease, congenital anomalies of the mouth or gastrointestinal tract, necrotizing enterocolitis, short gut syndrome, immune deficiency such as IgA deficiency, chromosomal anomalies, in utero drug/alcohol exposure, chronic renal failure, dialysis, postoperative cardiac/gastrointestinal/cleft lip/palate repair, irritable bowel syndrome, inborn errors of metabolism, cancers, candidiasis, graft versus host disease, liver transplant, Hepatitis C, chemotherapy induced mucositis, esophagitis, gastrointestinal ulceration, infectious diarrhea, inflammatory bowel disease, the elderly, sepsis, pediatric burn cases, and bronchopulmonary dysplesia. (Hayes, 2007; Lactation Education Resources Website www.leron-line/MilkBanking.htm 6/22/07; Williams, 2007; Boyd, 2007; Rehmeyer, 2006; Wang, 2006; Zimecki, 2005, Tully, 2004; Updegrove, 2004; Troost, 2003; Arnold, 2002; Playford, 2000; Morley, 2000; Schanler, 1999; Wiggins, 1998; Merhav, 1995; Anderson, 1993; Lucas, 1990; Trumpler, 1989; Asquith, 1987; and Rangecroft, 1978). For a summary of some of the nutritional/clinical, significant and long-term applications of human milk see Table 5 Recombinant forms of human lactoferrin are also currently in clinical trial[44]. Published clinical trials so far demonstrate only tolerance and safety of rhLF even when administered to the most vulnerable human populations and even following long-term exposure to significant doses. Some examples of clinical applications and significant, long-term exposures are discussed below.

Table 5
Clinical Long-term Exposure To Exogenous hLF By Adults

Study	Age	Indication	Duration	Total ml DBM	mg hLF total	mg hLF/k BW/d
(Tully, 2004)	6-18m	FTT GER Malrotation GI surgery Food intolerance	12 m	247,320	494,640	143
(Arnold, 1995)	6-30m	FTT Food intolerance FP solid food	24 m.			
(Tully, 1990)	4 y	IgA deficiency Food allergies	23 m	84,000	168,000	≥13 3

[44] As of August 25, 2005, rhLF has been administered to over 500 people and appears safe and well tolerated as reported on the Agennix website, www.agennix.com.

(Arnold, 1990)	School age	Acrodermatitis enteropathica	72 m	394,200	788,400	8 8-17 1

ALL = acute lymphocytic leukemia; FP = feeding problem; FTT = failure to thrive; GER = gastroesophageal reflux; DBM = donated banked milk

None of the above case histories report adverse events secondary to prolonged use of donor human milk, but in some instances, did indicate weight loss if tapered too fast from donor human milk. (Arnold, 1995).

A recent study at Baylor specifically examined the safety of oral recombinant hLF (rhLF) administered to 10 adult patients with metastatic, refractory cancer. (Hayes, 2006). Recombinant hLF was administered 14 days continuously alternating with 14 days off up to a maximum total of 105 days of rhLF consumption. Recombinant hLF was well-tolerated without evidence of hematological, hepatic, or renal toxicity. The most common symptom was mild diarrhea controlled by over the counter drugs which affected only 2 patients (one patient taking 1500 mg/day, one patient taking 9000 mg/day). One patient taking 4500 mg rhLF/day experienced 4-6 loose stools per day. There was no correlation between dose of rhLF and severity of diarrhea and any other complaint. Other constitutional symptoms noted are those commonly found in cancer patients (weakness, nausea, fatigue, vomiting, constipation, nasal congestion, taste perception with only patient complaining per symptom). The range of total doses of rhLF received over the longest study period of 105 days appears in Table 6.

Table 6
Clinical Long-term Exposure To Exogenous hLF By Adults

Study	Age	# days	mg rhLF/d	mg rhLF/k BW/d
(Hayes,2006)	median 63 yr			
		105	1500 mg	25
		105	4500 mg	75
		105	9000 mg	150

Oral administration of rhLF did not increase serum rhLF after 1 dose or following 14 consecutive days of dosing. Biologic activity of the rhLF was established by demonstrating an increase in serum and gut IL-18.

A randomized, multi-center, double-blind, placebo-controlled study of 110 chemo-naïve patients with advanced/metastatic, non-small cell, lung cancer was presented at the 2006 meeting of the American Society for Clinical Oncology. (Wang, 2006). Half of the patients received chemotherapy (carboplatin/paclitaxel) and oral rhLF for 1-3 cycles (35 days per cycle). The other 55 patients received chemotherapy alone. Treatment duration and total dose of rhLF appear in Table 7

Table 7

Study	# days	mg rhLF/d	mg rhLF/k BW/d
(Wang, 2006)	35	3000	50
	70	3000	50
	105	3000	50

Oral rhLF was well-tolerated with no drug-related adverse effects.

Patients with renal cell carcinoma have been treated for 14 days on 4500mg twice daily alternating with 14 days off for a maximum of 34 months. (Hayes, 2007). This schedule resulted in 476 days of recombinant hLF exposure at 9000mg/day, for a total exposure of 4,284,000 mg per patient and a daily exposure of 150 mg rhLF/k BW. The same group of investigators recently changed to continuous daily rhLf, 1500 mg twice daily to treat refractory patients with head and neck cancer. Thus far, the longest exposure is 4 months (120 days) at 3000 mg rhLF per day (or 50 mg rhLF/k BW), for a total exposure of 360,000mg per patient. (Hayes, personal communication, 2007). Most patients experienced no adverse events, but those who did had only minimal symptoms, including occasional mild diarrhea or nasal stuffiness which may or may not be related to rhLF.

e. Conclusion

As the foregoing information clearly indicates, it is definitely **not** the case that humans are not orally exposed long-term to exogenous hLF. Rather, long-term, oral exposure to exogenous hLF represents the norm. Indeed, such oral exposure begins very early in utero, continues to expand during the entire pregnancy (to hLF levels approximating 9 mg hLF/kg BW/day), continues to further expand during the first 12 months of life (to hLF levels of from 48-3077 mg hLF/kg BW/day) and may continue after infancy – from time to time – in large numbers of individuals for long-term periods – to include long-term exposures of from 9-150 mg hLF/kg BW/d, all of which exposures are deemed by qualified experts to be normal and safe. The above-reported clinical studies only further support this conclusion.

Moreover, such exposures almost universally expose the consuming individual to a form or forms of hLF that differ from his/her own. Again, all in normal and safe manner.

Finally, many such long-term, oral exposures are to individuals who are members of the most vulnerable populations (including the premature, the very old, infants, those with impaired immunity and/or impaired mucosal barriers secondary to use of drugs, or disease (including cancer, overwhelming infection or autoimmune disease), or inflammation), yet such exposures do **not** cause deleterious effects and are, in fact, broadly deemed safe and beneficial. And, most importantly, rather than such exposures causing any adverse, immunological effects, the actual effects observed and recorded represent only beneficial or positive immuno-effects[45] (even in the most vulnerable) such as decreased incidence hospitalization duration, less infections, decreased necrotizing enterocolitis, and increased growth

[45] For a comprehensive discussion of these effects, see pages 18-21 supra and Zimecki, 2007; Kruzel, 2007; and Fischer, 2006.

7. Subarea 4 (concerning oral exposure to endogenous hLF)

Describe the daily, long term, oral exposure that an adult human has to endogenous human lactoferrin resulting from, for example, saliva or other gastrointestinal secretions.

8. Pharming Update

a. Introduction

Endogenous human lactoferrin is naturally, continuously, and significantly present ubiquitously in human tissues throughout the entire human body throughout life. (Attachment 1, page 26; Nuijens, 1996). This discussion, however, focuses **only** on that portion of the above-referenced endogenous human lactoferrin that results from saliva and other gastrointestinal secretions and, thus, is the result of oral exposure.

b. Exposure via the oral route

Within a twenty-four hour period, significant quantities of endogenous human lactoferrin are secreted into the alimentary canal from one or more of several sources.

Perhaps the most obvious source of hLF is via the ingestion of saliva,[46] which begins to be produced (with hLF) in the fetus from 20 weeks gestation on. (Reitamo, 1981). In the adult oral cavity, saliva is produced under two, different, flow rate conditions, i.e., under unstimulated and stimulated conditions; each condition results in a different amount of saliva entering the alimentary canal. (Marino, 2003). The total daily amount of saliva introduced from both conditions

[46] Saliva is the clear, alkaline, somewhat viscid secretion secreted by the parotid, sublingual, submaxillary, and smaller mucus glands of the human mouth. It serves to moisten and soften the food, keep the mouth moist, and contains, among many other substances, lactoferrrin. (Dorlands, 2003; Troost, 2002; Nuijens, 1996).

is approximately 1500 milliliters per day (Yamada, 2003). Both flow-rates have been measured. Unstimulated saliva flows at a rate of approximately 0.5 ml/min. (Tanida, 2003). This basal rate typically produces approximately 720 ml saliva per day and contains from 19-47 micrograms of hLF per milliliter of saliva. (Tanida, 2003). Accordingly, approximately 14-34 milligrams (or more) of hLF could be introduced into the alimentary canal per day via production of unstimulated saliva Stimulated saliva flows at a rate of 1.5 to 2.4 ml/min. (Lin, 2001). This results in production of from 2.16-3 46 liters of saliva per day. Such saliva contains from 3.4 to 7.1 micrograms of hLF per ml of saliva. (Lin, 2001). Accordingly, from 2.4 to 5.1 milligrams of hLF could be ingested each day from stimulated saliva. Thus, one could ingest up to 39 mg endogenous hLF per day from oral consumption of saliva.

Distal to the tongue, the next source of endogenous hLF in the alimentary canal is the stomach[47]. In the fetal stomach, endogenous hLF first appears in mononuclear cells, presumably granulocytes, after 16 weeks gestation. (Reitamo, 1981). In the adult stomach, immunocytochemistry demonstrates lactoferrin expression in the gastric glands in the cardia (the region where the esophagus inserts into the stomach), and in the body (the largest region of the stomach between the lesser and greater curvatures) and in the antrum (that portion of the stomach between the body and the pylorus). (Luqmani, 1991). Within gastric glands, chief (peptic) cells in particular, strongly stain for hLF. (Luqmani, 1991). Chief cells are protein-secreting exocrine cells structurally similar to pancreatic acinar cells. (Weaver, 1991). Within the stomach, hLF may also be derived from neutrophils. In the absence of neutrophils, i e., when hLF is coming only from gastric glands, hLF measures approximately 0.12 ng/microgram of antral mucosa tissue protein and 0.08 ng/microgram of body mucosa tissue protein (Nakao, 1997). With mild neutrophil infiltration of the mucosa, hLF measures 0.33 ng/microgram in the antral mucosa tissue protein and 0.26 ng/microgram in the body mucosal tissue protein. (Nakao, 1997). In

[47] Please note that the tissues comprising the esophagus do **not** secrete any hLF into the alimentary canal. (Luqmani, 1991)

instances of significant neutrophil infiltration, e.g , during inflammation accompanying H. pylori infection, the foregoing hLF levels significantly increase to 2.7 ng/microgram of antral mucosal tissue protein and 2.61 ng/microgram of body mucosal tissue protein. (Nakao, 1997). There exists approximately 6400 milligrams of total protein in the gastric mucosa of the entire stomach. (Yamanaka, 1974). Of this amount, approximately eighty percent (i.e., 5120 milligrams) is associated with the body and antrum of the stomach, and approximately 75 percent (or 3840 milligrams) of that amount is from the antrum. All of this information – in the aggregate – indicates that the stomach provides (in non-pathogenic situations) – **at a minimum** – approximately .45-4.5 mg of hLF at any one time. No data is available indicating the maximum amount of hLF provided in any 24-hour period from the gastric mucosa; however, it is reasonable to assume that such 24-hour figure is multiple times the 4.5 mg cited. In addition, hLF is also present in the mucus layer which coats the gastric mucosa. No information is available on how much hLF resides within such mucus layer. (Clamp, 1986)

Within the duodenal lumen, endogenous hLF originates from the duodenum (i e., the first approximately 10 inches of the small intestine), the liver and the pancreas. The duodenum is the first portion of the small intestine connecting the pyloric stomach proximally and intestinal jejunum distally. Biliary and pancreatic ducts empty into the duodenum. (Weaver, 1991). Fetal expression of hLF occurs after 13 weeks gestation in the liver and small intestine, and after 20 weeks gestation in the pancreas. (Reitamo, 1981) In health, endogenous hLF immunolocalizes to enterocytes at the villus tips of the duodenum and jejunum (Tedeschi, 1987). In diseases such as celiac disease and postenteritis syndrome, endogenous hLF is increased in villus tips and crypts. (Tedeschi, 1987). During acute cholera, for example, gene expression for endogenous hLF in the duodenum is increased and has been interpreted as enhanced innate defense during acute infection. (Flach, 2007).

With regard to the hLF contribution from the liver which appears in bile (which originates in the liver, is stored and concentrated in the gall bladder, and

is secreted into the duodenum), basal hLF levels in bile have been measured in studies of inflammatory bowel disease where control subjects were post-colectomy patients cured of ulcerative colitis. Here, the mean hLF concentration was 1.2 mg/L. In active ulcerative colitis, hLF concentration was 2.8 mg/L. Crohn's disease patients in remission were also considered a control population; in these patients, hLF measured 1.1 mg/L. (Pereira, 1998). In active Crohn's disease, three fold higher concentrations of biliary hLF occur. (Pereira, 1998) Given that approximately 500 ml of bile is secreted daily (Yamada, 2003)., production of biliary hLF (in health) approximates 0.6 mg per day and (in inflammatory bowel disease) 3.1 mg per 24 hours.

In the pancreas, hLF immunolocalizes to the acinar cells bordering the lumen and are contained within zymogen granules and endoplasmic reticulum. (Lechene de la Porte, 1981; Colomb, 1976). Pancreatic hLF secretion is stimulated by a meal or administration of cholecystokinin alone or in combination with secretin, but not by secretin alone and parallels amylase secretion. (Hegnhoj, 1986). Basal pancreatic secretions obtained at the ligament of Treitz measured pancreatic hLF in healthy volunteers at 0.7 micrograms/ml of pancreatic secretions. (Brugge, 1988) Given that the pancreas secretes approximately 1500 ml/day, the pancreas produces 1050 micrograms (or 1.1 mg) hLF per day. (Yamada, 2003).

Endogenous hLF throughout the small intestine has been measured in whole gut lavage fluid (i.e., WGLF, a nonabsorbable liquid preparation routinely given to subjects to cleanse the bowel prior to colonoscopy or colorectal surgery or used for research purposes to measure ongoing production of intestinal proteins). (Kayazawa, 2002). "The rate of fluid passage along the gut during lavage is approximately 1 L/h (0.017 L/min), so the estimated daily loss (mg/day) of a certain substance can be obtained from the following equation: 24 (L) x the concentration in WGLF (mg/L)." (Kayazawa, 2002). By this formula, daily hLF production was calculated to be approximately 21.5 mg/day (24 x 1000 x 0.89) with a > 40 fold increase in active ulcerative colitis (i.e., 890 mg/day) and a > 30 fold difference in active Crohn's disease (i.e., 686 mg/day).

Estimates of colonic hLF are available from determinations of fecal hLF. Uchida et al. measured hLF using an enzyme – linked immunosorbent assay (ELISA). (Uchida, 1994). They found the fecal concentration of hLF to be approximately 0.75 microgram hLF/g feces. (Uchida, 1994) Since humans excrete approximately 200 g of feces per day, that would yield about 150 micrograms hLF/ day in feces (most of which is produced in the colon).

c. Conclusion

The foregoing information indicates that on a daily basis humans are naturally and normally exposed – via the alimentary canal – to significant quantities of endogenous hLF emanating from various internal sources. This is not surprising since virtually all other tissues of the human body are also constantly exposed to varying levels of endogenous hLF. More specifically, under non-disease (i.e., normal) conditions, during a twenty-four hour period and day after day (for a lifetime) one can be exposed to (conservatively estimated) up to 87 milligrams of endogenous human lactoferrin in the alimentary canal. In some normal cases, such exposure can be even greater. Moreover, during times of disease, such exposure can – as indicated above – increase by 30-40 fold. Thus, in certain instances, daily exposure to endogenous lactoferrin can significantly exceed 1 gram per day – indeed, possibly up to (again conservatively estimated) almost 3.5 grams. Most importantly, all such exposures are deemed – by the human body – to be natural, non-harmful, and beneficial.

9. Subarea 5 (concerning helpfulness of exposures to hLF)

Can the known safety of the exposures to human lactoferrin referenced in subareas 3 and 4 above be used to help address the safety of any daily, long term, oral exposure of infants or adults resulting from oral consumption of

transgenically-produced human lactoferrin? If yes, to what extent? If not, why not?

10. Pharming Update

Given the updates set forth to subareas 3 and 4 above, it is clear that the long-term exogenous and endogenous oral exposures to hLF that are documented in the informational updates to subareas 3 and 4 can be used to help address the safety of Pharming's human lactoferrin. As set forth in the updates to subareas 3 and 4, long-term, daily, oral exposure to **exogenous** human lactoferrin can approximate 48-3077 mg/kg BW in the first 12 months of life and 9-150 mg/kg BW thereafter for long periods **and** long-term, daily, oral exposure to **endogenous**, human lactoferrin can approximate 1.45-58 mg/kg BW. Together, these two types of oral exposures can approximate long-term, daily, oral exposures to hLF of (conservatively estimated) approximately 50-3100 mg/kg BW during the first 12 months of life and approximately 10-200 mg/kg BW during the rest of life after infancy – all of which the human body deems to be safe, non-harmful exposures Such daily, long-term, oral exposures far exceed any exposure that would result from consuming Pharming's hLF, i e., 1.91 mg/kg BW/day (90^{th} percentile user). (See Pharming's GRAS Notice, Attachment 1, pages 52-58).

11. Subarea 6 (concerning safety criteria)

Are the traditional, scientific criteria long-used by CFSAN to assess the long-term safety of substances to be added to food adequate to assess the long term exposure to such a substance when it may exhibit immunomodulatory properties? If so, explain why. If not, describe what other criteria are needed and why.

12. Pharming Update

a. Introduction

From a practical point of view the foregoing subarea can be subdivided into two subsidiary subareas, i.e., (1.) Whether there currently exists scientific criteria pertinent to assessing the safety of substances to be added to food (including GRAS substances)? and (2.) Whether such criteria – to the extent they currently exist – are sufficient to assess the long-term exposure to such a substance when it may exhibit known immunomodulatory properties? These two subareas are addressed below.

b. Scientific criteria

Numerous scientific criteria exist today for assessing the safety of substances appearing in or that have been added to food. They have been developed over the last 101 years since passage of the Food and Drugs Act of 1906. Following is a summary of those criteria and how they came to be

1. 1906-1958

Prior to 1958, the terms "food additive" and "GRAS substance" did not – from a legal point of view – formally exist, i.e., neither were separate categories under either the Food and Drugs Act of 1906 or the Food Drug and Cosmetic Act of 1938. Rather, such "added substances" – like all components of food – were regulated under the adulteration provisions of the law. In any particular instance, FDA paid close scrutiny to the individual safety characteristics of a given substance and then determined whether such characteristics amounted to a "poisonous" or "deleterious" substance. Safety characteristics were determined and evaluated – as they are today – via application of evolving and (at any given time) then applicable criteria, i.e., analytical and testing methodologies.

2. The 1958 Food Additives Amendment

Prior to 1958 numerous difficulties, uncertainties and problems arose when FDA attempted to regulate substances – of all kinds – to be added to food. (Such difficulties, etc. are best described at length in C. Dunn's "Legislative Record of the 1958 Food Additives Amendment", S Rep No 2422 and HR Rep No 2284, 85th Cong 2d Sess (1958)). After extensive debate, Congress unanimously passed the 1958 Food Additives Amendment to the FD&C Act and same was signed into law on September 6, 1958.

The Amendment created numerous legal/scientific criteria pertinent to evaluating the safety of substances to be added to food. These included that:

1. the term "food additive" was defined (see 21 USC § 321(s));
2. the term "generally recognized as safe" was defined (see 21 USC § 321(s));
3. both food additives and GRAS substances must be shown to be "safe" for each intended use (see 21 USC § 321(s)),
4. substances to be added to or appearing in food can be found to be adulterated if not either prior approved or generally recognized as safe (see 21 USC § 342);
5. substances to be added to or appearing in food can be unsafe if poisonous or deleterious and injurious to health, filthy, putrid, decomposed, or otherwise unfit for food (see 21 USC § 342);
6. food additives and GRAS substances not used pursuant to the specific requirements of the pertinent regulation can be found to be adulterated (see 21 USC § 342); and
7. no food additive may be approved (or remain approved) if FDA finds that such substance induces cancer in men or animals (see 21 USC § 348(c)(3)(A)).

These criteria form the initial core elements of evaluating safety and have been successfully applied now for approximately five decades.

3. **Regulatory enactments**

In 1977 numerous regulatory criteria were promulgated to flesh out the prior-enacted core criteria for evaluating food additives and GRAS substances. These include that:

1. GRAS substances can be found to be GRAS either via "experience based on common use in food" or "scientific procedures" (21 USC § 321(s) and 21 CFR § 170.3(f) and (h));
2. the term "common use in food" means a substantial history of consumption of a substance for food use by a significant number of consumers (21 CFR § 170.3(f));
3. the term "scientific procedures" is an evolving standard that means those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance (21 CFR § 170 3(h));
4. the term "safe or safety" means that there is a **reasonable certainty** in the minds of **competent scientists** that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance. Safety may be determined by scientific procedures or by general recognition of safety. In determining safety, the following factors shall be considered:
   (1) The probable consumption of the substance and of any substance formed in or on food because of its use;
   (2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet; and
   (3) **Safety factors** which, in the opinion of **experts qualified by scientific training and experience** to evaluate the safety of food

and food ingredients, are **generally recognized** as appropriate (emphasis added) (21 CFR § 170.3(i)); and

5. general recognition of safety shall be determined in accordance with 21 CFR § 170.30 which requires, among other things, that:
   a. general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food;
   b. the basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food;
   c. general recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food;
   d. general recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient;
   e. general recognition of safety through scientific procedures shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and information; and
   f. a GRAS substance should:
      (1.) generally comply with any applicable food grade specifications of the Food Chemicals Codex;
      (2.) perform an appropriate function in the food or food-contact article in which it is used; and
      (3.) be used at a level no higher than necessary to achieve its intended purpose in that food or, if used as a component of a food-contact article, at a level no higher than necessary to achieve its intended purpose in that article (21CFR § 170.30).

4. Publication of the "Red Book"

In 1982, FDA published the "Red Book" – a 240 page document formally entitled: "*Toxological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*". This document was intended:

1. to establish the boundaries of "reasonable certainty";
2. to set forth appropriate guidance regarding the scientific criteria to be used in assessing safety;
3. to establish a complete assessment system;
4. to ensure a cost-effective system;
5. to establish a flexible framework; and
6. to encourage use by all of the criteria-incorporating assessment system.

Such comprehensive document includes appropriate criteria, guidelines for conduct of studies (i.e., scientific procedures), standards for assessing testing results, decision elements and model testing protocols.

In 1993, FDA published the second version of the Red Book. It consists of 235 pages. It amounted a significant updating of the prior version – consistent with changing scientific standards – and was intended to indicate that a submission conforming to the Red Book's recommendations would provide **sufficient scientific information** to evaluate a substance's safety for the regulated intended use(s). It included, among many other protocols, testing pertinent to immunological end points.

Finally, in 2000, a third version of the Red Book – now called "Toxicological Principles for the Safety Assessment of Food Ingredients" – was published. It too amounted to a significant revision and is an ongoing process. Importantly, this third version was made applicable also to assessing the safety of GRAS substances.

5. **White House policy**

Due primarily to concerns about lack of coordination and uncertainty as to the manner in which biotechnology was going to be regulated in the United States, the Reagan Administration formed – in 1984 – an interagency working group to study, to establish, and to coordinate the federal government's regulatory policy pertinent to biotechnology. The group included at least thirteen member agencies, including FDA. Importantly, the group was to determine, among other things, whether the then current regulatory requirements were adequate for regulating biotechnology. The initial results of the working group were published on December 31, 1984. (See, 49 FR 50856 (1984)) This document was updated on December 14, 1985. (See, 50 FR 47174 (1985)).

Also in 1985 – specifically on October 31st – the President established the Biotechnology Science Coordinating Committee ("BSCC"), a scientific coordination body. (See, 50 FR 47174 (1985)). Importantly, the BSCC's responsibilities included establishing a common scientific approach to regulating biotechnology.

Finally, in June of 1986 a very lengthy, final notice was published indicating how – pursuant to White House requirements, specifically the Office of Science and Technology policy ("OSTP") – a number of Federal entities, including FDA, would regulate biotechnology. (See, 51 FR 23302 (1986)). Importantly, such notice stated that the then existing regulatory framework was deemed **adequate** and flexible enough to oversee modern biotechnology applications. Further, it made clear that **no** new laws, regulations or other regulatory burdens – such as new criteria – were necessary in order to adequately regulate such biotechnology.

c. The adequacy of the "criteria"

With regard to whether existing "criteria" for evaluating the safety of substances to be added to or that otherwise may become a component of food

are "adequate", such question has been legally answered since 1986 – that is, upon publication of the above-references OSTP notice. Such notice indicated that the answer is yes.

As a practical matter, as discussed below, such question has also been repeatedly answered in the affirmative by the approximately last thirty years of experience of federal entities authorizing use of biotechnology-related products in interstate commerce.

d. Application of the "criteria" to products of biotechnology

The above – referenced OSTP notice applies, of course, to **all** "criteria" pertinent to evaluating the safety of **all** products emanating from biotech sources regulated by federal entities – including FDA. To date, hundreds of products that "may" exhibit immunoregulatory properties have been reviewed by various US entities (such as EPA, USDA and FDA) and authorized for introduction into interstate commerce. All such reviews were conducted pursuant to the above-referenced criteria or very similar criteria coordinated by the BSCC.

More significantly, FDA has used virtually the same criteria to review and approve numerous drugs (and biologics) – for over twenty-five years – all of which were human proteins intended to be injected (the most potentially impactive route of exposure) and all of which were potentially immune modulators. In every case, such criteria were deemed adequate for evaluating the safety of such products. No new criteria were needed, although FDA has often provided pertinent guidance documents to indicate how such criteria would be applied to biotech products.

Even more specifically, CFSAN has been applying – without untoward incident – the criteria here in question (and even their predecessor criteria) to biotech products since 1990. All such products are proteinaceous in nature and might be immunomodulatory. The very first such product, i.e., chymosin (otherwise known as the active enzyme rennin) has been found to be GRAS when produced via three, different transgenic sources. (21 CFR § 184.1685).

Since this product, CFSAN has reviewed and authorized (i.e., found to be GRAS) via the criteria here in question numerous biotech enzyme products (as well as numerous enzyme products emanating from non-transgenic sources) both under the GRAS Affirmation process and the GRAS Notification process. All such products have been safely used since being found to be GRAS.

Finally and not least importantly, the criteria here in question were successfully applied to (and, thus, found to be adequate) three lactoferrin (also proteinaceous) products, i.e., bovine lactoferrin (see, GRN Nos. 67, 77 and 130). Especially with regard to GRN No. 77 whose intended uses are identical to those currently before CFSAN in GRN No. 189, the exact same immunomodulatory properties were before CFSAN since bLF induces every immuno-related effect that hLF does – and very often via the same testing common to both (testing that includes over 300 studies all cited in Pharming's GN documents). If the criteria here in question were deemed adequate by CFSAN for assessing the safety – including immunomodulatory properties – of bLF (and they were), then by definition they must be deemed adequate for assessing the safety of hLF under identical, requested pattern of use. There is no rational, scientific basis for concluding otherwise.

e. No need for additional criteria

The fact that the criteria here in question were successfully applied -- as discussed above – to the bLF-related GRAS Notifications indicates that no new additional criteria are needed. The bLF-related GRAS Notifications, especially GRN No. 77, raised the **very same** potential immunomodulatory issue(s) raised by Pharming's hLF GRAS Notification. No new questions have been raised by CFSAN with regard to Pharming's GRAS Notification that were not also raised when reviewing and assessing the safety of bLF Thus, such criteria should be deemed adequate as is.

C. Conclusion

As CFSAN has repeatedly acknowledged, Pharming has answered all questions posed to it via the scientific information forwarded to CFSAN. Such information includes – both qualitatively and quantitatively – more, better, and more current scientific information than has ever been submitted in any GRAS Notification to CFSAN. Pharming believes that such extensive information – as supported by numerous qualified experts, including those discussed above but unconnected to Pharming – demonstrates consistent with all pertinent assessment criteria that Pharming's hLF is GRAS for its intended uses and that such information is more than sufficient for CFSAN to base its review on Thus, Pharming encourages CFSAN to complete its review in the near future based on such information and to issue a "no questions" letter.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
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JAN 2 2008

Charles L. Morin
Morin & Associates
388 Market Street, Suite 1460
San Francisco, California 94111

Dear Mr. Morin:

This responds to your letter of November 1, 2007, to Dr. Robert Brackett regarding new Section 912 of the Food and Drug Administration Amendments Act of 2007. As you know by now, Dr. Brackett is no longer with the Agency.

I understand that you met with Mr. Michael Landa, Deputy Director for Regulatory Affairs, Center for Food Safety and Applied Nutrition (CFSAN), on December 11th and discussed certain aspects of Section 912. I have asked Mr. Landa to keep me informed of the discussions and any follow-up. In the meantime, I thank you for sharing your comments with us in this matter.

Sincerely yours,

David W. K. Acheson, M.D., F.R.C.P.
Acting Director
Center for Food Safety
and Applied Nutrition

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February 29, 2008

Stephen F. Sundlof, DVM, PhD (HFS-1)
Director (Room 4B-064)
Center for Food Safety and
Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Pharming Group NV
Notice of GRAS exemption for human lactoferrin derived from the milk of transgenic cows expressing a human gene encoding human lactoferrin
GRN No. 000189
Request for a meeting

Dear Dr. Sundlof:

First and importantly, congratulations on being appointed Director of CFSAN! Such appointment is, in my view (based on 33 years of experience), a distinct honor. We look forward to you exercising in timely fashion "the dedication, vision and expertise needed to tackle the challenges" at CFSAN.

To that end, we have a challenge for you that needs your **immediate attention**. Such challenge concerns the current regulatory status of Pharming's GRAS Notification (i.e., GRN No. 189) which GN speaks to use of human lactoferrin for certain food uses (as specifically set forth in the GN), i.e., uses that are identical to those already authorized by CFSAN in connection with use of bovine lactoferrin (see GN number 77 and its associated "no questions" letter dated 08/14/01), and results from the following events:

Date	Event	Running Clock
12/29/05	Pharming files GN (supported by qualified	NA

Date	Event	Running Clock
	experts).	
12/30/05	CFSAN receives Pharming's GN.	NA
01/03/06	CFSAN acknowledges receipt of GN.	NA
01/12/06	CFSAN formally "files" GN.	Day 0
05/17/06	Pharming receives email from CFSAN; CFSAN has no questions about the content of the GN; CFSAN has questions about whether hLF induces any adverse, non-allergic response by the adaptive immune system.	Day 125
09/01/06	CFSAN and Pharming hold teleconference concerning CFSAN's questions and related matters.	Day 232
12/22/06	Pharming files a qualified experts' comprehensive response to CFSAN's questions.	Day 344
12/26/06	CFSAN receives Pharming's response.	Day 348
03/09/07	CFSAN and Pharming hold teleconference concerning whether to hold a Part 15 hearing in the near future.	Day 421
07/26/07	Pharming updates its GN file.	Day 560
10/05/07	Pharming meets with Dr. Mattia to unstall review of its GN.	Day 631
10/12/07	Pharming meets with Dr. Tarantino to unstall review of its GN.	Day 638
11/15/07	Pharming requests meeting with Dr. Brackett to unstall review of its GN.	Day 671

Date	Event	Running Clock
12/11/07	Pharming meets with Mike Landa, Dr. Tarantino et al. to unstall review of its GN. CFSAN promises to reach a decision with regard to procedure by last week of January or the first week of February, 2008.	Day 697
12/12/07-02/27/08	Pharming communicates with CFSAN numerous times. No CFSAN decision is reached.	Day 775
02/29/2008	Pharming formally requests a meeting with Dr. Sundlof to unstall the review process.	Day 777

As you can see, CFSAN's process of reviewing Pharming's GN and making a final decision on the merits has been overwhelmingly stalled for over 14 months! And this is in addition to the prior 12 months CFSAN spent reviewing information and determining that no scientific questions remain. Such unnecessary and unwarranted delay has already significantly harmed Pharming, and such continuing delay is increasingly functioning to irrevocably and substantially further harm Pharming. Moreover, it is clear that unless you immediately intercede to make it happen in timely fashion, such delay will continue – perhaps indefinitely. Pharming has been more than patient, but it can no longer afford to do so. Thus, we respectfully request that you intercede **immediately** – and begin your involvement by granting a meeting as soon as is possible.

To make this all happen, Pharming respectfully and formally requests a meeting with you as soon as it can be arranged. Because cetain Pharming representatives will already be in the United States, Pharming respectfully suggests the following dates and times for your consideration – an afternoon meeting on March 10th or 14th at 1 or 2 p.m.

The suggested agenda for such meeting – in order to unstall this entire matter and bring it to a final decision in the near future – would include (subject to your input) a discussion of:

1. whether there needs to be some sort of unusual, public involvement in a GRAS Notice review (especially since neither the pertinent **proposed** regulation nor its predecessor calls for such involvement and CFSAN has never (in more than 50 years!), to my knowledge, conducted a hearing in association with any GRAS submission ever made to CFSAN);
2. if not, then number 5;
3. if so, whether such involvement must amount to a hearing (Part 15 or otherwise) or whether another, just-as-useful means – such as notice and comment – might suffice;
4. if there must be public involvement when and how such will take place; and
5. if no public involvement is necessary, when Pharming can reasonably expect a final decision, i.e., a "no questions" letter, on the merits of its GN.

As each delay day occurs, Pharming becomes more and more harmed by the ongoing stall. Thus, we hope that you will act quickly to accommodate Pharming's request for a meeting.

If after reviewing the foregoing you should have questions, please let me know.

Thank you in advance for your prompt attention to and consideration of Pharming's request.

Sincerely,

Charles L. Morin

Via email; hard copy to follow via Federal Express

MEMORANDUM OF MEETING

Date: March 14, 2008

Place: Center for Food Safety and Applied Nutrition, FDA, College Park, MD

Participants:

Industry
Frans de Loos, Pharming
Charles L. Morin, Morin and Associates
Anurag Relan, Pharming

FDA
Stephen Sundlof, Director, CFSAN
Michael Landa, Deputy Director for Regulatory Affairs, OCD/CFSAN
Laura Tarantino, Director, Office of Food Additive Safety, CFSAN
Antonia Mattia, OFAS/CFSAN
Jeremiah Fasano, OFAS/CFSAN
Anne Crawford, Executive Operations Staff, OCD/CFSAN

Subject: Pharming GRAS Notification - Lactoferrin

The meeting was held at the request of Mr. Morin to discuss issues related to Pharming's GRAS submission for human lactoferrin.

Mr. Morin noted previous meetings between Pharming and CFSAN to discuss the status of his client's (Pharming) GRAS submission, which was received and filed by FDA as GRAS Notice No. 000189 in January, 2006. He asked for this meeting with the Center Director to specifically request his involvement in reaching a final decision. Dr. Sundlof indicated he was in a listening mode for this meeting but that he would give the matter serious and prompt consideration and work to reach a decision as soon as possible following the meeting.

Mr. Morin discussed his view of the pros and cons of three options he presented for what CFSAN might do with regard to the GRAS submission. He indicated his client's preference to receive a "no questions" letter from the Agency. He is not in favor of actions that would result in seeking some sort of public participation before reaching a decision. However, if CFSAN believes public participation is necessary, he would prefer doing it via notice and comment, rather than a public hearing.

At the close of the meeting Mr. Morin noted that his clients want to receive a clear answer from CFSAN within 30 days on the process and timeline for moving forward on Pharming's GRAS submission, or they will need to consider what other options are available to them.

Dr. Sundlof noted he will not make a decision that is not based on sound science and indicated he will pay immediate attention to the matter and do his best to reach a decision as soon as possible.

Anne B. Crawford

Docname:H:\MEMORANDUM OF MEETING - Pharming031408.doc
Drafted:ABCrawford:HFS-022:03/14/08
Review/clear:SSundlof:HFS-001:3/14/08
Edit/clear:AMattia:HFS-255:3/14/08
Review/clear:JFasano:HFS-255:3/14/08
Edit/clear: MLanda:HFS-002:3/14/08
Review/clear:LTarantino:HFS-200:3/14/08
f/t:ACrawford:HFS-022:03/17/08

cc: SSundlof:HFS-001
MLanda: HFS-002
LTarantino:HFS-200
AMattia: HFS-255
JFasano:HFS-255
EHarden:HFS-022
RWheeler: HFS-022

LF_
8-2946

Agennix Incorporated

April 24, 2008

APR 2 9 2008

Andrew C. von Eschenbach, M.D.
Commissioner of Food and Drugs
Food and Drug Administration, HF-1
5600 Fishers Lane
Rockville, MD 20857

Re: Request for Meeting

Dear Commissioner:

I am writing on behalf of Agennix, Inc. ("Agennix") to seek a meeting with you and relevant senior staff concerning a submission by Ventria Biosciences, Inc. (Ventria) to find its rice-derived recombinant human lactoferrin ("rhLF") substance to be Generally Recognized as Safe (GRAS) for use in foods. We have learned through the FDA's public calendar that you have recently met with Ventria representatives on this subject. In light of that meeting, and because we have filed scientific objections with FDA's Center for Food Safety and Applied Nutrition (CFSAN) relating to the GRAS status of this substance, we wanted to be sure that we could communicate those concerns to you directly.

Agennix

Agennix is a small Houston-based biotechnology company. Agennix has been developing recombinant human lactoferrin ("rhLF") as a pharmaceutical drug since 1996 under FDA's IND process. Agennix is currently preparing to enter Phase III clinical trials with rhLF in advanced non-small cell lung cancer (NSCLC), for which Agennix has received Orphan Drug designation from the FDA, and has been granted Fast Track designation by the FDA for both first-line combination therapy and third-line monotherapy. Agennix has completed double-blind, placebo-controlled Phase II clinical trials supporting both of these NSCLC indications, and both of these trials met their primary endpoints. Agennix has also received approval of an SPA for the first-line NSCLC indication. In advanced renal cancer, Agennix is preparing for a Phase IIb trial, and has been granted Orphan Drug designation by the FDA for this indication as well.

Based on our experience in testing rhLF as a pharmaceutical product, we believe there are significant scientific reasons why it would not be appropriate to market this compound in food products. Agennix has assembled a panel of experts who have expressed their opinion that Ventria's rhLF is not GRAS and submitted

Eight Greenway Plaza Suite 910 Houston Texas 77046 Telephone 713/552-1091 Facsimile 73/552-0795

that information to CFSAN. We believe the potential availability of rhLF to the general population through food is also unwise, in light of the lack of medical supervision and pharmaceutical-grade good manufacturing practices. Similar concerns exist regarding a related GRAS notification submitted by Pharming, Inc. for its rhLF product.

We would plan to focus our discussion on the scientific issues presented with this GRAS issue. Accompanying me in my capacity as Board Chair of Agennix would be Rick Barsky, CEO of Agennix, Atul Varadhachary, M.D., Ph.D., COO of Agennix, and two or three outside experts, depending on availability. We will also be accompanied by our outside legal counsel, Joe Levitt of Hogan & Hartson, LLP, who would plan to speak briefly to the legal issues involving Section 912 of the recently enacted Food and Drug Administration Amendments Act of 2007 ("FDAAA"), with the intention to update you on discussions he has had with FDA Chief Counsel Gerry Masoudi on this subject.

As a former FDA Commissioner myself, I am most respectful of your time and recognize the many scheduling challenges. Towards that end, we have listed below a series of dates that we could be available over the coming months. Please let me know either by e-mail (frankcosmos@aol.com) or by telephone (301-908-3182) which of these dates would be acceptable.

Thank you very much for your consideration and I look forward to meeting with you.

Most sincerely,

Frank E. Young, M.D., Ph.D.
Chairman, Agennix, Inc.

Available Dates:

June 24-27
July 1-2
August 19, 27-29
September 2-3, 16, 23-24, 26, 30

Cc: Susan Winkler, RPh, Esq.
Chief of Staff, FDA

Stephen Sundlof, D.V.M., Ph.D.
Director, CFSAN

Michael Landa
Deputy Director for Regulatory Affairs, CFSAN

Laura Tarantino, Ph.D.
Director, Office of Food Additive Safety, CFSAN

Gerald Masoudi, Esq.
Chief Counsel

Richard Barsky, CEO
Agennix, Inc.

Atul Varadhachary, M.D., Ph.D., COO
Agennix, Inc.

Joseph Levitt, Esq.
Hogan & Hartson, LLP

Kaarina Marajh
Agennix
8 Greenway Plaza, Suite 910

Houston, TX 77046

fedEx Express

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Andrew von Eschenbach
Food and Drug Administration HF-1
5600 Fishers Lane

Rockville, MD 20857

Ref # Regulatory
Invoice #
PO #
Dept #

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TRK# 0201 7910 5255 4921

PRIORITY OVERNIGHT

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

June 4, 2008

Frank E. Young, M.D., Ph.D.
Chairman
Agennix, Incorporated
Eight Greenway Plaza
Suite 910
Houston, TX 77046

Dear Dr. Young:

I am pleased to accept your request to meet with you to discuss Ventria Biosciences Incorporated's submission that requests the agency to find its rice-derived recombinant human lactoferrin substance to be generally recognized as safe (GRAS) for use in foods. The meeting is confirmed for September 24, 2008, at 1 p.m., at the Parklawn Building, Room 14-68, Rockville, Maryland. Please contact Ms. Pam Pisner, my special assistant, at 301-827-2410, to make the necessary arrangements.

As a public servant, I may be called away at the last minute. Should such a situation arise, you will be notified, and I will make every attempt to find an FDA representative to serve as a substitute.

Sincerely,

Andrew C. von Eschenbach, M.D.
Commissioner of Food and Drugs

G:\Wp\Brian\meeting request\8-2946 Frank Young.doc
Drafted by B. Botsford 5/29/08
Edited by V. Jackson 5/29/08
B. Clarke 5/30/08

Cc: HF-1 (2)
HF-40

OFFICE OF THE COMMISSIONER
CORRESPONDENCE SUMMARY SHEET

Subject: A confirmation letter to Dr. Young

Control Number: 8-2946 **Date:** May 29, 2008

Action Required: Commissioner Signature

Summary/Background:
A letter confirming the Commissioner will meet with Dr. Young to discuss a submission by Ventria Biosciences INC. regarding its rice-derived recombinant human lactoferrin substance to be GRAS for use in foods. The meeting is confirmed for September 24, 2008, at 1 p.m., at the Parklawn Building, Room 14-68, Rockville, Maryland

Prepared By: B. Botsford **Reviewed/Cleared By: V. Jackson** 5/29/08 **Intranet: Yes[ ] No[ ]**

ExecSec Contact: B. Botsford **Correspondence Analyst:** B. Botsford

---

Office of the Commissioner
Review/Clearance

Please hand carry to next addressee

Reviewer	Signature	Date
Dotty Foellmer, HF-40	Beth Clarke for D.F.	5/30/08
Ms. Winckler, HF-1		
Ms. Pisner, HF-1	J. Pisner	6/3/08
Dr. Von Eschenbach, HF-1	/s/	6.4.08

COMMENTS:

Document Signature Date: 6.4.08

OK TO ISSUE
Before

AFTER SIGNATURE RETURN TO EXECUTIVE SECRETARIAT HF-40

851

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: July 3, 2008

TIME: 3:30 PM

PARTICIPANTS:

FDA

Jeremiah Fasano	HFS-255
Antonia Mattia	HFS-255
Laura Tarantino	HFS-200
William McConagha	HF-22

External

Charles L. Morin	Morin & Associates
Frans de Loos	Pharming
Samir Singh	Pharming

SUBJECT: Status of GRN 189

FDA staff met with representatives of Pharming, Inc. (Pharming), including Pharming's agent, Mr. Morin, to discuss the status of the firm's notice for the use of recombinant human lactoferrin from bovine milk as an ingredient in food, GRN 189.

We explained that FDA's review of GRN 189 had identified a number of significant issues related to the immunological properties of Pharming's ingredient. We had been unable to resolve these issues on the basis of the information provided by the notice and its amendments.

Pharming and FDA discussed these issues, and FDA agreed to provide them in writing. We stated our view that unless these issues could be resolved in a relatively short period of time, it would be appropriate to move towards closure of the notice. We noted that Pharming could choose to withdraw GRN 189 any time without prejudice to future submissions.

FDA concluded by reaffirming the agency's willingness to engage in continued dialogue on the issues we had identified.

Jeremiah Fasano

R/D:HFS-255:JMFasano:07/03/2008
Init:HFS-255:AMattia:06/01/2010
F/T:HFS-255:JMFasano:06/01/2010

FILE COPY

OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

Summary – Outstanding Questions Identified by CFSAN Regarding GRN 000189

Four lines of evidence addressing the safety of the intended use of Pharming's human lactoferrin (hLF-b) can be identified within the data and information presented in GRN 000189. These are:

1. infant consumption of hLF in human milk;
2. endogenous exposure to hLF secreted by the body in the alimentary canal;
3. bLF as a more potent equivalent of hLF; and
4. published studies with hLF and bLF in both animals and humans.

FDA continues to have questions about the adequacy of each line of evidence as a basis for a GRAS determination for the intended use of hLF-b. These questions concern hLF-b's immunomodulatory effects[1] when the substance is consumed chronically by the general population.

1. **FDA questions whether infant consumption of hLF in human milk is an appropriate model for demonstrating the safety of hLF in adults.** The infant immune system is qualitatively different from the mature immune system and these differences may alter the risk of adverse immune-mediated effects resulting from the consumption of hLF. The notice does not contain evidence supporting the premise that the immunological effects of hLF in infants are equivalent to those in adults.

2. **FDA questions whether endogenous hLF secreted by the body in the alimentary tract is an appropriate physiological model for exposure to hLF in food.** Pharming has not provided information to demonstrate that low-level background secretion is physiologically equivalent to consumption of a larger quantity of hLF in food. Furthermore, we do not agree that endogenous hLF levels in unhealthy individuals are an appropriate point of reference for safe consumption of hLF by the healthy general population.

3. **FDA questions whether a history of consumption of bLF in food is sufficient to demonstrate the safety of hLF for Pharming's intended use in food.** There is evidence suggesting that bLF and hLF are functionally distinct in the context of *in vivo* consumption by humans, as acknowledged in public statements attributed to Pharming. The notice does not provide evidence that differences in the complex functionality of these proteins are not relevant from the perspective of a safety assessment for chronic consumption by the general human population.

[1] During its evaluation, FDA also raised questions about the potential for adverse hLF-specific autoimmune effects as a consequence of consumption of an exogenous human protein. It remains unclear to FDA that the potential for disruption of specific immune tolerance in the context of this intended use is well understood.

4. **FDA questions whether studies with hLF and bLF cited by Pharming are sufficient to demonstrate the safety of hLF for Pharming's intended use in food**. The cited studies are almost entirely therapeutic and do not have endpoints appropriate to a food safety assessment. Furthermore, the notice does not provide evidence that the limited number of food toxicology studies cited employ animal models and endpoints suitable for assessing the safety of chronic consumption of an immunomodulatory human protein. We note that immune-mediated consequences of chronic modulation of the adult immune system by hLF consumption do not appear to be well-studied in a food safety context.

As you know, there was a recent session on the use of human protein food ingredients at the Summer Meeting of The Toxicology Forum. Although we mentioned during the session that it had been prompted in part by specific submissions to FDA, we did not focus on these submissions (including your notice) or discuss them. It was not the intent of the session to evaluate specific products. Speakers were selected both on the basis of expertise in issues related to the use of human proteins in food as well as availability. The session included multiple speakers with therapeutic backgrounds because existing experience with human proteins appears to be limited outside this area. As noted during the session, the therapeutic experience was discussed not because it was directly applicable to food, but in order to see what considerations might or might not be relevant.

We believe that the session accomplished its primary goal of promoting discussion and awareness with respect to the topic of human protein food ingredients. Given the complexity and cross-disciplinary nature of the issues and the limited time available during the session, we hope that the dialogue will continue more broadly in and beyond the food safety community.

Fasano, Jeremiah

From: Fasano, Jeremiah
Sent: Thursday, July 17, 2008 11:31 AM
To: 'charleslmorin@earthlink.net'
Subject: Summary of outstanding questions re: GRN 189 identified in July 3 phone call
Attachments: Outstanding Question Summary - GRN 189.pdf

Mr. Morin-

Per your request, we're providing a brief list of the issues we discussed on July 3rd, 2008 with respect to GRN 189. We have also briefly addressed the recent session on human protein food ingredients at The Toxicology Forum. I hope you will find the document useful.

Regards-

-Jeremiah Fasano

Jeremiah Fasano, Ph.D.
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

Phone: 301-436-1173
Fax: 301-436-2964
Email: jeremiah.fasano@fda.hhs.gov

Mailing Address:
HFS-255
5100 Paint Branch Parkway
College Park, MD 20740

MEMORANDUM OF MEETING
January 16, 2009
10:00 a.m. – 10:50 a.m.
White Oak, Building 1

Attendees: FDA: Andrew von Eschenbach, Susan Winckler, Randy Lutter, Stephen Sundlof, Stephen Mason, William McConagha, Michael Landa, Mitchell Cheeseman, Phil Broadbent, and Kristy Moran

Agennix, Inc.
Frank E. Young, M.D., Ph.D., Chairman of the Board
Rick G. Barsky, CEO of Agennix
Atul Varadhachary, M.D., Ph.D., COO of Agennix
Richard D. Cummings, Ph.D., Emory University School of Medicine
Arno Kromminga, Ph.D., IPM BIOTECH
Michael P. Sherman, M.D., Southern Illinois University School of Medicine
Joseph A. Levitt, Hogan & Hartson, LLP, Counsel to Agennix

Subject: The purpose of this meeting is to discuss scientific issues presented with the generally recognized as safe (GRAS) status of recombinant human lactoferrin (rhLF) for use in foods.

Highlights:

- Agennix introduced themselves and three independent scientists commenting on the scientific issues why rhLF would not be appropriate for use in foods.

- Issues discussed:
  - rhLF as a promising cancer therapy
  - Scientific concerns with food uses of rhLF, which demonstrate that rhLF is not generally recognized as safe (GRAS):
    - Risks specifically associated with the glycosylation of rhLF from rice and cows
    - Risks of immunogenicity and allergenicity with rhLF from rice and cows
    - Risks associated with feeding rhLF to young children, including infants
  - Experts at the Toxicology Forum Meeting support the conclusion that rhLF is not GRAS
    - There are unknown risks associated with recombinant human proteins
  - Scientific conflict exists among experts
    - The science is too new to support the safety of intended use for foods.

- The Office of Chief Counsel has no further questions at this time.

- FDA will continue its evaluation of the scientific issues.

Action Items:

- None.

Kristy Moran
Policy Analyst
FDA Executive Secretariat

History Page

Document Name: G:\Wp\KRISTY\Meetings Minutes\Agennix Joe Levitt rhLF GRAS 1 16 09.doc

Drafted: KMoran, 1/16/09
Sent to Susan Winckler and Bill McCongaha on 1/16/09 at 1:00 pm via e-mail.

Releasable: Yes _X_ No ____

Moran, Kristy

From: Moran, Kristy
Sent: Friday, January 16, 2009 1:00 PM
To: McConagha, William; Winckler, Susan
Subject: Draft Meeting Minutes - Agennix - Jan. 16, 2009

Attachments: Agennix Joe Levitt rhLF GRAS 1 16 09.doc

Attached are the draft meeting minutes from today's meeting with Agennix, Inc. Please let me know if you have edits or additions to these minutes.

Agennix Joe Levitt rhLF GRAS 1...

Thank you,

Kristy Moran
Policy Analyst
FDA/OES
301.796.4678
kristy.moran@fda.hhs.gov

10903 New Hampshire Avenue
Building 1, Rm 3318
Silver Spring, MD 20993

Agennix

A Biopharmaceutical Company Developing Drugs for Cancer and Diabetic Ulcers

FDA Commissioner's Meeting

January 16, 2009

Meeting Attendees

On Behalf of Agennix, Inc:

- **Frank E. Young, M.D., Ph.D**, Chairman of the Board
- **Rick G. Barsky**, Chief Executive Officer
- **Richard D. Cummings, Ph.D**, Emory University School of Medicine
- **Arno Kromminga, Ph.D**, IPM BIOTECH
- **Michael P. Sherman, M.D.**, Southern Illinois University School of Medicine
- **Atul Varadhachary, M.D., Ph.D**, President and COO
- **Joseph A. Levitt**, Counsel to Agennix

Introduction

Frank E. Young, M.D., Ph.D.

Chairman of the Board
Agennix, Inc.

Rick G. Barsky

Chief Executive Officer
Agennix, Inc.

Topics to be Covered

1. Recombinant human lactoferrin (rhLF) as a Promising Cancer Therapy
2. Scientific Concerns with Food Uses of rhLF
   - Glycosylation of Proteins
   - Immunology
   - Pediatric/Neonatal Issues
3. Toxicology Forum Summary
4. Legal Issues Involving rhLF GRAS Petitions
   - “Severe conflict” among qualified experts
5. Applicability of § 912 of FDAAA to rhLF

Glycosylation of Proteins

Richard D. Cummings, Ph.D.

William Patterson Timmie Professor
Chair of the Department of Biochemistry
Emory University School of Medicine

Academic Appointments

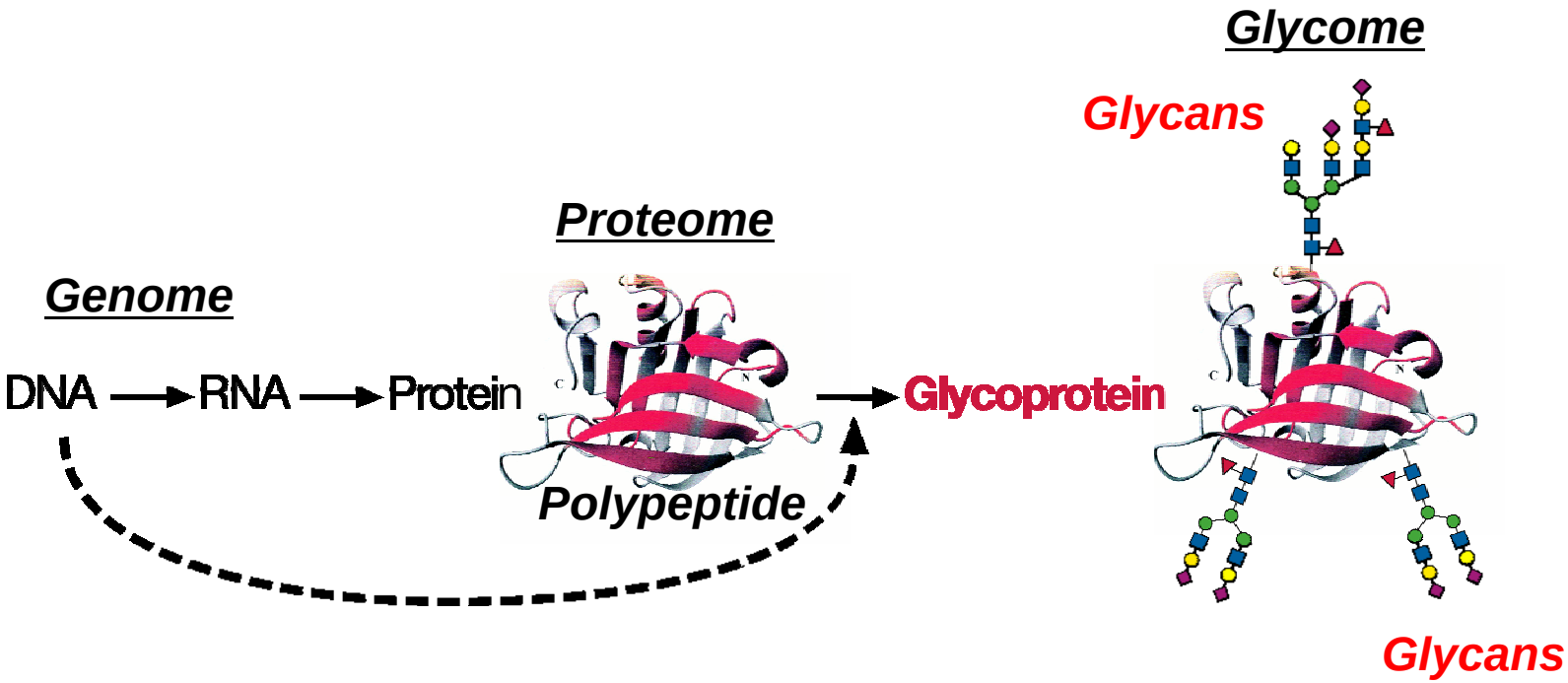
- William Patterson Timmie Professor and Chair, Dept. of Biochemistry, Emory University School of Medicine
- Director and Founder, Glycomics Center at Emory University School of Medicine
- George Lynn Cross Distinguished Research Professor of Biochemistry and Molecular Biology
- Ed Miller Endowed Chair in Molecular Biology; Professor of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center
- Director and Founder, Oklahoma Center for Medical Glycobiology

Scientific Publications

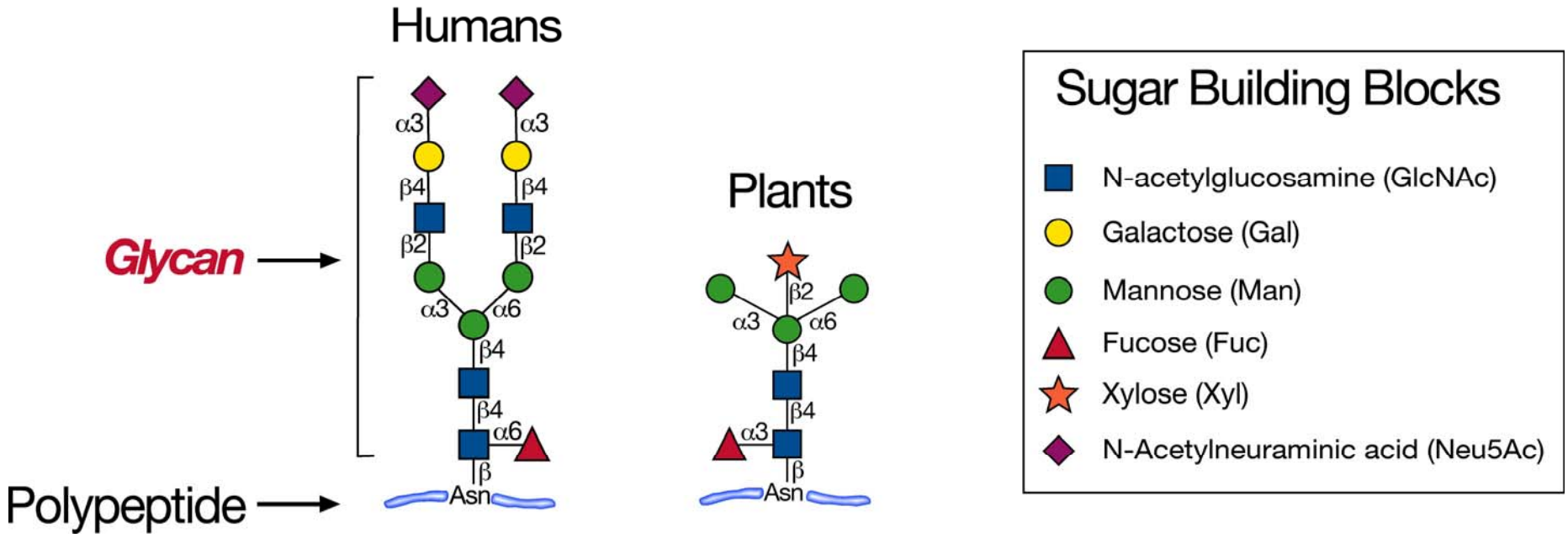
- Co-Editor of First Textbook on Glycobiology – 1st Edition of *Essentials of Glycobiology* (1999); 2nd Edition published in 2008

- Published over 180 peer-reviewed articles, over 30 review articles, eleven textbook chapters, and owner of 27 different U.S. patents

Modern Expansion of the Central Dogma of Biology - Post-Translational Modifications Greatly "Amplify" Genomic Information



Glycans in Glycoproteins from Humans and Plants are Very Different



Differ in Composition of Sugars, Linkage of Sugars, and Overall Glycan Structures

See new textbook "*Essentials of Glycobiology*" – 2nd Edition (2008) (Varki, A., Cummings, R.D., Esko, J., Freeze, H., Stanley, P., Bertozzi, C., Hart, G., and Etzler, M., Eds), Cold Spring Harbor Laboratory Press, Inc., Boston.

Agennix

These Differences in Glycans Between Humans and Plants Contribute to Antigenicity and Allergenicity of Glycoproteins

"Complex carbohydrates are potent inducers of Th2 responses, and carbohydrate antigens (Ags) can stimulate the production of different classes of glycan-specific antibodies (Abs), including Th2 associated IgG but also non-specific IgE."

"Plants, helminths, and other invertebrates such as insects and snails share "common" glycan determinants that are not found in humans…. Such glycan antigens, as well as non-human "species-specific" glycan antigens, are highly immunogenic and represent a major focus for the host immune response.."

Quoted from a review by Drs. Irma van Die and Richard D. Cummings (2006) Glycans Modulate Immune Responses in Helminth Infections and Allergy, in ***Parasites and Allergy*** (Eds. M. Capron and F. Trottein), pp. 91-112, Karger Publishers, Basel, SZ.

Plant Food Allergies (including Peanut Allergy) is to Proteins and Glycoproteins

*"During the initial exposure, which may occur in utero, during breast-feeding, or in early childhood, antibodies of the IgE isotype, which are highly specific for epitopes on the surface of the food allergen (usually proteins or **glycoproteins**), are elaborated.*

The propensity to produce IgE antibodies against commonplace substances is the hallmark of the allergic diathesis.

The factors underlying this propensity remain incompletely understood but appear to include exposure to allergens as well as a genetic predisposition.

Exposure through mucosal surfaces, such as those of nasal passages and the respiratory and gastrointestinal tracts, seems to increase the risk of sensitization, whereas parenteral exposure, such as through subcutaneously administered immunotherapy, seems to increase the likelihood of tolerance.

The threshold for sensitization most likely differs among patients."

Quoted from a review by Dr. Adrian Long (2002) The Nuts and Bolts of Peanut Allergy, *New Engl. J. Med.* **346**:1320-1322.

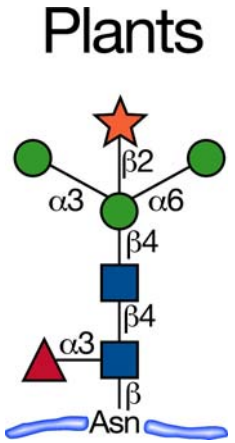
Other Allergies to Proteins and Glycoproteins

"Kolarich and Altmann (18) recently described the major glycan species of Ara h 1 [Note: this is the major glycoprotein allergen from the peanut *Arachis hypogaea*]*, as Man(3(-4))XylGlcNAc(2), a complex glycan containing a β1-2 xylose attached to the proximal mannose of the glycan core."*

"Individuals with IgE-mediated allergy to bee venom and plant pollen or foods have been shown to make specific IgE to these structures."

"Bardor et al. (28) also found that 25–50% of non-allergic individuals made a humoral immune response to these epitopes."

Quoted from a paper by Shreffler, et al (2006) The Major Glycoprotein Allergen from *Arachis hypogaea*, Ara h 1, is a Ligand of Dendritic Cell-Specific ICAM-Grabbing Nonintegrin and Acts as a Th1 Adjuvant in Vitro, *J. Immunol.* **177**:3677-3685.



Agennex

Bovine and Human Protein Glycosylations are Different

Carbohydrates are considered among the strongest antigens and allergens.

- Bovine milk-protein glycosylation includes N-glycolylneuraminic acid (NeuGc) ("Hanganutziu-Deicher antigen" (Asaoka 1994)) which is different from the human N-acetylneuraminic acid glycosylation.
- NeuGc is a potent antigen in humans; when attached to a *human* glycoprotein, it is considered as a “foreign” substance that invokes immunity.
- Bovine glycoproteins also contain LDN and alpha-Gal antigens, which are not present on human-milk glycoproteins (Coddeville 1992, Nakata 1993). LDN and alpha-Gal (also expressed by several parasites) are involved in host immunity to parasitic infections (Die I and Cummings RD 2006) and are potent antigens.
- The types of carbohydrates likely to be found on transgenic cow-derived human glycoproteins are potent inducers of antibody responses, including IgE (Leino 2006, Ahrazem 2006, Chow 2005).
- Bovine-type glycosylation of *human* lactoferrin (“foreign” glycosylation) is immunologically different from bovine glycosylation of bovine lactoferrin.

Bovine-type glycosylation of human lactoferrin is also of significant safety concern

Agennix

Summary

- There is clearly a pressing need to make recombinant glycoproteins for human therapies.
- However, it is now well recognized that the source of glycoprotein and the type of glycosylation contribute greatly to the biological activity and the immunogenicity and allergenicity of the products.
- Great care must be taken to avoid undesirable immune effects that can be introduced by producing recombinant human glycoproteins in non-natural host cells, especially in plants.
- It should be considered that the addition of altered carbohydrates to a recombinant glycoprotein [made in either rice (as for Ventria) or in cows (as for Pharming)] is akin to introducing modified amino acids, which we all agree would cause alarm and concerns in terms of biosafety and bioactivity.
- Thorough product testing through multi-year clinical trials with appropriate numbers of subjects of different genders, age groups, and ethnicities, would appear to be the only mechanism to ensure product safety to the general public.
- Neither Ventria nor Pharming have conducted the testing needed to assess these risks, and their products are not GRAS for use in foods.

Immunogenicity of recombinant human Lactoferrin

PD Dr. Arno Kromminga

IPM BIOTECH
Hamburg, Germany

Agennix

Experience and Credentials

- Director of IPM BIOTECH in Hamburg, Germany
- *Summa cum laude* Ph.D. from University of Münster
- Research at Albert Einstein College of Medicine (NY)
- Over 15 years experience in clinical autoimmunity and immunogenicity of biopharmaceuticals
- Co-founder European Immunogenicity Platform (EIP)
- My group was one of the first to establish a robust and sensitive assay for the detection of binding and neutralizing antibodies against erythropoietin
- Faculty member at University of Kiel, habilitation in Immunology
- Official Certificate as Clinical Immunologist
- Frequently published scientific articles, journals, book chapters and presenter at international conferences

Purpose

I am here to comment on the:

- Appropriateness of adding recombinant human lactoferrin to food products from the position of my specialty in immunogenicity
- To present some aspects of causes and consequences of immunogenicity of recombinant human therapeutic proteins including lactoferrin

My starting point:

- Most, if not all, recombinant therapeutic proteins have the potential of inducing an immune response

Immunoreactivity against Biopharmaceuticals

Class	Drug	Indication	Reactivity
Antibodies	anti-Il 2 R	Immune suppression	18%
	anti-TNFα	RA, M. Crohn	34%
Receptors	CD4	HIV	12%
	CD20	NHL, RA, SLE	0 - 40%
Cytokines	Interleukin 2	Cancer	52%
	Interleukin 3	Cancer	85%
Interferons	Interferon α 2a	HCV	60%
	Interferon β	Multiple Sclerosis	80%
Enzymes	Factor VIII	Hemophilia	30%
	DNase	Cystic Fibrosis	9%
Hormones	Insulin	Diabetes	60%
	HGH	Growth	16%
	Erythropoietin	Anemia	< 1%

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Causes of Immunogenicity

Structural Differences

- Amino acid sequence
- Post-translational modification
- Chemical changes

Administration

- Route
- Frequency
- Dosage

Manufacturing characteristics

- Contaminations and impurities
- Formulation
- Storage

Others

- Immune status
- Genetic background
- Assay format

Risk Management

Risk = Probability x Impact

Due to structural differences between the exogenous and endogenous lactoferrin, e.g. glycosylation pattern, the likelihood of antibody induction is moderate. However, the cross-reactivity with endogenous lactoferrin may contribute to the onset or exacerbation of a pre-existing autoimmune disorder. Furthermore, antibodies that neutralize the physiological immune stimulatory function of endogenous lactoferrin may lead to an immune-suppressive status.

Therefore, the clinical impact of anti-lactoferrin antibodies is considered to be high and the risk of immunogenicity is high.

IgEs Against Carbohydrate Moieties Lead to Anaphylactic Reactions

The NEW ENGLAND JOURNAL of MEDICINE

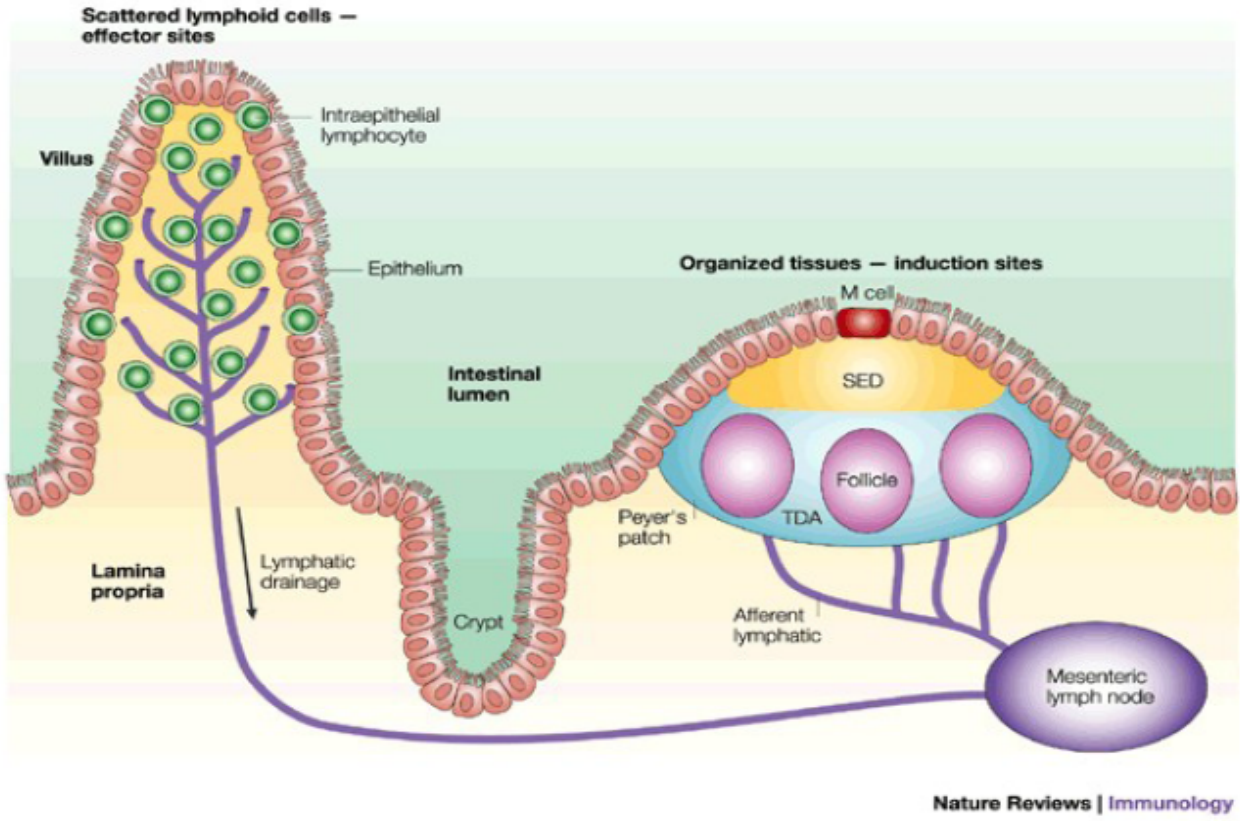
ORIGINAL ARTICLE

Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose

Christine H. Chung, M.D., Beloo Mirakhur, M.D., Ph.D., Emily Chan, M.D., Ph.D., Quynh-Thu Le, M.D., Jordan Berlin, M.D., Michael Morse, M.D., Barbara A. Murphy, M.D., Shama M. Satinover, M.S., Jacob Hosen, B.S., David Mauro, M.D., Ph.D., Robbert J. Slebos, Ph.D., Qinwei Zhou, Ph.D., Diane Gold, M.D., Tina Hatley, M.D., Daniel J. Hicklin, Ph.D., and Thomas A.E. Platts-Mills, M.D., Ph.D.

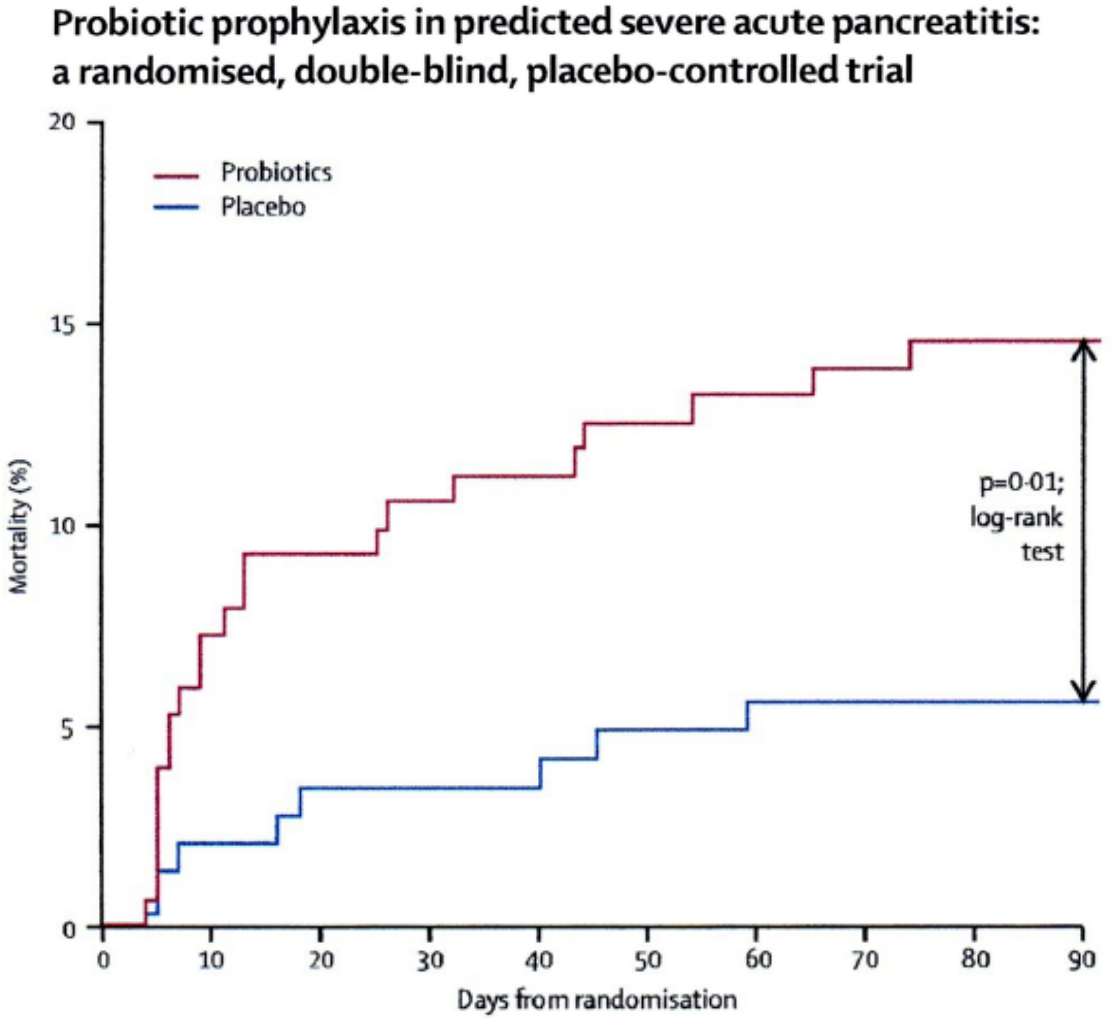
Chung et al, N Engl J Med, 2008;358:1109-1117

Intestinal Lymphoid System



Aside from all of its other functions, the **gastrointestinal tract is a lymphoid organ** (gut-associated lymphoid tissue or GALT).

Mortality in Acute Pancreatitis after Oral Administration of Probiotics



Besselink et al, The Lancet, 2008; 371.651-659

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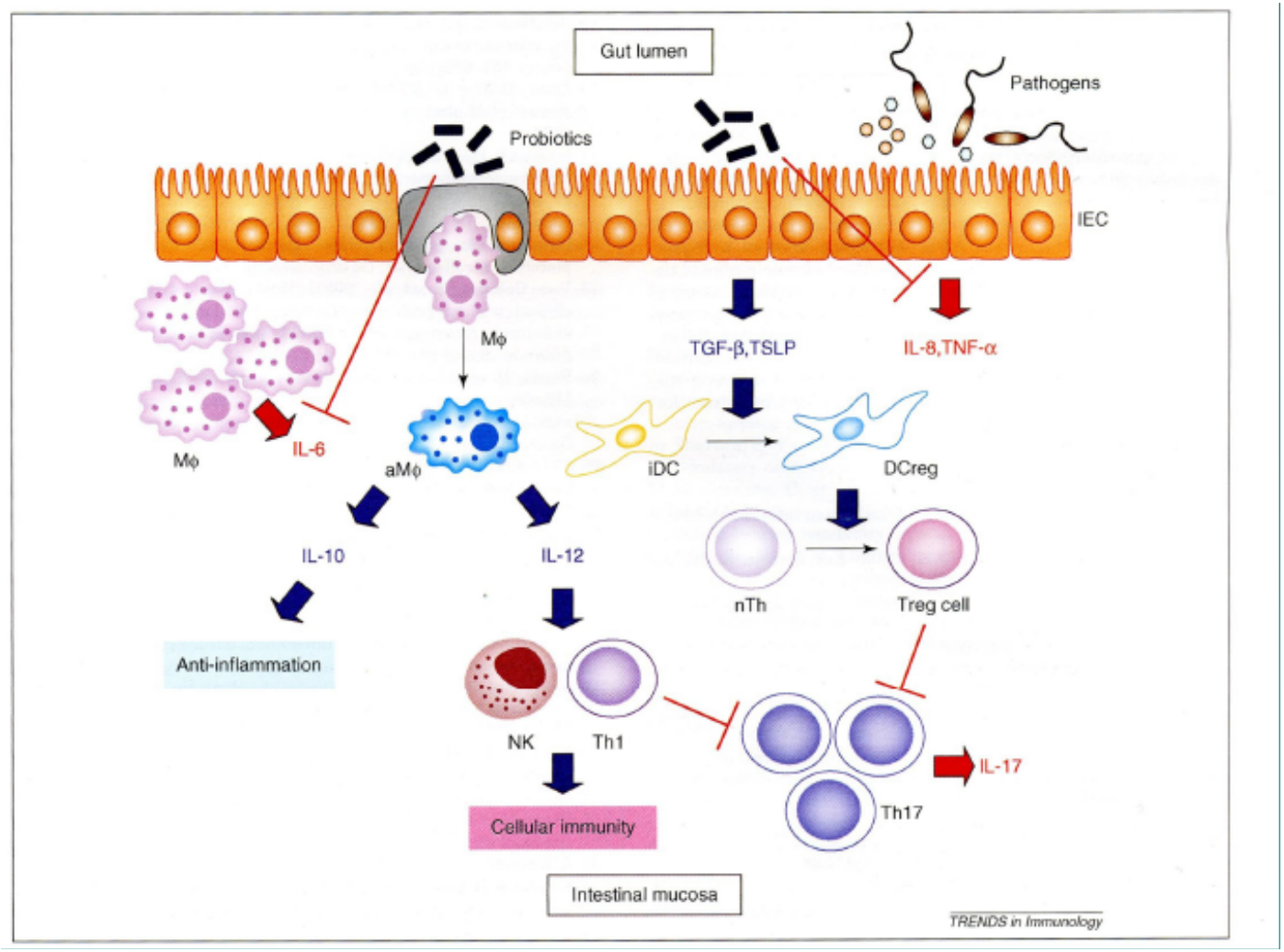
Immune Cell Circuits Modulated by Probiotics



Shida et al, Trends Immunol, 2008; 29.565-573

Lactoferrin

- Antibodies against recombinant human lactoferrin may cross-react with the endogenous lactoferrin causing an immune impairment by neutralizing the action of endogenous lactoferrin.
- Antibodies against lactoferrin are thought to be associated with some autoimmune diseases.
- Although it is not known yet whether auto-antibodies against lactoferrin have a direct pathogenic effect, these antibodies may contribute to a clinical exacerbation of a pre-existing autoimmune disorder.
- There is a recent report about the high prevalence of IgE response against glycans of rice-derived lactoferrin. It should be noted that it is not a general rule that IgE antibodies against glycans from non-human expression systems have no clinical impact. Recently, it was shown that IgE against the carbohydrate structure of the monoclonal antibody cetuximab lead to anaphylactic reactions.

Summary

- All recombinant human therapeutic proteins have the potential of being immunogenic independent of the route of administration.
- The risk of immunogenicity should be assessed by detailed and thorough risk management.
- Antibodies against rh-lactoferrin may cross-react with endogenous lactoferrin causing an impaired immune response and possibly an association with other autoimmune diseases.
- Anti-rhLF IgEs may lead to immune mediated allergic anti-drug reactions with severe anaphylactic consequences.
- Neither Ventria nor Pharming have conducted the necessary clinical testing to properly assess this risk, and their products are not GRAS for use in foods.

rhLF Produced in Rice

Concerns for Infants and Children

Michael P. Sherman, M.D., F.A.A.P.

Professor of Pediatrics
Southern Illinois University
School of Medicine

Agennix

Training and Experience

Training and Practice – Pediatrics/Neonatology
University of Michigan and University of California

1969 - 1981

University of California, Los Angeles
UCLA Medical Center
Associate Professor of Pediatrics
1982 - 1993

University of Kansas, KU Medical Center
Professor and Director of Neonatology
1993 - 1996

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Training and Experience

Baylor College of Medicine
Texas Children's Hospital
Professor of Pediatrics (1996 – 1998)

University of California, Davis
UC Davis Children's Hospital
Professor and Chief of Neonatology (1998 – 2005)
Professor Emeritus (2005 – present)

Southern Illinois University School of Medicine
St. John's Children's Hospital
Professor of Pediatrics (2005 – present)

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rhLF in Neonates – Discussion Overview

- Neonates, infants and children are vulnerable human subjects that face additional immunological risks when receiving rhLF for gastroenteritis
- Antibodies directed against chimeric rice- or bovine-derived rhLF could cross-react with endogenous or therapeutically administered lactoferrin or with other proteins present in rice- or bovine-based rhLF formulations
- Notwithstanding a published report describing rice-based rhLF, there is currently no credible evidence that rhLF administration provides a benefit to children with infantile gastroenteritis
- Breast-Milk Lactoferrin is a Natural Peptide Antibiotic and Immunomodulatory Protein. *IT IS NOT A NUTRIENT* and should not be treated as a food
- rhLF is not Generally Recognized As Safe (GRAS) in children

Elevated Risk of Antibody Formation in Children

- Neonates, infants and older children with gastroenteritis have macromolecular opening of intestinal barriers to foreign antigens or toxins
- The degree and duration of intestinal opening during intestinal illnesses increases exposure to antigens [or toxins]
- Intestinal opening can facilitate the formation of IgM or IgG antibodies that can cause:
  - hives
  - anaphylaxis
- There can also be formation of IgE antibodies that can cause:
  - eczema
  - respiratory symptoms (i.e., wheezing)
- There is also a risk of developing cross-reacting antibodies against rice- or cow- milk-based proteins that may react with endogenous or therapeutic LF

Effectiveness in Gastroenteritis: A Study Using LF & Lysozyme in Rice

- A report suggests a benefit when rhLF + rhlysozyme [Lz] combination is used to treat infantile gastroenteritis
  - The duration of diarrhea was reduced to 3.7 in the LF/Lz-R-ORS vs. 5.2 days in control group [WHO]
  - By 48 hours, a solid stool was achieved in 85% of LF/Lz-R-ORS-treated children vs. 69% in controls
  - The researchers report but do not qualify a reduction in diarrhea in treated compared to the control groups
- Careful scientific analysis of the article does not support the conclusion that rhLF improves outcomes in these patients or allay safety concerns

Concerns with Pediatric rhLF Study in Peru

- A small number of children received treatment with rice-produced rhLF over a limited dosing duration
- Methodological concerns with the published study:
  - No pre-defined primary endpoint that was quantitative; no information related to sample size and power calculations
  - The reported endpoints are not clinically significant and there is no evidence that morbidity was reduced
  - Subjects did not receive adequate follow-up
- Separate published reports of IgE cross-reactivity against rice-derived rhLF that were not evaluated in this study
- Use of a combined “control group” had half the cases of ORS known to be inferior compared to the ORS base received by all the infants in the rhLF/rhLZ “treatment group”
- No ability to separate out effects of rhLF versus rhLZ
- Difficult to draw conclusions relating to the safety or potential efficacy of rhLF in this patient population

Lactoferrin is a Milk Defense Protein

- Lactoferrin plays an important role in host defense
- Lactoferricin produced from Lactoferrin
  - Released by action of pepsin at pH ~2 in the stomach
  - Lactoferricin is a potent microbicide and binds endotoxin
- Lactoferrin fragments identified in urine of human infants – play a role in intestinal and systemic immune priming; there is also an anti-inflammatory effect
- Lactoferrin is not a nutritional protein; it is inappropriate to add LF to infant formula because LF is a therapeutic biologic agent that is still under human-subject investigation in infants and children

Conclusion from the Peruvian Pediatric Study

Clinical trials are required to ascertain the safety and effectiveness of rice- and bovine-derived recombinant human lactoferrin when treating infants and children.

The long-term (multi-year) trials should be:

- **Well-designed**
- **Multi-centered**
- **Well-controlled**

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Summary

- There is an enhanced risks of antibody formation in pediatric populations.
- Autoimmune diseases and other immunogenicity concerns pose a greater threat to infants and children with developing immune systems, particularly in neonates or in infantile gastrointestinal infections where gut permeability can be further increased.
- The published Peruvian trial does not establish a safe pediatric use of rice-derived rhLF or adequately demonstrate a benefit during its use
- Human lactoferrin in breast milk is not a nutrient, but a natural peptide antibiotic and immunomodulatory protein
- rhLF cannot be considered Generally Recognized As Safe

Toxicology Forum Summary

Atul Varadhachary, M.D., Ph.D.

President and COO
Agennix, Inc.

The Toxicology Forum Annual Summer Meeting July 7, 2008
Human Protein Food Ingredients

Chairperson: Antonia Mattia, FDA

Key speakers:

Daniela Verthelyi, FDA
Jeremiah Fasano, FDA
Rafael Ponce, ZymoGenetics
Gopi Shankar, Centocor Research and Development, Inc.
Richard Goodman, University of Nebraska
Marian Kruzel, University of Texas

Overall Conclusion: In addition to the known risks associated with recombinant human proteins, there are significant unknowns. These safety concerns must be adequately addressed **before** recombinant proteins can be considered to be GRAS.

The concerns expressed regarding the immunological and other risks associated with recombinant proteins are consistent with those previously articulated by our expert panel even though neither Agennix nor any of its experts had any involvement in this Toxicology Forum panel.

Concerns Articulated by the Speakers Include

- Immunogenicity, cross-reactivity, auto-immunity, and allergenicity remain major concerns even with orally administered proteins.

  Dr. Goodman: “The protein in the stomach is digested and peptides will be processed and presented through antigen presenting cells … Depending on the local tissue environment the T cell [may] provide tolerance to that specific food protein. However, under different circumstances, such as a different local cytokine profile, T cells produced [will] drive T cell-mediated reactions, some of which can be deleterious”

  Dr. Verthelyi: “just because the route is oral [one cannot] eliminate the possibility of inducing antibodies.”

- Recombinant proteins differ from their native counterparts and physiological context in many different ways which can pose additional safety risks.

  Dr. Shankar, “How do you really know that the human protein is as human as your body is making it? Are we really manufacturing it the way the Almighty is manufacturing it? I don’t know.”

Speakers at the Toxicology Forum Concluded

- Apparent safety in animal trials is no guarantee of safety in humans. Safety can only be evaluated in appropriately sized long-term clinical trials.

  Dr. Shankar: “When we are talking about mostly human proteins, in terms of immunogenicity [nonclinical animal studies] are generally not useful … A mouse reacts to a human protein differently from a human. It is just as simple as that.”

- The consensus from the meeting appears to be that there are significant concerns to using recombinant proteins as food.

  Dr. Fasano: “We don’t have a lot of experience with human proteins as food ingredients. This is sort of a new trend or issue.”

  Dr. Verthelyi: “That is pretty much the bottom line: The potential consequences of introducing recombinant human proteins is unknown. There are many questions.”

Thus, it appears clear that the use of a recombinant human protein in food is not Generally Recognized as Safe (GRAS)

Legal Issues

Joseph A. Levitt

Hogan & Hartson, LLP
Counsel to Agennix

Legal Issue regarding GRAS

- The GRAS standard requires a consensus among qualified experts regarding safety of rhLF
- Although unanimity is not required, a “severe conflict” among qualified experts precludes finding of GRAS
- Agennix has provided FDA with the opinions of 14 prominent physicians and scientists that rhLF is *not* GRAS for its intended uses
- These expert opinions create a “severe conflict” on the key issues affecting GRAS evaluation
- This reason alone requires denial of both Ventria’s and Pharming’s GRAS notifications

Section 912 of FDAAA

- Law intended to ensure safety and protect drug development, when drug development came first.
- rhLF meets all necessary criteria of Section 912, and none of the specific exceptions apply.
- Agennix submitted comments to FDA docket; Ventria and Pharming did not.
- Conclusion: Section 912 prohibits rhLF from being added to food.

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Conclusions

FDA should not permit recombinant human lactoferrin to be added to foods:

- Scientific basis for GRAS in food has not been demonstrated – long-term clinical studies are needed
- "Severe conflict" among qualified experts precludes GRAS finding as matter of law
- Section 912 of FDAAA prohibits addition of rhLF to foods

These scientific and legal bases are clear and compelling

Agennex

EXECUTIVE SUMMARY
AGENNIX PRESENTATION TO FDA COMMISSIONER
JANUARY 16, 2009

I. Main Points

FDA should not permit recombinant human lactoferrin (rhLF) to be added to foods because: (a) the scientific basis for GRAS status in food has not been demonstrated—long-term clinical studies are needed; (b) a "severe conflict" among qualified experts exists and precludes a finding of GRAS, as a matter of law; and (c) Section 912 of the FDAAA prohibits addition of rhLF to foods.

II. Background: Recombinant Human Lactoferrin is a Promising Cancer Therapy

Agennix is the pioneer innovator of rhLF as a pharmaceutical. Phase II trials for non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC) indications were conducted and each met its primary endpoint. The Phase III program for NSCLC is underway and Agennix has received Fast Track Designation from FDA for two NSCLC indications. If approved as a drug, rhLF would meet major unmet medical needs.

III. Significant and Unresolved Scientific Concerns Demonstrate that rhLF is Not GRAS for Use in Foods

A. Risks specifically associated with the glycosylation of rhLF from rice and cows

Comprehensive studies characterizing the long-term safety risks related to exposure to foreign glycans are necessary before any consensus on its safety can be reached. Glycosylation is of particular concern because glycans in glycoproteins in human, plants, and animals are very different. They differ in composition of sugars, linkage of sugars, and overall glycan structures. It is now well recognized that the source of glycoprotein and the type of glycosylation contribute greatly to the biological activity and the immunogenicity and allergenicity of the products.

Great care must be taken to avoid undesirable immune effects that can be introduced by producing recombinant glycoproteins in non-natural host cells. Thorough product testing through multi-year clinical trials with appropriate numbers of subjects of different genders, age groups, and ethnicities would appear to be the only mechanism to ensure product safety to the general public.

B. Risks of immunogenicity and allergenicity with rhLF from rice and cows

Most, if not all, recombinant therapeutic proteins have the potential of inducing an immune response in humans. Antibodies against rhLF may cross-react with the endogenous lactoferrin causing an immune impairment by neutralizing the action of endogenous lactoferrin. Antibodies against lactoferrin are thought to be associated with some autoimmune diseases. Although it is not known yet whether auto-antibodies against lactoferrin have a direct pathogenic effect, these antibodies may contribute to a clinical exacerbation of a pre-existing autoimmune disorder. Anti-rhLF IgEs may lead to immune-mediated allergic anti-drug reaction with severe anaphylactic consequences.

Insufficient human data have been presented to resolve the safety concerns relating to immunogenicity, induction of anti-lactoferrin antibodies and exacerbation of autoimmune diseases that may be associated with anti-lactoferrin antibodies.

C. Risks associated with feeding rhLF to young children, including infants

Vulnerable populations including neonates, infants and young children with gastroenteritis face additional immunological risks when receiving rhLF. Antibodies against chimeric rice- or bovine-derived rhLF could have cross-reactivity to endogenous or therapeutically administered LF or against other proteins in a rice- or cow's milk-based formulation administered to infants and children. Breast-milk Lactoferrin is a natural peptide antibiotic and immunomodulatory protein. It is *not* a nutrient and should not be treated as a food.

The trial conducted by Ventria in South America in a relatively small number of children receiving very short term administration did not establish the safety of the pediatric use of rice-derived rhLF, nor did it provide credible evidence that rhLF administration provides a benefit to children with infantile gastroenteritis. In fact, the study was so poorly designed and conducted that no meaningful conclusions can be drawn. Well-designed and well-conducted randomized, multi-year clinical studies are needed to adequately assess safety.

IV. Experts at Toxicology Forum Meeting Support Conclusion that rhLF is not GRAS

Scientific experts at the Toxicology Forum concluded that, in addition to the known risks associated with recombinant human proteins, there are significant unknowns. These safety concerns must be adequately addressed before recombinant proteins can be considered to be GRAS in food. In particular, these experts found: (1) immunogenicity, cross-reactivity, auto-immunity, and allergenicity remain major concerns; (2) immunological concerns exist with both oral and parenteral routes of administration; (3) recombinant proteins differ from their native counterparts and physiological context in many different ways which can pose additional safety risks; and (4) apparent safety of human proteins in animal trials is not a meaningful indicator of safety in humans.

The concerns expressed regarding the immunological and other risks associated with recombinant proteins are consistent with those previously articulated by Agennix experts, even though neither Agennix nor its experts played any role in this meeting.

V. "Severe Conflict" Among Qualified Experts Precludes Finding of GRAS

Agennix has contacted 14 prominent, highly qualified scientific and medical experts who all believe there are significant, unresolved safety issues and that rhLF has *not* been shown to be safe for its intended food uses. Moreover, as mentioned above, experts at the Toxicology Forum who have not had any contact with Agennix expressed the same views. This unequivocally demonstrates that a "severe conflict" exists among qualified experts. This reason alone requires denial of GRAS status, as a matter of law.

VI. Section 912 of the FDAAA Prohibits rhLF from Being Added to Food

This law was intended to ensure product safety and protect drug development, when drug development came first. rhLF meets all the necessary criteria of Section 912, and none of the specific exceptions apply. Agennix submitted comments to the FDA docket supporting this position, while Ventria and Pharming did not submit comments disagreeing. Section 912 should prohibit rhLF from being added to foods.

From: Levitt, Joseph A.
To: Tarantino, Laura M;
cc: Fasano, Jeremiah; Cheeseman, Mitchell A;
Subject: Background Materials from FDA Commissioner Meeting with Agennix
Date: Friday, January 23, 2009 2:16:38 PM
Attachments: Agennix FDA Meeting Jan 16 2009 slides.pdf
ExecutiveSummaryfromFDACommissionerMeeting.pdf

Laura--

Attached are copies of the slide deck and the two-page Executive Summary that Agennix presented last Friday to the FDA Commissioner and agency staff. As you know, Mitch Cheeseman attended from OFAS. I wanted to be sure that these materials were added to the relevant OFAS files for both the Ventria and the Pharming GRAS notices. I am copying Jeremiah Fasano so he will have these directly.

Thank for your continued attention to this matter.

Best regards,

Joe

Joseph Levitt, Partner
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Facsimile (713) 552-0795

March 9, 2009

Laura M. Tarantino, Ph.D. (HFS-200)
Director, Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Room 3044, University Station
5100 Paint Branch Parkway
College Park, Maryland 20740

Re: Request for Legal Conclusion that Recombinant Human Lactoferrin from Transgenic Cows GRN No. 000189 Submitted by Pharming Group N.V. is *not* Generally Recognized as Safe (GRAS) based on a "Severe Disagreement" among Qualified Experts

Dear Dr. Tarantino:

On behalf of Agennix, Inc. (Agennix) 1/, we write to urge the Food and Drug Administration (FDA) to reach the legal conclusion that recombinant human lactoferrin (rhLF) from transgenic cows is *not* generally recognized as safe (GRAS) for use in sports and functional foods and drinks, due to a "severe disagreement" among qualified experts as to whether it is safe for these food uses. For that reason alone, GRN No. 189, submitted by Pharming Group N.V.

1/ Agennix is a Houston, Texas-based biotechnology company and is the pioneer innovator of recombinant human lactoferrin as a pharmaceutical product. Agennix began clinical testing of rhLF in 1996 under the FDA's investigational new drug (IND) program. Agennix has completed blinded, placebo-controlled Phase II clinical trials with rhLF that met their primary endpoints in indications including non-small cell lung cancer and diabetic foot ulcers. In advanced renal cell carcinoma (RCC), rhLF has also been successfully tested in a Phase II open label trial to evaluate its effects in patients whose disease had progressed after receiving at least one prior regimen of systemic therapy. Additionally, the Company has initiated an NIH-funded, randomized, placebo-controlled, multi-center Phase II trial in patients with severe sepsis. Agennix obtained FDA Orphan Drug designation for rhLF for indications including graft versus host disease (Aug. 2003), non-small cell lung cancer (Aug. 2007), and renal cell carcinoma (Sept. 2006). Agennix also obtained Fast Track designation from the FDA for two different non-small cell lung cancer (NSCLC) indications (first-line in combination with chemotherapy [Sept. 2006] and third-line as monotherapy [Oct. 2007]), and has started Phase III trials. Agennix obtained approval of a Special Protocol Assessment (SPA) from the FDA for its first-line trial of rhLF in combination with chemotherapy in NSCLC patients (Dec. 2007).

("Pharming") to FDA's Center for Food Safety and Applied Nutrition (CFSAN), should be denied—based solely on legal grounds.

Agennix has already filed extensive scientific comments regarding significant, unresolved safety issues with the use of rhLF in food. 2/ That submission was supported by the opinions of 15 prominent scientific and medical experts that rhLF is *not* GRAS for these food uses. These scientific and medical experts are from disciplines directly applicable to the safety assessment of rhLF—including the fields of glycobiology, immunology, and medicine. Moreover, these scientific and medical experts are leaders in their respective fields, based on their many years of experience, prestigious academic posts, extensive publications, and numerous positions on government panels and editorial boards. They are regularly sought after as speakers at national and international conferences precisely because they are thought leaders whose opinions are highly respected.

More recently, the 2008 Annual summer meeting of the Toxicology Forum included an expert panel discussion on the use of human proteins as food ingredients. The panel, which was chaired by Dr. Antonia Mattia, included scientific experts from the FDA, academia and industry. These scientific experts concluded that, in addition to the known risks associated with recombinant human proteins, there are significant unknowns. According to these experts, these safety concerns must be adequately addressed before recombinant proteins can be considered to be GRAS in food. In particular, these experts found: (1) immunogenicity, cross-reactivity, auto-immunity, and allergenicity remain major concerns; (2) immunological concerns exist with both oral and parenteral routes of administration; (3) recombinant proteins differ from their native counterparts and physiological context in many different ways which can pose additional safety risks; and (4) apparent safety of human proteins in animal trials is not a meaningful indicator of safety in humans. The concerns expressed by the Toxicology Forum panel regarding the immunological and other risks associated with recombinant proteins are consistent with those previously articulated by our experts, even though neither Agennix nor any of its experts had any involvement in this Toxicology Forum panel.

Today's submission is tantamount to a "motion for summary judgment" because there are no material facts in dispute (i.e., it is a matter of record that there are two independent groups of experts expressing views diametrically opposed to those articulated by the Pharming expert panel) and so the Agency may rightfully decide this issue as a matter of law. Furthermore, this letter is based solely on the third prong of the GRAS test—namely, that there be a consensus among qualified experts that the food ingredient is safe. 3/ We are asking FDA to determine, as a matter

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2/ Agennix submitted to CFSAN its original Scientific Assessment on GRN No. 000189 on June 27, 2006.

3/ This letter does not rely on either of the first two prongs of the GRAS test—namely, that there be technical evidence of safety and that the data relied upon be publicly available. Those prongs are addressed in previous comments filed by Agennix to the scientific staff in CFSAN. Because all three prongs are required

of law, that rhLF from transgenic cows is *not* GRAS for use in sports drinks, functional foods, and any other food uses because Pharming has failed to demonstrate that there is a scientific consensus among qualified experts that the substance is safe.

The law is clear: a substance must meet all three prongs of the GRAS test to qualify as generally recognized as safe. An Agency determination that any one of the three elements is not met eliminates the need to evaluate and resolve the other two. As described below, Pharming so clearly fails to meet its burden of establishing a scientific consensus among experts that its GRAS notification for its transgenic cow-produced rhLF must be denied on this basis alone. 4/

I. The GRAS Standard Requires A Consensus Among Qualified Experts

As you are aware, a substance added to food is a "food additive" for which FDA pre-market approval is required unless the substance is GRAS or qualifies for another statutory exemption. The intended use of a substance is GRAS if it is—

> generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use . . . 5/

As the statutory language states, a GRAS determination may be based on "scientific procedures." FDA has advised that a GRAS determination based on scientific procedures requires three elements:

1. Evidence that a substance is safe for its intended use;
2. A basis for concluding that such evidence of safety is generally available; and
3. A basis for concluding that such evidence of safety is the subject of scientific *consensus among qualified scientific experts*.

---

for a GRAS determination, the Agency does not need to reach a conclusion on the first two prongs if FDA determines, as a matter of law, that the third prong of expert consensus is not met.

4/ Should the FDA agree that there is a severe disagreement among qualified experts, not only would there be no need for FDA to reach a conclusion on the complex scientific issues surrounding its technical evidence of safety, but FDA also would not have to reach a conclusion on the effect of Section 912 of the Food and Drug Administration Amendments Act of 2007 (FDAAA) on Pharming's GRAS notification (see letter of October 31, 2007 from Joseph A. Levitt, Counsel to Agennix).

5/ FFDCA § 201(s).

FDA refers to the first element as "technical evidence of safety"; the second and third criteria collectively constitute the "common knowledge" element of the GRAS standard. All three elements must be demonstrated or the GRAS notice is considered incomplete. 6/ Further, the common knowledge elements of scientific consensus and publication apply to all of the evidence that is the basis for the determination of safety. 7/

Technical evidence of safety requires a showing that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use." 8/ This is frequently paraphrased as demonstrating that there is a "reasonable certainty of no harm." The second element, general availability, requires publication of key data or information in peer-reviewed scientific journals, general reference materials, textbooks, or other appropriate sources. 9/ Although we believe that Pharming also fails on the first two counts of the GRAS standard, this submission is limited to the third prong of the GRAS standard—the common-knowledge element of scientific consensus among qualified experts.

II. A Scientific Consensus Does Not Exist If There Is A "Severe Disagreement" Among Qualified Scientific Experts

It is well-settled law that a "consensus" of qualified experts does *not* exist if there is a "severe disagreement" among such experts as to whether the food ingredient is safe for its intended use. The very fact that Agennix has identified 15 prominent, highly qualified scientific and medical experts who all believe there are significant, unresolved safety issues and that rhLF has *not* been shown to be safe for its intended uses, unequivocally demonstrates that a "severe disagreement" exists on this pivotal point. Further, the concerns expressed by the independent expert panel convened by the Toxicology Forum regarding the immunological and other risks associated with recombinant proteins are consistent with those previously articulated by our experts, even though neither Agennix nor any of its experts had any involvement in this Toxicology Forum panel. Accordingly, Pharming has failed to demonstrate that the safety of its proposed uses of rhLF produced from transgenic cows is the subject of expert consensus.

6/ 62 Fed. Reg. at 18937, 18948 (Apr. 17, 1997) (stating "A notice summary that fully describes the technical evidence of safety, but does not provide a basis to conclude that the technical evidence is generally available and accepted [by experts], would be incomplete").

7/ *Id.*

8/ 21 C.F.R. § 170.3(i); 62 Fed. Reg. at 18948.

9/ 21 C.F.R. § 170.30(b); 62 Fed. Reg. at 18942-43.

FDA's 1997 proposed rule on "Substances Generally Recognized as Safe" provides clear guidance on criteria for the basis of concluding expert consensus, 10/ and that the existence of a "severe conflict' among experts will preclude a GRAS determination. 11/

As discussed in FDA's GRAS proposal and the pertinent case law, a proponent of a GRAS claim bears the burden of establishing expert consensus (i.e., that experts "generally" consider the ingredient at issue to be safe). The courts and FDA have interpreted this to mean that, although a mere divergence of views will not necessarily preclude GRAS status (as "even properly conducted studies may produce disagreement" 12/) a "severe conflict" of expert opinion will *prevent* a finding of general recognition. 13/

Although there is no bright-line test for identifying what constitutes a "severe conflict," courts have readily found a "severe conflict" to exist after evaluating the facts at hand. In one case, even where the proponent of a GRAS claim presented the testimony of seven experts supportive of GRAS status, general recognition was found to be lacking in light of persuasive opposing views offered by "several" government experts. 14/ In another case, "sharply divided testimony" was found to present a severe conflict of opinion. 15/ Expert testimony critical of general recognition in that case suggested that the studies presented did not prove safety or meet other criteria contained in FDA's regulations. 16/ Another court failed to find a consensus where there was a "sharp difference of opinion" between experts regarding the methods and results of the available studies. 17/ Although these and other cases addressing expert consensus involve drug products, the expert consensus standard regarding safety is exactly the same for both food

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10/ 62 Fed. Reg. at 18948-49.

11/ *See* 62 Fed. Reg. at 18939 (citing *United States v. An Article of Drug . . . 4,680 Pails,* 725 F.2d 976, 990 (5th Cir. 1984); *Premo Pharma. Labs. v. United States*, 629 F.2d 795, 803 (2d Cir. 1980). Significantly, according to the Proposed Rule, "an ongoing scientific discussion or controversy about safety concerns . . . would make it difficult to provide a basis about the safety of a substance for an intended use." *Id.* at 18949.

12/ *See, e.g., United States v. Articles of Food and Drug . . . "Coli-Trol 80,"* 518 F.2d 743, 746 (5th Cir. 1975).

13/ 62 Fed. Reg. at 18939 (citing *United States v. Articles of Drug . . . 5,906 boxes*, 745 F.2d 105, 119 n. 22 (1st Cir. 1984); *4,680 Pails*, 725 F.2d at 990; *Coli-Trol 80*, 518 F.2d at 746 (5th Cir. 1975); *United States v. Articles of Drug . . . Promise Toothpaste*, 624 F. Supp. 776, 782 (N.D. Ill. 1985), *aff'd* 826 F.2d 564 (7th Cir. 1987)).

14/ *See, e.g., Pails*, 725 F.2d at 990 (holding that presentation by the United States of the views of "several experts" that a drug was not generally recognized as effective showed a "severe conflict" in the expert testimony and precluded general recognition).

15/ *United States v. An Article of Drug . . . X-Otag Plus Tablets*, 441 F. Supp. 105, 113-114 (D. Colo. 1977).

16/ *Id.* at 113.

17/ *Premo Pharma. Labs.*, 629 F.2d 795 at 804.

and drugs. 18/ For both food products and drugs, the key is whether there is a "severe disagreement" of views among qualified experts.

As described further below, these judicial characterizations of "sharply divided testimony" and "sharp difference of opinion" perfectly describe the current case—i.e., whether rhLF is generally recognized as safe for its intended food uses. The experts presented by Pharming express one view, and the experts presented by Agennix, as well as the independent experts at the Toxicology Forum that have never consulted with Agennix, express the very opposite view. Indeed, it is hard to imagine a scenario where the experts are any more "sharply divided." In such cases, the courts have consistently found that expert consensus does not exist, and FDA should reach the same conclusion here.

Expert credentials play an important role when assessing whether expert consensus exists. In one case evaluating the status of a drug for a particular treatment, the court gave great weight to the opinions of several chairmen of leading medical departments from that specialty area. The court stated that "it cannot be denied that the affidavits of five of the leading doctors in the field which deny general recognition creates more than a 'mere' conflict . . . [i]t is inconceivable that a drug such as this could be considered generally recognized in the face of such learned non-recognition." 19/

Once again, the court has very much described the current case. As detailed below, and reinforced in the collection of expert CVs already on file with CFSAN, the 15 scientific and medical experts presented by Agennix have national and international stature. They hold prestigious academic posts, direct cutting-edge scientific and medical centers, serve on important governmental committees, and publish extensively in leading journals. In short, they are quintessential examples of "leading doctors [and scientists] in the field" so that a finding of GRAS is virtually precluded "in the face of such learned non-recognition."

Agennix, the clear worldwide leader in research, development and production of rhLF, has consulted leading national and international experts on lactoferrin and issues relevant to the safety of rhLF from transgenic cows. These experts are primarily from the fields of: (a) glycosylation/glycobiology; (b) immunology; and (c) medicine. Included among these are experts who have conducted research directly with recombinant human lactoferrin, so they have first hand knowledge of its safety profile. These 15 highly-qualified experts have expressed serious and specific concerns regarding the safety of the Notifier's proposed uses of rhLF from transgenic cows, demonstrating a "severe conflict" with the expert opinions and conclusions submitted by Pharming. We feel strongly that all of our experts are qualified to opine on various issues related to the GRAS status of rhLF from cows and their credentials speak for themselves.

18/ *See, e.g., 62 Fed. Reg.* at 18938-18939 (citing drug and food precedent in discussion of meaning of GRAS standard under section 201(s) of the FFDCA).

19/ *United States v. An Article of Drug . . . "Mykocert,"* 345 F. Supp. 571, 575 (N.D. Ill. 1972).

These are notable opinion leaders in various fields of science and medicine expressing widely-held safety concerns.

The credibility of the Agennix experts is only strengthened by the concordance between their views and those articulated by the Toxicology Forum's independent expert panel. The Toxicology Forum is a highly respected organization with a focus on the scientific underpinnings of toxicology. The panel assembled by the Forum included experienced individuals representing a broad range of stakeholders including CFSAN, CDER, academia and industry.

We believe that the opinions of Agennix's experts and those of the expert panel convened by the Toxicology Forum — as contrasted to those of Pharming's experts — demonstrate there is a "severe disagreement" among qualified experts and that there is no "consensus" of the scientific community on the safety of rhLF for its intended uses.

III. Agennix has Provided the Opinions of 15 Prominent Physicians and Scientists that Cow-based Recombinant Human Lactoferrin is *Not* GRAS.

Agennix has provided FDA with the opinions of 15 prominent physicians and scientists that rhLF is *not* GRAS for its intended uses. These experts were selected based on their recognized subject matter expertise, professional reputation, and experience in areas that have a high degree of relevance to the safety, biologic activity, and mechanism of action of rhLF, including glycobiology, immunology, and medicine. These experts include renowned professors at universities in the United States, Europe and Australia, chairs of their respective departments or groups, directors of scientific or medical centers, and practicing physicians. Collectively, they have published over 1,500 scientific articles, abstracts or book chapters, including a number of studies on recombinant human lactoferrin. The background and experience of each of these 15 experts may be summarized as follows:

1. Richard D. Cummings, Ph.D.: Dr. Cummings is a preeminent leader in the field of glycobiology with over 30 years of research and academic experience. He is William Patterson Timmie Professor and Chair of the Department of Biochemistry at Emory University School of Medicine. He and his research labs have made numerous significant discoveries and contributions at the forefront of this emerging field. Dr. Cummings founded and directed two major centers for glycobiology at Emory University School of Medicine and the University of Oklahoma Health Sciences Center. He is co-editor of the first textbook on glycobiology. Dr. Cummings has published over 170 peer-reviewed articles, over 30 review articles, eleven textbook chapters, and owns 27 different U.S. patents.

Dr. Cummings is an internationally known lecturer and speaker on issues related to glycobiology. He has been an invited speaker of over 125 organizations and institutions. He has organized or chaired various national and international meetings and symposia on glycomics. He

is a former President of the Society of Glycomics and is active in numerous professional societies. Dr. Cummings has been awarded various prestigious research fellowships from the National Institutes of Health (NIH) and National Science Foundation. He has served in an editorial capacity on ten different scientific journals. Dr. Cummings and his labs have been the recipient of seven current and seventeen prior NIH research grants, and twelve other research grants from various public and private institutions. He has provided government service in many different roles as an NIH reviewer, panel member, and study section member.

2. James Michael Pierce, Ph.D.: Dr. Pierce is a Professor of Biochemistry and Molecular Biology at the University of Georgia and Director of the University of Georgia Cancer Center. He is a tenured professor with over 25 years in academia. Dr. Pierce's research focuses on the function of complex carbohydrates in human health with an emphasis on cancer progression and diagnosis. He has conducted extensive research in the area of glycobiology. He is the editor of the *Handbook of Glycomics* and an officer in the Society of Glycobiology. Dr. Pierce's work has been supported by the NIH and the National Cancer Institute. He has published over 65 peer-reviewed articles. He has served as a reviewer for various NIH, NCI, and American Cancer Society study sections and project reviews. Dr. Pierce is also a reviewer at leading publications including *Nature, Biochemistry, Gene, Glycobiology, Glycoconjugate Journal*, and the *International Journal of Cancer*. Dr. Pierce has been an invited speaker or lecturer at over 70 major seminars/symposia in the U.S. and abroad. He also holds eleven U.S. patents.

3. Irma van Die, Ph.D.: Dr. van Die is Head of the Glycoimmunology Group in the Department of Molecular Cell Biology & Immunology at Vrije University Medical Center in Amsterdam. She has written over 100 publications in the areas of glycobiology and immunology and has been a professor for over fifteen years. Dr. van Die has done extensive work for various sections of the Netherlands Organization for Scientific Research (NWO), the Dutch government organization that funds research at top universities and institutes. She is a regular reviewer for major journals including: *European Journal of Biochemistry, Glycoconjugate Journal, Glycobiology, Journal of Biological Chemistry* and a grant reviewer for the NWO. She has been a board member and is the current Secretary of the Dutch Society of Glycobiology, and is a member of various other professional societies. Dr. van Die's research is supported by numerous major public and private grants. Research at her glycoimmunology department has made a significant contribution to the present understanding and knowledge of glycan function.

4. Hubertus Schellekens, M.D.: Dr. Schellekens is a professor of Pharmaceutical Sciences at Utrecht University in the Netherlands. Dr. Schellekens has written more than 200 peer-reviewed journal articles concerning the preclinical development of biotechnology-derived therapeutic proteins. His most recent work focuses on immunogenicity of therapeutic proteins and biosimilars. He is the editor-in-chief of *Biotherapy*. Dr. Schellekens is very active in the Netherlands Commission on Genetic Modification (COGEM), serving as chairman of several subcommittees. COGEM provides scientific advice to the government on the risks to human

health and environment regarding the production and use of bioengineered compounds. He also serves as an expert in rDNA pharmaceuticals for the European Medicines Agency (EMEA), and as chairman of the Dutch Society of Microbiology's Committee for Biological Safety and deputy chair of its Committee on Biotechnology in Animals.

5. Arno Kromminga, Ph.D.: Dr. Kromminga serves as Director of Immunology at the Institute for Immunology, Clinical Pathology, and Molecular Medicine (IPM) in Hamburg, Germany. IPM's work focuses on resolving immunogenicity issues by antibody detection against biopharmaceuticals using a broad range of methods and different assay formats. It is dedicated to the development, validation and application of innovative methods in molecular and immunological diagnostics including immunogenicity. He is an international leader in the field of immunology. Dr. Kromminga has written over 25 publications and presented over 40 lectures at major symposia around the world.

6. **John Axford, D.Sc., M.D., FRCP**: Dr. Axford is the Chair of Clinical Rheumatology and Director of The Sir Joseph Hotung Centre for Musculoskeletal Disorders at St. George's University of London. He has an active rheumatology clinical practice and his academic research focuses on glycoprotein oligosaccharide characterization in rheumatic diseases and how sugar profiles are associated with specific disease entities. He has served on the editorial board of six journals including *Glyconjugate Journal* and *Journal of Therapeutic Biotechnology* and is a reviewer for seven journals. Dr. Axford has written 18 textbooks and chapters, including *Glycoimmunology I and II*, and "Glycobiology & Medicine" and won the British Medical Association Book of the Year commendation for *Medicine* in 1997. He has also written over 125 articles and been an invited presenter at twenty international symposia. Dr. Axford has served as a Past President, Secretary and Council Member of the Royal Society of Medicine, coordinating its Clinical Immunology and Allergy Section. He has been awarded several prestigious research fellowships, including a Fulbright Scholarship. Dr. Axford has received numerous private and government grants for research in areas including glycosylation and glycoimmunology studies.

7. David J.A. Goldsmith, M.D.: Dr. Goldsmith is a Consultant Nephrologist at Guy's Hospital in London and an Honorary Senior Lecturer at Guy's King's and St. Thomas' Hospitals Medical School at King's College in London. Dr. Goldsmith has over 20 years of medical teaching experience. He serves on the editorial board of four journals including the *Journal of Nephrology* and as a regular reviewer for seven journals. His publications include ten books and chapters, 172 papers, and 22 letters. Dr. Goldsmith has also presented abstracts at over 70 national and international meetings. He has received numerous private and public research grants. He is an Honorary Secretary and Trustee of the UK Renal Association, a Member of the Executive Council of the European Renal Association, and a Medical Advisor to the UK National Kidney Federation.

8. E.D. Weinberg, Ph.D.: Dr. Weinberg is Professor Emeritus in Microbiology at Indiana University and the Scientific Advisory Board Chair for the Iron Disorders Institute. He was a professor for over 40 years for more than 15,000 students. He has published over 150 research papers or book chapters. Two of his papers have been designated as Benchmark Papers in Microbiology. Dr. Weinberg has presented thirty-six invited lectures at national and international meetings and attended over forty invited seminars throughout the world.

Dr. Weinberg has conducted important research on lactoferrin over decades and is particularly qualified to advise on the safety issues related to human use of rhLF. Three of his publications include: "Human lactoferrin: a novel therapeutic with broad spectrum potential," "Therapeutic potential of human transferrin and human recombinant lactoferrin," and "The therapeutic potential of lactoferrin."

9. Sidney E. Grossberg, M.D.: Dr. Grossberg is Walter Schroeder Professor of Microbiology and Molecular Genetics and Professor of Medicine at the Medical College of Wisconsin. He served as Chairman of the Department of Microbiology for thirty-one years and has been a medical professor for over fifty years. Dr. Grossberg has been published in over 170 peer-reviewed publications. He has served as an advisor or reviewer for the National Cancer Institute, National Institute of Allergy and Infectious Diseases, the World Health Organization, and the National Board of Medical Examiners. His expertise includes microbiology and immunology.

10. Marco van de Weert, Ph.D.: Dr. van de Weert is a professor in the Department of Pharmaceutics and Analytical Chemistry and is Biomacromolecules Group Leader at the Danish University of Pharmaceutical Sciences. He has been a professor for six years and focuses his research on protein formulation and drug delivery. He has written over 20 publications and three book chapters. Dr. van de Weert is a regular reviewer for scientific journals, including the *European Journal of Pharmaceutical Sciences*, the *Journal of Pharmaceutical Sciences*, and the *International Journal of Pharmaceutics*. He is also a member of the European Working Party on Biosimilars, the group that advises the European Medicines Agency on issues related to comparability testing for follow-on biologics and any other clinical and non-clinical matters relating directly or indirectly to the safety and efficacy of biosimilar therapies.

11. Wolfgang E.B. Jelkmann, M.D.: Dr. Jelkmann is Professor of Physiology and Director of the Institute of Physiology at the University of Luebeck in Germany. He has over thirty years of academic medical experience and focuses his research on the production and action of inflammatory cytokines and hemopoietic growth factors, with an emphasis on erythropoietin. Dr. Jelkmann has written over 120 original publications, over fifty book chapters and reviews, and edited three books regarding the pathophysiology, pharmacology, molecular biology and clinical use of erythropoietin. He is on the editorial board of six journals.

12. Martin K. Kuhlmann, M.D.: Dr. Kuhlmann is an Associate Professor of Medicine and Nephrology and Director of Internal Medicine - Nephrology at Vivantes Clinical Center-Friedrichshain in Berlin. He has been a professor of nephrology for fifteen years, with research focusing on various issues related to hemodialysis, peritoneal dialysis, and cytoprotection from ischemic/toxic renal injury. Dr. Kuhlmann is a reviewer for fourteen different scientific journals. He has written thirty peer-reviewed publications, over forty review articles, and has been an invited presenter at over 100 international conferences and symposia.

13. Simon D. Roger, M.D.: Dr. Roger is a renal physician and Director of Nephrology at Gosford Hospital in Australia and a Clinical Associate Professor in the Department of Medicine and Health Sciences at Newcastle University. He has written over forty publications and a book chapter. Dr. Roger's research focuses on the management of anemia/chronic kidney disease, erythropoietin use and renal failure, and biosimilars.

14. Ashraf I. Mikhail, M.D.: Dr. Mikhail is a renal physician at Morriston Hospital and Senior Clinical Tutor at Swansea University in Wales. Dr. Mikhail's main areas of research include the impact of introducing biosimilar epoetins on the quality of anemia management in hemodialysis patients and the role of cytokines in modulating the response to erythropoietin therapy. He has published fourteen articles in peer-reviewed journals and two book chapters.

15. Nicole Casadevall, M.D.: Dr. Casadevall is Professor of Hematology at Saint Antoine Hospital in Paris. Her areas of research have centered on hemodialysis with special emphasis on erythropoiesis, erythropoietin, and myeloproliferative and myelodyspastic syndromes. She has served as a member of the Medical Committee for the French Health Products Safety Agency (AFSSAPS) and as Scientific President of the French Society of Hematology.

Agennix sought out these experts solely for the purpose of obtaining an independent evaluation of the safety of rhLF for use in food. In doing so, Agennix sought a broad range of perspectives and experience including: (1) topical experts from research and academia (experts who are leaders in their respective fields and who are familiar with state-of-the art in these fields); (2) seasoned medical professionals in academia (physicians from teaching hospitals and/or medical professors); (3) practicing medical doctors (providing a perspective from frontline clinicians); and (4) experts in proteins, in general, and recombinant human lactoferrin, in particular. The mix was selected to provide both technical and practical depth.

Although the opinions of just a few of these experts would be compelling, the opinions of this broad array of experts concurring in their scientific assessments unambiguously demonstrates a "severe conflict" that precludes GRAS status.

IV. These Expert Opinions Create a "Severe Disagreement" with those of Pharming's Experts on the Key Issues Affecting GRAS Evaluation.

The opinions of 15 prominent experts submitted by Agennix, supported by the independent opinion of the Toxicology Forum's expert panel, quite clearly demonstrate there is a "severe disagreement" among experts regarding whether the use of transgenic cow-produced rhLF in sports drinks, functional foods and other food uses is safe. These experts have raised legitimate concerns regarding important, unanswered safety questions as well as regarding the safety of long-term use of rhLF in food. Thus, given that these experts have expressed an opinion diametrically opposed to that offered by Pharming's experts, a consensus definitely does *not* exist in the medical and scientific communities.

The expert opinions provided by Agennix raise concerns in a number of areas, particularly concerning: (1) risks specifically associated with the glycosylation of rhLF from cows; (2) risks of immunogenicity and allergenicity with rhLF from cows; and (3) risks related to iron exposure. 20/ These 15 prominent scientific and medical experts have all endorsed the entire Scientific Assessment submitted to FDA on June 27, 2006. The summary below highlights particular expertise that certain experts bring to each of the major issues presented.

Fundamental to the concerns raised by these experts is the genuine opinion and belief that the safety of this compound cannot be established in the absence of appropriately powered long-term human clinical studies. Of particular concern was the need to determine (again through appropriately powered long-term human clinical studies) rhLF's safety in uniquely vulnerable patient populations, including children and immunocompromised subjects such as those with autoimmune disease. Moreover, the opinions of these experts, many of whom have been evaluating this particular issue since 2005, have not wavered during the intervening 3 years.

1. Risks specifically associated with the glycosylation of rhLF from cows.

Our experts strongly disagree with Pharming's experts on whether the safety profiles of transgenic cow-derived lactoferrin and native human lactoferrin are equivalent and whether the structural differences and major changes in glycosylation patterns can pose significant, long-term health risks. We have consulted some of the most prominent leaders in the field of glycobiology (Dr. Cummings, Dr. Pierce, and Dr. Schellekens) who concluded that the data presented in Pharming's GRAS notice did not substantiate the safety of transgenic cow-produced rhLF. Rather, comprehensive studies characterizing the long-term safety risks related to exposure to foreign cow glycans are necessary before any consensus on its safety can be reached. Pharming's rhLF product has the characteristics of bovine glycosylation rather than human glycosylation and substantial and material differences exist between these compounds. The glycosylation issue is of particular concern, according to these experts, because Pharming's rhLF consists of allergenic bovine glycans attached to a human protein sequence. As these experts

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20/ These experts have also expressed concern about the absence of adequate safety studies conducted with rhLF from transgenic cows; and other risks associated with extended dosing with any rhLF.

have explained in submissions to CFSAN, evaluation of the safety of bovine glycans and of human lactoferrin separately does not replace the need to evaluate the safety of rhLF that combines bovine glycosylation with the human protein sequence. Rather, these experts believe that the novel structure of bovine glycosylated human lactoferrin may create new risks relating to the recombinant protein's processing and recognition by the human immune system that can only be adequately assessed by long-term human clinical studies with the cow-derived recombinant protein.

Dr. Cummings is one of the preeminent scholars on glycosylation and the resulting effect on the function of and safety of therapeutic proteins. As noted above, he holds the prestigious position as the William Patterson Timmie Profession at the Emory University School of Medicine, where he also chairs the Department of Biochemistry. He founded and directed two major centers for glycobiology at leading universities. He has published over 170 peer-reviewed articles and is co-editor of the first textbook on Glycobiology. He is also a former President of the Society of Glycomics. In short, any "who's who" in the field of glycobiology would start with Dr. Cummings.

Dr. Pierce is also a prominent expert on glycobiology and carbohydrates. He has spent 25 years in academia and is currently a Professor of Biochemistry and Molecular Biology at the University of Georgia and is Director of the University's Cancer Center. He has published over 65 peer-reviewed articles, is the editor of the *Handbook of Glycomics* and is a reviewer for several leading scientific journals, including *Nature* and *Glycobiology.*

Dr. Schellekens is a physician and a professor of pharmaceutical sciences at Utrecht University in the Netherlands. He has extensive experience on the effect of glycosylation on the immunogenicity of proteins. He has published more than 200 peer-reviewed journal articles and is editor-in-chief of *Biotherapy.* He serves as an expert to the European Medicines Agency and is chairman of the Dutch Society of Microbiology's Committee for Biological Safety.

In the individual and collective opinions of these experts, there is no justification for Pharming's experts to ignore evidence that foreign glycoforms may have an effect on the safety of rhLF from transgenic cows. Further, our experts disagree with Pharming's experts' basic assertions that carbohydrates are not generally considered allergens and have poor biological activity. Moreover, according to our experts, risks related to bovine-derived glycans including IgE-mediated responses may even be amplified by the administration of transgenic cow-produced lactoferrin which could serve as a vector to deliver cross-reactive bovine glycans directly to immune cells in the gut.

These unresolved safety issues present an "ongoing scientific discussion or controversy about safety concerns" as stated in the Agency's 1997 Proposed Rule, that should clearly stand in the way of establishing the safety of an intended use. The impeccable credentials of our glycosylation experts should solidify the validity of their opinions and preclude a finding of

scientific consensus, as established by the *Mycocert* court. In that case, it was "inconceivable" that a substance could be GRAS given the "learned non-recognition" of several chairmen of leading specialty medical departments. 21/ Here, too, we have a severe disagreement among prominent experts and a sharp difference of opinion on the key issue of the potential consequences of the glycosylation of transgenic cow-derived rhLF. Failure of these learned experts to recognize Pharming's rhLF as GRAS is demonstrative of a "severe disagreement" in the scientific community.

2. Risks of immunogenicity and allergenicity with rhLF from transgenic cows.

Drs. van Die, Kromminga, and Schellekens are well-known and esteemed experts in the field of immunology, and Drs. Weinberg and van de Weert are notable researchers who have addressed protein immunogenicity in their published work. These experts all strongly disagree that Pharming has provided sufficient human data to resolve the safety concerns of immunogenicity, induction of anti-lactoferrin antibodies and exacerbation of autoimmune diseases that are associated with anti-lactoferrin antibodies. Indeed, these experts believe that Pharming and its experts may be basing their conclusions on a dated understanding of the mechanism and activity of human lactoferrin. Our experts have evaluated several published, peer-reviewed studies conducted by Agennix which have further strengthened their conclusion that long-term human studies are needed to accurately understand the actual safety profile of rhLF.

Dr. van Die is Head of the Glycoimmunology Group in the Department of Molecular Cell Biology & Immunology at Vrije University Medical Center in Amsterdam. She has been a professor for over 15 years and has written over 100 publications in the areas of glycobiology and immunology. She is a regular reviewer for major scientific journals and is a grant reviewer for the Netherlands Organization for Scientific Research. She is a former board member and current Secretary of the Dutch Society of Glycobiology.

Dr. Kromminga is Director of Immunology at the Institute of Immunology, Clinical Pathology, and Molecular Medicine in Hamburg, Germany, where he focuses on resolving important immunogenicity issues. He is an international leader in the field and has written over 25 publications and presented over 40 lectures at major symposia around the world.

Dr. Schellekens' extensive scientific and medical expertise is summarized in the previous section. Dr. Weinberg has researched lactoferrin for decades and has noted immunogenicity and other risks from lactoferrin administration in scientific publications. Dr. van de Weert's research and publications on the development of protein-based drugs include relevant concerns relating to immunogenicity.

21/ *United States v. An Article of Drug . . . "Mykocert"*, 345 F. Supp. 571, 575 (N.D. Ill. 1972).

Our experts strongly disagree with Pharming's experts' assertion that possible allergenic properties of rhLF cannot be the basis to deny a GRAS petition. Known allergenic/immunogenic properties are a significant safety concern and should be questioned when determining if a substance is GRAS. Our experts also believe there is a further increase in immunogenicity and allergenicity risk in the context of patients with conditions known to be associated with anti-lactoferrin antibodies (autoimmune liver disease, inflammatory bowel disease, Wegener's granulomatosis, rheumatoid arthritis, systemic lupus, and autoimmune pancreatitis). Our experts have provided the equivalent of the "sharply divided testimony" from the *X-Otag* case on the issue of whether exogenous lactoferrin can cause allergenic responses in humans. 22/ According to our experts, long-term studies are the only credible way to identify and quantify health risks associated with immunogenicity and allergenicity. Our experts fervently believe that the conclusions reached in Pharming's GRAS Notice are not supportable. Thus, an ongoing scientific controversy clearly exists on these issues, and the experts from Pharming and Agennix maintain an unresolved and severe conflict of opinion on these subjects.

3. Risks related to iron exposure.

Dr. E.D. Weinberg is Professor Emeritus in Microbiology at Indiana University and the Scientific Advisory Board Chair for the Iron Disorders Institute. As such, he is a renowned expert in the field of iron-related disorders. He has also conducted research with lactoferrin itself, and so he is well-versed in the potential for lactoferrin to impact individuals with iron-related disorders.

According to Dr. Weinberg and our other experts, dosing of pharmacologically active rhLF for extended periods of time can result in toxicity in individuals with iron overload. Iron overload can proceed asymptomatically for years, with the patient often presenting only after severe tissue damage has already occurred. Lactoferrin binds with a high avidity across a broad range of pH concentrations and its ability to deliver iron is an important biological property of the molecule. Iron delivery to iron-constrained pathogens in the gut is also a concern since a variety of bacteria have developed a mechanism for acquiring iron directly from human lactoferrin. Tumor cells are known to over-express receptors that bind lactoferrin with high affinity. Thus there is a risk that pre-cancerous or early stage GI tumors might also access iron from lactoferrin to accelerate their growth and metastasis.

These iron-related concerns have not been addressed adequately in Pharming's GRAS Notice and our experts strongly disagree with the assessment of Pharming's experts that the safety of transgenic cow-produced rhLF has been established. Thus, an ongoing scientific controversy clearly exists on these issues, and the experts from Pharming and Agennix maintain an unresolved and severe conflict of opinion on these subjects.

22/ *United States v. An Article of Drug . . . X-Otag Plus Tablets*, 441 F. Supp. 105, 113-114 (D. Colo. 1977).

In summary, the clear lack of scientific consensus that rhLF is GRAS is evidenced by the compelling opinions of these 15 prominent scientific and medical experts, further supported by the independent opinion of the expert panel convened by the Toxicology Forum. These experts raise legitimate questions about the safety of transgenic cow-based rhLF. That so many, and such highly qualified, experts have repeatedly expressed serious concern about the proposed uses of rhLF demonstrates a "severe conflict" of expert opinion and precludes GRAS status for rhLF from transgenic cows.

III. Conclusion

Based on Pharming's failure to demonstrate that there is a scientific consensus among qualified experts that rhLF from transgenic cows is GRAS, we are asking CFSAN to determine, as a matter of law, that rhLF from transgenic cows is *not* GRAS for use in sports drinks, functional foods or other food uses.

Agennix appreciates CFSAN's consideration of this important information as Pharming's GRAS notification for rhLF from cows is considered. Please do not hesitate to contact us if there are any questions or if additional information would be useful.

Sincerely,

Rick Barsky
Chief Executive Officer

cc: Jeremiah Fasano (HFS-255)
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review

Stephen F. Sundlof, D.V.M., Ph.D. (HFS-001)
Director, CFSAN

Michael M. Landa (HFS-001)
Deputy Director for Regulatory Affairs, CFSAN

Jeff Senger (GCF-1)
FDA Deputy Chief Counsel

From: Levitt, Joseph A.
To: Fasano, Jeremiah;
cc: Tarantino, Laura M;
Subject: Agennix "Summary Judgment" Letter on GRN 189
Date: Monday, March 09, 2009 2:55:20 PM
Attachments: GRAS Sum Judgment Pharming.pdf

Dear Jeremiah --

Attached is an electronic copy of a letter being delivered to your office tomorrow. The letter requests that FDA reach the legal conclusion that recombinant human lactoferrin (rhLF) from transgenic cows, GRN No. 000189 submitted by Pharming, Inc, is *not* Generally Recognized as Safe (GRAS) based on a "severe disagreement" among qualified experts.

Agennix has approached this as being tantamount to a legal motion for summary judgment because there are no material facts in dispute (i.e., it is a matter of record that there are two groups of experts expressing diametrically opposing views) and so the Agency may rightfully decide this issue as a matter of law. Furthermore, this letter is based solely on the third prong of the GRAS test—namely, that there be a consensus among qualified experts that the food ingredient is safe. Agennix is asking FDA to determine, as a matter of law, that rhLF from transgenic cows is *not* GRAS for use in foods solely because Pharming has failed to demonstrate that there is a scientific consensus among qualified experts that the substance is safe.

This letter is substantially the same as one filed last August in connection with GRN No. 000235, except that this letter contains an additional reference to the discussion held last summer at the Toxicology Forum meeting on transgenic proteins.

Like the previous letter, this letter also notes that, if FDA were to take this approach, then it would obviate the need to address the rhLF issue in the context of Section 912 of the FDAAA.

Please let me know if you have any questions. FYI, I am sending similar notes to others named as cc's on the letter.

Best regards,

Joe

Joseph Levitt, Partner
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telephone (+1-202-637-5600) or by electronic mail (PostMaster@HHLAW.COM) immediately.

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Agennix Incorporated

May 28, 2009

Laura M. Tarantino, Ph.D. (HFS-200)
Director, Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Room 3044, University Station
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Tarantino:

Attached is a summary of the scientific discussion by an expert panel convened at last summer's Toxicology Forum meeting concerning the safety of human proteins added to foods. This panel concluded that there are too many unresolved scientific questions to find human proteins to be Generally Recognized As Safe (GRAS) until extensive clinical trials have been conducted.

Specifically, these experts found:

(1) Immunogenicity, cross-reactivity, auto-immunity, and allergenicity remain major concerns.

(2) Immunological concerns exist with both oral and parenteral routes of administration.

(3) Recombinant proteins differ from their native counterparts and physiological context in many different ways which can pose additional safety risks.

(4) Apparent safety of human proteins in animal trials is not a meaningful indicator of safety in humans.

We believe these findings and conclusions are directly relevant to FDA's consideration of the GRAS status of recombinant human lactoferrin (rhLF)—specifically, rice-based rhLF (GRN-235), submitted by Ventria Biosciences, and transgenic cow-produced rhLF (GRN-189), submitted by Pharming, Inc. The expert panel's discussion and conclusions reinforce the findings and conclusions of Agennix's experts that rhLF is not GRAS for use in any food products.

Importantly, the Toxicology Forum panel was convened with no involvement by, nor even prior knowledge of, Agennix. Therefore, the concordance between the findings of the Toxicology Forum experts and Agennix's experts is even all the more compelling.

Agennix

Eight Greenway Plaza Suite 910 Houston Texas 77046 Telephone 713/552-1091 Facsimile 713/552-0795

These findings reinforce the views expressed by Agennix experts that rhLF is not GRAS, as a matter of science. Furthermore, these expert views are diametrically opposed to those expressed by Ventria's and Pharming's experts, thereby greatly exacerbating the severity of the conflict among qualified experts, and conclusively making rhLF not GRAS, as a matter of law.

We ask that a copy of this submission be placed in files of both GRN-235 and GRN-189. We again call upon FDA to reach the conclusion that rhLF is not GRAS for use in any food products.

Sincerely,

Rick Barsky
Chief Executive Officer

Attachment

cc: Jeremiah Fasano (HFS-255)
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review

Toxicology Forum – Summary of expert discussion on the use of Recombinant Human Proteins as Food Ingredients (July 2008)

The Toxicology Forum is an international, nonprofit organization that is devoted to conducting open dialogues among various segments of society concerned with problems in toxicology. Views are exchanged among experts from regulatory and health agencies, industry, academia, policymakers, and public interest groups to arrive at a balanced view of the topics discussed [http://www.toxforum.org/content].

The 2008 Annual summer meeting of the Toxicology Forum included an expert-panel discussion on the use of human proteins as food ingredients. The panel included scientific experts from the FDA, academia and industry.[1] These scientific experts concluded that, in addition to the known risks associated with recombinant human proteins, there are significant unknowns that directly impact a safety assessment. According to these experts, these safety concerns must be adequately addressed before recombinant proteins can be considered to be GRAS in food. The concerns expressed by the Toxicology Forum panel regarding the immunological and other risks associated with recombinant proteins are consistent with those previously articulated by Agennix's experts, even though neither Agennix nor any of its experts had any involvement in this Toxicology Forum panel.

A brief summary of the Toxicology-Forum discussions, arranged according to four key topics, is given below. Summaries of expert comments were taken from "Speaker Edits" for Session I of the Toxicology Forum meeting, on which each speaker's version starts on page 1, so multiple speakers may appear to have the same page reference. Statements from these individual experts have been paraphrased in some places to make the style of the narrative more consistent, but are intended to be as close to the actual statements as possible.

(1) Immunogenicity, cross-reactivity, auto-immunity, and allergenicity remain major concerns.

The first key scientific point made by the Toxicology Forum experts was a reminder that immunogenicity, cross-reactivity, auto-immunity and allergenicity are all major concerns with recombinant proteins. Thus, most, if not all, recombinant therapeutic proteins have the potential of inducing an immune response in humans. Agennix has argued previously that this is of particular concern with chimeric molecules like rice- or cow-derived recombinant human lactoferrin (rhLF) which combine a human-protein sequence with non-human glycosylation. Antibodies against rhLF may cross-react with the endogenous lactoferrin, causing an immune impairment by neutralizing the action of endogenous lactoferrin. Antibodies against lactoferrin are thought to be associated with some autoimmune diseases. Although it is not known yet

[1] David Longfellow, Ph.D., President, The Toxicology Forum; Jeremiah Fasano, Ph.D., FDA; Richard Goodman, Ph.D., University of Nebraska; Marian Kruzel, Ph.D., University of Texas; Antonia Mattia, Ph.D., FDA; Rafael Ponce, Ph.D., ZymoGenetics; Gopi Shankar, Ph.D., Centocor Research and Development, Inc.; Daniela Verthelyi, Ph.D., M.D., FDA. Dr. Mattia moderated the discussion.

whether auto-antibodies against lactoferrin have a direct pathogenic effect, these antibodies may contribute to a clinical exacerbation of a pre-existing autoimmune disorder. Anti-rhLF IgEs may also lead to immune-mediated allergic anti-drug reaction with severe anaphylactic consequences.

The points made by the Toxicology Forum experts on this topic include the following:

- It is important to recognize that "All exogenous proteins, including therapeutic ones, have the potential to cause antibody formation." (Dr. Shankar, quoting Dr. Huub Schellekens, an Agennix expert, page 2).

- One can also have different reactions occurring simultaneously with the same protein so there is really not an all or none phenomenon. If one tried to identify at an individual cellular level who is really tolerant, totally tolerant, to all proteins of a certain class, one would find mixed reactions and would conclude that nobody is totally tolerant. (Dr. Goodman, page 4)

- Antigenicity or immunogenicity of a substance is innate to it. Further, antigenicity is relative to the host, and is modulated by several factors, including the accompanying substances in a drug product, such as the formulants and impurities. Immunogenicity effects range from no adverse side effects to severe adverse reactions. The main concern with the immunogenicity of food-based proteins is allergenicity. Other concerns include consequences due to cross-reactivity of antibodies against that particular food protein or against other therapeutic products. Cross-reactivity with endogenous proteins is another concern. (Dr. Shankar, page 2)

- Immunological mediators involved extend beyond antibodies. In addition to the effect of antibodies, it is also cell-mediated immune responses and innate immunity and inflammation that are of concern. (Dr. Verthelyi, page 1)

- The available evidence, however imperfect, indicates that food allergy has increased two- to four-fold in the past 20 years. Why? This is not known. When one thinks about all of the complexity of this, one can imagine that it might be hard to predict what is going to happen. Further, complex matrices such as specific emulsions are being used. These may be taken up differently than soluble antigen. There have been changes in food processing treatments that will lead to aggregation of proteins, glycation, or denaturation. Those can impact how the antigen is processed. The different effects may be mostly in terms of elicitation, rather than sensitization or tolerance. (Dr. Goodman, page 3)

(2) <u>Immunological concerns exist with both oral and parenteral routes of administration.</u>

Agennix has also previously presented its experience with orally administered rhLF, including its presence in and its ability to modulate the Gut-Associated Lymphoid Tissue (GALT), the largest lymph organ in the body. RhLF has a direct effect on immature dendritic cells, the key antigen-presenting cell in the body, inducing their maturation. (Rosa 2008; Spadaro 2008) Following oral administration, rhLF has been shown to recruit immature DCs to the GALT, increase cellularity of the Peyer's patches, induce systemic activation of the innate and adaptive immunity, activate distant tumor-draining lymph nodes, and induce effector-cell infiltration into distant tumors with immune-dependent tumor killing. Thus, oral administration of rhLF is associated with a robust immunological response, which is not surprising considering the

GALT's importance to the body's immune defense. Agennix has also previously presented part of the extensive published data relating to the immunological effects of orally administered proteins. For example, studies with OVA, which has been cited by one of the Notifiers as an example of an orally tolerogenic protein, have shown that a cytotoxic T-lymphocyte response can be induced by oral administration and indeed trigger the onset of autoimmune disease, leading the investigators to conclude that "the intragastric route of antigen administration does not necessarily provide a default mechanism for tolerance induction" (Blanas 1996, Blanas 1999, Blanas 2000).

Statements made by the Toxicology Forum experts confirmed that these immunological concerns exist not just with parenteral routes of administration, but with oral administration as well, and contradict suggestions by the Notifiers of GRN 189 and GRN 235 that immunological concerns do not apply to orally administered proteins.

- Potential consequences of introducing recombinant human proteins are unknown. There are many questions. (Dr. Verthelyi, page 4)

- Such proteins should be considered as biological response modifiers and not as human food ingredients. (Dr. Adamson, page 6 in Dr. Ponce "Speaker Edits")

- Just because the route is oral, one cannot eliminate the possibility of inducing antibodies. Further, the status of the recipient may play a role. For example, if there were a GI inflammation, the absorption of the proteins may be different. If the immune system of the recipient is suppressed or immature, that may result in a different response. (Dr. Verthelyi, page 6)

- One gets antigen exposure and uptake through the airway, the nose or in the oral area. The stomach probably doesn't present much antigen, but in the lumen of the intestine, there is a tremendous immune system that is not as simple as many like to think. Some proteins or peptides are absorbed, go through the hepatic portal system and can flow through this way and be exposed to different immune cells in a variety of organs such as liver, spleen and peripheral lymph nodes. Some antigen can come through the M cells of the Peyer's patch and eventually become picked up by dendritic cells and go to the mesenteric lymph nodes. Some antigen will be picked up directly by dendritic cells (these cells directly sample out of the lumen with processes reaching into the lumen), and the antigen-loaded dendritic cells can go through the lymphatics. These antigen-presenting cells probably are exposing different T-cell populations in this environment that lead to IgA and IgG production, rather than to allergenic T cells that will lead to IgE. There are also epithelial cells that can take up and actually present antigen through either the lymphatics or capillary system. So, there are different systems, and they may lead to different outcomes. There are conditions such as inflammatory systems where you are going to set up T cells that are effectors or helpers that may drive IgE. There are conditions where some individuals have high dose tolerance, a lot of antigen presented, and maybe some people are allergic because they were not dosed with high-enough doses quickly enough in order to get deletion or anergy of the antigen-specific Th 2 cells. This is a theory, but maybe there are people who will never become tolerant to some proteins. I would suggest that we do not yet understand some of the critical factors in terms of which individuals will become spontaneously tolerant, which individuals will be

sensitized, which foods they will become tolerant to or sensitized to or why. That is an important fact that we have to live with. (Dr. Goodman, page 6)

(3) Recombinant proteins differ from their native counterparts and physiological context in many different ways which can pose additional safety risks.

Arguments presented below are especially important to the safety assessment of rhLF. Ventria and Pharming have each suggested that exposure to endogenously produced lactoferrin, including that produced in human milk and saliva, serves as an adequate basis for establishing the safety of exogenously administered rhLF. As already discussed, recombinant proteins, especially chimeric molecules with non-human glycosylation, can be treated very differently by the body. Beyond this, there are significant differences in terms of the types of exposure. Human exposure to lactoferrin from human milk is typically limited to the very young when the physiological context can be very different from that of older children or adults. Lactoferrin is also present in saliva but can be present at doses as low as 3.4 microgram/mL (Lentner 1981) and is presented continuously in a very different physiological context. Thus, it would not be at all appropriate to rely on the safety of native human lactoferrin expressed in milk or saliva to establish the safety of rhLF.

The Toxicology Forum experts explicitly articulated the point that recombinant proteins, in addition to differing structurally from their native counterparts, can also be presented in ways that can differ significantly from the normal physiological context of the native protein. These non-physiological presentations can themselves pose additional safety risks.

- For example, we try to make milk for babies similar to that of their mothers, easily digestible and so on, but maternal milk has a host of hormones, cytokines, chemokines, all sorts of things. These will modulate the response to the proteins present in milk, and putting those same proteins in a different context may result in a different response. (Dr. Verthelyi, page 2)

- Properties of proteins affect allergenicity. Stability to processing and digestion in the GI (gastrointestinal) tract makes a difference. If the protein gets unfolded or falls apart, it may have a higher propensity to have an immune response against it. The abundance of a protein: most food allergens actually comprise less than 1% of the protein content of foods, especially nuts and seeds and so on, whereas the highly abundant proteins, such as enzymes and so on, from leaves and other fruits and so on are less often reported as allergens. Interaction with lipid structures and aggregation: Food processing causes a propensity of food proteins to aggregate via association with lipids, and it is known that aggregation of proteins tends to give a greater incidence of immunogenicity. (Dr. Shankar, page 7)

- How does one really know that the human protein is as human as your body is making it? Are we really manufacturing it the way the Almighty is manufacturing it? I don't know. (Dr. Shankar, page 5)

- The problem is that in all expression systems, we have the glycosylation that is provided by the system in which it is expressed. So how do you make it really 100% compatible, not only at the level of protein, but also at the level of the glycoprotein? (Dr. Kruzel, page 3)

- Aggregates, from our point of view, are a very important factor in eliciting an immune response, both because they can change the way that the proteins are processed by cells, such as macrophages and dendritic cells, and because repetitive epitopes can activate B cells in the absence of specific T cells. Then there are a host of post-translational modifications: glycoforms, truncations, oxidation, deamidation, cystilation. All of those can contribute to product immunogenicity in their own way, either by revealing epitopes that were before hidden or by facilitating aggregation and so on. (Dr. Verthelyi, page 2)

(4) Apparent safety of human proteins in animal trials is not a meaningful indicator of safety in humans.

The crux of the position that Agennix has been communicating is that biological activity of human recombinant proteins cannot be adequately captured by testing in heterologous species. This is particularly true of immunomodulatory molecules whose full spectrum of activity cannot be accurately reflected in animal models and can only be fully observed following extended administration and surveillance in humans. Agennix believes that rhLF, with a low protein-sequence homology to its rodent counterpart (Pentecost et al. 1987; van Veen et al. 2002) and biological effects demonstrated in clinical trials, including Phase II trials in patients with cancer and diabetic foot ulcers, is one such substance. Although many GRAS determinations have been made, and should continue to be made, based on an established battery of animal toxicology studies and safety factors that establish safe conditions of use, this approach should not be applied to recombinant human proteins that must by necessity be subject to rigorous clinical testing in order to demonstrate a reasonable certainty of no harm, as required by the statute.

The need for human clinical trials to support the safety of administering a human protein was also emphasized by the experts in the Toxicology Forum meeting. These experts concluded that, although animal studies are helpful in terms of an overall safety assessment in humans, they cannot substitute for the conduct of human clinical studies as a necessary part of any safety assessment of human proteins, including rhLF.

The last point below made by Dr. Shankar is especially important, and reinforces the Agennix position that adequately powered, long-term human clinical studies are needed to properly assess the safety of rhLF in humans as part of the food supply.

- The use of human proteins in conventional foods raises a number of intriguing scientific questions, such as the adequacy of the existing animal models, the validity of data extrapolation from homologous proteins and the immunological significance of small variations in the homology of the proteins or in post-translational modifications. (Dr. Mattia, page 1)

- It has already been mentioned that animal studies do not predict immunogenicity in humans. Moreover, negative results do not abrogate the need for human immunogenicity testing. That is what that means. Just because we don't see a response in animals, it does not mean that we are not going to need to look at whether this is immunogenic in humans. On the other hand, positive results do not necessarily predict that it is going to be immunogenic in humans either. But this does not make animal models irrelevant. It may be very useful to look at what would happen were the animals to develop or were the humans to develop antibodies to that protein. What are the potential clinical consequences? What is the risk? Lastly, the absence

of effect on immunogenicity in animal models does not ensure that the structure is the same or that the factor does not impact on immunogenicity. We have had several companies come and say, "We have changed this and it hasn't changed immunogenicity in animals. It shouldn't change immunogenicity in humans." That is not the case. (Dr. Verthelyi, pages 3 and 4)

- It is artificial to talk of individual syndromes. The whole focus of immunotoxicity associated with immunostimulation is context, meaning what are the cellular players, in what tissues, at what levels are these various cytokines being expressed, and then one will see the diverse nature of the toxicity that will be elaborated in the host. (Dr. Ponce, page 2)

- When one looks at the picture of allergic responses in the human or in a mouse model, one can have a mixed response, and it is not easy to predict. Rodents rarely have allergies. So, they don't necessarily prove to be the best model. (Dr. Goodman, page 2)

- Clinical studies, obviously, are required. They support the safety and efficacy of the drug. (Dr. Shankar, page 4)

Conclusions

The Toxicology Forum panel concluded that there are significant risks attendant with the use of recombinant human proteins in foods. These include the risks that have been well characterized, such as immunogenicity, as well as additional lesser known or unknown risks that need to be better understood. The consensus was that these safety concerns must be adequately addressed through human clinical trials before recombinant proteins (such as rhLF) can be considered to be GRAS.

Agennix believes that the scientific concerns articulated by the expert panel convened by the Toxicology Forum, independent of any involvement by Agennix or its experts, make it abundantly clear that the use of recombinant human lactoferrin cannot be considered to be GRAS for use in any human food products at this time.

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From: Levitt, Joseph A.
To: Tarantino, Laura M;
cc: Fasano, Jeremiah;
Subject: Summary of Toxicology Forum Expert Discussion on Human Proteins being Added to Food
Date: Thursday, May 28, 2009 6:49:29 PM
Attachments: Tox Forum Submission.pdf

Dear Laura--

Attached please find a letter from Agennix, Inc., enclosing a summary of the Toxicology Forum expert discussion held last summer on the subject of human proteins being added to food. Even though Agennix had no participation in, or even prior knowledge of, this expert discussion, the views expressed there are remarkably consistent with those expressed by Agennix with respect to GRN 235 and GRN 189 in the context of the agency's GRAS review of rice-based and cow-based recombinant human lactoferrin (rhLF) -- particularly, that there are too many unanswered scientific questions and that human clinical studies are needed to properly assess its safety. Accordingly, this provides yet additional support for Agennix's position that neither rice-based nor cow-based rhLF is GRAS, either as a matter of science or as a matter of law.

We would ask that a copy of this letter and attachment (within the same PDF file) be added to both GRN 235 and GRN 198, and that the agency consider its contents as part of the GRAS review process. We will send hard copies to Jeremiah Fasano tomorrow.

Best regards,

Joe

Joseph Levitt, Partner
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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION
MEMORANDUM OF TELECONFERENCE

DATE: December 3, 2009

TIME: 4:00 PM

NUMBER: 415-957-0101

PARTICIPANTS:

FDA
Antonia Mattia HFS-255
Jeremiah Fasano HFS-255

External
Charles L. Morin Morin & Associates

SUBJECT: GRN 189

Mr. Morin is the agent for GRAS notice GRN No. 000189 (GRN 189), submitted to FDA on behalf of Pharming Group, N.V. (Pharming). GRN 189 describes intended food uses of transgenic human lactoferrin isolated from bovine milk. Mr. Morin contacted us at our request to discuss the status of this notice. In a previous discussion on July 3, 2008, we had identified significant obstacles to a 'no questions' response by FDA. In the absence of further substantive communication from Pharming, we stated that we were prepared to send a 'no basis' letter, but were offering Pharming an opportunity to withdraw their notice prior to issuance of the letter on December 17, 2009. We expressed our interest in continuing to discuss the scientific issues associated with this novel class of food ingredients as new data and information became available.

Mr. Morin stated that he would advise his clients of our request and inform us of their decision shortly.

Jeremiah Fasano

Drafted:HFS-255:JMFasano:12/09/2009
Comment/Init:HFS-255:AMattia:12/10/2009
Edit:HFS-255:JMFasano:12/10/2009
F/T:HFS-255:JMFasano:12/10/2009

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OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE
HFS-255		4/27/10						

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December 4, 2009

Antonia Mattia, PhD (HFS-255)
Director
Division of Biotechnology and
GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied
Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: Pharming Group, N.V
Notice of GRAS exemption for human
lactoferrin derived from the milk of
transgenic cows expressing a human gene
encoding human lactoferrin
GRN No. 000189

Dear Dr. Mattia:

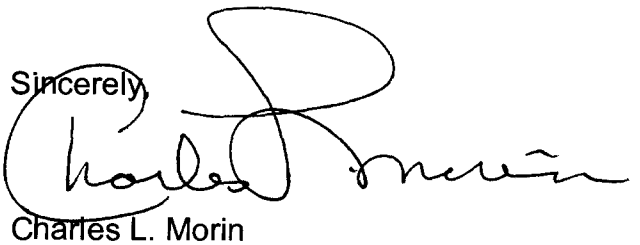
Thank you and Dr. Fasano for calling to discuss the status of Pharming's GRAS Notification, i.e., GRAS Notice Number 000189. Given CFSAN's request for certain – currently unavailable – information and the time needed to obtain such information, Pharming has decided to withdraw its GRAS Notice – effective this date – without prejudice. If and when such requested information becomes available in the future,

Pharming can then decide if it desires to refile (i.e., a new GRAS Notice) and, if so, make such filing.

Thank you for the professional and other courtesies extended to my client over the past more than fifteen years and, especially, more recently.

If you should have any further questions about Pharming's Notice, please do not hesitate to contact me.

Sincerely,



Charles L. Morin