



GRAS Notice (GRN) No. 464

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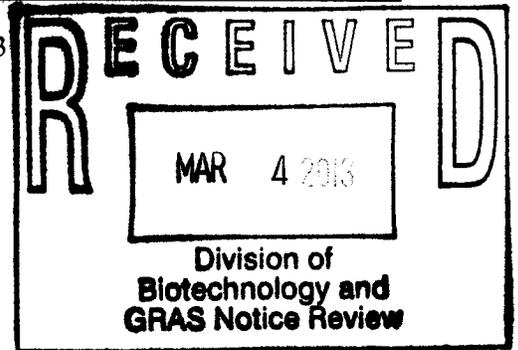
ORIGINAL SUBMISSION

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Spherix Consulting, Inc.

March 1, 2013

Office of Food Additive Safety
HFS-255
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740



To Whom It May Concern:

Enclosed please find three copies of the dossier entitled "Generally Recognized As Safe (GRAS) Determination for Cow's Milk-derived Lactoferrin as a Component of Cow's Milk Products and Chewing Gum," accompanying appendices and GRAS Panel Consensus Statement. We have prepared them for Morinaga Milk Industry Co., Ltd. We have also enclosed the original GRAS Panel Consensus Statement for GRN 423, the "Generally Recognized As Safe (GRAS) Determination for Cow's Milk-Derived Lactoferrin as a component of Cow's Milk-Based Infant Formulas, Cow's Milk Products, and Chewing Gum," which was submitted to OFAS on February 22, 2012 and withdrawn on May 22, 2012.

Per guidance obtained during our meeting on August 8, 2012 with OFAS and ONLDS about GRN 423, we have now separated the infant and adult uses into separate notifications and addressed the questions and comments submitted to us on May 30, 2012 and July 4, 2012. We would also like to inform you that Dr. Lloyd Mayer, an expert panel member that reviewed GRN 423, is now on medical leave and is unable to sign the new GRAS Panel Consensus Statement.

The data and information that serve as the basis for this GRAS determination is available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, Inc., 6430 Rockledge Drive, Suite 503, Bethesda, Maryland, 20817, Telephone: 301-897-0611; Facsimile: 301-897-2567; Email: clairek@chromadex.com, or will be sent to FDA upon request.

Should you have any questions or concerns, please contact me at the number listed above.

Sincerely,

(b) (6)

Claire L. Kruger, Ph.D., D.A.B.T.
President

Enclosures:

Three copies of the GRAS Panel Consensus Statement for the above-referenced GRAS Notification

Three copies of the GRAS Panel Consensus Statement for GRN 423

Three copies of the dossier entitled "Generally Recognized As Safe (GRAS) Determination for Cow's Milk-derived Lactoferrin as a Component of Cow's Milk Products and Chewing Gum" and accompanying Appendices

GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR COW'S MILK-DERIVED LACTOFERRIN AS A COMPONENT OF COW'S MILK PRODUCTS AND CHEWING GUM

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of food and food ingredients, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of the use of cow's milk-derived lactoferrin (cMDLf) in cow's milk-based yogurt (100 mg/100 g), powdered milk (400 mg/100 g), ice cream and sherbets (200 mg/ 100 g), and chewing gum (30 mg/g) is based upon scientific procedures as described under 21 CFR §170.30(b). The data and information are summarized in this GRAS determination document, Generally Recognized As Safe (GRAS) Determination for Cow's Milk-Derived Lactoferrin as a Component of Cow's Milk Products and Chewing Gum, prepared by Spherix Consulting, Inc., for Morinaga Milk Industry Co., Ltd., and that is appended herewith.

Based upon our review of the information and data available, we find that the intake of cMDLf from the intended uses specified has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that cMDLf is safe, and GRAS, under the intended conditions of use, the safety of the intake of cMDLf has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of cMDLf to supplement levels of this protein in selected cow's milk-based food products has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. In the United States, cMDLf has been determined safe and GRAS for use as an ingredient in sport and functional foods at concentrations of 100 mg/serving (GRN 77). cMDLf has also been determined safe and GRAS for use as a component of an antimicrobial spray for application to uncooked beef to prevent microbial contamination under GRN 67 and GRN 130.
2. cMDLf has a long and safe history of ingestion. Milk from cows has been consumed by the human population for centuries. Milk protein is a combination of caseins and whey proteins. Caseins account for 79.2% (27 mg/ml milk) of the total milk proteins.

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The remaining 20.8% (7.1 mg/ml milk) is whey protein. cMDLf, which is a whey protein, accounts for 0.3% (0.1 mg/ml milk) of the total protein or 1.4% of whey protein in milk.

3. cMDLf manufactured for Morinaga Milk Industry Co., Ltd. complies with established food grade specifications and utilizes only food grade raw materials and processing aids. The product consists of 4.2% moisture, 1.3 % ash, and 94.5% protein, of which $\geq 96\%$ is lactoferrin. The current production facilities comply with either the requirements of International Food Standard Version 5 or ISO 9001:2008 and ISO 14001:2004.
4. Morinaga's cMDLf has been listed on the "natural additive list" in Japan since 1989, was added to the "existing additive list" in Japan in 1995, and there is no specific restriction for cMDLf because it is considered a natural material.
5. The mean and 90th percentile EDIs by the total population, ages 1 year and older, of cMDLf from addition to proposed products are 142 mg/day and 273 mg/day (2.7 mg/kg/day and 5.8 mg/kg/day, respectively). The addition of cMDLf to the proposed products increases intake by three- to four-times background. (Non-milk products (chewing gum) containing cMDLf will be appropriately labeled as containing cow's milk proteins.)
6. The EDIs for cMDLf have been concluded to be safe and GRAS based on ADME studies, animal toxicology studies, studies evaluating physiologic effects, and clinical studies in adults, children, and infants. These published studies support the safety of intake of cMDLf at the proposed levels.
7. Digestion and metabolic fate of lactoferrin has been evaluated from studies of both human milk-derived and cow's milk-derived lactoferrin. Lactoferrin from both sources is handled similarly by the body. Lactoferrin that is absorbed from the gastrointestinal tract partitions into the lymph and then appears in the blood. Lactoferrin is rapidly removed from the systemic circulation by distribution into the spleen, liver, and kidneys while the iron portion is transferred to the liver for its transport into the bone marrow. Intact lactoferrin is detected in the feces and urine of infants.
8. While very few patients with cow's milk allergy (CMA) have antibodies to bovine lactoferrin, no evidence exists that cMDLf is a clinically relevant allergen.

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9. It has been shown that infants fed with formula containing added cMDLf developed anti-cMDLf antibodies. Exclusively breastfed infants develop anti-human lactoferrin antibodies, and autoimmune adults have antibodies that recognize both human and cMDLf. However, there is no evidence that cMDLf causes, promotes, exacerbates, or resolves autoimmunity in infants or adults.
10. The safety of cMDLf produced by Morinaga Milk Industry Co., Ltd. was evaluated in an acute toxicity study, a four-week oral toxicity study, a thirteen-week oral toxicity study, a chronic oral toxicity study and genotoxicity assays. cMDLf is not acutely toxic or genotoxic. cMDLf administered by oral intubation to rats for 13 weeks did not result in toxicologically significant treatment-related changes. Thus, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) of cMDLf was estimated to be in excess of 2,000 mg/kg/day. The chronic toxicity study was not available as a full report and therefore was not used to derive a NOAEL.
11. Numerous published studies on the effects of oral ingestion of cMDLf in adults, children, and infants corroborate the GRAS status of cMDLf for its proposed uses. Of the 37 studies in humans, 17 have been conducted using cMDLf supplied by Morinaga Milk Industry. cMDLf is safe and well-tolerated under the conditions of administration in the clinical studies at doses that range in adults and children from 100 mg/day to 3.6 g/day and with durations ranging from one week to one year. Studies carried out in both healthy and health-compromised adults and children reported no treatment related side effects attributed to oral ingestion of cMDLf. Administration of cMDLf at 200 mg/day for up to 90 days in pregnant women was well tolerated (highest dose tested in this target population). In term and preterm infants, exposure to cMDLf from infant formulas has been studied using concentrations ranging from 10 mg/100 mL (0.01%) to 285 mg/100 mL (0.285%), comprising durations ranging from two weeks to one year. Resulting intake of cMDLf is up to 150 mg/kg/day. No treatment related adverse effects have been reported in infants.

Determination of the GRAS status of cMDLf supplied by Morinaga Milk Industry under the intended conditions of use has been made through the deliberations of Dr. Stephen Taylor, Dr. Lloyd Mayer, Dr. A. Wallace Hayes, and Dr. Roger Clemens. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in

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this document, including the safety of cMDLf and the potential human exposure to cMDLf resulting from its intended use in cow's milk-based products and chewing gum, and concluded:

There is no evidence in the available information on cMDLf that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when cMDLf is used at levels that might reasonably be expected from the proposed applications. cMDLf is GRAS for use in foods as proposed by Morinaga Milk Industry Co., Ltd.

Therefore, cMDLf is safe and GRAS at the proposed levels of addition to foods. cMDLf is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

It is our opinion that other experts qualified by training and/or experience to evaluate the safety of food and food ingredients would concur with these conclusions.

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Roger A. Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
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School of Pharmacy

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Date:

Feb 22, 2013

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Harvard School of Public Health

Signature:

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Date:

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Lloyd Mayer, MD
GRAS Expert Panel Member
Mount Sinai School of Medicine

ON MEDICAL LEAVE
See attached, original Expert Panel
statement

Steve L. Taylor, MS, PhD
GRAS Expert Panel Member
University of Nebraska

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Date:

March 1, 2013

Claire L. Kruger, PhD, DABT
Scientific Advisor to the Panel
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Signature:

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Date:

3-1-13

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GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR COW'S MILK-DERIVED LACTOFERRIN AS A COMPONENT OF COW'S MILK-BASED INFANT FORMULAS, COW'S MILK PRODUCTS, AND CHEWING GUM

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of food and food ingredients, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of the use of cow's milk-derived lactoferrin (cMDLf) in cow's milk-based infant formulas [powdered (100 mg/100 g), liquid concentrates (26 mg/100 ml), and ready-to-feed formulas (13 mg/100 ml)], yogurt (100 mg/100 g), powdered milk (400 mg/100 g), ice cream and sherbets (200 mg/ 100 g), and chewing gum (30 mg/g) is based upon scientific procedures as described under 21 CFR §170.30(b). The data and information are summarized in this GRAS determination document, Generally Recognized As Safe (GRAS) Determination for Cow's Milk-Derived Lactoferrin as a Component of Cow's Milk-Based Infant Formulas, Cow's Milk Products, and Chewing Gum, prepared by Spherix Consulting, Inc., for Morinaga Milk Industry Co., Ltd., and that is appended herewith.

Based upon our review of the information and data available, we find that the intake of cMDLf from the intended uses specified has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that cMDLf is safe, and GRAS, under the intended conditions of use, the safety of the intake of cMDLf has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of cMDLf to supplement levels of this protein in selected cow's milk-based food products has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. In the United States, cMDLf has been determined safe and GRAS for use as an ingredient in sport and functional foods at concentrations of 100 mg/serving (GRN 77). cMDLf has also been determined safe and GRAS for use as a component of an antimicrobial spray for application to uncooked beef to prevent microbial contamination under GRN 67 and GRN 130.

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2. cMDLf has a long and safe history of ingestion. Milk from cows has been consumed by the human population for centuries. Milk protein is a combination of caseins and whey proteins. Caseins account for 79.2% (27 mg/ml milk) of the total milk proteins. The remaining 20.8% (7.1 mg/ml milk) is whey protein. cMDLf, which is a whey protein, accounts for 0.3% (0.1 mg/ml milk) of the total protein or 1.4% of whey protein in milk.
3. cMDLf manufactured for Morinaga Milk Industry Co., Ltd. complies with established food grade specifications and utilizes only food grade raw materials and processing aids. The product consists of 4.2% moisture, 1.3 % ash, and 94.5% protein, of which $\geq 96\%$ is lactoferrin. The current production facilities comply with either the requirements of International Food Standard Version 5 or ISO 9001:2008 and ISO 14001:2004.
4. Infant formulas with added cMDLf are approved for use and sold in Japan, Taiwan, Pakistan, Indonesia, and China by Morinaga Milk Industry Co., Ltd. In Japan, the formulas have been certified by the Japanese Ministry of Health, Labor, and Welfare as Special Nutritious Foods according to the Nutrition Improvement Law. Morinaga's cMDLf has been listed on the "natural additive list" in Japan since 1989 and was added to the "existing additive list" in Japan in 1995. In Japan, there is no specific restriction for cMDLf because it is considered a natural material. The proposed GRAS level of cMDLf (100 mg/100 g powdered infant formula) to be added to cow-milk based infant formula is similar to the level of cMDLf in currently marketed and consumed Morinaga Milk infant formula products (80 mg/100 g powdered infant formula).
5. Since the release of Morinaga's infant formula products in 1986 and 1989, Morinaga has sold annually approximately 3,200 metric tons (76,800 metric tons total) and 3,500 metric tons (73,500 metric tons total) in Japan for the 0- to 9-month formulation and 9-month to 3-year formulation, respectively. With consumption of these formulas with added cMDLf by over a million infants and toddlers in Japan since 1986, there have been no reported significant health problems, including allergenic reactions or autoimmunity attributable to cMDLf, associated with either of these two products containing cMDLf based on post-marketing surveillance.

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6. The mean EDIs of cMDLf from addition to infant formulas are 100.3 mg/day for infants 0 to 4 months and 87.4 mg/day for infants 5 to 11 months. The 90th percentile intakes for these age groups are 145.5 mg/day and 129 mg/day, respectively. After cMDLf addition to infant formulas, the cMDLf exposure to this protein for infants in these age categories is approximately two-times the background exposure from unsupplemented cow-milk based infant formula.
7. The mean and 90th percentile EDIs by the total population of cMDLf from addition to proposed products excluding infant formulas are 142 mg/day and 273 mg/day (2.7 mg/kg/day and 5.8 mg/kg/day, respectively). Similarly, for all other population groups, the exposure to cMDLf from addition to the proposed products increases exposure by two- to three-times background.
8. The EDIs for cMDLf have been concluded to be safe and GRAS based on ADME studies, animal toxicology studies, studies evaluating physiologic effects, and clinical studies in adults, children, and infants. These published studies support the safety of intake of cMDLf at the proposed levels.
9. Digestion and metabolic fate of lactoferrin has been evaluated from studies of both human milk-derived and cow's milk-derived lactoferrin. Lactoferrin from both sources is handled similarly by the body. Lactoferrin that is absorbed from the gastrointestinal tract partitions into the lymph and then appears in the blood. Lactoferrin is rapidly removed from the systemic circulation by distribution into the spleen, liver, and kidneys while the iron portion is transferred to the liver for its transport into the bone marrow. Intact lactoferrin is detected in the feces and urine of infants.
10. While very few patients with cow's milk allergy (CMA) have antibodies to bovine lactoferrin, no evidence exists that cMDLf is a clinically relevant allergen.
11. It has been shown that infants fed with formula containing added cMDLf developed anti-cMDLf antibodies. Exclusively breastfed infants develop anti-human lactoferrin antibodies, and autoimmune adults have antibodies that recognize both human and cMDLf. However, there is no evidence that cMDLf causes, promotes, exacerbates, or resolves autoimmunity in infants or adults.
12. The safety of cMDLf produced by Morinaga Milk Industry Co., Ltd. was evaluated in an acute toxicity study, a four-week oral toxicity study, a thirteen-week oral toxicity

study, a chronic oral toxicity study and genotoxicity assays. cMDLf is not acutely toxic or genotoxic. cMDLf administered by oral intubation to rats for 13 weeks did not result in toxicologically significant treatment-related changes. Thus, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) of cMDLf was estimated to be in excess of 2,000 mg/kg/day. The chronic toxicity study was not available as a full report and therefore was not used to derive a NOAEL.

13. Numerous published studies on the effects of oral ingestion of cMDLf in adults, children, and infants corroborate the GRAS status of cMDLf for its proposed uses. Of the 37 studies in humans, 17 have been conducted using cMDLf supplied by Morinaga Milk Industry. cMDLf is safe and well-tolerated under the conditions of administration in the clinical studies at doses that range in adults and children from 100 mg/day to 3.6 g/day and with durations ranging from one week to one year. Studies carried out in both healthy and health-compromised adults and children reported no treatment related side effects attributed to oral ingestion of cMDLf. Administration of cMDLf at 200 mg/day for up to 90 days in pregnant women was well tolerated (highest dose tested in this target population). In term and preterm infants, exposure to cMDLf from infant formulas has been studied using concentrations ranging from 10 mg/100 mL (0.01%) to 285 mg/100 mL (0.285%), comprising durations ranging from two weeks to one year. Resulting intake of cMDLf is up to 150 mg/kg/day. No treatment related adverse effects have been reported in infants.

Determination of the GRAS status of cMDLf supplied by Morinaga Milk Industry under the intended conditions of use has been made through the deliberations of Dr. Stephen Taylor, Dr. Lloyd Mayer, Dr. A. Wallace Hayes, and Dr. Roger Clemens. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of cMDLf and the potential human exposure to cMDLf resulting from its intended use in milk-derived products and infant formulas and have concluded:

There is no evidence in the available information on cMDLf that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when cMDLf is used at levels that might reasonably be expected from the proposed applications. cMDLf is GRAS for use in foods as proposed by Morinaga Milk Industry Co., Ltd.

Therefore, cMDLf is safe and GRAS at the proposed levels of addition to foods. cMDLf is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

It is our opinion that other experts qualified by training and/or experience to evaluate the safety of food and food ingredients would concur with these conclusions.

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GRAS Determination for Cow's Milk-Derived Lactoferrin
Morinaga Milk Industry Co., Ltd.

June 22, 2011

Roger A. Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
University of Southern California
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A. Wallace Hayes, PhD, DABT, FATS, ERT
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Claire L. Kruger, PhD, DABT
Scientific Advisor to the Panel
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Date:

6/22/11

**GENERALLY RECOGNIZED AS SAFE (GRAS)
DETERMINATION FOR COW'S MILK-DERIVED
LACTOFERRIN AS A COMPONENT OF COW'S MILK
PRODUCTS AND CHEWING GUM**

Prepared for:

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February 21, 2013

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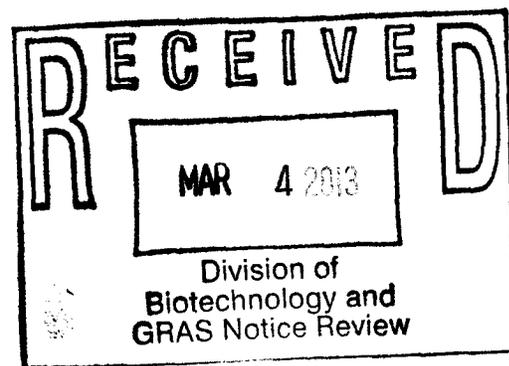


TABLE OF CONTENTS

I. GRAS EXEMPTION CLAIM.....	1
A. NAME AND ADDRESS OF THE SPONSOR	1
1. Business Address	1
2. Mailing Address	1
B. COMMON OR USUAL NAME OF GRAS SUBSTANCE	1
C. INTENDED USE AND CONSUMER EXPOSURE	1
D. BASIS FOR GRAS DETERMINATION	1
E. AVAILABILITY OF INFORMATION	4
F. SIGNATURE	5
II. DESCRIPTION OF SUBSTANCE	6
A. PHYSICAL AND CHEMICAL COMPOSITION	6
B. MANUFACTURING PROCESS	6
1. General Description of the Production Process	6
2. Starting Materials and Processing Aids	8
C. FINISHED PRODUCT SPECIFICATIONS	10
1. Product specifications and batch records	10
2. Parasiticides	16
3. Other whey proteins	17
D. STABILITY OF cMDLf	17
III. INTENDED EFFECT	18
IV. HISTORY OF USE, INTENDED USE, AND ESTIMATED DAILY INTAKE.....	19
A. HISTORY OF USE	19
1. Exposure to human lactoferrin	19
2. Exposure to cMDLf	20
B. INTENDED USE	25
C. ESTIMATED DAILY INTAKE OF cMDLf FROM PROPOSED USES	25
D. CONCLUSIONS	27
V. SAFETY ASSESSMENT	28
A. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF cMDLf	28
1. Introduction	28
2. Lactoferrin Fragments	28
3. Lactoferrin Receptors	28
4. Distribution and clearance of lactoferrin	29
5. Excretion of ingested lactoferrin	29
B. PHYSIOLOGICAL EFFECTS RELEVANT TO SAFETY OF INGESTION	29
1. Introduction	29
2. Immunomodulation	30
3. Cows milk allergy, hypersensitivity, and oral tolerance	30
4. Autoimmunity	59
5. Effects on Bone	60
C. ANIMAL TOXICOLOGY STUDIES	65
1. Acute Toxicity Study in Rats	66

2. Four-Week Oral Toxicity Study in Rats	66
3. Thirteen-week Oral Toxicity Study in Rats	68
4. Chronic Oral Toxicity Study in Rats	70
5. Genotoxicity	71
D. HUMAN STUDIES OF cMDLf	72
1. Introduction	72
2. Infants	72
3. Children	86
4. Adults	91
VI. REFERENCES	118

TABLES

Table 1. Physical and Chemical Properties of Cow’s Milk-Derived Lactoferrin.....	7
Table 2. Processing Aids and Chemicals Used in the Production of Cow’s Milk-Derived Lactoferrin (cMDLf).....	10
Table 3. Manufacturing Specifications for Cow’s Milk-Derived Lactoferrin (cMDLf)	11
Table 4. Batch Analyses of Cow’s Milk-Derived Lactoferrin (cMDLf)	13
Table 5. Batch Analyses of Radioactivity in Cow’s Milk-Derived Lactoferrin (cMDLf-1)	15
Table 6. Batch Analysis of Parasiticides in Cow’s Milk-Derived Lactoferrin (cMDLf)	16
Table 7. Concentration of Proteins in Cow’s Milk	20
Table 8. Foods Codes and Cow’s Milk-Derived Lactoferrin (cMDLf) Concentrations in Foods Used for Estimating Background Exposure.....	22
Table 9. All-User Estimated Daily Intake (EDI) of Cow’s Milk-Derived Lactoferrin (cMDLf) from Background Exposure to Cow’s Milk and Cow’s Milk-Based Products (U.S Population Groups; 2007-2008 NHANES Data).....	23
Table 10. Concentration and Recommended Daily Intake of Cow’s Milk-Derived Lactoferrin (cMDLf) in Dietary Supplements	24
Table 11. Intended Uses of Cow’s Milk-Derived Lactoferrin (cMDLf) and Its Maximum Use Levels.....	25
Table 12. Food Codes and Cow’s Milk-Derived Lactoferrin (cMDLf) Levels in Proposed Foods Used for Calculating Supplemental Intakes.....	26
Table 13. All-User Estimated Daily Intake (EDI) of Cow’s Milk-Derived Lactoferrin (cMDLf) from the Proposed cMDLf-containing Products (U.S Population Groups; 2007-2008 NHANES Data)	27

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity	36
Table 15. Clinical Studies of cMDLf in Infants	78
Table 16. Clinical Studies of cMDLf in Children.....	88
Table 17. Clinical Studies of cMDLf in Adults.....	100

FIGURES

Figure 1: Schematic of diferric Cow Lactoferrin at a Resolution of 2.8 Angstroms (Moore et al., 1997).....	7
Figure 2: Production Process of Cow's Milk-Derived Lactoferrin	9
Figure 3: Mean lactoferrin concentrations by country.....	19

APPENDICES

Appendix 1 – Raw Material Product Specification

Appendix 2A – HPLC Method

Appendix 2B – HPLC Method

Appendix 2C – HPLC Method

Appendix 3 – Stability of cMDLf

Appendix 4 – Grant Certificate by the Japanese Ministry of Health

Appendix 5A-1 – Specification monograph for cMDLf produced by Morinaga Milk Industry

Appendix 5A-2 – Specification monograph for cMDLf produced by Morinaga Milk Industry
(English Translation)

Appendix 5B-1 – List of Food Additives Other Than Chemically-Synthesized Compounds in
Japan

Appendix 5B-2 – List of Food Additives Other Than Chemically-Synthesized Compounds in
Japan (English Translation)

Appendix 5B-3 – List of Existing Food Additives in Japan for the Japan Food Chemical
Research Foundation

Appendix 5C-1 – cMDLf Notification in China

Appendix 5C-2 – cMDLf Notification in China - 2

Appendix 5C-3 - cMDLf Notification in China (English Translation)

Appendix 5D-1 – Certificate of Registration of cMFLf at the Ministry of Health in Taiwan

Appendix 5D-2 – Certificate of Registration of cMFLf at the Ministry of Health in Taiwan
(English Translation)

Appendix 5E – Specification Monograph for cMDLf for Korea

I. GRAS EXEMPTION CLAIM

A. NAME AND ADDRESS OF THE SPONSOR

1. BUSINESS ADDRESS

Morinaga Milk Industry Co., Ltd.
33-1, Shiba 5-Chome, Minato-ku
Tokyo 108-8384, Japan
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2. MAILING ADDRESS

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B. COMMON OR USUAL NAME OF GRAS SUBSTANCE

The substance that is the subject of this Generally Recognized As Safe (GRAS) determination is cow's milk-derived lactoferrin (cMDLf).

C. INTENDED USE AND CONSUMER EXPOSURE

Morinaga Milk Industry Co., Ltd. intends to supplement cow's milk-based products and chewing gum with cMDLf at levels of 100 mg/100 g yogurt, 400 mg/100g powdered milk, 200 mg/ 100 g ice cream and sherbets, 30 mg/g chewing gum.

D. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of cMDLf in cow's milk-based products and chewing gum is based upon scientific procedures as described in 21 CFR §170.30(b).

The intake of cMDLf from the specified intended uses has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that cMDLf is safe, and GRAS, under the intended conditions of use, the safety of the intake of cMDLf has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of cMDLf in selected cow's milk-based food products has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. In the United States, cMDLf has been determined safe and GRAS for use as an ingredient in sport and functional foods at concentrations of 100 mg/serving (GRN 77). cMDLf has also been determined safe and GRAS for use as a component of an antimicrobial spray for application to uncooked beef to prevent microbial contamination (GRN 67 and GRN 130).
2. cMDLf has a long and safe history of ingestion. Milk from cows has been consumed by the human population for centuries. Milk protein is a combination of caseins and whey proteins. Caseins account for 79.2% (27 mg/ml milk) of the total milk proteins. The remaining 20.8% (7.1 mg/ml milk) is whey protein. cMDLf, which is a whey protein, accounts for 0.3% (0.1 mg/ml milk) of the total protein or 1.4% of whey protein in milk.
3. cMDLf manufactured for Morinaga Milk Industry Co., Ltd. complies with established food grade specifications and utilizes only food grade raw materials and processing aids. The product consists of 4.2% moisture, 1.3 % ash, and 94.5% protein, of which \geq 96% is lactoferrin. The current production facilities comply with either the requirements of International Food Standard Version 5 or ISO 9001:2008 and ISO 14001:2004.
4. Morinaga's cMDLf has been listed on the "natural additive list" in Japan since 1989, was added to the "existing additive list" in Japan in 1995, and there is no specific restriction for cMDLf because it is considered a natural material.
5. The mean and 90th percentile EDIs by the total population, ages 1 year and older, of cMDLf from addition to proposed products are 142 mg/day and 273 mg/day (2.7 mg/kg/day and 5.8 mg/kg/day, respectively). The addition of cMDLf to the proposed products increases intake by three- to four-times background. (Non-milk products (chewing gum) containing cMDLF will be appropriately labeled as containing cow's milk proteins.)
6. The EDIs for cMDLf have been concluded to be safe and GRAS based on ADME studies, animal toxicology studies, studies evaluating physiologic effects, and clinical

000020

studies in adults, children, and infants. These published studies support the safety of intake of cMDLf at the proposed levels.

7. Digestion and metabolic fate of lactoferrin has been evaluated from studies of both human milk-derived and cow's milk-derived lactoferrin. Lactoferrin from both sources is handled similarly by the body. Lactoferrin that is absorbed from the gastrointestinal tract partitions into the lymph and then appears in the blood. Lactoferrin is rapidly removed from the systemic circulation by distribution into the spleen, liver, and kidneys while the iron portion is transferred to the liver for its transport into the bone marrow. Intact lactoferrin is detected in the feces and urine of infants.
8. While very few patients with cow's milk allergy (CMA) have antibodies to bovine lactoferrin, no evidence exists that cMDLf is a clinically relevant allergen.
9. It has been shown that infants fed with formula containing added cMDLf developed anti-cMDLf antibodies. Exclusively breast-fed infants develop anti-human lactoferrin antibodies, and autoimmune adults have antibodies that recognize both human and cMDLf. However, there is no evidence that cMDLf causes, promotes, exacerbates, or resolves autoimmunity in infants or adults.
10. The safety of cMDLf produced by Morinaga Milk Industry Co., Ltd. was evaluated in an acute toxicity study, a four-wk oral toxicity study, a thirteen-wk oral toxicity study, a chronic oral toxicity study and genotoxicity assays. cMDLf is not acutely toxic or genotoxic. cMDLf administered by oral intubation to rats for 13 wks did not result in toxicologically significant treatment-related changes. Thus, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) of cMDLf was estimated to be in excess of 2,000 mg/kg/day. The chronic toxicity study was not available as a full report and therefore was not used to derive a NOAEL.
11. Numerous published studies on the effects of oral ingestion of cMDLf in adults, children, and infants corroborate the GRAS status of cMDLf for its proposed uses. Of the 43 studies in humans, 17 have been conducted using cMDLf supplied by Morinaga Milk Industry. cMDLf is safe and well tolerated under the conditions of administration in the clinical studies at doses that range in adults and children from 100 mg/day to 3.6 g/day and with durations ranging from one wk to one yr. Studies carried out in both healthy and health-compromised adults and children reported no treatment-related side effects attributed to oral ingestion of cMDLf. Administration of cMDLf at 200 mg/day for up to 90 d in pregnant women was well tolerated (highest dose tested in this target

population). In term and preterm infants, exposure to cMDLf from infant formulas has been studied using concentrations ranging from 10 mg/100 mL (0.01%) to 285 mg/100 mL (0.285%), for durations ranging from two wks to one yr. Resulting intake of cMDLf is up to 150 mg/kg/day. No treatment-related adverse effects have been reported in infants.

Determination of the GRAS status of cMDLf supplied by Morinaga Milk Industry under the intended conditions of use has been made through the deliberations of Dr. Stephen Taylor, Dr. Lloyd Mayer, Dr. A. Wallace Hayes, and Dr. Roger Clemens. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of cMDLf and the potential human exposure to cMDLf resulting from its intended use in cow's milk-based products and chewing gum, and concluded:

There is no evidence in the available information on cMDLf that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when cMDLf is used at levels that might reasonably be expected from the proposed applications. cMDLf is GRAS for use in foods as proposed by Morinaga Milk Industry Co., Ltd.

Therefore, cMDLf is safe and GRAS at the proposed levels of addition to foods. cMDLf is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

E. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, Incorporated, 6430 Rockledge Drive, Westmoreland Bldg, Suite 503, Bethesda, Maryland, 20817, Telephone: 301-897-0611; Facsimile: 301-897-2567; Email: clairek@chromadex.com or be sent to FDA upon request.

000022

F. SIGNATURE

Pursuant to the criteria provided in proposed 21 CFR 170.36, Morinaga Milk Industry Co., Ltd. hereby notifies the Food and Drug Administration that the use of cMDLf in foods under the intended conditions of use is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Morinaga Milk Industry Co., Ltd. has determined that such use is Generally Recognized As Safe through scientific procedures.

(b) (6)



Signature
Claire L. Kruger, President, Spherix Consulting, Inc.
Authorized Representative of Morinaga Milk
Industry Co., Ltd.

February 22, 2013

Date

000023

II. DESCRIPTION OF SUBSTANCE

A. PHYSICAL AND CHEMICAL COMPOSITION

As cited in GRN 42, cow lactoferrin (cLf) is a 689 amino acid glycoprotein with a molecular weight of approximately 75 to 80-kDa (Mead and Tweedie, 1990; Pierce et al., 1991, and Table 1). The mature protein contains intramolecular disulfide bonds, is absent of free sulfhydryl groups, and folded into two symmetrical lobes (Spik et al., 1988; van Halbeek et al., 1981). Each lobe binds a metal atom in synergy with the carbonate ion (CO_3^{2-}) (Figure 1). Lactoferrin primarily binds Fe^{2+} or Fe^{3+} , but can also bind to Cu^{2+} , Zn^{2+} and Mn^{2+} (van der Strate et al., 2001). Because of its ability to bind Fe^{3+} reversibly, cLf can exist in iron (Fe^{3+})-free (apo-cLf) or iron-saturated (holo-cLf) states (Drago, 2006) and its three-dimensional conformation depends on whether or not it is bound to iron (Schanbacher et al., 1993). Apo-cLf has an open conformation, while holo-cLf has a closed conformation (Yeşim and Özgüneş, 2005). Holo-cLf is more resistant to proteolysis and thermal denaturation (Yeşim and Özgüneş, 2005; Paulsson et al., 1993).

Cow milk-derived lactoferrin contains four N-linked glycans, which are composed of different amounts of N-acetyllactosaminic acid, galactose, mannose, fucose, N-acetylglucosamine, N-acetylgalactosamine and N-acetylneuraminic acid residues (Coddeville et al., 1992; Spik et al., 1994). The isoelectric point (pI) of cMDLf is 8.2 to 8.9 by chromatofocusing (Shimazaki et al., 1993) and 9.5 to 10 by isoelectric focusing (Yoshida, 1991). Apo-cMDLf has an absorbance of 12.7 when measured at 280 nm and the absorbance of holo-cMDLf is 0.460 at 470 nm (Aisen and Leibman, 1972). cMDLf is also resistant to sequential heating at 70°C for 3 min and 130°C for 2 sec at low pH (Abe et al., 1991).

B. MANUFACTURING PROCESS

1. GENERAL DESCRIPTION OF THE PRODUCTION PROCESS

cMDLf is extracted either from sweet whey (cMDLf-1), a cheese production byproduct, or directly from skim milk (cMDLf-2). When sweet whey is the starting material, the entire production process is carried out at Milei GmbH located at Kemptener Strasse 91, 88299 Leutkirch, Germany. Milei GmbH has been certified for the development, production, and sales of products from whey and milk by the certifying body TUV SUD Management Service GmbH (Certificate registration number: 12 100/104 21695 TMS). When skim milk is used as the starting material, crude cMDLf is prepared at the Riedlingen plant of Allgäuland-Käsereien GmbH located at 88499 Riedlingen, Göffinger Straße 6, Germany. Crude cMDLf is then

000024

Table 1. Physical and Chemical Properties of Cow's Milk-Derived Lactoferrin		
Property	Values	Reference
Molecular mass (Da)		
<i>Sedimentation co-efficient</i>	77,100 ± 1,500	(Castellino et al., 1970)
<i>SDS-PAGE</i>	76,000 ± 2,400	(Castellino et al., 1970)
<i>Iron titration</i>	78,500	(Aisen and Leibman, 1972)
Isoelectric point (pH)		
<i>Chromatofocusing</i>	8.2-8.9	(Shimazaki et al., 1993)
<i>Isoelectric focusing</i>	9.5-10.0	(Yoshida, 1991)
Absorption spectra		(Aisen and Leibman, 1972)
<i>Apo-form at 280 nm</i>	12.7	
<i>Holo-form at 470 nm</i>	0.460	
Iron-binding		(Aisen and Leibman, 1972)
<i>Equilibrium dialysis ($K_1 \times 10^{-4}$)</i>	3.73	
Thermal denaturation		(Paulsson et al., 1993)
<i>Apo-Lf denaturation (T_{max}: °C)</i>	71 ± 0.3 and 90 ± 0.3	
<i>Apo-Lf enthalpy (ΔH_{cal}: J/g)</i>	12 ± 0.4 and 2 ± 0.5	
<i>Holo-Lf denaturation (T_{max}: °C)</i>	65 ± 0.3 and 93 ± 0.3	
<i>Holo-Lf enthalpy (ΔH_{cal}: J/g)</i>	2 ± 1.2 and 37 ± 1.3	

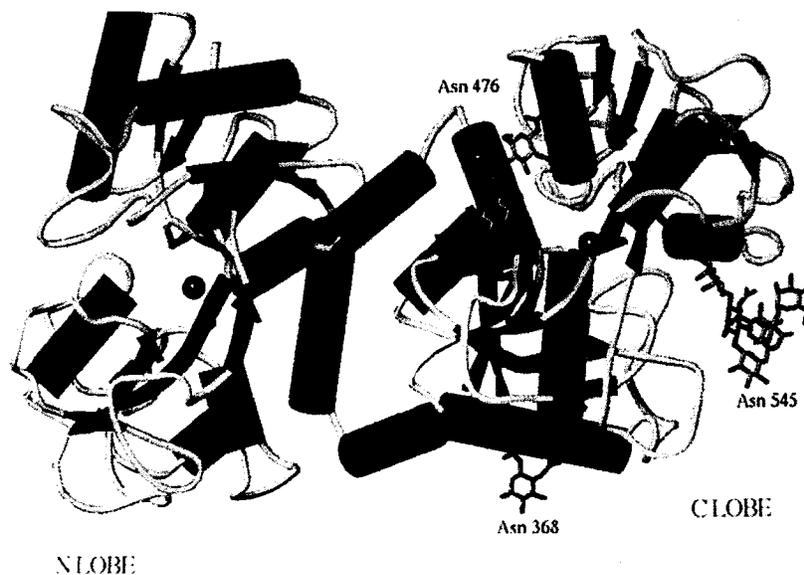


Figure 1: Schematic of diferric Cow Lactoferrin at a Resolution of 2.8 Angstroms
(Moore et al., 1997)

000025

transported to Milei GmbH for further processing. The Riedlingen plant of Allgäuland-Käsereien GmbH has been certified for processing, manufacturing, and sales of butter, cream cheese preparations, farmer's cheese (quark), yogurt, UHT milk, UHT cream and milk protein concentrate from dairy products as required by the International Food Standard, Version 5, August 2007 (Certificate registration number: F12683-2010-01) by the certifying body LACON GMBH on November 10, 2010.

The production process is summarized in Figure 2. Skim milk (unpasteurized) or sweet whey is cooled to below 8°C to prevent microbial growth (Step A), prefiltered with a 5 µM cloth (Step B1), and microfiltered with a 1 µM cloth (Step B2) to remove the insoluble material, contaminating microbes, fat, and fat-soluble drugs, organochlorine pesticides, and PCBs. The filtrate is bound to an ion exchange column containing CM Sephadex C-50 (currently used) or another food-grade resin such as SP Sepharose Big Beads (proposed) (Step C), rinsed thoroughly with demineralized water, and washed with a 1.6 % NaCl solution to remove contaminants weakly bound to the resin. cMDLf is then eluted with a 10 % NaCl solution and desalted by ultrafiltration using Tangential Flow Filtration (Steps D). Importantly, the ion exchange columns are designed to prevent carry-over of the resins into the cMDLf eluate and the ultrafiltration step removes the residual low molecular weight contaminants such as veterinary drugs and water-soluble pesticides eluted with cMDLf. The pH of the cMDLf ultrafiltrate is adjusted to approximately 5.8 and sterilized at 75 °C for 15 seconds, conditions that exceed the sterilization requirements for pasteurized milk described in the Grade "A" Pasteurized Milk Ordinance (Step E) (FDA, 2009). The sterilized cMDLf solution is then further concentrated with a second ultrafiltration step (Step F), freeze-dried (Step G), and pulverized to desired mesh size (Step H) before packaging (Step I). Critical quality control points are monitored routinely and procedures to correct out-of-specification issues are in place. Batches deemed out-of specification are removed from the production process and quarantined.

2. STARTING MATERIALS AND PROCESSING AIDS

The sweet whey and skim milk used as starting materials for the production of cMDLf conform to the European Union Food Hygienic Guidelines and The German Food Law ("Lebensmittel-und Bedarfsgegenstaendegesetz" - LMBG), which allow for the use of only US approved pesticides and veterinary drugs (<http://www.mrldatabase.com>). Product specifications for the sweet whey and skim milk starting materials are provided in Appendix 1. Furthermore, except for the demineralized water, all the remaining processing aids used in the production of cMDLF are food grade (Table 2).

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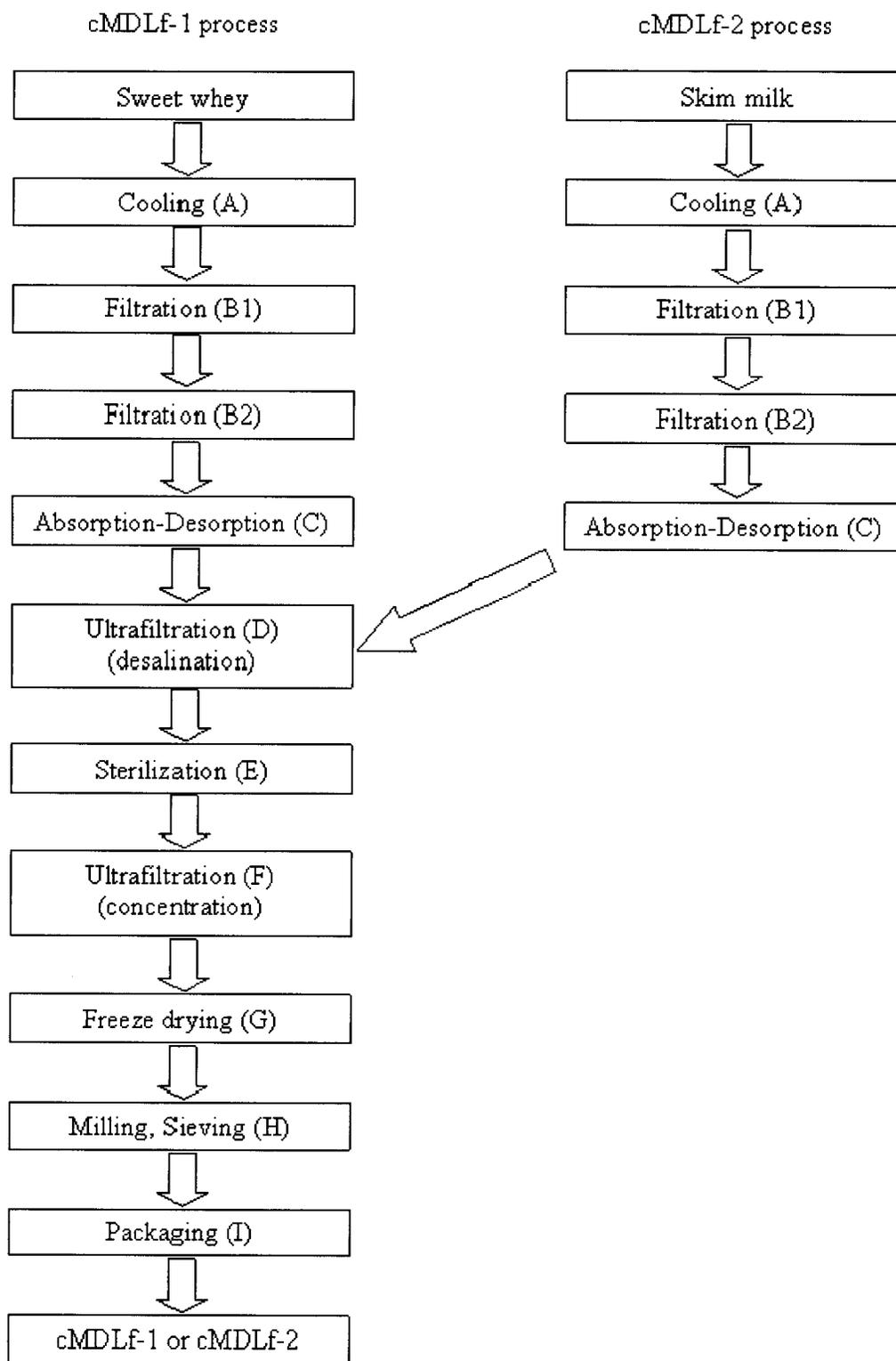


Figure 2: Production Process of Cow's Milk-Derived Lactoferrin (cMDLf)

Table 2. Processing Aids and Chemicals Used in the Production of Cow's Milk-Derived Lactoferrin (cMDLf)		
Process Aid or Chemical	Manufacturer	
	At Milei for cMDLf-1, cMDLf-2	At Riedlingen for cMDLf-2
Demineralized water	Milei	Riedlingen plant
Sodium chloride (NaCl)	Herkommer & Bangerter	Herkommer & Bangerter
Hydrochloric Acid (HCl)	Herkommer & Bangerter	Not used
CM Sephadex C-50 or SP Sepharose Big Beads	GE Healthcare	GE Healthcare
Filter cloth (1um)	Wolftechnik Filtersysteme	Wolftechnik Filtersysteme
Filter cloth (5um)	Wolftechnik Filtersysteme	Not used
GR61PP Membrane	Alfa Laval	Not used

C. FINISHED PRODUCT SPECIFICATIONS

1. PRODUCT SPECIFICATIONS AND BATCH RECORDS

The manufacturing specifications for cMDLf are listed in Table 3. The product consists of 94.5% protein, 4.2% moisture and 1.3 % ash, and lactoferrin accounts for 96% of the protein fraction. The pH of the finished product ranges between 5.5 and 7.2 with a water activity (a_w) of less than 0.2. Complete solubility is achieved when 2 g of lactoferrin are added to 100 ml of 20°C water. The bound iron content is less than 35 mg/100 g. Because cMDLf has two iron-binding sites, the iron binding capacity of the final product is $\geq 75\%$ ¹. Batch analyses of three non-consecutive lots of cMDLf from each source [sweet whey (cMDLf-1) and skim milk (cMDLf-2)] are provided in Table 4 and 5, and confirm that the production processes is capable of generating highly purified cMDLF. In addition, although Morinaga no longer manufactures cMDLF-2 and is unable to provide batch data for the radioactivity product specification, if Morinaga manufactures cMDLF-2 in the future, all batches will conform to the radioactivity product specification described in Table 3.

The amount of lactoferrin in cMDLF-1 and cMDLF-2 is determined by the Kjeldahl method ($N \times 6.38$) and HPLC, and verified by absorption at 280 nM (Appendix 2). Analytical methods for the other specifications include the AOAC official method 905.02 for atomic absorption spectrometry for iron content, ICP-MS for heavy metals, microbial analyses for total aerobic count, coliform bacteria, yeast and mold, coagulase positive *staphylococci* and *salmonella*, for *Bacillus cereus*, and for *Enterobacter sakazakii*, GC-MS for PCBs, GC-ECD for pesticides, HPLC-MS and microbial quantification for antibiotics, HPLC with a fluorescence detector for aflatoxin M1 and Germanium semiconductor detector for radioactivity. Parasiticides are also screened using an HPLC with a fluorescence detector.

¹ Theoretically, each lactoferrin can bind two irons with a maximum iron binding of 2x56 g iron/80,000 g lactoferrin = 140 mg/100g. Therefore, the calculated binding capacity of cMDLf product is $\geq 1-35/140 = 75\%$.

000028

Table 3. Manufacturing Specifications for Cow's Milk-Derived Lactoferrin (cMDLf)

Specifications	Limits	Method
Appearance	Pink and odorless powder	Visual check
Foreign matter	Absent	Visual checking in 10 g
pH (2% solution)	5.2 – 7.2	pH meter
Protein content (%)	≥ 94.5	Kjeldahl method (n x 6.38)
cMDLf Purity (%/protein)	≥ 96.0	HPLC-UV/VIS
Fat (% dry weight)	≤ 0.5	AOAC 905.02
Residue on ignition (%)	≤ 1.3	500-550°C for 1-2 d
Loss on drying (%)	≤ 4.2	Heat to 105°C, 5 hours
Water Activity (a _w) ^g	≤ 0.2	Water activity test device
Iron content (mg/100 g)	≤ 35	Atomic absorption spectrometry
Iron saturation (%)	≤ 25 ^a	Calculated iron content
Heavy Metals		
Lead (pb) ppm	≤ 1	ICP-MS ^b
Cadmium (Cd) ppm	≤ 0.05	ICP-MS ^b
Mercury (Hg) ppm	≤ 0.05	ICP-MS ^b
Arsenic (as As ₂ O ₃) ppm	≤ 1	Fluorescent X ray analysis
Solubility		
In water (2% at 20°C) (%)	= 100	Visual check
Transmittance (2% solution, 600nm) (%)	≥ 80	Spectrophotometer
Microbiological tests		
Total aerobic count	≤ 1,000 cfu/g	Standard Agar
Coliform bacteria	Negative/0.1 g	Desoxycholate Agar
Coagulase positive staphylococci	Negative/g	Mannitol salt agar with egg yolk
Yeast and Mold	≤ 10 cfu/g	Potato dextrose agar
Salmonella	Negative/25 g	DHL agar
Bacillus cereus ^g	Negative/20 mg	NGKG agar plate
Enterobacter sakazakii ^g	Negative/333 g	Violet red bile dexystrose agar
Listeria monocytogenes	Negative/25 g	Palukam agar
Clostridium perfringens	Negative/30 g	GAM Semisolid medium and CW agar with kanamycin
Yersinia enterocolytica	Negative/25 g	CIN Agar

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Table 3. Manufacturing Specifications for Cow's Milk-Derived Lactoferrin (cMDLf)		
Specifications	Limits	Method
PCBs (mg/kg) ^g	<0.1	GC-MS ^c
Pesticides (mg/kg) ^g	<0.1	GC-ECD ^d
Antibiotics ^g		
<i>Dihydrostreptomycin, streptomycin</i>	Not detected ^e	Microbial quantification method
<i>Cefazolin, ampicillin, chloramphenicol</i>	Not detected ^e	HPLC-MS
Aflatoxin M1 (µg/kg) ^g	<0.5	HPLC with a fluorescence detector
Radioactivity (¹³⁴ Cs + ¹³⁷ Cs) (Bq/kg) ^g	< 5	Germanium semiconductor detector ^f
Notes:		
<p>a. Theoretically, each lactoferrin can bind two irons with a maximum iron binding of 2x56 g iron/80,000 g lactoferrin = 140 mg/100 g. Therefore, the calculated binding capacity of cMDLf product is $\geq 1-35/140 = 75\%$.</p> <p>b. According to the Method of Analysis in Health Science (2010).</p> <p>c. PCBs include PCB #28, #52, #101, #118, #153, #138, #180. The detection limit is 0.01 ng/g.</p> <p>d. Pesticides include BHC, DDT, aldrin, dieldrin, endrin, heptachlor and hexachlorobenzene. The detection limit for BHC, DDT, heptachlor and hexachlorobenzene is 0.01 ppm. The detection limit for aldrin, dieldrin and endrin is 0.005 ppm.</p> <p>e. The detection limit for dihydrostreptomycin and streptomycin is 0.02 ppm. The detection limits for cefazolin, ampicillin and chloramphenicol are 0.01 ppm, 0.005 ppm and 0.0005 ppm, respectively.</p> <p>f. The detection limits for ¹³⁴Cs and ¹³⁷Cs are 2.3 Bq/kg and 2.3 Bq/kg (1,500 sec) respectively.</p> <p>g. Analyzed quarterly; meets the specifications set in GRN 67, 77, and 130.</p>		

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Table 4. Batch Analyses of Cow's Milk-Derived Lactoferrin (cMDLf)

Item	Specification Limits	Lot # (cMDLf-1 from Sweet Whey)			Lot # (cMDLf-2 from Skim Milk)		
		131110	151110	171110	101110	161210	211210
Appearance	Pink and odorless powder	Conform	Conform	Conform	Conform	Conform	Conform
Foreign matter	Absent	Conform	Conform	Conform	Conform	Conform	Conform
pH (2% solution)	5.2 - 7.2	5.53	5.58	5.75	5.50	5.59	5.20
Protein content (% dry weight)	≥ 94.5	98.8	99.4	99.3	99.2	98.8	98.7
cMDLf Purity (per protein)	≥96.0	97.3	96.8	96.8	97.0	97.7	97.2
Fat (% dry weight)	≤ 0.5	<0.5 ^a	<0.5 ^a	<0.5 ^a	<0.5 ^a	<0.5 ^a	<0.5 ^a
Residue on ignition (% dry weight)	≤1.3 %	0.13	0.17	0.11	0.07	0.09	0.05
Loss on drying (%)	≤4.2%	0.41	0.05	0.35	0.54	0.70	0.43
Water Activity (a _w)	≤0.2	0.03	0.03	0.03	0.02	0.03	0.03
Iron content (mg/100 g)	≤ 35 mg/100 g	21.1	21.7	19.7	8.20	9.59	9.51
Iron saturation (%)	≤ 25	15.1	15.5	14.1	5.9	6.9	6.8
Minerals							
Sodium (Na) (mg/100 g)		38.0	39.9	42.0	33.5	47.1	48.2
Potassium (K) (mg/100 g)		1.00	5.36	5.30	1.09	1.11	2.70
Magnesium (Mg) (mg/100 g)		0.49	0.51	0.55	0.56	0.55	0.53
Phosphorus (P) (mg/100 g)		3.25	3.87	3.99	2.95	3.14	3.57
Calcium (Ca) (mg/100 g)		8.14	8.87	9.15	7.81	7.93	7.69
Chlorine (Cl) (mg/100 g)		766	816	758	795	764	889
Copper (Cu) (mg/100 g)		0.28	0.09	0.31	ND ^b	ND ^b	ND ^b
Zinc (Zn) (mg/100 g)		0.15	0.64	0.32	0.32	0.29	0.25
Manganese (Mn) (mg/100 g)		ND ^b	ND ^b	ND ^b	0.01	0.01	0.01
Heavy Metals							
Lead (Pb) ppm	≤ 1	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
Cadmium (Cd) ppm	≤ 0.05	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
Mercury (Hg) ppm	≤ 0.05	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
Arsenic (as As ₂ O ₃) ppm	≤ 1	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c	ND ^c
Solubility							
In water (2% at 20°C) (%)	100	100	100	100	100	100	100
Transmittance (2% solution, 600 nm) (%)	≥80%	91.9	92.0	90.6	81.4	95.2	85.7

000031

Table 4. Batch Analyses of Cow's Milk-Derived Lactoferrin (cMDLf)

Item	Specification Limits	Lot # (cMDLf-1 from Sweet Whey)			Lot # (cMDLf-2 from Skim Milk)		
		131110	151110	171110	101110	161210	211210
Microbiological tests							
<i>Total aerobic count</i>	≤ 1,000 cfu/g	0	0	0	0	0	0
<i>Coliform bacteria</i>	Negative/0.1 g	Negative	Negative	Negative	Negative	Negative	Negative
<i>Coagulase positive staphylococci</i>	Negative/g	Negative	Negative	Negative	Negative	Negative	Negative
<i>Yeast and Mold</i>	≤ 10 cfu/g	0	0	0	0	0	0
<i>Salmonella</i>	Negative/25 g	Negative	Negative	Negative	Negative	Negative	Negative
<i>Bacillus cereus</i>	Negative/20 mg	Negative	Negative	Negative	Negative	Negative	Negative
<i>Enterobacter sakazakii</i>	Negative/333 g	Negative	Negative	Negative	Negative	Negative	Negative
<i>Listeria monocytogenes</i>	Negative/25 g	Negative	Negative	Negative	Negative	Negative	Negative
<i>Clostridium perfringens</i>	Negative/30 g	Negative	Negative	Negative	Negative	Negative	Negative
<i>Yersinia enterocolytica</i>	Negative/25 g	Negative	Negative	Negative	Negative	Negative	Negative
PCBs	< 0.1 mg/kg	ND ^d	NT	NT	ND ^d	NT	NT
Pesticides	< 0.1 mg/kg	ND ^e	NT	NT	ND ^e	NT	NT
Antibiotics							
<i>Dihydrostreptomycin, streptomycin</i>	Not detected	ND ^f	NT	NT	ND ^f	NT	NT
<i>Cefazolin, ampicillin, chloramphenicol</i>	Not detected	ND ^g	NT	NT	ND ^g	NT	NT
Aflatoxin M1	< 0.5 µg/kg	ND ^h	NT	NT	ND ^h	NT	NT

Notes:

- Detection limit for fat content is 0.5%.
- Detection limits are 0.05 mg/100 g for copper and 0.003 mg/100 g for manganese.
- Detection limits are 0.1 µg/g for Pb, 0.01 µg/g for Cd, 0.01 µg/g for Hg and 1 µg/g for As₂O₃.
- PCBs tested include PCB #28, #52, #101, #118, #153, #138, #180. The detection limit for each PCB is 0.01 ng/g.
- Pesticides tested include BHC, DDT, aldrin, dieldrin, endrin, heptachlor and hexachlorobenzene. The detection limit is 0.01 ppm for BHC, DDT, heptachlor and hexachlorobenzene and is 0.005 ppm for aldrin, dieldrin and endrin.
- Detection limit is 0.02 ppm.
- Detection limit is 0.01 ppm for cefazolin, 0.005 ppm for ampicillin, 0.0005 ppm for chloramphenicol.
- Detection limit is 0.5 ppb.

ND stands for "not detected", NT stands for "not tested" as these parameters are monitored periodically only.

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Table 5. Batch Analyses of Radioactivity in Cow's Milk-Derived Lactoferrin (cMDLf-1)

Item	Specification Limits	Lot # (cMDLf-1 from Sweet Whey)			
		120227068	120312068	120512068	120514068
Radioactivity (¹³⁴ Cs + ¹³⁷ Cs)	<5 Bq/kg ^a	ND ^b	ND	ND	ND
Notes:					
a. Detection limits are 2.3 Bq/kg for ¹³⁴ Cs and 2.3 Bq/kg for ¹³⁷ Cs.					
b. ND stands for "not detected".					

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2. PARASITICIDES

cMDLf was analyzed for the presence of parasiticides including abamectin, doramectin, moxidectin, eprinomectin (as eprinomectin B_{1a}) and ivermectin (as 22, 23-dihydroavermectin B_{1a}) because they are potential contaminants of raw milk. None of these parasites were detected (Table 6).

Table 6. Batch Analysis of Parasiticides in Cow's Milk-Derived Lactoferrin (cMDLf)		
Parasiticide	Lot # 131110 (cMDLf-1)	Lot # 101110 (cMDLf-2)
Abamectin	Not detected	Not detected
Doramectin	Not detected	Not detected
Moxidectin	Not detected	Not detected
Eprinomectin (as Eprinomectin B _{1a})	Not detected	Not detected
Ivermectin (as 22, 23-dihydroavermectin B _{1a})	Not detected	Not detected
cMDLf-1 is derived from sweet whey and cMDLf-2 is derived from skim milk. Parasiticides are analyzed by HPLC. The detection limits are 0.005 ppm		

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3. OTHER WHEY PROTEINS

The levels of bovine serum albumin, IgG, β -lactoglobulin, lactoperoxidase, and angiogenin in cMDLf-1 and cMDLf-2 were determined by HPLC. The amount of bovine serum albumin, IgG, β -lactoglobulin, and lactoperoxidase were negligible in both products. Angiogenin was found at low levels (1-2% peak area) in both products, but the ratio of angiogenin to lactoferrin in cMDLf-1 and cMDLf-2 is similar to that found in cow's milk. Thus, it is reasonable to assume that other milk proteins are not selectively concentrated during the production process. Casein, the main protein in milk, was not seen in either lactoferrin product.

D. STABILITY OF CMDLF

cMDLf products were packaged in polyethylene or aluminum bags, the standard materials for the storage of cMDLf. Appearance, flavor, solubility, moisture content, pH of a 2% solution, lactoferrin stability, microbial contamination and growth were monitored periodically (Appendix 3). cMDLf stability was quantified by HPLC (purified product) and by an agglutination immunoassay and/or enzyme-linked immunosorbent assay (in food matrices) (Yamauchi et al., 2004). When incubated at room temperature, cMDLf was stable for up to 96 mo when packaged in polyethylene bags and stable for up to 102 mo when packaged in aluminum bags. cMDLf was stable for up to 3 yr in powdered infant formulas and up to 46 mo in skim milk powder. In yogurt products maintained at less than 10°C, cMDLf was stable for up to 17 d. For evaluating the stability of cMDLf in sterile liquid formulations, a solution containing cMDLf was adjusted to pH 4, heated at 140 to 155°C for 2 to 5 seconds, and then added to the remaining sterilized formulation. Seventy percent of the added cMDLF was detectable after 34 months of storage at 25°C and 63% was detectable after 48 months of storage at 25°C.

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III. INTENDED EFFECT

The intended effect is to increase the intake of cMDLf from consumption of cow's milk-based products and chewing gum.

In the United States, cMDLf has been determined safe and GRAS for use as an ingredient in sport and functional foods at concentrations of 100 mg/serving (GRN 77). cMDLf has also been determined safe and GRAS for use as a component of an antimicrobial spray for application to uncooked beef (GRN 67 and GRN 130). In Europe, cMDLf has been determined safe and subsequently approved for use in foods at levels ranging from 60 to 100 mg/serving and infant formulas at levels ranging from 40 to 1000 mg/L (EFSA Panel on Dietetic Products, 2012a; EFSA Panel on Dietetic Products, 2012b).

Lactoferrin is a member of the transferrin family of iron-binding glycoproteins and is found in mucosal secretions, such as milk and saliva, and the secondary granules of neutrophils (Baggiolini, 1972; Masson and Heremans, 1971; Masson et al., 1966). Lactoferrin from cows (*Bos taurus*) is 69% homologous to human lactoferrin, and although cMDLf contains many of the same structural features as human lactoferrin, it contains approximately four times more iron than human lactoferrin (Crichton, 1990; Pierce et al., 1991; Baker et al., 1994; Wang et al., 1984). The lactoferrin level in human milk is approximately 2 mg/ml milk (GRN 235; Lien et al., 2004; Prentice, 1995). Cow's milk contains approximately 0.1 mg lactoferrin/ml milk (GRN 77).

As a natural defense protein, lactoferrin is known for its ability to inhibit the growth of certain strains of pathogenic bacteria by scavenging iron (reviewed in Adlerova et al., 2008). Some cell culture experiments indicate an inhibitory effect of cMDLf on osteoclasts (Cornish et al., 2004; Blais et al., 2009; Yamano et al., 2010), plus multiple stimulatory effects of cMDLf on osteoblasts (Cornish et al., 2004; Cornish et al., 2006; Takayama and Mizumachi, 2008; Blais et al., 2009). Other studies suggest that oral ingestion of cMDLf may be beneficial for maintaining iron homeostasis in infants and women (Chierici et al., 1992; Koikawa et al., 2008; Nappi et al., 2009; Paesano et al., 2006; Paesano et al., 2009; Paesano et al., 2010).

000036

IV. HISTORY OF USE, INTENDED USE, AND ESTIMATED DAILY INTAKE

A. HISTORY OF USE

Lactoferrin is a naturally occurring protein present in mucosal secretions, ie. milk and saliva, and the secondary granules of neutrophils. During infection and inflammation, lactoferrin is released from neutrophils and thus, is found in the plasma.

1. EXPOSURE TO HUMAN LACTOFERRIN

Human lactoferrin is encoded by one gene, located on chromosome 3 (Taylor et al., 2004). It is generally accepted that human milk-derived and neutrophil-derived lactoferrin differ only in the composition of their carbohydrate side chains; human milk-derived lactoferrin (hMDLf) contains fucose residues whereas neutrophil-derived lactoferrin does not (Teng, 2002; Derisbourg et al., 1990; Taylor et al., 2004). Functionally, both types of lactoferrin appear to be equivalent (Wu et al., 1995; Broxmeyer et al., 1986) and no reports have investigated how the carbohydrate side chains and/or their composition contribute to lactoferrin immunogenicity. Infants and adults can be exposed to endogenous/neutrophil-derived lactoferrin, but the first exposure to exogenous lactoferrin is through consumption of breast milk and/or cow's milk-based infant formula. Although the amount of lactoferrin in human milk varies from country to country (Figure 3), the mean levels of lactoferrin are approximately 5 mg/ml in colostrum, 2.5 mg/ml in mature milk (day 15 to 84 postpartum), and 1 mg/ml in mature milk up to two yr postpartum (GRN 235; Lien et al., 2009, Prentice, 1995).

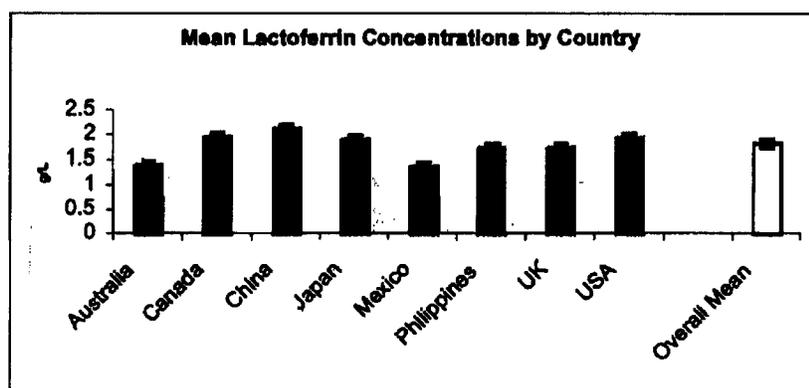


Figure 3: Mean lactoferrin concentrations by country (Lien et al., 2009)
Mature milk was collected from women who had delivered full-term healthy infants and analyzed by HPLC.

2. EXPOSURE TO cMDLf

Milk, by definition is the lacteal secretion practically free of colostrum obtained by the milking of one or more healthy cows (21CFR131.110). cMDLf is component of cow's milk and therefore humans have been exposed to cMDLf for centuries. Whole cow's milk is composed of approximately 86.6% water, 4.1% fat, 3.6% protein, 5.0% lactose, and 0.7% ash (Swaisgood, 1985). Because the concentration of cMDLf in cow's milk is approximately 0.1 mg/ml (Table 7) (Barth and Behnke, 1997), one serving of whole milk (240 ml) contains approximately 24 mg of lactoferrin.

Protein components		Absolute concentration	Relative concentration to total milk protein
Whey proteins	Lactoferrin	0.1 mg/ml milk	0.3%
	Others	7.0 mg/ml milk	20.5%
Caseins		27.0 mg/ml milk	79.2%
Total milk protein		34.1 mg/ml milk	100%

Note: Adapted from (Barth and Behnke, 1997)

a) From currently marketed infant formulas and cMDLf-supplemented formulas

Cow's milk-based infant formulas typically contain whey proteins or cow's milk. Thus, infants and a small percentage of toddlers consuming cow's milk-based infant formulas are exposed to small amounts of cMDLf. In 1986 Morinaga Milk Industry Co., Ltd introduced infant formulas with additional cMDLf into the Japanese market under the product name "BF-L". BF-L was intended to be used 0- to 9-mo-old and contained 50 mg of cMDLf/100 g powdered milk. The cMDLf level was then increased to 80 mg/100 g powdered milk to be consistent with the levels used in competitors' products and the new product was named "Hagukumi". Three yr later, Morinaga launched "Chil-Mil", a follow-up infant formula for toddlers of 9 mo to 3 yr that contains cMDLf at 45 mg/100 g powdered milk. Morinaga has subsequently introduced and sold cMDLf-supplemented infant formulas and follow-up formulas in Taiwan (2000), Pakistan (2001), mainland China (2004), and Indonesia (2004). Infant formulas containing cMDLf are also sold in other companies in Japan, Korea, and China.

Both cMDLf-supplemented infant formulas sold in Japan by Morinaga Milk Industry Co., Ltd. have been certified by the Japanese Ministry of Health as Special Nutritious Foods according to the Nutrition Improvement Law (Appendix 4). The specification monographs for these two products are found in Appendix 5A. Morinaga's cMDLf has been listed on the "natural

additive list” in Japan since 1989 and was added to the “existing additive list” in Japan in 1995 (<http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/pages/list-exst.add>) (Appendix 5B). In Japan, there is no specific restriction for cMDLf because it is considered a natural material. Regulatory approval documents for the use of cMDLf in infant formulas and follow-up formulas in China (since 2004) and for the use of cMDLf as a food additive in Taiwan (since 2000) and in Korea are provided in Appendices 5C, D and E, respectively.

Since the release of cMDLf-containing formulas, Morinaga has sold approximately 3,200 metric tons and 3,500 metric tons annually for the 0- to 9-month formulation and 9-month to 3-yr formulation in Japan, respectively. With consumption of Morinaga's cMDLf-supplemented formulas by over a million infants and toddlers in Japan since 1986, there have been no reported significant health problems, including allergenic reactions attributable to cMDLf, associated with either of these two products containing cMDLf based on post marketing surveillance by Morinaga.

Although current sales volumes are lower in Pakistan (700 and 500 metric tons/yr for 0- to 6-mo and 6-mo to 3-yr formulas, respectively), Indonesia (sales volume not available), and China (sales volume not available), no health problems, including allergenic reactions, have been reported based on post marketing surveillance by Morinaga. Information on sales volume and health status for Korea are not available.

b) cMDLF exposure from food

To understand the historical exposure of toddlers, children, teens, and adults to cMDLf, estimated daily intakes (EDIs) of cMDLf from the consumption of currently available foods was calculated using consumption data obtained from the National Center for Health Statistics' 2007-2008 National Health and Nutrition Examination Surveys (NHANES) database, and the food codes and assumed cMDLf concentrations in food presented in Table 8 (CDC, 2006; USDA, 2010; Bodner-Montville et al., 2006). Mean and percentile intake estimates represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2007-2008 data, and

000039

Table 8. Foods Codes and Cow's Milk-Derived Lactoferrin (cMDLf) Concentrations in Foods Used for Estimating Background Exposure

Food code ^a	Food description	Average cMDLf level (mg/g) ^b
111xxxxx	Milk, fluid (regular; filled; buttermilk; and dry reconstituted)	0.1
112xxxxx	Milk, fluid, evaporated and condensed	0.21
114xxxxx	Yogurt	0.1
115xxxxx	Flavored milk and milk drinks, fluid	0.1
116xxxxx	Milk-based meal replacements, fluid	0.05
118xxxxx	Milk, dry, and powdered mixtures with dry milk, not reconstituted	0.75
13xxxxxx	Milk desserts, sauces, gravies	0.05
140xxxxx	Cheese, not specified as to type	0.75
141xxxxx	Natural cheeses	0.75
142xxxxx	Cottage cheeses	0.75
143xxxxx	Cream cheeses	0.75
144xxxxx	Processed cheeses and cheese spreads	0.75
146xxxxx	Cheese mixtures	0.75
147xxxxx	Cheese soups	0.075

- a. Food codes obtained from NHANES database. "x" denotes that the food category contains multiple product listings.
- b. Estimates reflect consumption of milk and milk products, including all fluid and flavored milks (excluding imitation milks), powdered, concentrated and reconstituted milks, yogurt, milk-based meal replacements, milk deserts/sauces/deserts, and cheeses. cMDLf concentrations in these products used for calculating EDI are listed below.
- 0.1 mg cMDLf/g of fluid milks including regular, filled, buttermilk and dry reconstituted (Barth and Behnke, 1997).
 - 0.21 mg cMDLf/g of fluid milk (evaporated and concentrated). The average composition of milk consists of 86.6% water, 4.1% fat, 3.6% protein, 5.0% lactose, and 0.7% ash (Swaisgood, 1985). Approximately 60% water in milk is removed when milk is concentrated giving a cMDLf level of $0.1 \text{ mg} / (0.134 \text{ g solid} + 0.866 \text{ g} \times 40\% \text{ water}) = 0.21 \text{ mg cMDLf/g}$ of concentrated milk.
 - 0.1 mg cMDLf/g of yogurt.
 - 0.1 mg cMDLf/g of flavored milk and milk drinks (fluid).
 - 0.05 mg cMDLf/g of milk-based meal replacement (fluid) assuming they contain 50% milk.
 - 0.75 mg cMDLf/g of dry milk powder (not reconstituted) because $0.1 \text{ mg} / 0.134 \text{ g dry matter in milk} = 0.75 \text{ mg cMDLf/g dry milk powder}$.
 - 0.05 mg cMDLf/g milk deserts, sauces, gravies, assuming they contain 50% milk.
 - 0.75 mg/g cheese (excluding imitation cheeses). In addition to water, cheeses contain proteins and fats from milk. Some cheeses contain reduced fat. Cheese products contain approximately 25% protein (USDA National Nutrient Database for Standard Reference <http://ndb.nal.usda.gov/>). Milk protein contains 0.3% cMDLf (Barth and Behnke, 1997), giving cMDLf concentration in cheese of $0.3\% \text{ cMDLf} \times 25\% = 0.75 \text{ mg/g cheese}$. 0.075 mg cMDLf/g cheese soup assuming cheese soups contain 10% cheese.

000040

were generated incorporating sample weights to provide representative intakes for the entire U.S. population². All-person intake refers to the estimated intake of cMDLf averaged over all individuals surveyed, regardless of whether they consumed food products containing cMDLf, and therefore includes “zero” consumers (those who reported no intake of food products containing cMDLf during the 2 survey days). All-user intake refers to the estimated intake of cMDLf by those individuals consuming food products containing cMDLf on day 1 or 2 or both. Individuals were considered users if they consumed 1 or more food products containing cMDLf on either Day 1 or Day 2 of the survey.

In general, toddlers were the highest consumers with mean and 90th percentile EDIs of 59 and 105 mg cMDLf/d, respectively. Adults were the lowest consumers with mean and 90th percentile EDIs of 36 and 74 mg cMDLf/d, respectively (Table 9). For the entire population, the mean and 90th percentile EDIs were 39 and 80 mg cMDLf/d, respectively.

Table 9. All-User Estimated Daily Intake (EDI) of Cow's Milk-Derived Lactoferrin (cMDLf) from Background Exposure to Cow's Milk and Cow's Milk-Based Products (U.S Population Groups; 2007-2008 NHANES Data)

Population	N _u /N _p ^{a, b}	Percent users ^c	Absolute EDI (mg cMDLf/d)		Weight-based EDI (mg cMDLf/kg bw/d)	
			Mean	90 th Percentile	Mean	90 th Percentile
Toddlers, 12-35 mo	437/446	97.6	59	105	4.8	8.6
Children, 3-11 yr	1310/1335	97.7	45	79	1.8	3.5
Teen, 12-19 yr	863/963	90.4	44	96	0.7	1.5
Adult, 20+ yr	4045/4628	88.7	36	74	0.5	1.0
Total population	6655/7372	90.2	39	80	0.8	1.9

- a. Individuals were considered users if they consumed one or more food products containing cMDLf on either Day 1 or Day 2 of the survey.
- b. Number of users over the number of people surveyed.
- c. Percent of user when sample weights were considered.

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² A sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, non-response adjustment, and adjustment to independent population controls.

c) cMDLf exposure from the consumption of dietary supplements

Lactoferrin is also marketed as a dietary supplement. Example products and the manufacturers' recommended daily intakes are presented in Table 10. These products are not included in the NHANES database and, therefore, not included in estimations of cumulative daily intake.

Table 10. Concentration and Recommended Daily Intake of Cow's Milk-Derived Lactoferrin (cMDLf) in Dietary Supplements				
Product	Manufacturer Country	cMDLf Concentration	Label Instructions	Manufacturer's Recommended Daily Intake
Lactoferrin	Jarrow Formulas, Canada	250 mg/capsule	1 capsule/d	250 mg
Immune Ultra	AOR, Canada	4820 mg/rounded scoop	1 or 2 scoops/d	4820-9640 mg
Laktoferrin with Colostrum	Allergy Research Group, USA	100 mg/capsule	1-4 capsules/d	100-400 mg
NutriCology - Laktoferrin	Allergy Research Group	1050 mg/3 capsules	3 capsules/d	1050 mg
High Potency Lactoferrin	Swanson Health Products, USA	100 mg/capsule	1-3 capsules/d	100-300 mg
Lactoferrin	Symbiotics, USA	250 mg/capsule	1-2 capsules/d	250-500 mg
Lactoferrin With Colostrum Plus	Symbiotics, USA	29 mg/2 capsules	4 capsules/d	58 mg
Lactoferrin Caps	Life Extension, USA	300 mg/capsule	1 capsule/d	300 mg
Laktoferrin with Colostrum	Nutricology, USA	100 mg/capsule	1-4 capsules/d	100-400 mg
Immunity Boost, Natural Tangerine Flavor	Beveri, USA	30 mg/stick	1 stick/d	30 mg
Lactoferrin	Immunecare, UK	250 mg/capsule	1-3 capsules/d	250-750 mg

Note: Data retrieved from the World Wide Web on May 23, 2011

000042

B. INTENDED USE

Morinaga Milk Industry Co., Ltd. intends to use cMDLf as an ingredient in selected foods including powdered milks, yogurts, ice creams and sherbets, and chewing gums. The product categories to which cMDLf will be added and the corresponding maximum use-levels are summarized in Table 11.

Table 11. Intended Uses of Cow's Milk-Derived Lactoferrin (cMDLf) and Its Maximum Use Levels		
Food group^a	Foods with cMDLf added	Maximum use level
Yogurt	All yogurt products including yogurt drinks and those as baby foods.	100 mg cMDLf/100 g
	Non-fermented milk fortified with probiotics in addition to lactoferrin. ^b	
Powdered milk	All powdered, not reconstituted, mixtures with dry milk	400 mg cMDLf/100 g
Milk dessert	Ice creams and sherbets	200 mg cMDLf/100 g
Sugars and sweets	Chewing gums	30 g cMDLf/g
a. Foods are grouped based on the US Food and Nutrient Database for Dietary Studies (FNDDS). b. Non-fermented milk fortified with probiotics in addition to cMDLf is new to US consumers. Yogurt drinks were used as surrogate products for the intake estimate for cMDLf.		

C. ESTIMATED DAILY INTAKE OF cMDLf FROM PROPOSED USES

To determine the EDIs of cMDLf by toddlers, children, teens, and adults from the proposed uses of cMDLf in cow's milk-based products and chewing gums, mean and 90th percentile all-user intakes were calculated using the proposed use levels (Table 11), the NHANES database, and the food codes presented in Table 12. Because non-fermented milk fortified with probiotics and containing cMDLf are not currently sold in the United States, yogurt drinks were used as surrogate products for estimating the intake of cMDLf from the consumption of probiotic fortified unfermented milk.

Table 12. Food Codes and Cow's Milk-Derived Lactoferrin (cMDLf) Levels in Proposed Foods Used for Calculating Supplemental Intakes		
Food code^a	Food description	Maximum cMDLf level^b (mg/g)
114xxxxx	Yogurt	1
118xxxxx	Milk, dry, and powdered mixtures with dry milk, not reconstituted	4
13110000 to 13140900 13150000 to 13160420 13161630	Ice cream and sherbet	2
53112000	Cake, ice cream and cake roll, chocolate	0.2
53112100	Cake, ice cream and cake roll, not chocolate	0.2
53222020	Cookie, cone shell, ice cream type, wafer or cake	0.2
53222100	Cookie, cone shell, ice cream type, brown sugar	0.2
53430300	Crepe, dessert type, ice cream-filled	0.2
91611050	Ice pop filled with ice cream, all flavor varieties	0.2
92510730	Fruit punch, made with soda, fruit juice, and sherbet or ice cream	0.2
918xxxxx	Chewing gum	30
a) Food codes obtained from NHANES database. "x" denotes that the food category contains multiple product listings. b) Presented in Table 7.		

Approximately 39 % of the total U.S. population was identified as consumers of the additional cMDLf from the proposed uses (Table 13). On an individual population basis, the highest percent users were children (54.5%). The greatest absolute intake of supplemental cMDLf was found in teenagers (12- to 19-yr-old), with EDIs of 164 mg/day on average and 336 mg/day for heavy consumers (90th percentile) (Table 13). Consumption of foods supplemented with cMDLf by the total U.S. consumers resulted in an estimated mean intake of supplemental cMDLf of 142 mg/person/day or 2.7 mg/kg bw/day. The 90th percentile intake of supplemental cMDLf by all users was estimated to be 273 mg/person/day or 5.7 mg/kg bw/day (Table 13).

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Table 13. All-User Estimated Daily Intake (EDI) of Cow's Milk-Derived Lactoferrin (cMDLf) from the Proposed cMDLf-containing Products (U.S Population Groups; 2007-2008 NHANES Data)						
Population	N _u /N _p ^{a, b}	Percent users ^c	Absolute EDI (mg cMDLf/d)		Weight-based EDI (mg cMDLf/kg/d)	
			Mean	90 th Percentile	Mean	90 th Percentile
Toddlers, 12-35 mo	197/446	51.0	81	166	6.4	12.5
Children, 3-11 yr	687/1335	54.5	125	260	4.8	10.0
Teen, 12-19 yr	346/963	37.9	164	336	2.7	5.3
Adults, 20+ yr	1643/4628	37.3	146	275	1.9	3.8
Total population	2873/7372	39.5	142	273	2.7	5.7

a. Individuals were considered users if they consumed one or more food products supplemented with cMDLf on either Day 1 or Day 2 of the survey.

b. Number of users over the number of people surveyed.

c. Percent of user when sample weights are considered.

Maximum supplemental cMDLf concentrations in food products for EDI calculation are:

- 1 mg cMDLf/g in yogurts and probiotic fortified unfermented milks.
- 4 mg cMDLf/g in dry milk powders (not reconstituted), excluding infant formulas.
- 2 mg cMDLf/g in ice creams and sherbets.
- 30 mg cMDLf/g in chewing gums.

D. CONCLUSIONS

Toddlers, children, teens, and adults are currently exposed to cMDLf with the consumption of cow's milk, cow's milk-based products, and dietary supplements. According to the EDIs from the proposed uses, the daily exposure of those people consuming cow's milk-based products and chewing gums containing additional cMDLF will increase three- to four-fold from an average of 38 mg/d to an average of 142 mg/d. Importantly, the proposed use levels of cMDLf in this GRAS determination are similar to those that have been already determined safe and approved for use in Europe (EFSA Panel on Dietetic Products, 2012a; EFSA Panel on Dietetic Products, 2012b).

V. SAFETY ASSESSMENT

A. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF cMDLf

1. INTRODUCTION

Ingested lactoferrin is handled by the body like any other dietary protein; it enters the gastrointestinal tract and can be digested by proteolytic enzymes (Kuwata et al., 1998c; Kuwata et al., 1998a). Although it is not known what fraction of ingested lactoferrin is absorbed, studies indicate that a small amount of full-length lactoferrin survives the stomach (Troost et al., 2001), passes into the small intestine, and is absorbed intact (Takeuchi et al., 2004). Because the enzymatic activities in the gastrointestinal tract increase with age (Lindberg et al., 1997), infants may have the greatest opportunity for exposure to a small amount of undigested lactoferrin. After absorption in the small intestine, lactoferrin and its fragments partition into the lymph and then are rapidly transferred to the blood. Ingested lactoferrin may also be systemically absorbed via enterohepatic cycling (Fischer et al., 2007; Talukder et al., 2003; Harada et al., 2002; Harada et al., 1999). The mechanisms that mediate absorption of lactoferrin and its fragments are unclear but may include passive and active transport across the brush border of the intestinal mucosa (Tomé and Debabbi, 1998). Once in circulation, lactoferrin can then be absorbed or phagocytosed by cells (Adlerova et al., 2008; Graham et al., 2007), or excreted in the urine (Hutchens et al., 1991; Goldman et al., 1990). It should be noted that the iron bound to absorbed lactoferrin is ultimately deposited in the bone marrow (Bennett and Kokocinski, 1979).

2. LACTOFERRIN FRAGMENTS

Proteolysis of full-length ingested lactoferrin generates fragments such as lactoferricin, a bactericidal protein that contains the N-region of lactoferrin (Hamosh, 1998). Lactoferricin has been detected in the gastrointestinal tract of mice, rats and humans that were administered bovine, porcine, and human lactoferrin (Kuwata et al., 1998c; Kuwata et al., 1998a; Kuwata et al., 1998b; Kuwata et al., 2001). Information is lacking regarding the susceptibility of lactoferricin and other lactoferrin fragments to further proteolysis in the gut, but one study in rats suggests that the C-lobe of lactoferrin persists longer than the N-lobe in the small intestine (Yoshise et al., 2007).

3. LACTOFERRIN RECEPTORS

The uptake of lactoferrin in the gastrointestinal tract and throughout the body may be mediated by its binding to lactoferrin receptors. Recognition of lactoferrin by its receptor is

000046

somewhat species-specific, as cMDLf is reported to bind to suckling and adult rat lactoferrin receptors (Kawakami et al., 1990), but not to primate (Lönnerdal, 1994) or porcine (Gislason et al., 1993) lactoferrin receptors isolated from the gastrointestinal tract. Uptake of lactoferrin by the liver may occur via receptor-mediated endocytosis (Adlerova et al., 2008; Graham et al., 2007); however, lactoferrin is also bound by at least two non-specific binding sites on hepatocytes, the low-density lipoprotein receptor-related protein (LRP) and the major subunit of the asialoglycoprotein receptor (RHL-1) (Graham et al., 2007). The exact role of lactoferrin receptors in the in vivo distribution and processing of lactoferrin has not been comprehensively explored.

4. DISTRIBUTION AND CLEARANCE OF LACTOFERRIN

Any ingested lactoferrin that enters the systemic circulation is rapidly removed from the blood by distribution into the spleen, liver, kidneys, cerebrospinal fluid and the choroid plexus epithelium of the brain (Ji et al., 2006; Talukder et al., 2003; Harada et al., 1999; Bennett and Kokocinski, 1979). In liver cells, lactoferrin is targeted to lysosomes for degradation (Graham et al., 2007). In the liver, the bound iron atoms on lactoferrin are also extracted for transport to the bone marrow (Bennett and Kokocinski, 1979).

5. EXCRETION OF INGESTED LACTOFERRIN

Ingested lactoferrin that is absorbed into the bile (Regoeczi et al., 1994) will either be reabsorbed (Harada et al., 1999) or leave the body in the feces (Spik et al., 1982; Davidson and Lönnerdal, 1985; Davidson and Lönnerdal, 1987; Goldman et al., 1990); lactoferrin and its fragments may also appear in the urine (Spik et al., 1982; Goldman et al., 1990). Any systemically absorbed lactoferrin that is not excreted is presumed to be completely degraded at a cellular level into amino acids.

B. PHYSIOLOGICAL EFFECTS RELEVANT TO SAFETY OF INGESTION

1. INTRODUCTION

cMDLf represents approximately 0.3% of the total protein in cow milk and because it is present at much lower levels than the caseins, α -lactalbumin, and β -lactoglobulin in cow milk (Crittenden and Bennett, 2005), consumers are exposed to very low quantities when they ingest cow-milk derived products. With the consumption of cMDLf from the proposed uses, cMDLf intakes will increase by three to four-fold.

000047

A review of the publically available databases has revealed that the ingestion of cMDLf can have immunomodulatory and bone remodeling effects. Thus, the body of evidence supporting the roles of cMDLf in immune responses, hypersensitivity reactions, autoimmunity, and bone remodeling have been evaluated to assess the potential links between the consumption of cMDLf and its reported effects. Importantly, cMDLf may promote bone growth and is not a causative agent of cow's milk allergy (CMA) or autoimmunity. Furthermore, because additional cMDLf will be added to products already containing other cow milk proteins, should an individual be hypersensitive to cow's milk, these products should be avoided to prevent the onset of symptoms associated with CMA. Non-milk products (chewing gum) containing cMDLF will be appropriately labeled as containing cow's milk proteins.

2. IMMUNOMODULATION

Studies in animals have shown that orally administered cMDLf reduces the severity of dextran sulfate sodium-induced colitis, zymosan-induced inflammation, rotavirus-induced diarrhea, and *Staphylococcus aureus*, *Candida albicans*, *Toxoplasma gondii*, and influenza infections (reviewed in Table 14; Zimecki and Kruzel, 2000; Togawa et al., 2002a; Togawa et al., 2002b; Hartog et al., 2007; Perez-Cano et al., 2008; Bhimani et al., 1999; Takakura et al., 2003; Takakura et al., 2004; Shin et al., 2005; Mossallam, 2009). These results suggest that cMDLF may reduce inflammation and promote immunity in those individuals that consume it. However, the exact mechanisms by which ingested cMDLf contributes these responses are unclear. Importantly, 43 clinical trials involving approximately 1911 healthy and health-compromised infants, children, and adults consuming cMDLF from 14 mg to 7.2 g/d for up to 1 year have been conducted to evaluate the benefits and tolerability of cMDLF (reviewed in section V.D. and Table 15, 16, and 17) and the weight of the evidence shows that prolonged consumption of cMDLf does not promote the development of and/or exacerbate disease.

3. COW'S MILK ALLERGY, HYPERSENSITIVITY, AND ORAL TOLERANCE

CMA is often confused with lactose intolerance and broadly characterized as an inflammatory or hypersensitivity response to cow's milk proteins. CMA affects approximately 2 to 6 % of infants and 0.1 to 0.5% of adults, and for unknown reasons, a large majority (85 to 90%) of afflicted infants lose their hypersensitivity later in life (Crittenden and Bennett, 2005).

Hypersensitivity to food antigens results from the failure of the immune system to tolerate an otherwise innocuous ingested antigen (reviewed in Brandtzaeg, 2010; Mayer et al., 2001; Faria and Weiner, 2005; Mayer and Shao, 2004). Unlike "natural" or "self" tolerance,

where the immune system is unresponsive to self- or auto-antigens and responsive to foreign antigens, "oral" tolerance is an active state of immunosuppression whereby unwanted responses to the gut flora and the millions of foreign antigens ingested each day are prevented.

In humans, the mechanisms that contribute to oral tolerance are largely unknown. Studies in animal models suggest that they may involve the neutralization of foreign antigens with secreted IgA antibodies and suppression of mucosal immunity either by the induction of "suppressive" T regulatory cells, or clonal anergy and clonal deletion, which are mediated by low and high doses of antigen respectively. Furthermore tolerance can be terminated by prolonged absence of exposure to a particular antigen. In newborns, the "playing field" is different. The immune system is naïve with regard to its exposure to foreign antigens, the number of circulating IgA-secreting B cells is limited, and the gut epithelium is more permeable to ingested antigens. Breast milk contains IgA, the anti-inflammatory cytokines interleukin 10 (IL-10) and transforming growth factor β (TGF- β), and small amounts of antigens that the mother is exposed to everyday (Brandtzaeg, 2010). Thus, maternal breast milk not only provides the infant with the necessary nutrients, but may also promote the development of oral tolerance by neutralizing potentially dangerous antigens with IgA, reducing inflammation with IL-10 and TGF β , and inducing suppressor T cells with low doses of antigen. Why one individual is hypersensitive to an otherwise innocuous food antigen is unclear, but it is currently believed to involve a combination of genetics, age, dose and timing of antigen exposure, the integrity of the gut epithelium, and properties of the antigen itself.

Hypersensitivity reactions can be categorized into 4 groups, allergic or immediate (type 1), cytotoxic (type 2), immune complex (type 3), and delayed-type (type 4) (Janeway et al., 2005). Allergic responses are the product of an aberrant humoral CD4⁺ T helper (Th) 2 response that has skewed B cells to produce antigen-specific IgE. They are unlike cytotoxic, immune complex, and delayed-type responses because they occur in minutes, can persist for hours, and are triggered when antigen-specific IgE-bound F_c receptors expressed on the surface of mast cells are cross-linked. IgE cross-linking causes the mast cells to degranulate, releasing the inflammatory mediators histamine and leukotriene, which dilate capillary venules, activate the endothelium, and increase vascular permeability. If the antigen is systemic or rapidly absorbed, histamine and leukotriene release is widespread and can result in anaphylaxis and potentially death. *In vivo* diagnostic tests used to determine if an individual has antigen-specific IgE antibodies include oral challenges, and patch and skin prick tests (SPTs), which detect antigen-specific IgE antibodies bound to dermal mast cells. *In vitro* tests include RASTs (radioallergosorbant tests), and enzyme-linked immunosorbent assays (ELISAs) (Vanto et al., 1999). Passive cutaneous anaphylaxis (PCA) is also used, but only in animals.

Cytotoxic, immune complex, and delayed-type hypersensitivity reactions develop over the course of days or wks, and can lead to diseased states. Cytotoxic reactions are triggered when antigen-specific IgM or IgG bind cognate antigens bound to or found on the surface of cells. This activates the complement cascade, which releases the inflammatory mediator C5a, and results in the recognition and lysis of the antibody/complement-bound cell by macrophages. Immune complex reactions are similar, but are triggered when antibodies encounter soluble antigen. Aggregates of the antibody and antigen then form, are deposited in tissues, and cause complement activation and Fc receptor-mediated leukocyte activation. Clinically, an individual's sensitivity to this reaction can be determined by the Arthus reaction.

Delayed-type hypersensitivity reactions are unlike allergic, cytotoxic, and immune complex reactions because they are antibody independent. These reactions primarily result from an aberrant Th1 or cell-mediated immune response. They are triggered when antigen-specific T cells re-encounter antigen presented by antigen presenting cells. The T cells are activated and produce cytokines that promote inflammation. An individual's sensitivity to delayed-type hypersensitivity reactions can be determined clinically by oral challenges and SPTs but unlike an allergic response, the symptoms develop slowly. The prototypical delayed-type hypersensitivity test is the tuberculosis test.

Cow's milk allergy can be categorized into IgE-mediated and non-IgE-mediated responses (reviewed in Crittenden and Bennett, 2005). The mechanisms that drive non-IgE-mediated CMA are poorly defined, but are thought to include immune complex and delayed-type hypersensitivity reactions. The types of CMA, however, are not mutually exclusive, and, when exposed, CMA patients usually develop one or more cutaneous, gastrointestinal, and/or respiratory signs, including eczema, urticaria, angioderma, nausea, vomiting, diarrhea, rhinoconjunctivitis, and asthma. Anaphylactic reactions are rare. Currently, ELISAs for anti-bovine milk IgE antibodies in the serum or SPTs with whole milk are used as follow-up to discriminate between IgE- and non-IgE-mediated CMA. Also, milk avoidance is the most successful means of treating CMA, and milk oral challenges are the standard means of diagnosing CMA (Vandenplas et al., 2007). The following sections address the role of orally administered cMDLf in IgE- and non-IgE-mediated CMA (Table 14).

a) IgE-mediated hypersensitivity

Sixty-percent of people with CMA have IgE antibodies to cow's milk proteins and studies suggest that less than 4% these individuals have IgE antibodies to only cMDLf (Sampson, 1999; Crittenden and Bennett, 2005; Host et al., 1992; Wal et al., 1995a; Wal et al.,

000050

1995b; Natale et al., 2004; Gaudin et al., 2008). Anti-cMDLf antibodies have also been found in infants fed cMDLf-supplemented formula (1 mg/ml cMDLf) for six months and no adverse effects were reported (Brock et al., 1997; Lönnerdal and Hernell, 1994). Importantly, blinded oral challenges or SPTs with cMDLf have never been performed. In 1994, Atkinson and colleagues intraperitoneally sensitized Brown Norway rats with semi-skimmed cow's milk in the presence of the adjuvant carrageenan and elicited cow's milk protein-specific PCAs 21 days later to assess the ability of various cow's milk proteins to elicit IgE antibodies (Atkinson and Miller, 1994). Although Brown Norway rats were capable of developing IgE antibodies to a number of cow's milk proteins, lactoferrin immunoreactivity required both the removal of milk proteins from the diet prior to the sensitizing dose and an intraperitoneal booster of semi-skimmed milk seven days later. A follow-up study, designed to compare the relative allergenicity of orally or intraperitoneally administered lactoferrin in Brown Norway rats, found that orally administered lactoferrin was approximately 3000-times less potent at eliciting lactoferrin specific PCAs than intraperitoneally administered lactoferrin and occurred in only 25% of the sensitized rats (Meredith and Atkinson, 2000). Ishikado et al. (2005) performed a similar study in guinea pigs found that orally administered cMDLf was approximately 100-fold less potent at eliciting PCAs than subcutaneously administered cMDLf. Importantly, these models have limited predictive value because they do not replicate how humans would be exposed to ingested lactoferrin. Thus, although cMDLf can induce the development of IgE antibodies, it is well tolerated and its clinical relevance as an allergen is unknown.

In contrast, a variety of studies have also indicated that cMDLf may inhibit IgE-mediated hypersensitivity reactions: cMDLf and peptic cMDLf can reduce histamine release from IgE-cross-linked primary mast cells *in vitro* (Otani and Yamada, 1995); oral administration of cMDLf can reduce serum and antigen specific IgE levels (Kuhara and Hayasawa, 2002); oral administration of cMDLf increases the production of the anti-inflammatory cytokine IL-10 from the intestinal epithelium and the mesenteric lymph nodes (Takakura et al., 2006). Notably, IL-10 is present in breast milk and has been shown to inhibit other types of IgE-mediated hypersensitivity (Grimbaldeston et al., 2007). Thus, while ingested lactoferrin may be antigenic, it may promote oral tolerance by inhibiting mast cell degranulation and the inducing the production of IL-10.

b) Non-IgE-mediated hypersensitivity

The remaining 40% of people with CMA have non-IgE-mediated hypersensitivity reactions. Although the mechanisms that contribute to non-IgE-mediated hypersensitivity are

poorly defined, it is currently thought that non-IgE-mediated CMA may be mediated by immune complex and/or delayed-type hypersensitivity reactions.

Studies in animal models have shown that oral administration of cMDLf can increase the development of anti-cMDLf IgA, IgM, and IgG antibodies (Debbabi et al., 1998; Miyauchi et al., 1997), and IgA and IgG immune complexes have also be found in mice fed high levels of cMDLf for prolonged periods of time (Fischer et al., 2007). IgA is primarily found in serum and mucosal secretions and not thought to mediate hypersensitivity reactions because it poorly activates complement. IgM is primarily bound to and activates B cells when it binds antigen. IgM also circulates in the blood, binds antigen, activates complement, and can induce cytotoxic reactions. IgG is found primarily in the serum and, when it encounters an antigen, activates complement. Importantly, cMDLf does not elicit inflammatory responses when injected subcutaneously or intraperitoneally in lactoferrin-sensitized mice, and can inhibit complement-mediated cytotoxicity *in vitro* and be safely fed to animals and humans for extended periods of time (Otani and Yamada, 1995; Fischer et al., 2007; Zimecki and Kruzel, 2000; Iigo et al., 2004; Artym and , 2003; Prgomet et al., 2007; Hellweg et al., 2008; Handl et al., 2009; Brock et al., 1997; Lönnerdal and Hernell, 1994). Thus, it is highly unlikely that consuming cMDLf-supplemented infant formula will sensitize otherwise healthy infants to immune complex and/or cytotoxic hypersensitivity reactions later in life.

Delayed-type hypersensitivity (DTH) reactions are mediated by the cytokines produced by activated T cells. Unfortunately, the effects of cMDLf on T cell activation *in vitro* are varied, making it difficult to unambiguously define a role for cMDLf in regulating T cell activation and differentiation (Wilk et al., 2007; Actor, 2002; Rejman et al., 1992; Wong et al., 1997; Miyauchi et al., 1997; Debbabi et al., 1998; Artym and , 2003; Kobayashi et al., 2008; Hwang et al., 2007). However, cMDLf can promote the activation of antigen presenting cells *in vitro* and studies in mice have shown that cMDLf is a potent adjuvant, promoting DTH responses to ovalbumin, sheep red blood cells, and *Mycobacterium bovis* (Zimecki and Kruzel, 2000; Zimecki et al., 2002). Importantly, an experiment was performed by Zimecki and Kruzel (2000) to address the adjuvanticity of cMDLf. Mice were sensitized by subcutaneously administering sheep red blood cells in incomplete Freund's adjuvant and then subcutaneously injecting lactoferrin with the sensitizing agent, or administering it orally or intraperitoneally. Four days later the DTH responses were elicited with incomplete Freund's adjuvant, incomplete Freund's adjuvant and sheep red blood cells, or incomplete Freund's adjuvant and cMDLf. Compared to the DTH response elicited by incomplete Freund's adjuvant alone, cMDLf administered by all routes increased sheep red blood cell-specific DTH responses. Interestingly, oral and intraperitoneal administration of cMDLf inhibited the cMDLf-specific DTH response by approximately 50

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percent. Sensitization with subcutaneous cMDLf increased cMDLf-specific DTH response. These results indicate that orally administered cMDLf can promoting DTH response to other antigens, consistent with it being an adjuvant, but not to itself.

c) Conclusions

Although it is well established that infants with CMA have anti-cMDLf IgE antibodies, there is no evidence to support a role for lactoferrin as a causative agent. Moreover, the presence of IgE antibodies to multiple milk proteins in individuals with CMA indicates that this condition is a more generalized abnormality, possibly reflecting a failure of these individuals to establish oral tolerance. Because oral tolerance to antigens like those in milk is established and maintained by a complicated network of genetic, extracellular, intracellular, and intercellular events, reactivity to lactoferrin or any other milk protein, is likely a symptom of the abnormality rather than the cause. Importantly, given that oral administration reduces an antigen's immunoreactivity, providing small amounts of cMDLf may, in fact, contribute to the development of oral tolerance.

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Pierce et al., 1991	Cloned and sequenced cow-derived lactoferrin.	Cloned cow-derived lactoferrin from an λ gt 10 cDNA library constructed from salivary gland poly(A)-rich RNA and sequenced cMDLf using an Applied Biosystems 470A gas phase sequencer. Then compared the sequences of cMDLf, human, and mouse lactoferrin.	Bovine lactoferrin is 69% and 64% homologous to human and mouse lactoferrin.
Host et al., 1992	Characterized the anti-ovalbumin and cows milk protein (CMP) IgE, IgG, and IgG isotypes in infants with cow's milk protein allergy (CMPA).	Followed the levels of antibody isotypes in patients with or without CMPA for 1 yr from birth (via cord blood). CMPA diagnosis was established by the disappearance of symptoms after each of 2 dietary eliminations of cow's milk and cow's milk products, reoccurrence of identical symptoms after one challenge and exclusion of lactose intolerance and coincidental infection. SPTs and RASTs were used to determine if the CMPA was IgE-mediated or not. Measurements were made at birth, at the time of diagnosis, and before and after milk challenge at the age of 12 mo. Mothers were not on a diet without milk or eggs during pregnancy or lactation. Anti-cMDLf IgE antibodies were quantified by crossed radioimmuno-electrophoresis.	IgE antibodies to all the milk proteins tested were found in both cow's milk allergy (CMA; IgE-mediated) and cow's milk intolerant (CMI; non-IgE-mediated) patients. Anti-cMDLf IgE antibodies were undetectable in cord blood; detectable in the serum in one individual with CMI at 6 mo, and developed in 5/20 patients with IgE-mediated CMPA only after being challenged with cow's milk at 12 mo of age. This study suggests that cMDLf IgE antibodies are relatively rare compared to those specific for other milk antigens and are generated after milk challenge. The relevance of these antibodies to the development of CMPA was not addressed.
Rejman et al., 1992	Determined the components of whey that are responsible for inhibiting the proliferation of peripheral blood mononuclear cells.	Peripheral blood mononuclear cells were harvested from cows and stimulated with concanavalin (ConA) in the presence or absence of increasing concentrations of the different components of whey. The degree of proliferation was quantified by 3 H-thymidine incorporation.	Apolactoferrin, lactoferrin, and apotransferrin inhibited ConA-induced proliferation whereas transferrin, IgG, and serum albumin did not. This study shows that lactoferrin inhibits the proliferation of lymphocytes.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Atkinson and Miller, 1994	To establish an animal model for screening potentially allergenic food proteins.	Semi-skimmed cow's milk was injected intraperitoneally with cargeenan (an adjuvant) into Brown Norway rats. The rats were bled wkly and antigen-specific IgE mediated responses were assessed by passive cutaneous anaphylaxis (pCA). IgG levels were determined by ELISA.	Rats maintained on a diet containing milk and ovalbumin were relatively less sensitive to the milk challenge (1/5) than ovalbumin challenge (4/5). However, if ovalbumin and milk products were removed, the number of responders to milk challenge increased (3/5). IgG antibodies to all cow's milk proteins were detected and PCAs were the greatest for α -casein and lactoferrin. Based on these results, this study suggests that cow's milk proteins, and more specifically cMDLf, can induce the production of antigen-specific IgE antibodies when administered subcutaneously. However, positive PCAs were seen to all milk proteins and whether or not oral administration of cMDLf alone could induce the symptoms associated with cow's milk allergy in this experimental model was not determined.
Lönnerdal and Hernell, 1994	Evaluated the effect of selenium supplementation on the selenium status in formula-fed infants.	Compared the hematological status of infants (6-wk-old) that were either breast-fed or infant formula supplemented with different combinations of selenium, iron, and copper over time. For purposes of this review of supplementing infant formula with cMDLf, focused on Group A, which received 4 mg iron (FeSO_4); group B, 10 μg of selenium; Group C contained 4 mg of iron (1.4 mg of cMDLf and 2.6 mg of FeSO_4) and 10 μg of selenium.	Infants fed the different combinations of selenium, iron and cMDLf gained weight and grew at rates comparable to infants fed breast milk. Also, although there was less α 2-macroglobulin in all the formula fed groups, there were no differences in the hematological indices. Thus, supplementing infant formula with selenium, iron, and cMDLf did not dramatically affect homeostasis.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Otani and Yamada, 1995	Is bovine lactoferrin anti-inflammatory?	Tested the ability of cMDLf to inhibit inflammatory reactions, i.e. vascular permeability, histamine release from mast cells; complement-mediated cell lysis, Arthus reaction (hypersensitivity III reaction in the skin), picryl chloride-induced contact dermatitis, and SRBC-induced delayed-type hypersensitivity.	cMDLf increased vascular permeability when injected intraperitoneally into guinea pigs but inhibited IgE-mediated histamine release from mast cells and complement-mediated cell lysis. cMDLf had no effect on the Arthus reaction or picryl chloride-induced contact dermatitis, two measurements of hypersensitivity III reactions, or SRBC-induced delayed-type hypersensitivity. The results suggest that cMDLf has anti-inflammatory activities and no effect on delayed-type hypersensitivity reactions. cMDLf was administered either intravenously or intraperitoneally. The effect of orally administering cMDLf in any of these models was not addressed. Importantly, cMDLf did inhibit IgE-mediated histamine release from mast cells suggesting that it may prevent allergic reactions in the gut when administered orally.
Wal et al., 1995b	Developed an ELISA to detect anti- β -lactoglobulin, α -lactalbumin, bovine serum albumin, lactoferrin, and the whole casein fraction IgE antibodies.	Coated plates with purified proteins derived from cow's milk and incubated the plates with serum taken from patients with CMA. Determine the presence of anti-cow's milk proteins with a secondary antibody that recognized human IgE. All patients used in this study presented with clinical symptoms of food allergy and positive RASTs to whole milk. The use of other <i>in vivo</i> diagnostic tests such as SPTs or oral challenges was not noted.	Competitive inhibition tests using purified proteins and antibodies that specifically recognized each antigen validated the specificity of ELISA. IgE antibodies that recognized all milk proteins tested were found in the sera of patients with CMA and the reactivity to each of the antigens was varied, i.e. some patients were monoreactive, some were reactive to more than one antigen, and some were reactive to all antigens. Interestingly, one patient had IgE antibodies to only cMDLf. The relevance of anti-cow's milk protein reactivity to the pathogenesis of CMA was not addressed.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Wal et al., 1995a	Characterized the reactivity of patients with CMA to the different components in cow's milk.	Collected sera from patients presenting with clinical symptoms of food allergy and positive RAST to whole milk. The average age of the patients was 11 mo. Importantly, the number of patients tested was not noted nor was the method used to determine reactivity and the isotype of the reactive antibodies. The different components of cow's milk were purified by cation exchange.	Thirty-five percent of the patients had IgE antibodies that recognized cMDLf and less than 4% of these patients were mono-reactive cMDLf. The exact number was not noted. Approximately 20% of the patients also had IgA antibodies to α -lactoglobulin, and lactoferrin. IgG1 and IgG4 antibodies to lactoferrin were very rare and the few patients that did have these antibodies had them at barely detectable quantities. The relevance of these antibodies to the development of CMA was not determined nor was the reactivity of the patients to the purified proteins confirmed by SPTs or oral challenge.
Brock et al., 1997	Are there antibodies to cMDLf in the serum of infants fed infant formula supplemented with cMDLf (1 mg/ml)? How does this differ from infants that are breast-fed?	Analyzed serum collected from normal infants, cow's milk intolerant (CMI) infants, breast-fed infants, formula supplemented with cMDLf (1 mg/ml) fed infants, adults, and autoimmune patients for the presence of anti-bovine and anti-human lactoferrin antibodies by ELISA. The criteria used to determine cow's milk intolerance was not described nor were the isotypes of the antibodies detected in the sera.	CMI infants have anti-cMDLf antibodies whereas breast-fed infants have anti-human lactoferrin antibodies. Importantly, infants fed formula supplemented with cMDLf had high amounts of antibodies to cMDLf and also had antibodies to human lactoferrin. Antibodies to β -lactoglobulin were also detected and elevated in the CMI infants (noted as data not shown). The relevance of anti-cMDLf and anti- β -lactoglobulin antibodies to the development of CMI was not determined.

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Miyauchi et al., 1997	Does hydrolyzed bovine lactoferrin have immunostimulatory effects?	Stimulated splenocytes and enriched populations of T and B cells with cMDLf, cMDLf hydrosylate (H), concanavalin A (ConA), and lipopolysaccharide (LPS). The cMDLf was prepared from the whey protein fraction of bovine milk and cMDLf hydrosylate was prepared by digesting the bovine lactoferrin with Pepsin. Cellular responses were quantified by measuring the proliferative rates (³ H-thymidine incorporation) and antibody production.	cMDLf inhibited the proliferation of splenocytes whereas cMDLf H induced proliferation. Both responses occurred in a dose-dependent fashion and the ability of cMDLf H to induce proliferation did not require adherent cells. cMDLf and cMDLf H inhibited ConA-induced proliferation, lactoferrin inhibited PHA-induced proliferation while cMDLf H increased it, and cMDLf inhibited and LPS-induced proliferation while cMDLf H had no effect. cMDLf H induced the proliferation of enriched T and B cells and increased the production of IgM, IgG, and IgA from cultured splenocytes. The same effect was seen with Peyer's patch cells. This report suggests that cMDLf H is mitogenic to both B and T cells and while intact cMDLf may induce the cell death. cMDLf H may also induce B cells to produce immunoglobulin. It is noteworthy that the amount of proliferation observed when either cMDLf or cMDLf H was used to stimulate the cells was relatively small compared to the proliferative responses to ConA, PHA, and LPS. These results indicated that neither cMDLf nor cMDLf H are potent mitogens.
Debbabi et al., 1998	Does bovine lactoferrin affect immune homeostasis?	cMDLf (17% iron saturated; LactoBretagne) was administered to four groups of BALB/c mice (10 to 15 wk-old; n=10 for each group). Two groups received two different doses of cMDLf (0.5 mg/g or 1 mg/g body weight/d) administered through a stainless steel feeding tube for 5 consecutive d each wk for 4 wk. The control group received sterile saline using the same experimental conditions as the fed groups and the fourth group received an immunizing intramuscular injection of 0.01 mg of cMDLf in complete Freund's adjuvant and three wks later received an intramuscular injection of	Neither oral administration nor intramuscular injection of cMDLf affected the body weights of the mice. Also, no clinical abnormalities were observed over the course of the experiment. Oral administration of cMDLf increased total IgA and IgG levels in the saliva and intestinal fluid in dose-dependent fashion; IgA and IgG levels in the serum did not change. The cMDLf-fed mice also developed anti-cMDLf IgA and IgG antibodies in the intestinal fluid and serum. cMDLf stimulated splenocytes harvested from the cMDLf-fed mice produced more IgA and IgG; proliferated more when incubated either in medium alone, or restimulated with cMDLf

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
650000		0.01 mg of cMDLf in incomplete Freund's adjuvant. A control for the immunization with cMDLf was not performed. The mice were routinely observed for clinical signs of distress and body weights were recorded over the course of the experiment. Total and anti-cMDLf specific IgA and IgG antibodies in the saliva, intestinal fluid, serum, and media harvested from cultured Peyer's patch and splenocyte cell suspensions were quantified by ELISA. The proliferative responses of Peyer's patch and splenocyte cell suspensions to cMDLf and concanavalin A (ConA) were also determined by ³ H-thymidine incorporation.	or ConA. The effect of the cMDLf immunization on antibody production has been excluded from this analysis because the experiment lacked an appropriate control. This paper suggests that orally administering cMDLf to mice generates antigen-specific B cells that are capable of producing anti-cMDLf IgA and IgG antibodies.
Bhimani et al., 1999	Can lactoferrin promote immunity to <i>Staphylococcus aureus</i> ?	Human and bovine lactoferrin (85% and 97%, iron saturated, respectively) and human and bovine apo-lactoferrin (17% and 10% iron saturated, respectively) were incubated with <i>Staphylococcus aureus</i> (<i>S. aureus</i>) and their antibacterial effects were determined by zone-inhibition on agar plates. Bovine lactoferrin and bovine apo-lactoferrin was also intravenously (1 mg) or orally (20 mg/ml <i>ad libitum</i>) administered to NIH/PLCR mice one day prior to infection with <i>S. aureus</i> , and fourteen d later, the kidneys were removed, homogenized, and analyzed for the number of <i>S. aureus</i> colonies.	Apo-lactoferrin (human and bovine) directly inhibited the growth of <i>S. aureus</i> and the iron-saturated forms did not. <i>In vivo</i> , intravenous administration of bovine apo-lactoferrin prior to the <i>S. aureus</i> infection reduced both the bacterial burden in the kidneys and the percentage of infected kidneys in a dose-responsive manner. Intravenous administration of apo-lactoferrin after the infection did not affect the bacterial burden. Despite its inability to inhibit <i>S. aureus</i> growth <i>in vitro</i> , iron saturated bovine lactoferrin inhibited <i>S. aureus</i> growth <i>in vivo</i> . Importantly, oral administration of either bovine apo- or iron-saturated lactoferrin inhibited <i>S. aureus</i> growth <i>in vivo</i> . This study suggests that oral administration of bovine lactoferrin can protect mice from staphylococcal infections.

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Vanto et al., 1999	Evaluated the value of the patch test, skin prick test (SPT), and milk-specific IgE by CAP RAST to the diagnosis of CMA.	Performed skin prick tests, patch tests, and CAP RASTs on 305 infants with suspected hypersensitivity to cow's milk. Double-blind, placebo-controlled milk challenges were performed prior to testing determine the presence of true milk allergy.	Positive reactions were observed in 176 individuals following milk challenge (immediate reactions were observed in 100 infants and delayed reactions were observed in 76 infants). Serum milk-specific IgE levels and wheal size of the SPT were significantly greater ($p < 0.05$) in patients with immediate positive reactions to milk challenge. There were no significant differences in serum milk-specific IgE or SPTs between infants with delayed positive reactions and those with a negative milk challenges. Positive SPTs were significantly associated with positive patch tests whereas positive patch tests were not significantly associated with the presence of serum milk-specific IgE. Positive patch test results were also not significantly associated with positive cow's milk challenges. However, wheal size of the SPT was also found to be discriminatory measure of immediate, delayed, and negative reactions to milk challenge. Thus, the authors propose that the SPT is the best way to determine infants with immediate-type hypersensitivity followed by anti-milk protein IgE ELISAs.
Zimecki and Kruzel, 2000	Can bovine lactoferrin act as an adjuvant?	CBA mice (8- to 12-wk-old) were sensitized with sheep red blood cells (SRBC), Bacillus Calmette-Guerin (BCG), or ovalbumin (OVA) in complete or incomplete Freund's adjuvant at the base of the tail and delayed-type hypersensitivity reactions were elicited 4 d later by subcutaneously administering the sensitizing antigen into the footpad. Bovine lactoferrin (<25% iron saturated) or human lactoferrin (< 20% iron saturated) was administered intraperitoneally, subcutaneously, or orally at the time of sensitization. The intensities of the DTH responses were	When bovine lactoferrin was administered intraperitoneally, subcutaneously, or orally to mice that had been sensitized to SRBC or OVA in complete Freund's adjuvant, it did not dramatically (<1.25-fold) affect the DTH response. However, when mice were sensitized to SRBC in incomplete Freund's adjuvant, orally administered lactoferrin increased the DTH response approximately 9-fold at a low dose of immunizing SRBC (10^6), 2-fold at a medium dose (10^7 SRBC) and, <1.25-fold at a high dose (10^7 SRBC). Oral administration of lactoferrin also increased OVA-induced DTH responses approximately 2-fold when the mice were sensitized

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
000061		determined by measuring footpad swelling. The duration of lactoferrin oral administration was not commented on.	with OVA in incomplete Freund's adjuvant. When mice were sensitized to BCG in complete Freund's adjuvant, subcutaneous and oral administration of lactoferrin increased the DTH response, 2-fold and 1.25-fold respectively. Importantly, lactoferrin alone was unable to elicit a DTH response when mice were immunized with SRBC in incomplete Freund's adjuvant and either treated with lactoferrin intraperitoneally or orally at the time of immunization (Figure 7). When mice were treated with lactoferrin subcutaneously at the time of immunization, the lactoferrin elicited DTH response was approximately 1.3-fold greater than the incomplete Freund's adjuvant elicited response. This study indicates that bovine lactoferrin is an adjuvant. Furthermore, the control studies investigating the ability of lactoferrin to elicit a DTH response strongly suggests that oral administration of lactoferrin does not induce DTH.
Meredith and Atkinson, 2000 - <i>only abstract is available only</i>	To establish an animal model for screening potentially allergenic food proteins.	Increasing amounts of lactoferrin were injected intraperitoneally (IP) into Brown Norway rats in the presence of the adjuvant carrageenan (CGN). IgE-specific immunoreactivity was measured by passive cutaneous anaphylaxis (PCA) and antigen-specific IgE immunoblotting 28 d later. Increasing amounts of lactoferrin were also orally administered to Brown Norway rats by gavage twice a week with once a week IP injections of CGN for six weeks. Protein-specific immunoreactivity was determined by PCA and antigen-specific IgE immunoblotting.	IP administered lactoferrin was able to produce 50% responders at 40-50 ng. Oral administration of lactoferrin was able to produce 25% responders at 0.5 mg/kg. Assuming that an average Brown Norway rat weighs 0.3 kg, the IP dose would be 0.0016 mg/kg, which is approximately 3000-fold less than 0.5 mg/kg. Orally administered lactoferrin is much less immunogenic than IP administered lactoferrin.

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Actor, 2002	Can cMDLf promote delayed-type hypersensitivity (DTH) responses in mice?	CBA mice (8- to 12-wk-old) were sensitized with sheep red blood cells (SRBC) by injecting them subcutaneously into the base of the tail with or without cMDLf (<25% iron saturated) in complete or incomplete Freund's adjuvant. Four d later DTH responses were elicited by subcutaneously injecting SRBC in the hind footpads. Swelling was quantified 24 h later. The effect of lactoferrin on the proliferation and cytokine production (TNF α , IL-12, IL-15, IL-10, MIP-1 α , and MIP-2) of the macrophage/monocyte cell line J774A.1 was also analyzed.	Subcutaneous administration of 50 and 250 μ g cMDLf with a high dose of immunizing antigen in complete Freund's adjuvant modestly increased the DTH response (approx. 2-fold). When cMDLf was administered in incomplete Freund's adjuvant with a low dose of sensitizing antigen, lactoferrin increased the DTH response (approx. 20-fold). When higher doses of immunizing antigen were used with lactoferrin and incomplete Freund's adjuvant, the DTH response increased but the effect of cMDLf was not as dramatic. Intraperitoneal injection of cMDLf alone modestly increased the number of cells recruited to the peritoneal cavity. cMDLf also increased the proliferation of J774A.1 cells and increased their production of TNF α production, decreased their production IL-10, and increased their IL-15, MIP-1 α and MIP2 gene expression <i>in vitro</i> . Although the physiological significance of the <i>in vitro</i> findings are unclear, the DTH results support the notion that cMDLf is an adjuvant.
Togawa et al., 2002b	Is bovine lactoferrin anti-inflammatory?	Induced colitis in rats by injecting 2,4,6-trinitrobenzenesulfonic acid (TNBS) into the colon. Bovine lactoferrin (200 mg/kg/day; Morinaga Milk Industry) was administered directly to the stomach by gavage and inflammation was evaluated macroscopically, histologically, and biochemically.	Lactoferrin reduced the severity of TNBS-induced colitis by all parameters tested, and reduced the amount of TNF α , IL-1 β , and IL-6 and increased the amount of IL-4 and IL-10 in the colonic tissue. The results of this study are similar to that published by the same group addressing the anti-inflammatory activity of bovine lactoferrin in dextran sulfate sodium-induced colitis.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Togawa et al., 2002a	Does bovine lactoferrin have anti-inflammatory effects in dextran sulfate sodium (DSS)-induced colitis?	Administered bovine lactoferrin (100 mg/kg or 200 mg/kg/day; Morinaga Milk Industry) through a gavage, and 3 d later induced colitis by feeding rats DSS in the drinking water. Evaluated disease severity by a disease activity index, macroscopic and histological assessment of the colon, myeloperoxidase activity, and cytokine production. Plasma and fecal concentrations of lactoferrin and the number of colonic bacteria were also determined.	Administration of bovine lactoferrin protected against DSS-induced colitis in a dose-dependent manner. Lactoferrin inhibited the DSS-induced upregulation of TNF α , IL-1 β , and IL-6. It also induced IL-4 and IL-10 in the colon. Administration of bovine lactoferrin alone had no effect on any of the cytokines tested. This report indicates that lactoferrin has anti-inflammatory properties and because the development of inflammation in this model is not dependent on T or B cells, the anti-inflammatory activities of lactoferrin may directly affect on the colonic tissues. The allergenicity of bovine lactoferrin was not addressed.
Zimecki et al., 2002	Is the adjuvanticity of cMDLf dependent on a receptor that binds mannose/sugars?	CBA mice (10- to 12-wk-old) were sensitized with sheep red blood cells (SRBC) or ovalbumin (OVA) in complete or incomplete Freund's adjuvant and delayed type hypersensitivity responses were elicited with the sensitizing antigen 4 d later. cMDLf (<25% iron saturated) was administered intravenously, intraperitoneally, subcutaneously, or orally, either during sensitization or the elicitation. Mice were also treated or not with methyl- α -D-mannopyranoside.	This study contains experiments that extend the findings of Zimecki et al., 2000. The experiment in figure 4 is relevant to supplementing infant formula with cMDLf. Here the DTH-promoting effect of orally administered cMDLf when given with the sensitizing dose of OVA was inhibited by intraperitoneal administration of methyl- α -D-mannopyranoside 2 h prior to sensitization. This suggests that cMDLf acts as an adjuvant by binding a mannose-binding receptor but because the administration of cMDLf was for the 4 day prior to eliciting the DTH response the mechanisms by which this inhibition occurs is not straightforward.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Artym et al., 2003	Can cMDLf augment the reconstitution of humoral responses in previously immunocompromised mice?	Induced lymphopenia in mice (10 to 12 wk-old) by administering cyclophosphamide (an alkylating agent) intraperitoneally. The mice were then fed cMDLf in water (~20 mg/day; <25% iron saturated; Morinaga Milk Industry) for 30 d and the effect of cMDLf on humoral responses (immunization with sheep red blood cells (SRBC) followed by the isolation of splenocytes and the determination of antibody forming cell number), reconstitution of T and B cells in the spleen and macrophages in the peritoneal cavity, and the proliferative responses of splenocytes to concanavalin A (ConA) and pokeweed mitogen (pWM) were analyzed.	cMDLf partially reconstituted the SRBC-mediated humoral response, and partially reconstituted the numbers of splenic T cells and peritoneal macrophages in cyclophosphamide treated mice. The treatment of mice with cMDLf alone led to marginal increases in the percentage of splenic T cells and B cells but did not affect the percentage of peritoneal macrophages. Although cMDLf treatment <i>in vivo</i> also did not dramatically increase the basal and mitogen-induced proliferation of <i>ex vivo</i> splenocytes harvested from treated animals. This study shows that a diet containing cMDLf may promote the homeostatic proliferation of lymphocytes, which may or may not be due to its direct affect on T and B cells <i>in vivo</i> .
Takakura et al., 2003	Can orally administered cMDLf prevent oral candiditis in immunocompromised mice?	<i>Candida albicans</i> infections were established in ICR mice (6 wk-old) by injecting prednisolone (100 mg/kg) subcutaneously 1 day prior to swabbing of their oral cavities with cotton pads soaked in a <i>C. albicans</i> cell suspension. Prednisolone (100 mg/kg) was then reinjected subcutaneously 3 d later. The severity of the infection was evaluated macroscopically, microbiologically, and histologically over the course of the infection. cMDLf was continuously administered orally via the drinking water (0.3 % ~0.5 g/kg/d) from 1 day before the infection.	The number of viable <i>C. albicans</i> increased in the mice that received orally administered cMDLf to the same degree as the controls by three d after the infection. Thereafter, the numbers of viable <i>C. albicans</i> continued to rise in the controls but declined slightly in the mice that received orally administered cMDLf. The number of tongue lesions was also slightly reduced over the course of the infection in the cMDLf -fed mice. Small reductions in the number of viable fungi and tongue lesions were also obtained when cMDLf hydrosylate was used and/or cMDLf was delivered by intragastric intubation. Lactoferricin B did not have any prophylactic effects. Although this study shows that orally administering bovine cMDLf reduces the severity of oral candidiasis, it is difficult to draw conclusions about the effect of cMDLf on different cellular aspects of this type of infection because its infectivity is dependent on a depressed immune system. The direct effects of cMDLf on prednisolone-induced suppression of the immune system was not addressed.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Iigo et al., 2004	Does orally administrated of cMDLf induce cytokine production in the small intestine?	30 - 300 mg/kg/day of cMDLf (96% pure; iron content 143 ng/mg cMDLf), cMDLf hydrosylate (H; made from the cMDLf; iron content 100 ng/mg), and bovine apotransferrin (iron content 13 ng/mg; Morinaga Milk Industry) was orally administered to BALB/c mice (5 wk-old) for 24 h or for seven d and IL-18 and proinflammatory cytokine production was determined by western blot and ELISA respectively, and caspase 1 activity in the small intestine was determined by a colorimetric protease assay. Cytokine production from peritoneal macrophages treated <i>in vitro</i> with cMDLf was also analyzed by ELISA.	A single administration of cMDLf, cMDLf H, and apo-transferrin orally led to a significant and acute (within 5 hours) increase in IL-18 in the small intestine that slowly returned to baseline but did not appear to significantly affect the levels of IL-2, IL-12, IL-1 β , and TNF α . In the large intestine, all three proteins increased IL-18 but the levels did not return to baseline. In the serum, cMDLf increased IL-18 acutely and its levels slowly diminished thereafter. When administered over seven d, cMDLf significantly increased IL-18 and IFN γ expression but not in a dose-responsive manner, cMDLf H and apotransferrin also increased IL-18 and IFN γ but the increase was not significant nor dose-dependent, and cMDLf H significantly reduced the amount of IL-1 β and TNF α . cMDLf also significantly increased the amount of caspase 1 in the small intestine following either a single administration or seven d of continuous treatment. <i>In vitro</i> , cMDLf specifically induced IL-18 production and caspase 1 expression in peritoneal macrophages. Because IL-18 and IL-12 promote cell-mediated immunity, this study suggests that feeding mice cMDLf may induce a Th1 environment in the gut.
Natale et al., 2004	Characterized the cow's milk antigens recognized by the IgE antibodies in patients with cow's milk allergy (CMA).	Separated cow's milk proteins by 2D electrophoresis, blotted with serum obtained from patients with CMA, and developed with an anti-human IgE secondary antibody. Then isolated with immunoreactive spots and performed mass spectrometry to determine the immunoreactive protein.	Found IgE antibodies to almost all the milk proteins (except α -lactalbumin). Anti- cMDLf IgE antibodies were found in ~50% of the patients. The pathophysiological relevance of these antibodies in CMA was not addressed.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Sfeir et al., 2004	Does orally administered cMDLf affect immune homeostasis?	cMDLf (16.2 % iron saturated; Solarec-Soladec Recogne) was administered to BALB/c mice (4 wk; n=10 mice/group) by intramuscular injection (IM; 10 µg in complete Freund's adjuvant followed by a booster dose in incomplete Freund's adjuvant 4 wk later), intragastric gavage (IG; 0.5 ml of cMDLf (8 g/L (~ 4 mg/mouse/d))), buccal dose (BD; 0.5 ml of cMDLf (8 g/L) dropped into their mouth with the tip of an Eppendorf pipette (~ 4 mg/mouse/d)), ad libitum via the drinking water (DK; 1 or 25 g/L cMDLf solution at a level of 4 or 100 mg/mouse/day), or ad libitum as a powder in the diet (DT; 100 mg/d). Control mice received standard diet and had free access to sterile water. The amount of IgA, IgG, and IgM in the serum and intestinal secretions and secreted from cultured Peyer's patch cells and splenocytes harvested from the different groups was quantified by ELISA. <i>In vitro</i> proliferative responses and cytokine production to cMDLf and concanavalin A (ConA), and antibody production to cMDLf and lipopolysaccharide (LPS) were also determined for the Peyer's patch and splenocyte cell suspensions.	DT, BD, or IG administration of cMDLf significantly increased the amount of total IgA and IgG in the intestinal secretions 28 d post administration. At day 48 only IgG remained significantly increased in the DT, BD, and IG groups. Anti-cMDLf specific IgM, IgG, and IgA in the pooled serum and intestinal secretions increased overtime and reached a plateau at approximately 28 d post-administration. Anti-cMDLf specific IgM, IgG, and IgA also increased in the IM group but only after the booster dose. Although the analysis of IgG ₁ and IgG _{2a} subtypes in the serum and intestinal secretions lacked untreated/control mice, all methods of delivery appeared to increase anti-cMDLf IgG ₁ antibodies. Only IG and IM of cMDLf increased IgG _{2a} . Splenocytes harvested from DT and BD groups proliferated more in response to cMDLf and secreted more IFN γ , IL-4, and IL-5. Splenocytes harvested from the IG and IM groups proliferated more in response to cMDLf and secreted more IL-2, IFN γ , IL-4, and IL-5. cMDLf stimulated Peyer's patch cells from the DT, BD, and IG groups produced more IL-4 and IL-5. In response to polyclonal stimulus ConA, the splenocytes harvested from the DT, BD, IG, and IM groups proliferated more and secreted more IL-4. The IG and IM group also produced more IL-2. The Peyer's patch cells from the DT, BD, and IG groups secreted more IL-4 and IL-5. Splenocytes from the IG and IM group secreted more total IgA in response to cMDLf and those from the BD, IG and IM group secreted more IgG. LPS reduced total IgA production from only the splenocytes taken from the IG group and had no effect on the IgA production by other splenocytes or on IgG production. Peyer's patch cells from the IG and IM group produced significantly

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
000067			<p>more of IgG spontaneously than the BD and control groups and cMDLf treatment increased IgA production by the cells taken from the IG group. LPS treatment also increased the production of IgG from the IG and IM group. Total IgA and IgG production from splenocytes and Peyer's patch cells from either the DT and DK were not tested. This report suggests that mice fed bovine cMDLf develop a Th2 environment in the gut that presumably generates cMDLf-specific B cells that produce IgA, IgM, and IgG antibodies specific for cMDLf. Whether or not anti-cMDLf IgA, IgM, and IgG antibodies resulted in hypersensitivity to cMDLf was not addressed nor was the development of anti-cMDLf specific IgE antibodies. Also the general health of the mice was not noted.</p>
Takakura et al., 2004	Can oral administration of cMDLf prevent oral candidiasis in immunocompromised mice?	Oral candidiasis in mice was induced in mice (6 wk-old) by injecting prednisolone, infecting them with <i>Candida albicans</i> 1 day later, and then reinjecting prednisolone 4 d later. cMDLf was administered orally (0.5 g/kg/day continuously; Morinaga Milk Industry Co.) 1 day prior to inducing experimental oral candidiasis. The extent and severity of the infection was determined macroscopically. The cellular profile of the circulating leukocytes was determined by smears, and cervical lymphocyte cytokine production was quantified by flow cytometry.	Oral administration of cMDLf appeared to prevent the large decrease in peripheral blood leukocytes and cervical lymphocytes following the initial administration of prednisolone. After infection with <i>C. albicans</i> , cMDLf significantly inhibited number of viable fungi in the oral cavity and the tongue lesions. There were also small increases in the numbers of peripheral blood leukocytes and cervical lymph node cell and varied effects on the different cytokines produced by the cervical lymph node cells in the cMDLf -fed mice over the course of the infection but the significance of these differences is unclear because these mice all received an additional dose of prednisolone during the infection.

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Ishikado et al., 2005	Does liposomalization of cMDLf enhance its anti-inflammatory and immunomodulatory activities?	Prepared multi-lamellar liposomal cMDLf (Morinaga Milk Industry) and determined whether its liposomalization affected the prophylactic effect of orally administered cMDLf in CCl ₄ -induced hepatic injury and lipopolysaccharide (LPS)-induced TNF α production from peripheral blood mononuclear leukocytes (pBML). Its absorbability into the venous system, uptake into the thoracic duct, and antigenicity was also compared to cMDLf. CCl ₄ -induced hepatic injury was performed in rats by orally administering CCl ₄ , sacrificing the rats 24 h later, and quantifying the levels of glutamic pyruvate transaminase and glutamic oxaloacetate transaminase in the serum. cMDLf (300 mg/kg) was fed at 80, 56, 32 and 8 h prior to the CCl ₄ administration. The effects of cMDLf on LPS-induced TNF α production was assessed by feeding mice cMDLf or its liposomal derivative (300 mg/kg) once a day for seven d. PBML were then harvested and stimulated with LPS <i>in vitro</i> and the amount of TNF α in the supernatants was quantified by ELISA. The absorbability of cMDLf into the blood stream was determined by infusing cMDLf or its liposomal derivative into the duodenum of rats, collecting the venous blood from the jugular vein at different time points, and determining its concentration. cMDLf uptake by the lymph was determined by using a similar method but lymph fluid was collected from the thoracic duct. The antigenicity of cMDLf was determined by feeding it 5 d a wk for three wks to 6 wk-old guinea pigs (4.5 or 45 mg/body) or by injecting	Orally administered liposomal cMDLf reduced the levels of serum glutamic pyruvate transaminase and glutamic oxaloacetate transaminase in CCl ₄ -treated rats to a greater extent than cMDLf. Orally administered liposomal cMDLf also reduced the levels of TNF α secreted by LPS-stimulated PBMLs to a greater extent than cMDLf, indicating that liposomalization makes cMDLf more potent at inhibiting inflammation. However, liposomalization did not appear to affect the absorption of cMDLf into the blood stream or lymph. The antigenicity studies showed that liposomal cMDLf was just as antigenic as cMDLf when either fed orally or administered subcutaneously once a wk for three wks. It is noteworthy that the antigenicity of orally administered cMDLf was 10- to 100-fold less than if it was subcutaneously administered and 100- to 1000-fold less than ovalbumin if it was subcutaneously administered. These results indicate the liposomalization of cMDLf increases its prophylactic effects, does not increase its already weak antigenicity, and does not perturb its absorption.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
		it subcutaneously (4.5 or 45 mg/body) once a wk for three wk. The animals were then sacrificed and antibody titers were determined by passive cutaneous anaphylaxis using recombinant cMDLf or liposomal cMDLf (4.5 mg/body).	
Shin et al., 2005	Can orally administered cMDLf affect the progression of the influenza infection in mice?	0.5 ml of a 12.5% solution of cMDLf (Morinaga Milk Industry) or bovine lactoperoxidase was orally administered to BALB/C mice (6 wk-old) once daily by gavage (~62.5 mg/mouse) 1 day prior to infection and continued to 1 day prior to sacrifice. Mice were infected intranasally with 6.6×10^2 p.f.u. of the A/PR/8/34 strain of the influenza virus. Body weights were monitored daily and the number of bronchioalveolar lavage (BAL) cells, cytokines in the BAL fluid and serum, and viral particles were quantified.	Oral administration of cMDLf did not affect the wasting associated with the influenza infection. Although the oral administration of cMDLf did not affect the number viral particles in the BAL fluid, it appeared to reduce the severity of the infection as determined by the consolidation score, a previously described means of measuring the severity of associated pneumonia. cMDLf also significantly reduced the total number infiltrating BAL cells, including macrophages and neutrophils, at 6 d after infection.
Takakura et al., 2006	Determined the effects of cMDLf on lymphocytes harvested from the intraepithelial lining of the intestines and the mesenteric lymph node.	Orally administered cMDLf or bovine serum albumin (500 mg/kg/d) by intragastric intubation for 3 d to BALB/c mice (7 to 9 wk-old). Then isolated the lymphocytes from the intestinal intraepithelium or mesenteric lymph node and determined the cellular composition and cytokine profiles by flow cytometry and ELISA, respectively.	cMDLf did not dramatically affect the distribution of CD4 ⁺ , CD8 ⁺ , and $\gamma\delta$ T cells in either the intraepithelium or mesenteric lymph node. The absolute numbers of the different cell types was not calculated. Cultured lymphocytes harvested from the intestinal epithelium and mesenteric lymph nodes from the cMDLf-fed animals secreted more IFN γ and IL-10 spontaneously and they also secreted more IFN γ and IL-10 in response to T cell receptor crosslinking with agonistic antibodies. Although the time course for this study is relatively short and total cell suspensions were used, the results suggest that the oral administration of cMDLf induces the production of IL-10, which inhibits allergic and inflammatory events such as mast cell function, and IFN γ , which promotes cell-mediated immunity.

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Wakabayashi et al., 2006	Does orally administered cMDLf affect immune homeostasis?	Administered cMDLf (2.5 mg/g; 8.2% iron saturated; Morinaga Milk Industry) by gavage to mice (8-9 wk-old) and 24 h later analyzed the cellularity of the blood and spleen by flow cytometry. Also measured cytokine gene expression in the small intestine by quantitative RT-PCR.	cMDLf treatment caused a modest increase in the total numbers of circulating cells in the blood, which was accompanied by small but significant increases in the CD4, $\gamma\delta$, and granulocyte subtypes. In the spleen, cMDLf treatment caused a modest reduction in the total number of cells and CD4 ⁺ cells. Also found the cMDLf treatment increased the NOD, IFN β , IL-12p40 gene expression in the small intestine. Some of the effects reported in this paper are consistent with previous reports and some are not (i.e., the reduction in splenocyte cell numbers). However, the time course of cMDLf treatment is relatively short. Therefore, these effects are acute and not entirely relevant to understanding the allergenicity of bovine cMDLf in a diet.
Hartog et al., 2007	Does cMDLf have anti-inflammatory effects?	Administered cMDLf (0.1 to 25 mg/kg/day; 16% iron saturated; DMV International) by gavage and induced inflammation in the ear by injecting zymosan intradermally. The degree of inflammation was quantified by measuring the ear thickness. Also quantified the proinflammatory cytokine production (IL-6, IL-1 β , and TNF α) in the ear homogenates and from LPS-induced splenocytes harvested from the differently treated animals by ELISA. To corroborate their findings they also injected zymosan into the knee joints to induce inflammation.	Low doses of cMDLf (0.1 and 1.0 mg/kg) inhibited zymosan-induced ear inflammation whereas the high doses were ineffective. The anti-inflammatory activity of cMDLf correlated with reduced amounts of TNF α , IL-6, and IL-1 in the ear. cMDLf inhibited zymosan-induced TNF α production in the splenocytes that had been harvested from the different animals and stimulated with LPS-stimulated <i>in vitro</i> . Only the highest dose of cMDLf was able to significantly inhibit the joint swelling associated with zymosan injections. These results agree with the notion that administration of cMDLf into the gut inhibits/prevents inflammation.
Hwang et al., 2007	To examine the <i>in vitro</i> effect of cMDLf on cytokine production from activated and non-activated leukocytes.	Analyzed the production of cytokines from a variety of cell types (splenocytes, macrophages, purified CD4 ⁺ T cells etc.) stimulated with lipopolysaccharide (LPS) or Bacillus Calmette-Guerin (BCG) in the presence or absence of cMDLf (95% pure;	cMDLf increased TNF α production but not IL-12p40, IL-10 or IL-6 production from splenocytes; reduced IL-10, but did not affect IL-12p40, TNF α , or IL-6 produced from LPS-stimulated splenocytes. In macrophages, cMDLf reduced LPS-induced IL-10 and possibly IL-12p40 but the later was not dose

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
1200021		<20% iron saturated; PharmaReview Corporation).	dependent. cMDLf increased IL-12p40 and reduced IL-10 from BCG-stimulated bone marrow derived macrophages, and possibly increased the number IFN γ -producing BCG specific CD4 ⁺ T cells in the spleens of mice immunized with BCG although the differences were very small. These results suggest that cMDLf promotes the development of Th1/cell-mediated immune responses but the majority of the effects were observed <i>in vitro</i> and very marginal. Furthermore, the effects of cMDLf on the generation of BCG-specific CD4 ⁺ T cells was only observed at one time point following BCG infection, with only one immunizing dose of BCG. Other parameters such as sensitivity of the mice to the BCG infection were not analyzed or commented on.
Kobayashi et al., 2008	Does cMDLf have immunomodulatory activities on peripheral blood mononuclear cells (pBMC)?	PBMC were harvested from cats with and without feline immunodeficiency virus (FIV) and stimulated <i>in vitro</i> with concanavalin A (ConA) in the presence or absence of cMDLf (Morinaga Milk Industry). Proliferation, apoptosis, and IFN γ and IL-2 gene expression were then quantified.	cMDLf inhibited ConA-induced proliferation of PBMCs harvested from both uninfected and infected cats and prevented the upregulation of IFN γ and IL-2. cMDLf also appeared to prevent ConA-induced apoptosis, although it is difficult to conclude anything from this experiment because ConA, a known mitogen, did not induce cell cycle progression. This study suggests that cMDLf inhibits proliferation.

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Prgomet et al., 2007	Does orally administered cMDLf affect immune homeostasis in calves?	Calves were given <i>post natum</i> 3-2-1 colostrum 2-times a day for 6 d. On day three the cMDLf fed group received colostrum supplemented with 0.54 g cMDLf (DSM Nutritional Products) at each feeding until day 6. On day 6, the calves received non-medicated milk replacer (+/- 0.16% cMDLf), water, and hay. At wk 2-3 corn was also fed. Experiment was carried out for 10 wk. The animals were weighed and sacrificed and the amount of IgG in the colostrum and blood was measured, haematocrits and cell differentials in the blood were performed, the bacterial composition in the chyme from the colon was analyzed, histological evaluation of the intestine was performed, and genes expressed in the blood leukocytes and intestine were quantified.	Calves receiving the cMDLf -containing diet gained weight normally but had larger Peyer's patches and smaller villi in the jejunum and illeum. The levels of serum IgG rose initially after treatment with cMDLf and then returned to normal. Although there were acute increases in IL-8 and IL-10 (approximately 2-fold from 2 to 4 wk after cMDLf was introduced), the trend was that the diet containing cMDLf tended to reduce IL-10, IL-8, IL-1 β , and IFN γ expression in blood leukocytes. There were also varied effects of the cMDLf diet on the expression of the genes analyzed in the different parts of the intestinal tract (increased IL-10 and IL-6 in the omasum, reduced IL-6 in the illeum, and increased IFN γ in the abomasum). The cMDLf diet did not appear effect the types bacterial strains in the chyme of the colon. This study suggests cMDLf does not appear to significantly affect the development of calves. The allergenicity of cMDLf was not addressed.
Wilk et al., 2007	Does cMDLf activate antigen presenting cells?	Bone marrow macrophages were purified from mice that had been infected or not with Bacillus Calmette-Guerin (BCG) in the presence of cMDLf. BCG uptake, MHC class I, and MHC class II levels were quantified by flow cytometry. The effects of cMDLf on apoptosis and IL-12 and IL-10 secretion were quantified using the macrophage cell lines U937 and J774A.1.	cMDLf modestly increased BCG uptake and led to a greater increase in BCG-induced class II upregulation (approximately 1.5- to 2-fold). Class I upregulation was unaffected. cMDLf also modestly increased BCG-induced cell death and the IL-12:IL-10 ratio in U937 and J774A.1 cells, respectively. These results shows that cMDLf can promote the activation antigen presenting cells <i>in vitro</i> and suggests that it may be a good adjuvant.
Gaudin et al., 2008 000072	Characterized the spectrum of anti-cow's milk antibodies in patients with cow's milk allergy (CMA).	Used a protein microarray to quantify the amount of cow's milk protein-specific IgE antibodies in serum harvested from patients that presented with various symptoms of CMA. Although the CMA patients had milk-protein specific IgE antibodies, the exact criteria used to select patients with CMA was not reported.	IgE antibodies to bovine serum albumin (BSA), cMDLf, caseins, and other milk proteins were present in the CMA serum. Reactivity to the caseins and cMDLf was the greatest, whereas reactivity to BSA was less intense, and none of the sera contained IgE antibodies that were specific to β -lactoglobulin

Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
			or α -lactalbumin. Subsequent SPTs using purified components of cow's milk to corroborate the <i>in vitro</i> findings were not performed nor was the pathophysiological relevance of these antibodies to CMA addressed.
Handl et al., 2009	Does orally administered cMDLf affect immune homeostasis in beagle puppies?	Thirty-six beagle puppies were separated from their mothers three d after birth and subsequently fed a milk substitute with and without cMDLf (30, 60, and 120 mg/kg dry matter (DM)) every three h in the first wk and every four h in the second and third, four times daily in the fourth, and three times daily from wk five to wk eight. From day 32 on the dogs were also offered complete dry diet that had been sprayed with cMDLf at 0, 30, 60, and 120 mg/kg DM. After weaning at day 56, the dogs were exclusively fed the dry diet. Intestinal biopsies from the proximal duodenum and the proximal and middle colon were taken on day 14 and histologically scored for infiltrating lymphocytes in the villus and crypt structures of the duodenum, and for the presence of inflammation and disorganized architecture in the colon. Immunohistochemistry on the biopsies was also performed using antibodies that recognized IgA, IgG, IgM, CD3, CD4, and CD8 to enumerate infiltrating plasma cells and T lymphocytes.	Histologically, oral administration of cMDLf did not significantly alter the structure of the villus or crypts in the duodenum, nor did it cause any inflammation in the colon or alter the architecture of the colon. There was a small increase, although not significant, in lymphocytes and plasma cells in the mucosa of the group that received 30 and 60 mg/kg DM of cMDLf. The immunohistochemistry showed that orally administering cMDLf did not alter the numbers of IgA ⁺ or IgM ⁺ plasma cells in the duodenum and colon. IgG ⁺ cells tended to be lower (not significant) in the lamina propria at the villus bases of the duodenum and were significantly reduced in colon of the 30 and 60 mg group (30 and 25% respectively) but not in the 120 mg group. The distribution of CD3 ⁺ and CD4 ⁺ cells in the duodenum and colon was unaffected. Although the numbers of CD8 ⁺ cells in the lamina propria were unaffected by the treatment, the number of CD8 ⁺ cells in the intraepithelium of the colon were significantly increased (approximately 35 to 40%) in all the treated groups. This study suggests that although oral administration of cMDLf may cause shifts in cellular profiles in the duodenum and colon, it does cause any noticeable inflammation or alter the architecture of the colon.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Hellweg et al., 2008	Does oral administration of cMDLf have immunomodulatory effects in adult beagles?	Orally administered cMDLf to adult beagles (2.5 yr-old) for 3 wk (120 mg/kg or 1800 mg/kg diet) and evaluated the cellular composition of the blood and the proliferative capacity of the circulating lymphocytes. Also analyzed the fecal concentration of anaerobes and aerobes.	Oral administration of cMDLf did not affect the weight or general health of the dogs but led to a small and significant increase in the numbers of monocytes, CD4 ⁺ , and CD8 ⁺ cells. The peripheral blood mononuclear cells harvested from the cMDLf-fed animals also proliferated more in response to concanavalin A (ConA) at the highest dose tested. The allergenicity of cMDLf was not addressed.
Kanwar et al., 2008	Does cMDLf have anti-cancer properties?	8-9 wk-old mice were fed a diet containing cMDLf (11.6 g/kg of 100% saturated; 93% pure; prepared from skim milk; Fonterra Co-Operative Group). Tumors were then introduced subcutaneously into the left flank of mice and, once the tumors were established, the mice were injected chemotherapeutics. Tumor size, vascularity, and anti-tumor cytotoxicity were evaluated, leukocyte infiltration and cellularity of the lymphoid tissue was determined, and the cytokine and nitrile production in the small intestine was quantified.	Oral administration of cMDLf sensitized the tumors to chemotherapeutics and prevented the reduction in lymphocytes associated with the chemotherapeutic treatment. Control mice that receive cMDLf diet alone had splenic and lamina propria hypercellularity and Th1 and Th2 cytokines in the small intestine. cMDLf was also found to bind cells in the epithelium of the small intestine, i.e. the Peyer's patch and the lamina propria. This study shows that oral administration of cMDLf sensitizes tumors to chemotherapy, which may be due to its ability to prevent the death of lymphocytes associated with the administration of chemotherapeutics. Importantly mice fed the cMDLf diet alone had hypercellularity of the spleen and lamina propria, two results that are consistent with other reports. The allergenicity of the bovine cMDLf was not addressed.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Perez-Cano et al., 2008	Can oral administration of cMDLf ameliorate rotavirus-induced diarrhea?	The diet of newborn Lewis rats was supplemented with either 0.3 g/kg whey protein concentrate (WPC; contained 0.92% cMDLf), 0.3 g/kg WPC plus 0.1 g/kg cMDLf, or standard infant formula by oral gavage over the course of latency (d 3 to 21). On day 8, the rats were infected intragavagally with SA-11 rotavirus. The overall health and weight was monitored as was the incidence, period, and severity of diarrhea, and fecal viral load. A variety of immune parameters were also analyzed such as the amount of IFN γ in the intestinal mucosa, serum levels of anti-SA-11 IgG antibodies, the proliferation and IFN γ production by splenocytes activated with SA-11, and the composition of lymphocytes within the intraepithelial lining of intestine.	Although the differences were not significant, WPC and the WPC supplemented with the cMDLf appeared to reduce the severity and incidence of diarrhea. There was also a corresponding increase in the fecal viral load. cMDLf supplemented-WPC with the also reduced the amount of circulating anti-SA-11 antibodies, reduced the proliferation of and IFN γ production from SA-11 restimulated splenocytes <i>in vitro</i> , increased the amount of IFN γ in the intestine, and had varied effects of the lymphocyte populations in the intestinal mucosa. The significance of these findings, however, is unclear because uninfected controls that received the different supplements were not included. Otherwise, this study shows that cMDLf has prophylactic activities and can modestly reduce rotavirus-induced diarrhea in rats.
Schulmeister et al., 2008	Analyzed the specificity of anti-human milk IgE antibodies.	Determined the presence and reactivity of anti-human milk IgE antibodies in the sera of 17 patients (infants and adults) with established CMA. CMA was diagnosed by milk challenge, positive skin prick tests (SPTs), and the presence of anti-cow's milk IgE antibodies. The presence of anti-human IgE antibodies was determined by immunblotting human milk resolved by SDS-PAGE with the sera collected from the different patients. SPTs were also performed using human and other types of milk and the mean wheal diameter was determined.	Sera from all 17 patients with CMA reacted specifically with cow's milk and human milk, although reactivity to human milk was less than cow's milk. The sera were also tested against breast milk obtained from four genetically unrelated mothers to rule out alloreactivity and in all cases tested the sera reacted with the milk obtained from the different mothers. Competitive inhibition experiments revealed that the sera from one patient that contained anti-human and anti-cow's milk IgE specifically recognized both human and cow milk antigens with minimal cross-reactivity whereas others recognized the same antigens. Subsequent analyses revealed that the some of patients' sera cross-reacted to a-lactalbumin and casein. Cross-reactivity to cMDLf was not determined. Human milk was able to elicit positive SPTs in 2 individuals known to have anti-human milk IgE antibodies.

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Table 14. The Role of cMDLf in IgE and Non-IgE Mediated Hypersensitivity

Reference	Objective	Methods	Results
Mossallam, 2009	Determined the immune-potentiating effects of orally administered cMDLf in immunocompetent and immunosuppressed mice.	cMDLf was orally administered (1 mg/ml) on alternate d by a feeding syringe for 14 d (7 doses) to immunocompetent and immunosuppressed mice (swiss albino mice 4 to 6 wk-old), which received a single injection of cyclophosphamide (250 mg/kg) to induce lymphopenia. The mice were then infected with <i>Toxoplasma gondii</i> (<i>T. gondii</i>) and the mortality of the mice, and the numbers, viability, and infectivity of the tachyzoites, and the numbers of CD4 ⁺ T cells in the spleen were quantified.	In uninfected immunocompetent mice, oral administration of cMDLf did not induce morbidity and led to approximately 2-fold increase in numbers of splenic CD4 ⁺ T cells. In <i>T. gondii</i> -infected immunocompetent mice, oral administration of lactoferrin reduced <i>T. gondii</i> -induced morbidity from 80% to 5%, increase splenic CD4 ⁺ T cells 2-fold, and significantly reduced the overall numbers and viability of the tachyzoites. cMDLf treatment in uninfected immunocompromised animals significantly increased the numbers of CD4 ⁺ T cells. In <i>T. gondii</i> -infected immunocompromised mice, the oral administration of cMDLf also increased the numbers CD4 ⁺ T cells, reduced morbidity, and significantly reduced the overall number and viability of tachyzoites. This study shows that cMDLf facilitates the immune response to <i>T. gondii</i> , possibly by increasing the numbers of CD4 ⁺ T cells. Importantly and with regard to the allergenicity of cMDLf, cMDLf alone increased the numbers of splenic CD4 ⁺ T cells but did not induce morbidity, suggesting that it was not toxic to the mice.

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4. AUTOIMMUNITY

Autoimmunity results from a breakdown of self-tolerance because self-antigens, once regarded as safe, are recognized by the immune system as foreign and an immune response ensues (Janeway et al., 2005). Unfortunately, because self-antigens are ubiquitous, they are never completely eradicated. The result is a chronic inflammatory state whereby the immune system continues to respond to the never-ending supply of antigen. Although it is not known what causes autoimmunity, it is clear that genetics and environmental factors play important roles. Interestingly, evidence from animal and human studies has suggested that oral administration of antigens involved in autoimmunity may be therapeutic (Weiner et al., 2011; Mayer and Shao, 2004; Faria and Weiner, 2005).

Antibodies to human lactoferrin have been found in the serum of patients with autoimmune/chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, reactive arthritis, ulcerative colitis, and autoimmune pancreatitis (Nässberger et al., 1994; Penco et al., 1998; Lochter et al., 2000; Wong et al., 2009; Okazaki et al., 2000; Taniguchi et al., 2003), and, although orally administered cMDLf and human lactoferrin are well tolerated, they are immunogenic (Brock et al., 1997; Debbabi et al., 1998; Miyauchi et al., 1997; Sfeir et al., 2004). In a study conducted by Brock et al. (1997) it was found that infants fed cMDLf-supplemented formula developed anti-cMDLf antibodies, those exclusively breast-fed developed anti-human lactoferrin antibodies, and autoimmune adults had antibodies that recognize both human and cMDLf. Thus, it was proposed that bovine lactoferrin may sensitize the immune system to human lactoferrin and initiate autoimmunity. These findings have not been corroborated and, considering that lactoferrin is immunogenic and its blood levels rise during inflammation and infection, it is not surprising that individuals with autoimmune diseases have high titers of anti-human lactoferrin antibodies. The presence of antibodies that recognize cMDLf, however, is notable but could be explained by antibody cross-reactivity because of the high degree of homology between cMDLf and human lactoferrin. Unfortunately, this possibility was not ruled-out in this report and thus, the validity of these findings is questionable. In addition, it is conceivable that the anti-cMDLf antibodies found in these patients resulted from the breakdown in tolerance because autoimmunity is a breakdown in tolerance. The authors did note that antibodies to β -lactoglobulin were not present in the sera of the autoimmune patients, but this protein represents one of many present in cow's milk. Importantly, an extensive literature search has not revealed any subsequent reports showing a causal link between the consumption of cow's milk or cMDLf and the pathogenesis of these autoimmune/chronic inflammatory states.

An accumulating body of evidence has also suggested that the consumption of cow's milk during infancy is one factor in the development of type 1 diabetes (T1D). Type 1 diabetes, also known as diabetes mellitus type 1, IDDM, or juvenile diabetes, is an autoimmune disease whereby the body mistakenly destroys the β -islet cells of the pancreas resulting in insulin deficiency.

The cause of T1D is largely unknown, but as an autoimmune disorder, it is thought to depend on genetics and a variety of environmental factors. The environmental factors include dietary components, such as cow's milk and gluten consumption during infancy, viral infections, certain chemicals and drugs, and even the environment itself. Genetics is the greatest contributing factor with approximately 100% of affected individuals carrying at least one of the now 26 susceptibility loci (reviewed in Knip et al., 2005; Bluestone et al., 2010). However, among the genetically predisposed, the likelihood of developing this disease is approximately five percent and among genetically predisposed identical twins, the concordance ranges from 30 to 70% (Knip et al., 2010a; Kasper and Harrison, 2005).

In 1984 a case control study of diabetic children found that there was an inverse relationship between the duration of breastfeeding and the risk of developing type 1 diabetes (Borch-Johnsen et al., 1984). These findings were later confirmed by a study of Finnish children also diagnosed with T1D (Virtanen et al., 1991). Since then, numerous studies have been conducted, but a role for the consumption of cow's milk and/or cow's milk products by normal infants in the pathogenesis of disease is still unclear. Recent evidence from the Trial to Reduce IDDM in the Genetically at Risk (TRIGR) suggests that the consumption of cow's milk products, as opposed to hydrolyzed cow's milk products, by genetically predisposed infants may accelerate the onset of disease (Knip et al., 2010b). There are no studies addressing a specific role for cMDLf in promoting the development of T1D in normal and/or genetically predisposed individuals.

5. EFFECTS ON BONE

Although human studies on the effects of cMDLf on bone structure and turnover rate are rare in the literature, *in vitro* and *in vivo* studies have been carried out which provide some insight into the potential biological mechanisms of action of this protein. Some cell culture experiments indicate an inhibitory effect of cMDLf on osteoclasts (Cornish et al., 2004; Blais et al., 2009; Yamano et al., 2010) which can actively resorb bone, plus multiple stimulatory effects of cMDLf on osteoblasts (Cornish et al., 2004; Cornish et al., 2006; Takayama and Mizumachi, 2008; Blais et al., 2009) which promote bone growth. Ovariectomized rodent models of bone growth and resorption *in vivo* have also reported positive results for orally administered cMDLf

(Guo et al., 2009; Blais et al., 2009). One published investigation of the effects of cMDLf on bone turnover in postmenopausal women reported positive results on certain serum and urine markers (Bharadwaj et al., 2009).

a) *In vitro* effects on bone cell cultures

In cell culture, cMDLf is reported to exert various effects on bone cells, including the dose-dependent increase of thymidine incorporation into primary cultures of human osteoblasts at concentrations ranging from 10-1000 $\mu\text{g/mL}$ ($p < 0.05$) (Cornish et al., 2004), indicating a growth stimulatory effect on these bone-building cells. In this study, cMDLf was isolated from fresh milk and purified to $\geq 98\%$ purity, as evaluated by high-performance liquid chromatography (HPLC). This cMDLf preparation, at 100 and 1000 $\mu\text{g/mL}$ ($p < 0.05$), also stimulated bone nodule formation and mineralization in primary cultures of rat osteoblast-like cells during three wks in culture and also inhibited tartrate-resistant acid phosphatase expression in mouse bone marrow osteoclasts at 10-100 $\mu\text{g/mL}$ ($p < 0.05$), a measure of osteoclast activity. However, cMDLf did not affect the ability of isolated mature osteoclasts to cause resorption pits at concentrations up to 100 $\mu\text{g/mL}$. This differential effect of cMDLf on newly formed versus mature osteoclasts may suggest that cMDLf is only effective in actively dividing cells; however, this finding needs to be confirmed. cMDLf at 10 and 100 $\mu\text{g/mL}$ prevented serum starvation-induced apoptosis of primary rat osteoblast-like cells ($p < 0.05$), suggesting a protective effect that could occur via many potential mechanisms. In a similar study published by the same authors (Cornish et al., 2006), the effect of various cMDLf preparations on primary rat osteoblast-like cells was studied. The authors isolated cMDLf from fresh skim milk and refined it to a final purity of $\sim 98\%$ via HPLC; from this starting material, a deglycosylated cMDLf was produced enzymatically, as were both an apo- and holo-cMDLf. Full-length native cMDLf and deglycosylated cMDLf significantly ($p < 0.05$) and dose-dependently stimulated the growth of primary rat osteoblast-like cells, as measured via thymidine incorporation into the cells. The degree of iron-loading of cMDLf is apparently not involved in the growth stimulatory effect on these cells, as apo- and holo-cMDLfs exhibited equivalent potencies in this regard ($p < 0.05$ for a significant growth stimulatory effect by both proteins, versus control). Although full-length cMDLf has the most potent ability to stimulate growth of primary rat osteoblast-like cells in culture, the N-lobe, C-lobe, and various lactoferricin internal protein fragments also retain some of this biological activity, suggesting that cMDLf and its various protein fragments may operate via diverse pathways, possibly interacting with multiple target receptors. Additionally, this evidence suggests that proteolytic break down products of cMDLf in the gut may be able to stimulate bone growth *in vivo*. Another study by different authors (Takayama and Mizumachi, 2008) tested the effect of cMDLf (purity unstated; source: Fonterra, Auckland, New Zealand) on

MG63 human osteoblast-like cells cultured on collagen-coated plates. Addition of cMDLf at 1 μ M in culture caused a significant ($p < 0.01$) increase in both calcium deposition and osteocalcin production at three wks' culture, versus untreated cells. At two and three wks in culture, 1 μ M cMDLf also statistically significantly ($p < 0.01$) increased alkaline phosphatase activity of the MG63 human osteoblast-like cells. However, cMDLf had no effect on the growth of these cells, via measurement of cellular metabolic activity using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent. Yamano and co-workers report that cMDLf at 1 and 10 μ g/mL (purity unstated; source: Morinaga Milk Industry, Tokyo, Japan) tended to inhibit tartrate-resistant acid phosphatase in mouse preosteoclasts and osteoclasts, although only 10 μ g/mL had statistically significant results in osteoclasts ($p < 0.05$) (Yamano et al., 2010). Taken together, these *in vitro* results indicate that cMDLf can stimulate the growth and matrix deposition activity of some osteoblast populations and inhibit either the function or growth of osteoclasts, which are considered beneficial effects.

b) *In vivo* bone effects

Ovariectomized rodent models (*e.g.*, rat, mouse) are used in the study of agents thought to exert an effect on bone loss in the context of osteopenia, which is demineralization and loss of skeletal bone due to the withdrawal of endogenous estrogen by removal of the ovaries. This model mimics the postmenopausal human state and is used to evaluate substances that may exert a protective effect that results in sparing of bone demineralization and loss over time. Guo and co-workers reported results from seventy three-month-old virgin female Sprague-Dawley rats that received either ovariectomization or sham operation and were then randomized to the following treatments (n=10/group): 1) untreated sham operated, 2) untreated ovariectomized, 3) ovariectomized plus serum albumin (BSA; protein control, 85 mg/kg body weight (bw)), 4) ovariectomized plus 0.85 mg cMDLf/kg bw, 5) ovariectomized plus 8.5 mg cMDLf/kg bw, 6) ovariectomized plus 85 mg cMDLf/kg bw, and 7) ovariectomized plus estradiol (positive control) (Guo et al., 2009). cMDLf was 95% pure and 20% iron-saturated (source: Australian Yosica Holding). cMDLf and BSA were dissolved in physiological saline and given as a single oral dose every day for three mo after ovariectomization. Sham and ovariectomized untreated rats were orally intubated with 1 mL of 0.9% physiological saline/kg body weight daily. The positive control group received 10 μ g 17 β -estradiol (in corn oil)/kg body weight intraperitoneally every other day. After three mo of treatment, serum was collected for analysis and the uterus, femur, tibia and vertebrae were collected. cMDLf did not exert an effect on uterine weight at any of the three doses tested, versus either the untreated ovariectomized rats or ovariectomized rats receiving BSA, indicating that cMDLf does not demonstrate estrogenic activity *in vivo*. cMDLf, at 8.5 and 85 mg/kg/d for 3 mo, significantly prevented loss of bone

mineral density in the proximal femur and L₂₋₅ vertebra ($p < 0.05$), versus untreated or BSA-treated ovariectomized rats. The maximum load of the L₅ vertebra and the femur was also preserved, versus untreated or BSA-treated ovariectomized rats ($p < 0.05$), at these two doses of cMDLf. Serum calcium levels decreased with increasing dose of cMDLf, possibly due to the incorporation of calcium into newly forming bone. This possibility is supported by the concomitantly observed elevation in serum osteocalcin, a marker for bone formation, in the cMDLf-treated groups compared with the other treatment groups.

Blais and co-workers isolated cMDLf from fresh skimmed milk using cation exchange chromatography and purified it to $> 98\%$ by HPLC, and administered it in the diet of twelve-wk-old female C3H ovariectomized mice for 27 wks (Blais et al., 2009). One wk after surgery, the ovariectomized mice were divided into five groups ($n=8/\text{group}$): 1) control diet (included 140 g total milk protein/kg of diet), 2) 1 g cMDLf/kg diet (0.1%, w/w), 3) 5 g cMDLf/kg diet (0.5%), 4) 10 g cMDLf/kg diet (1.0%), and 5) 20 g cMDLf/kg diet (2.0%). Total protein was kept constant across all the diets as 140 g of milk-derived protein/kg diet. Diets were fed for 27 wks, and bone mineral density was measured at 9, 17, and 27 wks. Serum levels of cMDLf exhibited a dose-dependent increase across the groups at two mo, indicating it was at least partially absorbed intact after oral administration. Specifically, serum levels of cMDLf in mice consuming diets containing 0.1, 0.5, 1.0, and 2.0% (w/w) were approximately 0.1, 0.5, 0.9, and 1.45 $\mu\text{g/mL}$, respectively, indicating a dose-related increase in the absorption and systemic availability of cMDLf in mice. Bone mineral density in the femur and lumbar vertebrae, plus calcium content of the left femur, were measured at 27 wks; the maximum break load and yield load for the right femur were also determined. Ovariectomized mice receiving 0.5, 1.0, or 2.0% (w/w) cMDLf in the diet maintained femur bone mineral density, versus untreated ovariectomized mice (statistically significant, $P < 0.05$). The ovariectomized group receiving 2.0% cMDLf in the diet exhibited a statistically significant ($p < 0.05$) increase in femur bone mineral density versus the sham-operated controls, which suggests that cMDLf can improve bone mineral density even in non-estrogen-depleted states in mice. The 0.5, 1.0, and 2.0% cMDLf groups also maintained calcium content of the left femur, versus untreated ovariectomized controls ($p < 0.05$). However, only the 1.0 and 2.0% cMDLf groups maintained vertebral bone mineral density, versus untreated ovariectomized controls ($p < 0.05$). In contrast, the maximum break load and yield load of the right femur was maintained in all cMDLf groups, versus the untreated ovariectomized control group ($p < 0.05$). The effect of dietary cMDLf is both dose- and time-dependent, with higher doses manifesting results earlier on, such as prevented loss of whole body bone mineral density, where a protective effect was observed as early as 9 wks in the 1.0 and 2.0% cMDLf dietary groups, versus untreated ovariectomized controls ($p < 0.05$). Although this study did not include a positive control (ovariectomized) group receiving estradiol, as the Guo study (2009)

did, its results were indicative of a positive effect of dietary cMDLf on maintaining various physical strength and mineral parameters of bone in ovariectomized mice. Additionally, this study was valuable in that it allows correlation of effective dietary doses ($\geq 0.5\%$ cMDLf, w/w) with the physiologically achieved serum levels of cMDLf ($\geq 0.5 \mu\text{g/mL}$) in mice.

Cornish and co-workers demonstrated a direct effect of cMDLf on new bone formation in both neonatal and adult mouse calvariae (Cornish et al., 2004). Direct injection of 4 mg cMDLf over the right hemicalvaria of adult male mice for five consecutive d caused increased new bone growth as observed via fluorochrome labeling experiments for calcein and alizarin that were carried out ten d later; specifically, new bone marrow formation was observed within the recently formed bone. A dose-dependent response was observed in the 0.4 and 4.0 mg cMDLf treatment groups, versus the control and 0.04 mg groups ($p < 0.05$), for the depth of new bone formed ten d after the last injection of cMDLf. cMDLf at 100 $\mu\text{g/mL}$ also significantly increased thymidine incorporation into neonatal mouse calvariae ($p < 0.05$), indicating a growth stimulatory effect on these cells. The cMDLf used in this study was isolated and purified by the study authors from fresh milk and was reported to be $\geq 98\%$ pure by HPLC.

The only published study documenting the bone-related effects of orally administered cMDLf in humans was carried out by Bharadwaj and co-workers, who measured the effect of ribonuclease-enriched cMDLf on bone turnover markers in postmenopausal women (Bharadwaj et al., 2009). The ribonuclease (angiogenin)-enriched cMDLf used in the study was either co-isolated from milk or separately admixed to a 1:1 ratio of angiogenin:cMDLf; therefore the purity of this preparation is estimated as $\sim 50\%$ cMDLf. The researchers enrolled 35 healthy, ambulatory postmenopausal women aged 45- to 60-yr old who had no menses for at least 12 mo, and randomized them into one of two groups: 1) 250 mg ribonuclease-enriched cMDLf/day ($n=20$; estimate 125 mg cMDLf/d) or 2) control capsules ($n=15$) for 180 d. All subjects received an oral calcium supplement providing 100% of the Recommended Daily Allowance (RDA) for calcium. Blood and urine samples were collected throughout the study. At the study's end, the percent change from baseline for the median levels of bone resorption markers (serum N-telopeptides, NT_x , and urine deoxypyridinoline, Dpd) were significantly decreased ($p < 0.001$ and < 0.01 , respectively) and the percent change for bone formation markers (serum bone-specific alkaline phosphatase, BAP, and urine osteocalcin) was significantly increased ($p < 0.001$ and < 0.001 , respectively), which are considered beneficial effects in postmenopausal women. However, it is not possible to attribute these activities solely to the cMDLf portion of the administered protein formulation as it also contained $\sim 50\%$ angiogenin. Future studies utilizing cMDLf preparations of higher purity will aid in establishing whether oral administration of this protein is useful in maintaining bone mineral density in humans.

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c) Conclusions

From analysis of published studies, it was noted that the concentrations of cMDLf that are reported to elicit biologically meaningful effects in human cells *in vitro* in the Cornish et al. (2004) (10-1000 µg/mL) and Takayama and Mizumachi (2008) (1 µM; approximately 80 µg/mL, based on 80 kDa molecular mass for cMDLf) studies are much higher than the biologically effective *in vivo* serum cMDLf levels reported in the Blais et al. (2009) mouse study (0.5, 0.9, and 1.45 µg/mL in serum), indicating that additional studies of orally administered cMDLf (dietary and gavage) are necessary. Yamano and co-workers (2010) report that 10 µg/mL of cMDLf caused a statistically significant decrease in enzyme activity of mouse osteoclasts, which is also higher than the biologically active serum levels that were reported in the Blais et al. (2009) mouse study (≥ 0.5 µg/mL). The two published *in vivo* studies on the effects of cMDLf on bone strength and mineralization in the context of estrogen-depletion have reported a positive effect at a minimum dose of 8.5 mg cMDLf/kg/d in ovariectomized rats for three mo duration (Guo et al., 2009) and at a minimum of 0.5% (w/w) in the diet of ovariectomized mice for 27 wks (Blais et al., 2009). Additionally, preliminary results on the effects of orally administered cMDLf on bone turnover markers in postmenopausal women are favorable, but future studies are needed that utilize more highly purified preparations in order to more clearly define the role of exogenously administered cMDLf on the maintenance of bone mineral density.

C. ANIMAL TOXICOLOGY STUDIES

The safety of cMDLf produced by Morinaga Milk Industry Co. was evaluated in an acute toxicity study, a four-wk oral toxicity study, a thirteen-wk oral toxicity study, a chronic oral toxicity study and an Ames assay. Morinaga Milk Industry Lot no. MLF160996 with reported protein purity of 95.0% as determined by Kjeldahl and HPLC with iron content of 14.7 mg/100 g powder as determined by atomic absorption spectrometry was used in the thirteen-wk study and a chronic oral toxicity study. The lot number for cMDLf used was not cited. cMDLF was not acutely toxic or genotoxic in these assays. cMDLf administered by oral intubation to rats for 13 wks did not result in toxicologically significant treatment-related changes in the appearance, general condition, body weight, feed consumption, ophthalmology, hematology, blood chemistry, gross pathology, or histopathology of the animals. Thus, under the conditions of this study, the no-observed-adverse-effect level (NOAEL) of cMDLf was estimated to be in excess of 2,000 mg/kg/day. The chronic toxicity study was not available as a full report and therefore without a full data set, could not be used to derive a NOAEL.

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1. ACUTE TOXICITY STUDY IN RATS

Nishimura (1991, as cited in GRN 77, and Yamauchi et al., 2000b) evaluated the acute toxicity of cMDLf in rats. Male and female Crj:CD(SD) SPF rats were exposed to single oral doses of 1,000 or 2,000 mg/kg cMDLf (MONL-01) or iron-saturated cMDLf (MONL-02) via stomach intubation. Control animals received vehicle alone (2,000 mg/kg water). Animals were observed for mortality, clinical signs, and any changes in general condition during a 14-day observation period following administration. Body weights were measured prior to the study and periodically throughout the observation period. After 14 d, the animals were euthanized and the organs examined macroscopically for any abnormalities.

Exposure to 1,000 and 2,000 mg/kg MONL-01 or MONL-02 resulted in no deaths or abnormal clinical signs or effects on the general condition of the animals and there were no significant differences in body weights throughout the study period in treated animals compared to controls. No abnormal gross pathological findings were observed in any organ in the cranial, thoracic, and abdominal cavities. A single oral dose of 1,000 or 2,000 mg/kg cMDLf or iron-saturated cMDLf resulted in no adverse effects or deaths. Based on these results, the lethal dose of cMDLf exceeds 2,000 mg/kg.

2. FOUR-WEEK ORAL TOXICITY STUDY IN RATS

The safety of cMDLf was assessed in a four-wk oral toxicity study in rats (Nishimura 1997, cited in GRN 77 as an unpublished report, and Yamauchi et al., 2000b). Four-wk-old male and female Sprague-Dawley rats were exposed by oral intubation to 200, 600, or 2,000 mg/kg/day bovine cMDLf once daily for 4 wks (28 d). Animals in the control group received water by the same route of administration. Animals were observed daily for any changes in appearance or behavior. Body weight and feed consumption were measured prior to the start of treatment and twice weekly every 3 or 4 d prior to dosing. Ophthalmology, urinalysis, hematology, and blood chemistry analyses were conducted in wk 4 or at necropsy. Animals in each group were euthanized on day 29 and observed for external abnormalities. Absolute and relative weights were determined for all organs and tissues in the cephalic, thoracic and abdominal cavities. Organs and tissues of animals in the control group and high-dose group were examined histopathologically.

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There were no deaths or changes in the general condition, behavior or appearance of the animals due to administration of the test article. Body weight and feed consumption were similar in all groups throughout the study; no significant differences were observed between groups. No changes in males or females or significant differences between test and control groups were observed in urinalysis (pH, protein, ketone body, glucose, occult blood, bilirubin, urobilinogen,

color, urinary sediments, 24-hour urine volume, osmolarity, sodium, potassium, chloride, or water intake); hematology (red cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, platelet count, white blood cell count, differential leukocyte count, prothrombin time, activated partial thromboplastin time, or fibrinogen), or blood chemistry parameters (GOT, GPT, LDH, ALP, total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, A/G ratio or protein fractions).

Absolute and relative body weights did not change significantly throughout the treatment period or differ significantly between groups. Unilateral and/or bilateral persistence of the hyaloid artery in the eye was observed in at least one animal in each group, including the controls group. This effect was not considered treatment-related or abnormal because it occurs naturally during the development of the eyeball and disappears with growth. Gross pathological findings observed included excoriation in the neck of 2 males and 1 female in the 200 mg/kg/day group and 1 male in the 600 mg/kg/day group, pneumatosis-like enlargement of the lung in 1 male in the 200 mg/kg/day group, a dark-red spot in the lung of 2 males in the 2,000 mg/kg/day group, and dark-red spots in the glandular stomach in 1 male and 1 female in the control group and 1 female in the 600 mg/kg/day group. Corresponding macroscopic findings were mild ulcer, mild over-inflation of the lung, and slight erosion of the stomach, respectively. One female administered 600 mg/kg/day had a fracture of the incisors. These effects are considered incidental since they were not dose-related or consistently observed among the animals. A few microscopic changes were observed in male and female animals that were not considered to be treatment-related. These were cellular infiltration and focal hemorrhage of the lung; erosion in the glandular stomach; cellular infiltration of the cecum; microgranuloma in the liver; ectopic thymus; tubular basophilia, eosinophilic body in tubular epithelium, and cellular infiltration in interstitium of the kidney; degeneration and necrosis of spermatocyte; decrease of sperm in the epididymus duct; fibrosis in the muscle layer of the esophagus; hyperplasia of ductal epithelium in the sublingual gland; and disarrangement of the retina. These changes, which were slight to mild in severity, occurred sporadically in one or two animals, and were considered incidental.

Administration of 200, 600, and 2,000 mg/kg/day cMDLf to male and female rats resulted in no deaths or treatment-related changes in body weight, feed consumption, organ weight, ophthalmology, hematology, blood chemistry, urinalysis, or gross; pathology and histology examinations. Therefore, the NOAEL of cMDLf was estimated to be in excess of 2,000 mg/kg/day.

3. THIRTEEN-WEEK ORAL TOXICITY STUDY IN RATS

Repeat dose toxicity of cMDLf was investigated in a 13-wk oral toxicity study in rats (Yamauchi et al., 2000b and Nishimura 1997, cited in GRN 77 as an unpublished report). Groups of 12 male and 12 female Sprague-Dawley rats, 4 wks of age, were exposed to 200, 600, and 2,000 mg/kg/day cMDLf by oral intubation once daily for 13 wks. Control animals received vehicle (water) alone. Animals were examined daily for changes in appearance, nutrition condition, or behavior. Body weight and feed consumption measurements were taken prior to the start of treatment and twice weekly every 3 or 4 d prior to dosing. At the final wk of the study, ophthalmology examination and fasting urinalysis (4-hr urine sample; one day's water consumption was calculated at the same time), fasting (16-hour) hematology (mean corpuscular volume, red cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte ratio, platelet count, white blood cell count, differential leukocyte count, prothrombin time, activated partial thromboplastin time, or fibrinogen), and fasting (16-hour) blood chemistry determinations (glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, blood urea, nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin:globulin (A/G) ratio or protein fractions) were completed (urinalysis was also done at wk 6). Animals were euthanized after 91 d and observed for external abnormalities. Absolute and relative weights were determined for all organs and tissues in the cephalic, thoracic, and abdominal cavities. Histopathological examinations were conducted of all organs and tissues in the control and high-dose animals, the pancreas of males at all dose levels, and in any animal that died or exhibited macroscopic lesions.

There were two deaths during the treatment period. One male in the 200 mg/kg/day group died at wk 10 without any overt clinical signs of disease. Examination revealed perforation in the esophagus and hydrothorax with feed. The death was attributed to an error in intubation. One female in the 2,000 mg/kg/day group exhibited swelling of the subcutis in cervical, axillary and inguinal regions at wk 12 and died at wk 13. At necropsy, enlargement of the lymph nodes, thymus, spleen, and liver and white spots in the kidney were observed. Upon histologic examination, malignant lymphoma and tumor cells in the brain, harderian gland, heart, lung, pituitary, adrenal, ileum, cecum, ovary, uterus and bone marrow were observed. Slight extramedullary hematopoiesis was seen in the adrenal gland. Death of the animal was attributed to the presence of malignant lymphoma not related to test article administration.

000086

No abnormal clinical signs due to administration of cMDLf were seen in surviving animals. However, a few clinical signs deemed incidental because of their sporadic occurrence were observed. These were subcutaneous mass in the axillary mass accompanied by hemorrhage and paleness of skin in 1 male in the control group, fracture of incisors in 1 female in the control group, and excoriation in the neck in 1 female in the 200 and 600 mg/kg/day groups and 2 females in the 2,000 mg/kg/day group. One female in the 2,000 mg/kg/day group had decreased spontaneous movement and oligopnea. Perforation in the esophagus, hydrothorax, and dark-reddening of the lung was observed at necropsy suggesting that the effects were due to an error in administering the compound.

No significant differences were observed in body weight or feed consumption between groups during the treatment period. No ophthalmologic abnormalities were observed in any animal. Hematology and blood chemistry determinations did not indicate any statistically significant differences in any of the parameters between the control and test groups. No test article related statistically significant changes were seen in the urinalysis parameters at wks 6 or 13 with the exception of lowered urinary pH in males and females of the 2000 mg/kg group. At wk 13, significant increases in urine volume and daily excretion of sodium, potassium, and chloride in males from the low-dose group only were noted. These changes, however, are not considered test related because they occurred in only one gender and were not dose-related. The change in urinary pH, while possibly related to test article administration, is not thought to be of toxicological significance because there were no significant changes in any other urinary parameters such as volume and content of electrolytes. In addition, there were no corresponding adverse findings in the kidney or blood chemistry values.

No changes in absolute or relative organ weights were observed in males or females administered 200 or 600 mg/kg/day. At the highest dose administered, significant decreases in absolute and relative thyroid weights compared to controls were observed in females only ($p \leq 0.05$). These changes were not considered to be test article related since they were slight, observed in only one sex and no corresponding findings of toxicity were observed upon histopathological examination.

Gross pathology findings in a few animals and histopathologic changes in several organs were noted but these findings were not consistently observed among animals or were also noted in control animals. No histopathological findings were considered to be treatment related, but were judged incidental in view of their occurrence and the nature of the lesions. Slight or mild islet fibrosis of islet acinar cells was observed in 3/12 males in the control group and 7/12, 6/12 and 6/12 males each in the 200, 600, and 2,000 mg/kg/day groups, respectively. This finding was not seen in females. Although the incidence and severity of the finding in each treated group was

higher than that of the controls, these findings were not considered to be treatment related. No morphological differences in the fibrosis of islets between the control and treated groups were observed and the distribution of lesions in animals in the treated groups, were limited to the same small section of tissue as in the control animals. In addition, an age-dependent increase in the incidence of non-neoplastic pancreatic islet lesions, particularly fibrosis and hyperplasia, in male Sprague-Dawley rats has been noted in the literature (Imaoka et al., 2007). Incidence of fibrosis in pancreatic islets of male Sprague-Dawley rats fed standard diet at 8, 12, 18, and 26 wks of age was 0/20, 3/20, 14/20 and 18/20, respectively. In females, the incidence was 0/20, 1/20, 0/20 and 4/20 for the respective time points.

cMDLf administered by oral intubation to rats for 13 wks did not result in toxicologically significant treatment-related changes in the appearance, general condition, body weight, food consumption, ophthalmology, hematology, blood chemistry, gross pathology, or histopathology of the animals. Thus, under the conditions of this study, the NOAEL of cMDLf was estimated to be in excess of 2,000 mg/kg/day.

4. CHRONIC ORAL TOXICITY STUDY IN RATS

In a research communication (Tamano et al., 2008), results of long-term feeding studies of cMDLf in two experiments were reported. Male and female F344/DuCrj (F344) rats, 5 wks of age were purchased. In Experiment 1, starting at 6-wks-of-age, groups of 15 male rats were given a diet containing 0% (control) or 0.2% cMDLf for 40 wks. Animals were observed for general condition every day and weighed once wkly for the initial 4 wks and once every 4 wks thereafter. Feed consumption by cage was performed at the same time as body weight measurement. Test material intake was calculated. At the end of treatment, all animals were fasted overnight and in the morning, whole blood samples were collected by determination of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltranspeptidase, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, total cholesterol, triglyceride, total protein, albumin and serum iron. Gross inspections for any lesions were made at necropsy and liver, kidneys and spleen were weighed and relative organ weights calculated. In Experiment 2, starting at 17 (males) or 11 (females) wks of age, groups of 25 rats (groups 1 and 5) and 10 rats (groups 2, 3,4) of each sex were given diet with 0, 0.02, 0.2, 2.0, and 5.0% cMDLf (groups 1-5, respectively) for 60 wks in males and 65 wks in females. The animals were observed for general condition daily and weighed 8 times during the study. Measurement of feed consumption and water intake by cage were performed once every 2 wks for the first 16 wks and once every 4 wks thereafter. Test material intake was calculated for each group. Gross examination was done at necropsy. Liver, kidney, spleen, adrenals and pituitary were weighed for each animal. Samples of these organs, thymus, lungs, salivary glands, esophagus, stomach, duodenum, jejunum ileum,

000088

cecum, pancreas, urinary bladder, testes, prostate, seminal vesicle, ovaries, uterus, vagina, spinal cord and grossly visible lesions were fixed and processed for histopathological examination.

In Experiment 1, no adverse treatment-related clinical signs, effects on body weight, or macroscopic changes were reported (data not shown). Slight but significantly decreased relative (but not absolute) liver weights were reported (data not shown). Selected blood biochemistry data were reported: AST, ALT, ALP, BUN and TG were significantly lower in the 0.2% group compared to control. No treatment-related histopathological lesions were observed (data not shown).

In Experiment 2, no adverse clinical signs or deaths were reported during the study period. No adverse effects on body weight, feed or water consumption was reported (data not shown). There were no significant treatment-related adverse effects on final body weight, organ weight, gross or histopathology reported (data not shown).

5. GENOTOXICITY

The genotoxic potential of cMDLf was examined in the reverse mutation assay (Yamauchi et al., 2000b). The assay was performed using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537 and *Escherichia coli* strain WP2uvrA, with and without metabolic activation. Metabolic activation was provided by an Aroclor-induced, rat liver microsome fraction (S9 mix). The tester strains were exposed to cMDLf via the preincubation method. cMDLf was tested at six concentrations: 160, 320, 630, 1,250, 2,500, and 5,000 µg/plate, based on the results of a dose range finding test. Physiological saline was used as the vehicle control. In the absence of metabolic activation, the positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide for tester strains TA100, TA98, and WP2uvrA; sodium azide for tester strain TA1535, and 9-aminoacridine for tester strain TA1537. In the presence of metabolic activation, the positive controls were benzo(a)pyrene for tester strains TA100, TA98, and TA1537 and 2-aminoanthracene for tester strains TA1535 and WP2uvrA. cMDLf was evaluated in duplicate for each concentration in the presence or absence of metabolic activation.

No precipitation or crystallization was observed at any cMDLf concentration. The positive controls for the tester strains yielded the expected number of revertants/plate of at least a 2-fold increase in the number of revertants relative to the vehicle control. For the test article to be classified as producing a positive result, there had to be a proportional increase in the number of revertant colonies relative to the increase in the concentration of cMDLf and the ratio of the number of revertant colonies to that of the control group had to be at least 2.0. For all concentrations of cMDLf in all tester strains, with or without metabolic activation, the ratio of

the number of revertant colonies to that of the control group was 1.4 or lower. A second assay produced similar results.

cMDLf did not cause a positive response with any of the tester strains in the presence or absence of S9 activation at concentrations up to 5,000 µg/plate. Thus, under the conditions of this study, cMDLf was not found to be genotoxic in the *Salmonella typhimurium* reverse mutation assay or *Escherichia coli* mammalian microsome reverse mutation assay.

D. HUMAN STUDIES OF cMDLf

1. INTRODUCTION

The safety of mean and 90th percentile EDIs of approximately 142 mg/day and 273 mg/day in adults is supported by twelve studies involving 488 infants consuming from up to 2.9 g cMDLf/d for up to one year (Table 15), four studies involving 307 children consuming 100 mg to 1 g cMDLf/d up to nine months (Table 16), 28 studies involving 1116 adults consuming 100 mg to 7.2 g cMDLf/d for up to 12 months (Table 17). The types of studies reviewed include those that assessed the effects of cMDLf in term healthy and low-birth weight infants; healthy, *Helicobacter pylori*- and human immunodeficiency virus (HIV)-infected children; healthy, pregnant, *H. pylori*- and hepatitis C-infected adults; adults with tinea pedis, acne vulgaris, and periodontal disease; and *H. pylori*-infected adults receiving antibiotics. Previous GRAS Notifications supporting mean and 90th percentile EDIs of 100 mg/day and 196 mg/day in adults cited six studies involving a total of 123 infants consuming 1.4 mg to 2.9 g/day for up to 6 months, four studies involving 55 adults consuming 100 mg to 3.6 g/d for up to 8 weeks (GRN 77). Although some adverse events were reported, none were related to the ingestion of cMDLf and in no instance was cMDLf reported to promote disease, allergy or exacerbate existing infections. Moreover, since 1986, over a million infants and toddlers in Japan have consumed cow's milk-supplemented formulas and post-marketing surveillance by Morinaga found no reports cMDLf-related adverse effects.

2. INFANTS

Eleven studies have been published that evaluated the effects of cMDLf ingestion in infants (Table 15; Fairweather-Tait et al., 1987; Balmer et al. 1989; Chierici et al., 1992; Roberts et al., 1992; Hernell and Lönnerdal, 2002; King et al., 2007; Lönnerdal and Hernell, 1994; Schulz-Lell et al., 1991; Kawaguchi et al., 1986; Kawaguchi et al., 1989; Manzoni et al., 2009). Eight studies were performed in healthy term infants (Fairweather-Tait et al., 1987; Balmer et al. 1989; Chierici et al., 1992; Roberts et al., 1992; Hernell and Lönnerdal, 2002; King et al., 2007; Lönnerdal and Hernell, 1994; Schulz-Lell et al., 1991); three studies were performed in low birth

weight infants (Kawaguchi et al., 1986; Kawaguchi et al., 1989; Manzoni et al., 2009); five reported the effects of cMDLf on the establishment of healthy gut/fecal flora or prevention of infection (Kawaguchi et al., 1986; Kawaguchi et al., 1989; Roberts et al., 1992; King et al., 2007; Manzoni et al., 2009); four reported the effects of cMDLf on iron homeostasis (Hernell and Lönnerdal, 2002; Chierici et al., 1992; Fairweather-Tait et al., 1987; Schulz-Lell et al., 1991); two administered Morinaga Milk Industry's cMDLf (Kawaguchi et al., 1986; Kawaguchi et al., 1989); and all studies administered cMDLf via infant formula. Intake levels ranged from 10 mg/100 mL (0.01%) to 285 mg/100 mL (0.285%) and durations of intake ranged from two wks to one yr. Results from the studies suggest that supplementation of infant formula with cMDLf is safe and well tolerated by infants at levels up to 285 mg/100 mL.

Fairweather-Tait et al. (1987) administered the highest amount of cMDLf to infants. Thirty-six healthy, full-term (36-41 wk) infants weighing 2060-3800 g received either milk-based formula with (285 mg/100 mL; 86 µg total iron) or without (40 µg total iron) cMDLf for 14 d (Fairweather-Tait et al., 1987). This was a stable isotope feeding study that was part of a larger investigation into the effects of cMDLf on fecal flora. After 7 d on control formula, these infants were orally administered either 40 or 86 µg ⁵⁸Fe as ⁵⁸Fe-ferric chloride plus ascorbic acid (n=8 and n=8, respectively) and all subsequent diapers were collected for the following 3 d. Infants who received cMDLf-supplemented formula for 7 d were administered either 40 or 86 µg ⁵⁸Fe as ⁵⁸Fe-cMDLf (n=8 and n=5, respectively). Reported study exclusions included noncompliance (one subject), administration of incorrect feed (one subject), inadvertent pooling of fecal material (three subjects), and chronic constipation (two subjects). The methods did not specify recording of adverse effects, however, there was no report of treatment-related adverse effects.

Balmer et al. (1989) determined the effects of cMDLf on fecal flora patterns in newborn infants receiving 1) control formula with no cMDLf or iron (0.4 mg Fe/L; n = 20), 2) formula with added cMDLf at 2.8 g/L (0.8 mg Fe/L; n = 18), or 3) formula with added cMDLf and iron (9.16 mg Fe/L; n = 20) for 14 d. Addition of cMDLf to the formula had no effect on the fecal microflora pattern versus the pattern which was observed for the control formula group. More infants in the cMDLf plus iron group were colonized with *Escherichia coli* and fewer with staphylococci, versus the other groups. Lactoferrin excretion in feces was greater in the cMDLf-fed infants versus controls, but observed excretion levels were less than expected. The authors state that lactoferrin excretion in breast-fed infants is in the range of 5.7-23.6 mg/d, whereas infants in this study excreted a mean of 4.5 and 3.67 mg/d in the cMDLf and cMDLf+Fe groups, respectively. No adverse health effects of lactoferrin consumption were mentioned.

Lonnerdal and Hernell (1994) studied the effects of iron, copper, and selenium supplementation of infant formula on growth, and iron, zinc, and copper status in infant from 6-wk- to 6-mo-old. The target iron level for each formula was 4 mg/L delivered either as FeSO₄ or bovine lactoferrin in combination with FeSO₄ (1.2 mg/L bovine lactoferrin and 2.6 mg/L FeSO₄). There were no differences in weight or height at 6 wk or 6 mo between the breast-fed and formula-fed groups. However, serum selenium, α₂-macroglobulin, and glutathione peroxidase levels were reduced in the supplemented formula fed groups compared to those fed breast milk, but there were no differences in any of these parameters among the different formulas. All other hematological indices were similar between infants consuming breast milk or infant formula supplemented with bovine lactoferrin. All infants had a satisfactory iron status at the end of the treatment period.

Hernell and Lonnerdal (2002) studied the effects of cMDLf administration on iron parameters in healthy term infants that were initially breast-fed and then allocated to either a breast-fed group (n=16) or various formula-fed groups (n=10-12), according to parent choice. Experimental formulas contained 1.6, 1.8, 2.2, or 4.0 mg iron/L, where iron was present as FeSO₄, except in the 1.8 mg iron group, where 1.3 mg Fe was contributed from added cMDLf and 0.5 mg was present as FeSO₄. The 2.2 mg Fe group also received 40 mg/L monophosphate nucleotides. The cMDLf ingredient was reported to have a protein content of 95% (w/w), of which cMDLf constituted > 95%. The cMDLf was iron-saturated and had an iron content of 1.24 mg/g protein. Therefore, the concentration of cMDLf present in the supplemented formula was calculated as 0.104 g/100 mL, or 0.104% (w/v). Experimental formulas were fed to the infants for six mo. cMDLf supplementation had no effect on body weight, height, serum zinc and copper concentrations, or iron parameters, including serum iron, ferritin, hemoglobin, mean corpuscular volume, total-iron-binding capacity, and transferrin receptor levels. However, the Fe plus nucleotide-supplemented formula group had significantly lower serum Fe concentrations compared to other formula groups at 1 month and the infants in the Fe²⁺ with cMDLf group weighed significantly more than the group fed 2.2 mg Fe plus nucleotides (p<0.05) at 6 mo of age. Otherwise, all formulas were well tolerated.

000092
Chierici et al. (1992) studied the effect of cMDLf supplementation on serum hemoglobin, hematocrit, ferritin, iron and zinc levels in healthy, full-term infants. Fifty-one infants were assigned (protocol not provided) to one of four groups: breast milk (n=10), control formula (n=13), formula containing 10 mg cMDLf/100 mL (0.01%, w/v; n=14), and formula containing 100 mg cMDLf/100 mL (0.1%, w/v; n=14). The cMDLf was non-heat-treated and 20% iron-saturated. The iron content of the 100 mg cMDLf formula was slightly higher (98 μg/100 mL) versus the control formula (70 μg/100 mL) and the 10 mg cMDLf formula (72.8 μg/100 mL). Breast milk or formulas were fed for 150 d and serum samples were taken at 0, 7, 30, 90, and

150 d. Two breast-fed infants had low hemoglobin levels at 90 d. There were no statistically significant differences in hemoglobin or hematocrit between the groups at any sampling time (data not shown). At 150 d, the high-dose cMDLf group had significantly higher serum ferritin versus the control formula group ($p=0.02$), in spite of an across the board drop in serum ferritin in all groups. However, the high-dose cMDLf group received slightly more iron versus the other two formula groups, which may or may not account for the difference. Breast-fed infants had lower serum iron levels compared with infants fed the control formula ($p=0.012$) and the high-dose cMDLf group ($p=0.041$). There also were no significant differences in serum zinc levels across all groups at all sampling times. Although the methods did not specify recording of adverse effects, there was no report of treatment-related adverse effects.

Roberts et al. (1992) evaluated the effects of cMDLf on the fecal flora of healthy, full-term infants. cMDLf was administered as an experimental formula containing either 10 (0.01%, w/v; $n=15$) or 100 mg (0.1%, w/v; $n=14$) cMDLf/100 mL and unsupplemented formula ($n=14$) and breast milk ($n=12$) were fed to the two control groups. The cMDLf was 20% iron-saturated. Formulas were fed for 3 mo. At the end of the study, 57% of infants in the high-dose cMDLf group exhibited a "bifidus fecal flora," which was defined as a flora where bifidobacteria outnumber all other components of the fecal flora by 1 \log_{10} CFU/g of feces, versus 0% at baseline (no statistics). This level was similar to the prevalence of bifidus flora observed in the breast-fed group (50%); the effect was not observed in the low-dose cMDLf group. Although the methods did not specify recording of adverse effects, there was no report of treatment-related adverse effects.

King, Jr. et al. (2007) examined the impact of cMDLf supplementation on the incidence of infection in healthy infants 0- to 4-wks-old (≥ 34 wks' gestation; ≥ 2000 g birth weight) who were strictly bottle-fed. Infants were randomized to receive powdered Similac with iron formula (3 mg/L elemental iron) with (850 mg/L; 0.085%, w/v; $n=26$) or without (102 mg/L; background cMDLf content of cow's milk, 0.0102%, w/v; $n=26$) added cMDLf for twelve mo. The cMDLf contained 120 μg of iron/gram of powder. The authors noted that, as protein content was not normalized across the two infant formulas, the cMDLf group received ~5% additional protein versus the control group, which may or may not have influenced the growth of these infants. There was a trend for increased weight over time, up to 6 mo, in the cMDLf-supplemented group, but this increase did not reach statistical significance ($p=0.06$). Infants given cMDLf-supplemented formula had a significantly increased hematocrit at 9 mo ($p<0.05$), a trend toward increased mean corpuscular volume (MCV) at 12 mo ($p=0.06$), and significantly lower incidence of lower respiratory infections versus ($p<0.05$) than the control group. Twenty-seven infants dropped out of the study; 13 of these received the cMDLf-supplemented formula. Of the 27 drop-outs, nineteen withdrew due to intolerance; of these 19 intolerant infants, 10 received

cMDLf-supplemented formula. Three infants were lost due to withdrawal of consent and five were lost to follow-up. These results suggest that, when supplemented at 0.085% (w/v) in infant formula, cMDLf does not increase the risk for intolerance in healthy infants.

Kawaguchi et al. (1986 and 1989) conducted two studies on the effects of cMDLf in low birth weight infants. In the earlier study, 16 low birth weight infants weighing > 1500 g were fed with commercially available standard infant formula products until stable feeding of 150 mL/kg/day was achieved (Kawaguchi et al., 1986). Then the standard formula was replaced with formula containing Morinaga Milk Industries' cMDLf at 50 mg/100 g powder (assuming 13 g powder + 93 g water to reconstitute, the calculated final concentration is 6.5 mg cMDLf/100 mL formula) for 2 wks, after which the formula was switched back to a commercially available infant formula. Assuming the intake pattern of formula was stable over the course of the study, an intake of cMDLf of 9.75 mg/kg body weight/day can be calculated. The authors reported that cMDLf was detected in the feces at a concentration of 20-750 µg/gram of feces and during the cMDLf feeding period, feces tended to become soft, fecal pH declined (second wk), fecal lysozyme activity increased, fecal organic acid content increased (especially acetic acid during the second wk), the ratio of Bifidobacteria among total bacteria tended to be higher, and the Staphylococcus detection ratio tended to be lower (no statistics provided). There was no mention of adverse events. In the later study, nine premature, low birth weight infants (1454-2034 g; 29-36 wk gestational age) who were being bottle fed were supplemented with Morinaga's cMDLf in infant formula at 100 mg/100 mL (0.1%, w/v) for 2 wks (Kawaguchi et al., 1989). Stable bottle-feeding was reported to be achieved again at approximately 150 mL/kg body weight/day, or 150 mg cMDLf/kg body weight/day. There was a one-wk follow-up period after the experimental formula was discontinued. By the end of the 2 wk feeding period, 8-9 mg of cMDLf was detected/gram of feces, which is higher than reported in the previous study due to the larger dose of cMDLf administered. Furthermore, the composition ratio of *Bifidobacterium* and *Veillonella* culturable species in the fecal flora had increased relative to baseline levels (no statistics) and the composition ratio of *Enterobacteriaceae* and *Clostridium* declined during the same period, and both effects were observed 1 wk after discontinuation of the cMDLf supplementation. *Staphylococcus* and *Clostridium* species were not detected at 1 wk post-completion of the study. The relative ratio of *Bacteroidaceae* species was increased at the end of the study but this was a transient effect that disappeared by the end of the one-wk period after discontinuing cMDLf. No other effects were noted on fecal pH or organic acid content (acetic, lactic acids) and no adverse events were mentioned.

000094

Manzoni et al. (2009) studied the ability of cMDLf to affect the incidence of late-onset sepsis in very low birth weight neonates. Very low birth weight neonates (<1500 g) younger than 3-d-old were enrolled and randomized to receive placebo (2 mL of a 5% glucose solution;

n=168), 100 mg cMDLf/day (n=153) or 100 mg cMDLf plus 6×10^9 CFU *Lactobacillus rhamnosus* GG/day (n=151) for either four (birth weight 1001-1500 g) or six (birth weight < 1000 g) wks. Effectively, infants were treated from birth until day 30 or 45 of life. Treatments were diluted in prepared milk prior to administration. Breastfeeding was encouraged and supplemented when necessary with a very low birth weight formula that did not contain cMDLf. Adverse events were recorded until 2 d after the end of the study and liver function was evaluated wkly. Mortality attributable to sepsis was significantly reduced in the cMDLf-supplemented group, relative to control ($p=0.008$). No adverse effects or intolerances to treatment occurred. One infant in the cMDLf group discontinued treatment after eight doses (reason not provided), but was included in the intent-to-treat analysis. Nine infants had incomplete data on some variables but not for analysis of study outcomes. The incidence of hyperbilirubinemia requiring phototherapy was similar across the three groups and no infants exhibited signs of hepatotoxicity or cholestasis. Due to the range of birth weights of the infants that were enrolled in this study, the/body weight exposure to cMDLf can be estimated at 66.7 to greater than 100 mg/kg body weight/day.

Schulz-Lell et al. (1991) carried out iron balance studies in seven infants that were supplemented with 100 mg cMDLf/100 mL formula. Nine infants received control formula without cMDLf. The cMDLf-supplemented group received 169 μg iron per kg body weight per day, and iron retention in this group was 63 μg iron/kg per day. Iron retention was 43 μg iron/kg per day in the control group, which was not significantly different from the treatment group. The mean percent retention of iron in the supplemented group was 36% and in the unsupplemented group was 28% (not significantly different). No adverse effects of cMDLf consumption were mentioned.

Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Kawaguchi et al., 1986	To study the effect of cMDLf-enriched infant formula on premature, low birth weight infants. <i>Study design:</i> Intervention-only <i>Source:</i> cMDLf was from Morinaga Milk Industries Co., Ltd., Japan	<u>16 low birth weight infants (> 1500 g weight)</u> who were being bottle fed were enrolled in the study. After reaching stable bottle feeding of approximately 150 mL/kg/d, the infant formula was changed to one that was supplemented with <u>50 mg cMDLf/100 g powder and the supplemented formula was administered for 2 wk (calculated exposure to cMDLf = 9.75 mg/kg/d)</u> . Fecal samples were taken and measures were taken of fecal pH, lysozyme activity, organic acids content, cMDLf level, and intestinal flora.	<ul style="list-style-type: none"> • During cMDLf supplementation: <ul style="list-style-type: none"> -- Feces tended to become soft -- Fecal pH declined (second wk) -- Fecal lysozyme activity increased -- Organic acid content of feces increased (especially acetic acid during the second wk) -- The ratio of Bifidobacteria among total bacteria tended to be higher, while the Staphylococcus detection ratio tended to be lower (no statistics provided). • 20-750 µg of cMDLf was detected/g of feces, indicating the relative stability of cMDLf in the low birth weight infant intestinal tract. 	<ul style="list-style-type: none"> • None
Fairweather-Tait et al., 1987	To measure the effects of cMDLf on iron retention in infants. <i>Study type:</i> Stable isotope feeding; part of a larger study on cMDLf and fecal flora; group assignment method not reported <i>Source:</i> cMDLf was from Nestlé Research Department (nestec, Vevey, Switzerland). Study authors prepared their own ⁵⁸ Fe-labeled cMDLf from this source.	<u>36 healthy, full-term (36-41 wk) infants (16M/20F) weighing 2060-3800 g</u> were recruited. After the study 7 infants were excluded. The remaining 29 were fed <i>ad libitum</i> from birth either either milk-based formula without (40 µg Fe/100 mL; basic formula; n=16) or with cMDLf (<u>285 mg cMDLf/100 mL</u> ; 86 µg Fe/100 mL, additional iron content was contributed by the added cMDLf; cMDLf formula; n=13) on d 1-14. On 7d after birth, infants were then given either a single dose of ⁵⁸ Fe-labeled cMDLf or ⁵⁸ Fe-labelled ferric chloride plus ascorbic acid. Four groups received the following treatments on d7: 1) those previously fed basic formula who were given 40 µg ⁵⁸ Fe as FeCl ₃ (n=8), 2) those previously fed cMDLf-	<ul style="list-style-type: none"> • There were no differences observed across groups for fecal iron concentration or total iron excreted during the 3-day period following dosing of ⁵⁸Fe-labeled substrates. • There was no significant difference between the two iron sources, nor did previously fed formula influence iron retention from either FeCl₃ or cMDLf. 	<ul style="list-style-type: none"> • Study exclusions included: noncompliance (1), administration of incorrect feed (1), inadvertent pooling of fecal material (3), chronic constipation (2).

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Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
		<p>supplemented formula who were given 40 µg ⁵⁸Fe as iron-saturated cMDLf (n=8), 3) those previously fed basic formula who were fed 86 µg ⁵⁸Fe as FeCl₃ (n=8), and 4) those previously fed cMDLf-supplemented formula who were fed 86 µg ⁵⁸Fe as iron-saturated cMDLf (n=5). All subsequent diapers were collected for 3d after dosing of labeled Fe. Iron retention was calculated by subtracting fecally excreted ⁵⁸Fe from the administered dose, while adjusting for naturally occurring ⁵⁸Fe and loss due to leftover formula or vomiting.</p>		
<p>Balmer et al., 1989</p>	<p>To determine the effects of lactoferrin on fecal flora of infants.</p> <p>[Note: The source of the cMDLf was not provided.]</p>	<p>Formula was given to <u>infants</u> for 14 d and contained either: 1) no added iron/no added lactoferrin (n=20), 2) <u>no iron, with added lactoferrin (n=18), or 3) added iron and lactoferrin (n = 20).</u> <u>When present, lactoferrin was added at 2.8 g/L formula.</u> Fecal samples were collected on d 4, 11, and 14. Specimens collected on days 4 and 14 were used to measure the bacterial flora and the 24 h specimen on d 11 was used to estimate fecal lactoferrin concentration. Blood samples were taken on d 14. Infants weighed approximately 3 kg. Using this weight, and assuming the mean intake of formula at 1031 mL/d, lactoferrin intake is estimated at 2.9 g/d.</p>	<p>The addition of lactoferrin had no effect on the fecal microflora versus the pattern observed for the control formula group. More infants in the lactoferrin plus iron group were colonized with <i>Escherichia coli</i> and fewer with staphylococci, versus the other groups. Lactoferrin excretion in feces was greater in the lactoferrin-fed infants versus those fed the basic formula; however, observed lactoferrin excretion was far below that expected. Reports of lactoferrin excretion in breast-fed infants are in the range of 5.7-23.6 mg/d. The infants in this study excreted a mean of 4.5 and 3.67 mg/d in the lactoferrin and lactoferrin plus iron groups, respectively.</p>	<p>No adverse health effects of lactoferrin consumption were reported.</p>

Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Kawaguchi et al., 1989	<p>To study the effect of cMDLf-enriched infant formula on premature, low birth weight infants.</p> <p><i>Study design:</i> Intervention-only</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industries Co., Ltd., Japan</p>	<p>9 low birth weight infants (1454-2034 g weight; 29-36 wk gestational age) who were being bottle fed were enrolled in the study. After reaching stable bottle feeding of approximately 150 mL/kg/d and allowing 10 or more d to elapse following the discontinuation of any antibiotic treatments, the infant formula was supplemented with 100 mg cMDLf/100 mL and the supplemented formula was administered for 2 wk (calculated exposure to cMDLf = 150 mg/kg/d). There was an additional 1 wk follow-up period after the study formula was discontinued. Fecal samples were taken and measures were taken of fecal pH, lysozyme activity, organic acids content, cMDLf level, and intestinal flora.</p>	<ul style="list-style-type: none"> • By wk 2, the composition ratio of <i>Bifidobacterium</i> and <i>Veillonella</i> culturable species in the fecal flora was increased relative to baseline levels (no statistics). This effect persisted and was still observed 1 wk after cMDLf discontinuation. • There was a transient increase in the composition ratio of <i>Bacteroidaceae</i> species at 2 wk, versus baseline levels, but this effect disappeared by 1 wk post-study. • By 2 wk, the composition ratio of <i>Enterobacteriaceae</i> and <i>Clostridium</i> culturable species had declined, relative to baseline levels. This effect persisted and was still observed 1 wk after cMDLf discontinuation. • 8-9 mg of cMDLf was detected/g of feces, indicating the relative stability of cMDLf in the low birth weight infant intestinal tract. • No other significant effects were noted for fecal pH and organic acids (acetic, lactic acids) content. 	

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Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Schulz-Lell et al., 1991	To carry out iron balance studies in infants. [Note: The source of cMDLf was not provided.]	Iron balance studies were carried out in 16 <u>term infants</u> from their 3 rd until their 17 th week of life. The balance studies were comprised of five periods with an interval of 3 to 4 weeks, each consisting of three 24-hr collections of milk and stool samples. 7 infants were fed a formula supplemented with 100 mg cMDLf/100 mL formula and nine infants received the same formula without cMDLf.	The cMDLf-supplemented group received 169 µg iron/kg body weight per day. Iron retention in the cMDLf-supplemented group was 63 µg/kg/d and in the unsupplemented group it was 43 µg/kg/d. Mean percentage retention of iron in the supplemented group was 36% and in the unsupplemented group was 28% (groups not significantly different). A comparison with previous work on ingestion of human milk indicated that, although iron bioavailability from human milk was significantly better than formula, the absolute iron retention in the breast-fed infants (32 µg/kg/d) was lower versus the formula groups.	No adverse effects of lactoferrin consumption were reported.
Chierici et al., 1992	To study the effect of cMDLf supplementation in infant formula on serum hemoglobin, hematocrit, ferritin, iron and zinc levels in infants. <i>Study type:</i> Not specified; randomization unclear <i>Source:</i> cMDLf was from Oleofina, Brussels, Belgium, non-heat-treated, 20% iron-saturated	<u>Healthy, full-term Italian infants</u> were recruited with parental consent (both sexes; 51 parents consented). Infants were assigned (protocol not stated) to one of four groups: 1) breast milk (n=10), 2) control formula (n=13), 3) <u>formula containing 10 mg cMDLf/100 mL (n=14)</u> , 4) <u>formula containing 100 mg cMDLf/100 mL (n=14)</u> . Iron content of the control formula was 70 µg/100 mL; of the low-cMDLf-supplemented formula was 72.8 µg/100 mL; of the high-cMDLf-supplemented formula was 98 µg/100 mL. <u>Formula was fed for 150d</u> and serum samples were taken at 0, 7, 30, 90 and 150 d.	<ul style="list-style-type: none"> • There were no statistically significant differences in hemoglobin or hematocrit between the groups at any sampling time. • Byd30, breast-fed infants had significantly lower serum iron levels versus control formula-fed infants (p=0.012) and infants in the high cMDLf group (p=0.041). • Serum ferritin increased in all groups fromd0 tod7, held steady atd30, then declined byd30. Atd150, the high cMDLf group had significantly higher serum ferritin versus the control formula group (p=0.02). (<i>note:</i> infants in the high cMDLf group received slightly more iron versus the other two formula groups.) However, by this time 	• Two breast-fed infants had low hemoglobin levels atd90.

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Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
			<p>all serum ferritin levels had significantly declined versus d0 across all groups.</p> <ul style="list-style-type: none"> • No statistically significant differences in serum zinc levels were observed between the groups at any sampling time. 	
<p>Roberts et al., 1992</p>	<p>To study the infant fecal flora of infants consuming an adapted formula containing cMDLf.</p> <p><i>Study design:</i> Unknown</p> <p><i>Source:</i> cMDLf from Oleofina, Brussels, Belgium; 20% iron-saturated.</p>	<p><u>Healthy full-term infants</u> were recruited and <u>fed one of four diets from birth to at least the end of the 3rd mo of life:</u> 1) breast milk (n=12), 2) Preaptamil adapted formula (Milupa AG, Friedrichsdorf, Germany) (n=14), 3) Preaptamil + 10 mg cMDLf (cMDLf)/100 mL (n=15), 4) Preaptamil + <u>100 mg cMDLf/100 mL</u> (n=14). Protein content was normalized across the cMDLf-supplemented formulas by removal of an equivalent amount of whey protein. <u>Formula was fed for 3 mo.</u> Fecal samples were collected at 1 wk, 1 mo, and 3 mo after birth, and cultured for fecal flora content. A “bifidus flora” was defined as a flora where bifidobacteria outnumbered all other measured components of the fecal flora by 1 log₁₀ CFU/g of feces. Fifty-one infants finished the study. <i>Note:</i> introduction of outside weaning food was not restricted.</p>	<ul style="list-style-type: none"> • 57% of infants in the 100 mg cMDLf/100 mL formula-fed group exhibited a bifidobacteria-dominated spectrum (no statistics) at d90, which was similar to the prevalence of bifidobacteria-dominated flora observed for the breast milk-fed group (50%) at this time point. However, the “bifidus flora” effect did not appear until d90 and was not observed in the 10 mg cMDLf/100 mL formula-fed group; therefore, the authors did not consider cMDLf to have provided a beneficial effect of increasing early fecal colonization by bifidobacteria. • The authors commented that the 20% iron-saturation level of the cMDLf utilized may have negated the expected inhibition of the non-bifidobacteria species. 	

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Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Lönnerdal and Hernell, 1994	To study the hematologic effects of iron, selenium, and copper supplementation on infant growth, and iron and copper status. <i>Study type:</i> Randomized, double-blind <i>Source:</i> Not specified	<u>Healthy term infants</u> were either exclusively breast-fed or fed milk-based infant formula from <u>6-wk-of-age to 6-mo-of-age (n=10/group)</u> . Treatment groups are: A) 4.3 mg/L FeSO ₄ , 5 µg /L selenium, 0.4 mg/L copper; B) 4.4 FeSO ₄ , 15.6 µg /L selenium, 0.4 mg/L copper; C) <u>1.3 mg/L bovine lactoferrin</u> , 2.5 mg/L FeSO ₄ , 15.6 µg /L selenium, 0.7 mg/L copper; D) 4.7 mg/L FeSO ₄ , 3.9 µg /L selenium, 0.46 mg/l copper; E) 6.9 mg/L FeSO ₄ , 5.0 µg /L selenium, 0.4 mg/L copper; F) breast milk Anthropometric measurements and venous blood samples were obtained on entry into the study and at 6-mo-of-age. Only comparison between infants that received breast milk and infant formula supplemented with bovine lactoferrin are relevant.	<ul style="list-style-type: none"> • No significant differences in birth weight or height at 6 weeks or 6 mo of age. • There were no differences among the formula-fed groups for serum albumin, BUN, hemoglobin, ferritin, serum transferrin receptor concentration, zinc, • Although the copper, a2-macroglobulin, selenium, and glutathione peroxidase levels were higher in the breast-fed infants, there were no differences in these parameters among the formula-fed groups. 	<ul style="list-style-type: none"> • No significant differences in birth weight or height at 6-weeks or 6-mo-of-age. • No significant differences in hematological indices; all infants had satisfactory iron status.
Hernell and Lönnerdal, 2002	To study the hematologic effects of iron supplementation at various levels and with or without cMDLf or nucleotides in infant formula. <i>Study type:</i> Not specified <i>Source:</i> cMDLf was from SMR, Malmö, Sweden; cMDLf was iron-saturated	<u>Healthy term infants</u> were initially breast-fed and then allocated to either a breast-fed group (n=16) or various formula-fed groups (n=10-12/group), according to parent choice. Infants in the formula-fed groups were completely weaned from breast milk. The formula groups were as follows: 1) formula containing 1.6 mg Fe/L (n=12; Fe as FeSO ₄), 2) formula containing 1.8 mg Fe/L, <u>1.3 mg Fe</u> was provided as cMDLf, 0.5 mg was	<ul style="list-style-type: none"> • At 6 mo, mean body weight and height of the cMDLf-supplemented group was not significantly different from that of the breast-fed, 1.6 mg Fe group, or 4.0 mg Fe group. Weight and height were significantly higher (p<0.05) versus that of the 2.2 mg Fe plus nucleotide group. • cMDLf supplementation did not affect serum iron, ferritin, hemoglobin or other hematological parameters at any time point. 	<ul style="list-style-type: none"> • No effect of cMDLf on body weight or height at 6 mo, except versus that of the 2.2 mg Fe plus nucleotide group (p<0.05). • No effect of cMDLf on iron parameters at any time point.

Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
	with Fe content of 1.24 mg/g protein; purity was 95% protein, of which 95% was cMDLf	FeSO ₄ (n=10; calculated as 1.05 g cMDLf/L), 3) formula containing 2.2 mg Fe/L (Fe as FeSO ₄) plus 40 mg monophosphate nucleotides/L (n=10), 4) formula containing 4.0 mg Fe/L (Fe as FeSO ₄) (n=11). <u>Formula was fed for 6 mo.</u> Anthropometric measures and blood samples were taken at 1, 4, and 6 mo.	<ul style="list-style-type: none"> cMDLf supplementation did not affect serum zinc, copper, or the fatty acid composition, except docosahexanoic acid (DHA), of the erythrocyte membrane at any time point. All formula groups exhibited significantly lower levels of DHA in the erythrocyte membrane at 4 and 6 mo, versus the breast-fed group (p<0.05). 	
King et al., 2007	<p>To examine the impact of cMDLf supplementation in infants over one yr.</p> <p><i>Study type:</i> Randomized, placebo-controlled, double-blind</p> <p><i>Source:</i> cMDLf was provided by DMV International, Delhi, NY. Iron content of cMDLf was 120 µg/g powder.</p>	<p><u>Healthy infants aged 0-4 wk, born at ≥ 34 wk gestation at ≥ 2000 g weight, and who were strictly bottle-fed were eligible for the study.</u> Infants were randomized to receive powdered Similac with iron formula (3 mg/L elemental iron) either with (850 mg/L) or without (102 mg/L; basal cMDLf present in cow's milk-derived formula) added cMDLf for 12 mo. <u>Weight, length, and head circumference were measured at 1, 2, 4, 6, 9, and 12 mo of age.</u> Incidence and duration of infectious disease endpoints were tracked, including diarrhea, upper respiratory infection, acute otitis media, lower respiratory tract infection; plus, other endpoints were followed such as colic, hemoglobin, hematocrit, mean corpuscular volume, and antibody response to various vaccines.</p> <p>79 infants were enrolled in the study;</p>	<ul style="list-style-type: none"> CMDLf-supplemented infants had statistically significantly decreased occurrence of lower respiratory tract infections versus the control group (p<0.05). 	<ul style="list-style-type: none"> There was a trend for increased weight over time up to 6 mo in the cMDLf-supplemented group, but it did not reach statistical significance (p=0.06). <i>Note:</i> Authors state that the added cMDLf slightly increased the total protein content of the treatment formula by ~5%, and this may have influenced the growth and weight change differences between groups. CMDLf-supplemented infants had a statistically significantly increased hematocrit at 9 mo (p<0.05), and a trend toward increased mean corpuscular volume (MCV) at 12 mo (p=0.06), versus control. 13/27 dropouts received cMDLf-supplemented formula. Of the 27 total dropouts, 19 withdrew due to intolerance (10 received cMDLf), withdrawal of

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Table 15. Clinical Studies of cMDLf in Infants

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
		52 completed the full-yr study period. Of the 52 completers, <u>26 received the cMDLf-supplemented formula</u> and 26 received the control formula.		consent (n=3), or were lost to follow-up (n=5).
Manzoni et al., 2009 000103	To establish whether cMDLf, alone or in combination with <i>Lactobacillus rhamnosus</i> GG, reduces the incidence of late-onset sepsis in very low birth weight neonates. <i>Study type:</i> Prospective, multicenter, double-blind, placebo-controlled, randomized <i>Source:</i> LF100; Dicofarm SpA, Rome, Italy	<u>Very low birth weight (VLBW) neonates younger than 3 d</u> were enrolled and randomly allocated into one of three groups: 1) <u>100 mg cMDLf/d (n=153)</u> , 2) <u>100 mg cMDLf/d plus 6×10^9 CFU <i>Lactobacillus rhamnosus</i> GG/d (n=151)</u> , or 3) placebo (2 mL of a 5% glucose solution) (n=168). <u>Treatment lasted 6 (birth weight <1000 g) or 4 (birth weight 1001-1500 g) wk, beginning on d 3 of life</u> with one daily dose. All treatments were diluted in prepared milk. Breast-feeding was encouraged, and supplemented with a VLBW formula when necessary (did not contain cMDLf). Adverse events were recorded until 2 d after the end of the study. Liver function was evaluated wkly.	<ul style="list-style-type: none"> • Infants in the cMDLf group had statistically significantly decreased incidents of total late-onset sepsis (p=0.002); Gram-positive bacteria (p=0.007); and Gram-positive bacteria, including episodes diagnosed with ≥ 1 positive blood culture for coagulase-negative <i>Staphylococcus</i> spp. (p=0.002), versus controls. • The reduction in late-onset sepsis was statistically significant for extremely low birth weight infants (p=0.002), neonates weighing ≤ 750 g (p=0.003), and neonates ≤ 27 wk gestational age at birth (p=0.01) in the cMDLf group, versus controls. • Invasive fungal infection was reduced in total incidents (p=0.004) and in extremely low birth weight neonates (p=0.03) in the cMDLf group, versus controls. • Progression rate from colonization to invasive fungal infection was significantly reduced in all neonates for the cMDLf group versus control (p=0.002). • Threshold retinopathy of prematurity requiring surgery was also reduced in the cMDLf group versus control (p=0.02). 	<ul style="list-style-type: none"> • No adverse effects or intolerances to treatment occurred. • One infant in the cMDLf group discontinued treatment but was included in the intent-to-treat analysis. • Nine infants had incomplete data on certain variables but not for analysis of study outcomes. • At age 4 wk, liver enzyme values were within reference ranges but were significantly lower (p value not provided in main article) in both treatment groups versus control. • Incidence of hyperbilirubinemia requiring phototherapy was similar in the three groups. • No infants exhibited signs of hepatotoxicity or cholestasis. • Mortality attributable to sepsis was significantly reduced in the cMDLf group, versus control (p=0.008).

3. CHILDREN

Four studies have been published that evaluate the effects of cMDLf ingestion in children (Table 16; Okuda et al., 2005; Egashira et al., 2007; Zuccotti et al., 2007; Ochoa et al., 2008). cMDLf intake levels ranged from 100 mg/day to 3 g/day for durations up to 9 months. Two studies administered Morinaga Milk Industry's cMDLf (Okuda et al., 2005; Egashira et al., 2007).

Okuda et al. (2005) investigated the effects of cMDLf alone on *H. pylori* colonization in children and adults. They enrolled 25 healthy children and 34 healthy adults having *H. pylori* infection either without or with minimal upper gastrointestinal symptoms and who were not currently being treated. Infection was diagnosed by positive reactions in both the ¹³C-urea breath test and serum- or urine-based enzyme-linked immunosorbent assay (ELISA), although it was not stated what value was considered the cutoff for a "positive" result in the breath test, and baseline values varied widely across all groups. Subjects who were milk intolerant were excluded and the treatment groups received either 1) two 100 mg cMDLf tablets/twice a day (400 mg/d total) (n=17, adults), 2) placebo tablets (n=17, adults), 3) two 100 mg cMDLf tablets/twice a day (400 mg/d total) (n=14, children), or 4) placebo tablets (n=11, children) for 12 wks. cMDLf was supplied by Morinaga Milk Industry. After 12 wks supplementation, 10 of 31 combined subjects (6 adults, 4 children) receiving cMDLf treatment had a > 50% decrease in their urea breath test value, versus baseline (no statistics). In most responders receiving cMDLf, the urea breath test values returned to baseline levels by 4 wks after the end of the study. The methods did not specify recording of adverse effects, however, there was no report of treatment-related adverse effects.

Egashira et al. (2007) and co-workers enrolled 298 children under 5-yr-old who were attending nursery school or kindergarten and assigned them to one of two groups: 1) 100 mg lactoferrin/day for 12 wks (n=136) or 2) no cMDLf-containing products (n=98) for 12 wks. Milk allergic children were excluded. cMDLf was supplied by Morinaga Milk Industry. Although the number of children with rotaviral gastroenteritis was similar between treatment groups, the frequency and duration of vomiting (p=0.0106, 0.0137) and diarrhea (p=0.0446, 0.0285) were significantly reduced in the cMDLf group, versus control. Two children in the control group were hospitalized due to dehydration, compared with none in the cMDLf group.

Zuccotti et al. (2007) examined the immune modulating effects of cMDLf in eleven human immunodeficiency virus (HIV)-infected, antiretroviral-therapy-naïve children aged 4-17 yr. Subjects received 1 g cMDLf every 8 hours daily for 4 wks (3 g/day total). The methods did not specify recording of adverse effects, however, there was no report of treatment-related

adverse effects due to cMDLf. However, cMDLf supplementation did reduce (no statistics) the percent naïve and central memory CD4+ and CD8+ T cells and increase populations of terminally differentiated lymphocytes at 4 wk, versus baseline. cMDLf supplementation also significantly improved the ability of CD13+ cells to ingest and kill ($p=0.01$ and 0.009 , respectively) labeled *Candida albicans* blastospores at 4 wk, versus baseline. CMDLf also significantly increased the IL-12:IL-10 ratio in lipopolysaccharide (LPS)-stimulated CD14+ cells ($p=0.001$) at 4 wk, versus baseline. These immune modulations may be beneficial in HIV positive subjects.

Ochoa et al. (2008) enrolled previously weaned children 12- to 36-mo-old in a study and randomized the subjects to receive either 0.5 g cMDLf twice/day for six d/wk (daily average: 0.86 g/d) ($n=146$) or 0.5 g maltodextrin twice/day for six d/wk ($n=174$) for nine mo. Children with personal or family history of cow's milk or infant formula allergy, milk intolerance or moderate-to-severe allergic rhinitis or asthma were excluded from the study. There were no adverse events related to cMDLf ingestion reported in this study and height-for-age scores were significantly higher in the cMDLf group when analyzed by group ($p=0.03$) and by interaction of group and month ($p=0.03$). There was no difference in weight for age scores. At nine mo, there was a significant reduction in the number of positive stool samples for *Giardia lamblia* in the cMDLf group, versus the control group ($p<0.05$). This was the only study to report an effect of oral ingestion of cMDLf on colonization by a protozoal parasite.

Table 16. Clinical Studies of cMDLf in Children

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Okuda et al., 2005	<p>To study the effect of oral supplementation of cMDLf on <i>Helicobacter pylori</i> colonization in humans.</p> <p><i>Study type:</i> Randomized, double-blind, placebo-controlled</p> <p><i>Source:</i> cMDLf tablets were from Morinaga Milk Industry, Tokyo, Japan</p>	<p><u>25 healthy children and 34 healthy adults having <i>H. pylori</i> infection either without upper gastrointestinal symptoms or with minimal upper gastrointestinal symptoms who were not being treated were enrolled. <i>H. pylori</i> infection was diagnosed when both the ¹³C-urea breath test (UBT) and serum- or urine-based enzyme-linked immunosorbent assay (ELISA) were positive. [Note: It was not stated what a cutoff for a "positive" value in the UBT test might be.] Subjects who were milk intolerant were excluded. The four treatment groups were: 1) adults consuming two 100 mg cMDLf tablets twice/day (400 mg cMDLf/d) (n=17), 2) adults consuming placebo tablets (n=17), 3) children consuming two 100 mg cMDLf tablets twice/day (400 mg cMDLf/d) (n=14), and 4) children consuming placebo tablets (n=11) for 12 wk.</u></p>	<ul style="list-style-type: none"> • The mean UBT values were significantly different at wk 0 between the two child groups (p<0.01), which may have introduced a greater tendency for a change in UBT to be observed, versus the adult groups. • After 12 wk supplementation, 10 of 31 (32.3%) subjects in the combined cMDLf groups had a >50% decrease of their UBT value, versus baseline. One of 28 (4%) combined control subjects had a >50% decrease in their UBT value, versus baseline. [Note: Baseline UBT values appeared to vary widely across all groups, and it was unclear what constituted a positive result.] • In most of the responders in the cMDLf groups, the UBT values returned to baseline levels by 4 wk after the end of the study. 	<ul style="list-style-type: none"> • None
Egashira et al., 2007	<p>To demonstrate <i>in vivo</i> effects of cMDLf on rotaviral gastroenteritis in children in a day-care setting.</p> <p><i>Study type:</i> Open-label, non-randomized</p> <p><i>Source:</i> cMDLf was provided as cMDLf Active® dietary food</p>	<p><u>298 children under 5-yr-old attending either nursery school or kindergarten (Saga Prefecture, Japan) were enrolled. Milk allergic infants were excluded, and none had chronic illness. Subjects were assigned to one of two groups: 1) 100 mg cMDLf/d for 12 wk (n=136, final analysis) or 2) no cMDLf-containing products (n=98, final analysis). Incidence, duration, and severity of fever, diarrhea, vomiting, and fecal rotaviral antigen</u></p>	<ul style="list-style-type: none"> • Frequency (p=0.0106) and duration (p=0.0137) of vomiting and frequency (p=0.0446) and duration (p=0.0285) of diarrhea were all statistically significantly decreased in the cMDLf group versus control group. • Number of children with rotaviral gastroenteritis was similar between treatment groups. • There was no significant difference in the duration of fever between the two groups. 	<ul style="list-style-type: none"> • Two children in the control group were hospitalized due to dehydration, compared with none in the cMDLf group.

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Table 16. Clinical Studies of cMDLf in Children

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
	<p>supplement tablets (100 mg cMDLf/tablet; Morinaga Milk Industry Co, Ltd., Tokyo, Japan) and CMDLf Yoghurt® (120 g cup yogurt containing 100 mg cMDLf/cup; Morinaga Milk Industry).</p>	<p>were recorded during the study. Subjects were excluded from data analysis if they had diarrheal disease but failed to get the rotavirus antigen test, if the parents did not submit the symptom records, if they left the day-care center during the study, or if they took < 50% of the scheduled dose of cMDLf. 234 subjects were subjected to further analyses.</p>		
<p>Zuccotti et al., 2007</p>	<p>To study the immune modulating effects of oral cMDLf supplementation in HIV-infected, antiretroviral-therapy-naïve children.</p> <p><i>Study design:</i> Not stated</p> <p><i>Source:</i> cMDLf from Dicofarm, Rome, Italy</p>	<p><u>11 HIV-infected, antiretroviral-therapy-naïve children aged 4-17 yr</u> were orally administered <u>1 g cMDLf every 8 h daily for 4 wk</u>. Blood samples were taken before and after 4 wk of supplementation to measure lymphocyte subsets, intracellular cytokines and phagocytosis and killing by whole-blood leukocytes.</p>	<ul style="list-style-type: none"> • cMDLf supplementation had no effect on CD4 and CD8 cell counts or HIV plasma viraemia. However, the study was not designed to detect this. • cMDLf supplementation reduced (no statistics) the percent naïve and central memory CD4+ and CD8+ T cells and increased populations of terminally-differentiated lymphocytes at 4 wk, versus baseline. • cMDLf supplementation significantly improved the ability of CD13+ cells to ingest and kill (p=0.01 and 0.009, respectively) labeled <i>Candida albicans</i> blastospores at 4 wk, versus baseline. • cMDLf supplementation significantly increased the IL-12:IL-10 ratio in lipopolysaccharide (LPS)-stimulated CD14+ cells (p=0.001) at 4 wk, versus baseline. 	

Table 16. Clinical Studies of cMDLf in Children

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Ochoa et al., 2008	<p>To investigate whether supplementation with cMDLf can prevent diarrhea in children and affect colonization by pathologic microbial species.</p> <p><i>Study type:</i> Randomized, double-blind, placebo-controlled</p> <p><i>Source:</i> Tatua Nutritionals</p>	<p><u>Previously weaned children aged 12-36 mo</u> were randomly assigned to receive 1) <u>0.5 g cMDLf ×2/d (1 g/d × 6 d/wk; average = 0.86 g/d) (n=146)</u> or 2) <u>0.5 g maltodextrin ×2/d (n=174), on six d/wk for 9 mo.</u></p> <p>Exclusion criteria included children with personal or family history of allergy to cow's milk or infant formula and those who had moderate-to-severe allergic rhinitis or asthma, or milk intolerance.</p> <p>Children were evaluated monthly by a physician, and stool samples were collected during diarrheal episodes and on a monthly basis to detect asymptomatic infection.</p>	<ul style="list-style-type: none"> • There was a statistically significant reduction in the number of positive stool samples for <i>Giardia lamblia</i> in the cMDLf group versus the control group (p<0.05). 	<ul style="list-style-type: none"> • Height-for-age scores were significantly greater in the cMDLf group versus the control group (p=0.03). • There were no adverse events related to cMDLf.

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4. ADULTS

Twenty eight studies have been published that evaluate the effects of cMDLf ingestion in adults (Table 17; McMillan et al., 1977; Yamauchi et al., 1998; Tanaka et al., 1999; Yamauchi et al., 2000a; Iwasa et al., 2002; Okada et al., 2002; Di Mario et al., 2003; Ishii et al., 2003; Okuda et al., 2005; Zullo et al., 2005; Di Mario et al., 2006; Konishi et al., 2006; Paesano et al., 2006; Ueno et al., 2006; Tursi et al., 2007; Koikawa et al., 2008; Kondo et al., 2008; Mulder et al., 2008; Bharadwaj et al., 2009; Kozu et al., 2009; Nappi et al., 2009; Paesano et al., 2009; Bharadwaj et al., 2010; Ishikado et al., 2010; Ono et al., 2010; Paesano et al., 2010; Shin et al., 2010; Mueller et al., 2011). Eleven studies administered Morinaga Milk Industry's cMDLf (Yamauchi et al., 1998; Yamauchi et al., 2000a; Iwasa et al., 2002; Okada et al., 2002; Ishii et al., 2003; Okuda et al., 2005; Konishi et al., 2006; Ueno et al., 2006; Koikawa et al., 2008; Kondo et al., 2008; Kozu et al., 2009; Ishikado et al., 2010; Shin et al., 2010). In general, cMDLf intake levels ranged from 100 mg/day to 7.2 g/day for durations up to 1 year.

Yamauchi et al. (1998) enrolled ten healthy males to study the effect of consuming 2 g/day of cMDLf for 4 weeks on the phagocyte activation. Although the effects of cMDLf were variable, no adverse events were reported.

Ono and co-workers investigated the effect of cMDLf in healthy, overweight Japanese adults with a body mass index (BMI) > 25 kg/m² and a visceral fat area > 100 cm² (Ono et al., 2010). Thirty subjects were enrolled; two did not meet inclusion criteria. The remaining 28 subjects were randomized to either lactose tablets (control; n=14) or enteric-coated cMDLf tablets (300 mg cMDLf/d; n=14) for eight wks, following a two-wk run-in period. No adverse events were observed; cMDLf did not adversely affect systolic or diastolic blood pressure, pulse rate, or blood lipid parameters, versus control. Favorable effects on weight and BMI were also observed in the cMDLf group.

Bharadwaj and co-workers administered a unique, ribonuclease (angiogenin)-enriched preparation of cMDLf to postmenopausal women (Bharadwaj et al., 2010). Thirty-eight healthy postmenopausal women aged 45- to 60-yr old with no menses for at least 12 mo were enrolled. Three were excluded based on their history of treatment for bone health or hypothyroidism. The remaining women were randomized to receive either 250 mg of the cMDLf preparation (n=20; estimate 125 mg actual cMDLf, based on reported purity of ~50%)/day or placebo capsules (n=15) for 180 d. One subject in the treatment group was dropped for non-compliance; three from the control group dropped out as well. All subjects had > 95% compliance with supplements. The body weight and blood pressures of all the subjects were maintained within ±

3% of their baseline values. No adverse events were reported by the authors to have occurred during the study or the 3-month follow-up period.

Kozu et al. (2009) reported effects of cMDLf ingestion, enrolling two hundred fifteen patients aged 40-75 yr who had adenomatous colorectal polyps. Patients were randomized and assigned to placebo (n=35) or 1.5 g/d (n=37) or 3.0 g/d (n=34) cMDLf (supplied by Morinaga Milk Industry; 10-20% iron-saturated) for twelve mo. Two subjects were excluded due to not having a target polyp (placebo group) and use of statins (3.0 g cMDLf group). Two placebo group subjects withdrew from the study for reasons not stated. Two subjects were excluded from the full analysis set due to use of non-steroidal anti-inflammatory drugs. Two subjects in the 3.0 g group were found to have lung and liver metastases; both had a history of colon cancer. A mild increase in alkaline phosphatase was observed in one subject in the 1.5 g group and a moderate increase in total bilirubin was observed in one subject in the 3.0 g group. Both biochemical parameters returned to normal at the end of the study and the authors reported that no other serious adverse events occurred during the study.

Okuda and co-workers investigated the effects of cMDLf alone on *H. pylori* colonization in adults and children (Okuda et al., 2005). They enrolled 25 healthy children and 34 healthy adults having *H. pylori* infection either without or with minimal upper gastrointestinal symptoms and who were not currently being treated. Infection was diagnosed by positive reactions in both the ¹³C-urea breath test and serum- or urine-based enzyme-linked immunosorbent assay (ELISA), although it was not stated what value was considered the cutoff for a "positive" result in the breath test, and baseline values varied widely across all groups. Subjects who were milk intolerant were excluded and the treatment groups received either 1) two 100 mg cMDLf tablets/twice a day (400 mg/d total) (n=17, adults), 2) placebo tablets (n=17, adults), 3) two 100 mg cMDLf tablets/twice a day (400 mg/d total) (n=14, children), or 4) placebo tablets (n=11, children) for 12 wks. cMDLf was supplied by Morinaga Milk Industry. After 12 wks supplementation, 10 of 31 combined subjects (6 adults, 4 children) receiving cMDLf treatment had a > 50% decrease in their urea breath test value, versus baseline (no statistics). In most responders receiving cMDLf, the urea breath test values returned to baseline levels by 4 wks after the end of the study. The methods did not specify recording of adverse effects, however, there was no report of treatment-related adverse effects.

000110

Di Mario and co-workers carried out two large studies in *H. pylori*-positive patients who received triple antibiotic therapy either with or without simultaneous oral cMDLf supplementation (Di Mario et al., 2003; Di Mario et al., 2006). The first study enrolled 150 patients having dyspeptic symptoms, gastritis and peptic ulcer disease and randomized patients to receive either 1) Group A: rabeprazole (20 mg 2×/d), clarithromycin (500 mg 2×/d), and

tinidazole (500 mg 2×/d), plus cMDLf (200 mg 2×/d; 400 mg/d total) for 7 d (n=51), 2) Group B: the triple antibiotic regimen alone for 7 d (n=52), or 3) Group C: the triple antibiotic regimen alone for 10 d (n=47) (Di Mario et al., 2003). Subjects were considered noncompliant if < 90% of study medication was taken. Major side effects leading to treatment discontinuation were observed in six patients: two patients in Group A (dizziness, headache, fatigue, nausea), one patient in Group B (nausea, hypotension and taste disturbance) and three patients in Group C (fatigue, nausea and diarrhea). All but 27 patients had a negative ¹³C-urea breath test or *Helicobacter pylori* stool antigen test by two mo after the end of therapy. The eradication rates in the groups, based on intent-to-treat analysis, were as follows: Group A, 92.2% (p=0.01, versus Groups B and C); Group B, 71.2%; Group C, 70.2%. The second study enrolled 402 patients who were randomized to receive either 1) Group A: esomeprazole (20 mg 2×/d), clarithromycin (500 mg 2×/d), and tinidazole (500 mg 2×/d) for 7 d (n=136), 2) Group B: cMDLf (200 mg 2×/d; 400 mg/d total) for 7 d, followed by the triple antibiotic regimen for 7 d (n=132), or 3) Group C: concurrent treatment with both the triple antibiotic regimen plus cMDLf for 7 d (n=134) (Di Mario et al., 2006). Of the 402 patients, 389 completed the study. Six patients were discontinued due to side effects, one patient in Group B died because of a street incident and six patients were lost to follow up. The incidence of side effects was 9.5% in Group A, 9% in Group B and 8.2% in Group C. The *H. pylori* eradication rate was significantly higher in group 3 versus groups 1 and 2 (p=0.01, intent-to-treat analysis). These two studies suggest that concurrent administration of cMDLf at 200 mg twice/day (400 mg total) may have favorable effects in combination with standard triple antibiotic therapy for the elimination of *H. pylori*.

Zullo and co-workers also investigated the effect of adding cMDLf to triple antibiotic therapy for *H. pylori* infection in 133 patients with dyspepsia who underwent endoscopy and a rapid urease test for positive diagnosis (Zullo et al., 2005). Patients were randomized to receive 1) esomeprazole (20 mg 2×/d), clarithromycin (500 mg 2×/d), amoxicillin (1 g 2×/d) for 7 d (n=68) or 2) the antibiotic regimen plus cMDLf (200 mg 2×/d; 400 mg total) for 7 d (n=65). Bacterial eradication was checked at 4-6 wks after commencement of treatment by using a ¹³C-urea breath test, and there was no significant difference in eradication rates of *H. pylori* infection among the groups. Another group of researchers studied cMDLf in combination with quadruple antibiotic therapy in *H. pylori*-infected patients who experienced failure of a first antibiotic therapy (Tursi et al., 2007). Seventy patients were enrolled in the study and randomized into one of two groups: 1) Group A: ranitidine bismuth citrate (400 mg 2×/d), esomeprazole (40 mg/d), amoxicillin (1 g 3×/d), tinidazole (500 mg 2×/d) without cMDLf for 7 d (n=35) or 2) Group B: the quadruple antibiotic therapy plus cMDLf (200 mg 2×/d; 400 mg/d total) for 7 d (n=35). Elimination of *H. pylori* infection was achieved in 88.57% of patients in group A and 94.28% of those in group B; the difference between groups was not statistically significant. Regarding the

overall tolerability of the therapy, 16/68 patients (23.53%) exhibited side effects, but analysis of the side effects in the two groups revealed that cMDLf supplementation reduced the side-effect incidence. One group A patient withdrew from the study due to severe side effects (vomiting, diarrhea, abdominal pain) and nine other patients from this group (26.47%) experienced side effects but completed the study. Six (17.64%) group B patients experienced side effects and all completed the study.

Kondo and co-workers administered either a placebo (n=10) or 1.8 g of Morinaga Milk Industries' cMDLf (n=8)/day to patients with chronic periodontitis for three mo (Kondo et al., 2008). Only an English abstract is available; however, no adverse effects of cMDLf are reported. At one wk, *Porphyromonas intermedia* was significantly reduced in the subgingival plaque in the cMDLf group, versus control (p<0.05) and *P. gingivalis* was also reduced at 1 and 3 mo in the treatment group, versus control (p<0.05). There was also a reduction in the total number of bacteria observed in the subgingival plaque after one month in the cMDLf group, versus control (p<0.01).

Ishikado and co-workers studied the effect of cMDLf in patients with periodontal disease in an open intervention study (Ishikado et al., 2010). Fourteen patients aged 37-to 59-yr-old having periodontal disease were given 180 mg cMDLf/day as tablets that were formulated using liposomes from soy phosphatidylcholine for four wks (the authors report that soy phosphatidylcholine may contribute synergistically to the anti-inflammatory effects of cMDLf). cMDLf was supplied by Morinaga Milk Industry. Two subjects were dropped from the study due to lack of compliance. There were no clinically adverse effects on biochemical or hematological parameters examined, and any variations that occurred were reported to be within normal historical ranges (data for these parameters not reported). Levels of monocyte chemoattractant protein-1 (MCP-1) in gingival crevicular fluid were significantly decreased at 2 and 4 wks, versus baseline (p<0.001 and P<0.001, respectively). At four wks, the inflammatory cytokines tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) were reduced (p<0.05), as were IL-1 β and MCP-1 (p<0.01), in the culture supernatant of *Porphyromonas gingivalis* lipopolysaccharide (LPS)-stimulated peripheral blood monocytes from subjects, versus baseline levels.

Yamauchi and co-workers report the only published results on the effects of oral cMDLf on a topical fungal infection, *Tinea pedis* (Yamauchi et al., 2000a). Adults having mild or moderate *Tinea pedis* were assigned to one of three groups: 1) 600 mg cMDLf/day (n=14), 2) 2000 mg cMDLf/day (n=12), or 3) placebo (n=11) for 8 wks. Although a mycological cure was not observed in any of the subjects, the dermatological symptoms score was decreased for both

cMDLf groups versus control ($p < 0.05$). There were no adverse events reported and no subjects withdrew from the study because of an adverse event.

Mueller and co-workers administered cMDLf to subjects having mild to moderate facial acne vulgaris in an open label study (Mueller et al., 2011). Forty-three healthy subjects aged 17.5 ± 3.8 -yr-old, were enrolled in the study and those with known milk intolerance or allergy were excluded. Subjects ingested 200 mg of cMDLf in the form of chewable tablets/day for eight wks. Compliance was $> 95\%$ and no serious adverse events or adverse events leading to discontinuation of the cMDLf ingestion were reported. None of the subjects experienced an adverse event considered to be related to the test material. The total count of acne lesions was significantly decreased from baseline at 2, 4, and 8 wks' time ($p = 0.005$, 0.007 , and < 0.001 , respectively). In particular, inflammatory lesions were reduced at 2 wks ($p = 0.002$), while non-inflammatory lesions were reduced at 4 and 8 wks ($p < 0.001$ for both), versus baseline.

Tanaka and co-workers administered cMDLf for 8 wk in eleven patients having chronic hepatitis C (1999). An initial seven subjects received 1.8 g cMDLf/d for the 8 wk, while the following four subjects received 3.6 g cMDLf/d for 8 wk. At the end of treatment, a decrease in serum alanine transferase and HCV RNA concentrations was observed in three of the four subjects having low pretreatment serum concentrations of HCV RNA. The seven patients having high pretreatment serum concentrations of HCV RNA did not experience a change in the two indices. The authors report that no serious complications occurred during or after the treatment. No increases in serum alkaline phosphatase, lactate dehydrogenase, or total bilirubin levels were observed, and mentioned that iron studies were normal in all subjects.

Iwasa and co-workers enrolled 27 patients who had elevated ALT levels, were serum-positive for anti-HCV antibody, plus high serum titers of HCV RNA and serum HCV genotype 1b and randomized subjects to receive either 1) 0.4 g cMDLf/day ($n = 10$) or 2) 3.6 g cMDLf/day ($n = 15$) for 6 mo (Iwasa et al., 2002). All subjects completed the study and the authors reported no serious complications occurred during treatment. At 2, 4, and 6 mo, HCV RNA levels were significantly reduced ($p < 0.05$, 0.01 and 0.01 , respectively) in the high-dose cMDLf group, versus baseline levels. Two mo after the study ended, HCV RNA levels had increased but not yet returned to baseline levels.

Okada and co-investigators treated 45 patients with confirmed chronic hepatitis C (based on biopsy, genetic and biochemical criteria) with 1.8 ($n = 15$), 3.6 ($n = 15$), or 7.2 g ($n = 15$) cMDLf/day for 8 wks (Okada et al., 2002). Authors reported that there were no clinically significant abnormalities in laboratory values observed during or after treatment in this study (methods state that a complete blood count, biochemistry tests, urinalysis and serum tests were

performed, but give no details or data). No serious adverse events were reported. Adverse events that appeared to be dose-related occurred in four subjects and included diarrhea in one person in the 3.6 g group, plus three people in the 7.2 g group who experienced skin eruption, anorexia, fatigue, chills and constipation. No adverse effects were found in persons at the lowest dose. A virological response was observed in four patients in the 1.8 g group at the end of treatment but they still had persistent and detectable serum HCV RNA levels. The duration of response ranged from 6-10 mo. All patients who responded to cMDLf relapsed during the follow-up period but an age-associated effect was observed wherein patients \geq 60-yr-old had higher response rates versus those younger than 60-yr-old ($p < 0.01$). A biochemical response in the form of lowered ALT levels was observed in two patients, one in the 1.8 g/d group and one in the 3.6 g/d group, and the effect persisted 5-6 mo. None of the subjects achieved ALT normalization.

Ishii and co-authors reported on the effect of supplementation with cMDLf plus lactulose plus *Bifidobacterium longum* in patients with chronic hepatitis C (Ishii et al., 2003). Sixty-three patients who were serum-positive for anti-HCV antibodies and HCV RNA were randomized to receive either 1) 600 mg cMDLf plus 600 mg lactulose plus 3 billion active *B. longum*/day ($n=36$) or 2) no cMDLf ($n=27$) for 12-36 mo. It was assumed that the control group received no interventions, including the lactulose and *B. longum* supplement, although this was not explicitly stated in the published report for this study. The methods did not specifically mention recording adverse effects; however, no adverse findings were reported. At three mo, there was a significant increase in serum IL-18 levels, versus baseline, in the treatment group ($p < 0.01$); this effect was transient. There was no effect on serum ALT, HCV RNA, and IL-10 levels, or on CD4+ Th1 and CD4+ Th2 cells.

Konishi and co-workers examined the effect of cMDLf on lipid peroxidation in chronic hepatitis C patients having high serum HCV RNA, HCV genotype 1b and who did not have other causes of liver dysfunction (Konishi et al., 2006). Ninety patients received either 3.6 g cMDLf/day ($n=47$) or no intervention ($n=43$) for 8 wks. By the end of the study, the cMDLf-treated group had reduced serum ALT levels ($p < 0.05$) and reduced plasma levels of 8-isoprostane ($p < 0.05$) (a marker of lipid peroxidation), versus baseline. The methods did not specifically mention recording adverse effects; however, no adverse findings were reported.

Ueno and co-investigators carried out the largest study of cMDLf in chronic hepatitis C patients, enrolling 199 patients with adequate bone marrow and renal function, and assigning them to receive either 1) 1.8 g cMDLf/day ($n=97$) or 2) placebo ($n=101$) for 12 wks (Ueno et al., 2006). Three subjects withdrew for reasons that were unrelated to adverse effects but not specified. Minor adverse events occurred with similar frequency and intensity across groups and included neutropenia, γ -GTP elevation and hyperglycemia. CMDLf was well tolerated and no

serious adverse events were reported during treatment. cMDLf treatment did not significantly affect the virologic or biochemical response rates; nor did it alter serum IL-18 levels or CD4+, CD8+, CD16+ or CD56+ peripheral blood lymphocyte populations, versus control.

Koikawa and co-workers carried out a double-blind, randomized trial that examined the ability of cMDLf to improve or prevent anemia in female long distance runners at high risk of iron-deficiency anemia (Koikawa et al., 2008). Sixteen women (approximately 20-yr-old) were randomized to either 1.8 g cMDLf (as tablets containing 0.45 g cMDLf each) plus 6 mg iron (as ferric pyrophosphate)/day (n=8) or iron supplement only (n=8) for eight wks. All subjects also received 30 g of hydrolyzed milk protein in 700 mL water daily. The cMDLf was supplied by Morinaga Milk Industry and was 17% iron-saturated, with 0.23 mg iron/gram of protein. At the end of the study, cMDLf-supplemented runners did not have the significant decrease in serum iron and ferritin levels, as was observed in the control group. Compared with the control group, cMDLf-supplemented runners had significantly increased red blood cell (RBC) count ($p<0.01$) by 8 wk. At 8 wk, the cMDLf-supplemented runners had significantly elevated mean corpuscular volume (MCV) ($p<0.01$) and mean corpuscular hemoglobin (MCH) ($p<0.05$) values, versus baseline levels, although final values did not differ significantly from those observed in the control group.

Nappi and co-workers studied the effect of cMDLf on iron status and tolerability in pregnant women with iron deficiency anemia (Nappi et al., 2009). The investigators evaluated 139 pregnant women having a physiological course of a singleton pregnancy and who were between 12 and 36 wks gestation with iron deficiency anemia as defined by hemoglobin (Hb) value < 11 mg/dL, serum iron < 30 μ g/dL, serum ferritin < 12 μ g/dL and total iron binding capacity (TIBC) > 450 μ g/dL. One hundred women were enrolled and randomized to receive either 100 mg cMDLf/day (n=50) or 520 mg ferrous sulfate (n=50) for 30 d. Both cMDLf and ferrous sulfate supplementation caused a significant increase in Hb, serum ferritin, serum iron and a concurrent decrease in TIBC at 30 d, versus baseline levels ($p<0.01$). Median scores of abdominal pain and constipation were significantly higher in the ferrous sulfate group compared with the cMDLf group, and cMDLf was reported to be well tolerated. In the cMDLf group, gastrointestinal side effects including epigastric pain, vomiting and constipation were reported for one subject for each side effect. One patient in the cMDLf group was excluded due to miscarriage and two subjects from the ferrous sulfate group discontinued treatment due to severe constipation.

Paesano and co-workers have studied the effects of cMDLf on iron parameters in pregnant and non-pregnant women (Paesano et al., 2006; Paesano et al., 2009; Paesano et al., 2010). One study included 300 pregnant women of various ages, parities and trimesters of

pregnancy who were offered oral iron supplementation (Paesano et al., 2006). Pregnant women who refused treatment represented the control group (n=54), and the remaining subjects were randomly divided into two groups, receiving 520 mg ferrous sulfate/day (n=98) or 100 mg cMDLf twice/day (200 mg total/d) (n=107). The cMDLf was 30% iron-saturated. The interventions were administered for 30 d and hemoglobin and total serum iron levels were measured at 0 and 30 d. It should be noted that, although the study authors define iron deficiency as $Hb \leq 11$ g/dL and iron deficiency anemia as total serum iron of ≤ 30 mg/dL, most of the women enrolled in this study met neither of these criteria. However, oral ingestion of 200 mg cMDLf/day caused a significant increase in the delta mean values for both Hb and total serum iron in this group, versus control ($p < 0.01$ for both) and versus ferrous sulfate ($p = 0.02$ for Hb effect; $P < 0.01$ for total serum iron effect) groups. The authors report that no side effects were observed in the 107 women who received cMDLf. In 98 women receiving ferrous sulfate, the authors report that 95% had stomach pain, cramps and constipation; 2% had at least one episode of diarrhea. Thirty-one subjects were excluded or lost to analysis (reason not specified), 4 moved or were lost to follow-up, 3 had miscarriages, and 3 were excluded for other reasons not stated.

A second study by the same investigators was reported as part of a review paper, and thus many details are not available, such as the inclusion and exclusion criteria, study protocol, randomization scheme, and any adverse effects (Paesano et al., 2009). There was no reference made in the review article to a published report that could be retrieved. One hundred forty-three pregnant women either refused therapy (n=33), or were given cMDLf (n=60; dose not stated, presume 200 mg/d, as in previous study) or ferrous sulfate (n=50) for 30 d. Mean values for red blood cell count (RBC), Hb, total serum iron and serum ferritin were increased in the cMDLf group at 30 day, versus baseline levels ($p < 0.001$ for all).

A third report from Paesano and co-workers was a publication of results from two studies, one in pregnant women and the second in non-pregnant women (Paesano et al., 2010). The first study involved pregnant women in their third trimester of pregnancy who met at least one of the following hematological criteria: $< 4 \times 10^6$ RBC/mL, $Hb \leq 11$ g/dL, total serum iron ≤ 30 mg/dL or serum ferritin ≤ 12 ng/mL. Twelve pregnant women refusing iron therapy were designated as the control group and the remaining 75 women were assigned to receive either 520 mg ferrous sulfate once/day (n=33) or 100 mg cMDLf twice/day (200 mg total/d) (n=30) for 30 d. After 30 d, mean RBC, Hb, total serum iron, serum ferritin and hematocrit levels increased significantly in the cMDLf group, versus baseline levels ($p < 0.001$ for all parameters). The authors reported that there were no dropouts in the cMDLf group. The second study enrolled non-pregnant women meeting the same hematological criteria as the first study and excluded subjects who were allergic to milk proteins. Women were offered iron therapy and the nine women refusing intervention constituted the control group. The remaining 189 women

000116

consenting to iron therapy were randomized to receive 520 mg ferrous sulfate once/day (n=90) or 100 mg cMDLf twice/day (200 mg total/day) (n=90). Of the subjects receiving ferrous sulfate, therapy was administered for 30 (n=25), 60 (n=25), or 90 d (n=26). Of the non-pregnant women receiving cMDLf, the intervention was carried out for either 30 (n=34), 60 (n=22), or 90 d (n=34). After 30, 60 and 90 d, mean RBC, Hb, total serum iron, serum ferritin and hematocrit levels increased significantly in the cMDLf group, versus baseline levels ($p < 0.001$ for all parameters). The study authors report that there were no drop-outs in the cMDLf group due to side effects.

A separate report comparing the absorption of ^{59}Fe from proprietary infant formulas and human milk was published by McMillan and co-workers in 1977. Eight healthy (gender and age unspecified), iron-sufficient adult subjects were fed a single dose of 100 mL of either human milk, simulated human milk, simulated human milk containing 100 mg purified human lactoferrin (Calbiochem)/100 mL, or two commercial infant formulas containing 10 μCuries of ^{59}Fe . Incorporation of the isotope into red blood cells was determined at 14 d post-feeding. The percent iron incorporation was significantly higher ($P = 0.05$) from the human milk (15.4%) versus simulated human milk (9%), simulated human milk containing lactoferrin (4.7%), SMA (not defined in the study; a proprietary formula; 3.08%), or Similac (2.6%). The most iron was obtained from the iron-fortified formulas. Absolute iron incorporation was approximately 0.038 mg/100 mL of formula ingested. Addition of human lactoferrin to simulated human milk increased the actual iron incorporation to 0.010 mg/100 mL, from 0.006 mg/100 mL. No adverse events were mentioned by the authors.

Results from the above studies suggest that cMDLf may exert favorable effects on iron homeostasis in women, although in some of the published studies non-standardized hematological inclusion criteria were utilized. Administration of up to 1.8 g cMDLf/day for 8 wks in female long distance runners and 200 mg cMDLf/day for up to 30 d in pregnant women was well tolerated.

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
McMillan et al., 1977	<p>To compare the absorption of iron from several proprietary formulas with that observed for human milk, in an attempt to identify the factors responsible for the enhanced iron availability of human milk.</p> <p>[Note: Human lactoferrin from Calbiochem containing 140 mg Fe/g was used.]</p>	<p><u>8 healthy iron-sufficient adult subjects were fed a single dose of 100 mL of a test milk (human milk, simulated human milk, simulated human milk containing 100 mg lactoferrin/100 mL milk (total dose of 100 mg lactoferrin), and two commercial infant formulas. Feedings were conducted at intervals over two weeks. The iron content of each milk was 66, 70, 210, 1250, and 1250 µg/100 mL milk, respectively. Blood samples were obtained 14 d following sample ingestion, just prior to ingestion of the next milk sample to be studied. Incorporation of ⁵⁹Fe into red blood cells was determined 14 d post-feeding.</u></p>	<p>The percent iron incorporation was significantly higher ($P = 0.05$) from the human milk (15.4%) compared with simulated human milk (9%), simulated human milk with added human lactoferrin (4.7%), SMA (3.08%) and Similac (2.6%). The most iron was obtained from the iron-fortified formulas, as absolute iron incorporation was approximately 0.038 mg/100 mL of formula ingested. Iron incorporation from human milk was 0.010 mg/100 mL, and from simulated human milk was 0.006 mg/100 mL. The addition of human lactoferrin to the simulated human milk increased actual iron incorporation to 0.010 mg/100 mL.</p>	<p>No adverse events were reported.</p>
Yamauchi et al., 1998	<p>To study the effects of orally administered cMDLf on the immune system of healthy volunteers.</p> <p><i>Study type:</i> Intervention-only</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industry Co., Ltd., Japan</p>	<p><u>10 healthy male volunteers, aged 31-55 yr, were given 2 g cMDLf/day for 4 wk. Blood samples were drawn before, during and after the intervention. Phagocytic activity and superoxide production activity of polymorphonuclear leukocytes (PMN) were evaluated and the expression levels of CD11b, CD16 and CD56 on leukocytes were quantified on leukocytes using flow cytometry.</u></p>	<ul style="list-style-type: none"> • Phagocytic activity of PMN increased (no statistics) in 3/10 subjects during the cMDLf intervention. • In two of three subjects in which the phagocytic activity increased, PMN expressed CD16 at higher levels as well. • Superoxide production of PMN increased in only 1 of the 10 subjects. • CD16+ lymphocytes increased in 3/10 subjects. • CD11b+ lymphocytes and CD56+ lymphocytes increased in four subjects, including the same three subjects who showed an increase in CD16+. 	<ul style="list-style-type: none"> • None

000118

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
			<ul style="list-style-type: none"> • Overall, 7/10 subjects had changes in immune parameters during the cMDLf treatment. 	
000119 Tanaka et al., 1999	<p>To test the hypothesis that lactoferrin inhibits hepatitis C virus (HCV) viremia in patients with chronic hepatitis C.</p> <p><i>Source:</i> cMDLF was from Morinaga Milk Industry Co., Tokyo, Japan.</p>	<p><u>11 patients with chronic hepatitis C (5M/6F; 35-66 yr old) received an 8 wk course of lactoferrin. The initial 7 subjects received 1.8 g cMDLf/d for 8 wk and the following 4 subjects received 3.6 g cMDLf/d for 8 wk.</u></p>	<p>At the end of lactoferrin treatment a decrease in serum alanine transaminase and HCV RNA concentrations was apparent in three (75%) of the four patients having low pretreatment serum concentrations of HCV RNA. Seven patients with high pretreatment concentrations showed no significant changes in these indices.</p>	<p>No serious complications occurred during or after the treatment. No increases in the serum alkaline phosphatase, lactate dehydrogenase, or total bilirubin levels were observed in any subject. Iron studies were normal in all patients.</p>
Yamauchi et al., 2000a	<p>To evaluate the effectiveness of cMDLf in the treatment of <i>Tinea pedis</i>.</p> <p><i>Study type:</i> Double-blind, placebo-controlled, randomized</p> <p><i>Source:</i> Milei GmbH, Leutkirch Adrazhofen, Germany</p>	<p><u>Adult subjects (age not stated) having mild or moderate <i>Tinea pedis</i> confirmed by microscopy and culture were assigned to one of three groups: 1) 600 mg cMDLf (cMDLf)/d (n=14), 2) 2000 mg cMDLf/d (n=12), and 3) placebo (n=11). Four tablets of either low- (75 mg/tablet) or high-dose cMDLf (250 mg/tablet) were administered twice a day for 8 wk. Each subject's feet were inspected 1 wk prior to initiating treatment, at 2, 4, and 8 wk after beginning treatment, and 4 wk after ending treatment. Clinical symptoms were evaluated and recorded, and cultures were taken. Immune parameters, hematology tests, blood chemistry and urinalysis were also measured.</u></p>	<ul style="list-style-type: none"> • The dermatological symptoms score was statistically significantly decreased for both cMDLf treatment groups (p<0.05), versus control, at 12 wk. • The above improvements were noted in a subgroup of patients that excluded those having an initial symptom score of under five, or those having "hyperkeratosis type" <i>Tinea pedis</i>. Eight subjects comprised the control group, eight subjects were included from the 600 mg cMDLf/d group and five subjects were included from the 2000 mg cMDLf/d group. • Mycological cure was not observed in any of the subjects. • At 8 wk treatment, levels of serum IgG specific for cMDLf were significantly higher than at pretreatment (data not shown), but serum levels of IgE specific for cMDLf were below the detection 	<ul style="list-style-type: none"> • There were no adverse events and no subjects withdrew from the study due to adverse events.

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
			limit. Serum levels of cMDLf were also below the detection limit (10 ng/mL) that was also lower than the minimum inhibitory concentrations of cMDLf against <i>Trichophyton rubrum</i> and <i>T. mentagrophytes</i> (> 6 µg/mL; unpublished data).	
Iwasa et al., 2002	<p>To assess the effect of cMDLf on hepatitis C virus viremia in chronic hepatitis C patients.</p> <p><i>Study design:</i> Double-blind, randomized</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industry Co., Ltd., Japan</p>	<p><u>27 patients with chronic hepatitis C (16M/11F)</u> were enrolled. Patients all had elevated ALT levels above the upper normal limit for ≥ 6 mo, were serum-positive for anti-HCV antibody, had high serum titers of HCV RNA (> 100 kIU/mL), and serum HCV genotype 1 b, plus absence of other causes of chronic hepatitis. None had responded to interferon therapy. <u>25 patients were randomized</u> to receive either 1) 0.4 g cMDLf/d (n=10) or 2) 3.6 g cMDLf/d (n=15). cMDLf was administered for 6 mo. Patients were followed up for 2 mo after the study.</p>	<ul style="list-style-type: none"> • HCV RNA was significantly reduced at 2 mo (p<0.05) and 4 and 6 mo (both p<0.01) in the high cMDLf dose group, versus baseline levels at 0 mo. By 2 mo post cessation of therapy, HCV RNA levels had increased but not yet returned to baseline levels. 	<ul style="list-style-type: none"> • No serious complications occurred during the treatments and all subjects completed the study. • Two patients in the low cMDLf dose group were excluded from the study due to detection of hepatocellular carcinoma soon after entry. • Neither cMDLf treatment had an effect on AST or ALT levels at any time point during the study. • Serum levels of aminotransesterase (? Spelling error in paper), iron and ferritin did not change at any time point in either cMDLf group (data not shown).

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Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Okada et al., 2002	<p>To assess the effect of cMDLf on serum alanine aminotransaminase (ALT) and hepatitis C virus (HCV) RNA levels in patients with chronic hepatitis C.</p> <p><i>Study type:</i> Dose-finding, uncontrolled, treatment-only cohort study</p> <p><i>Source:</i> Morinaga Milk Industry Co., Tokyo, Japan</p>	<p><u>Patients 20- to 74-yr-old who had confirmed chronic hepatitis C and who met biopsy, genetic and biochemical criteria, were enrolled. cMDLf tablets (450 mg/tablet) were administered orally 2-3×/d for 8 wk on an outpatient basis and were followed up for an additional 8 wk. cMDLf intake and adverse events were self-recorded in a diary daily. 45 were enrolled at three dose levels (n=15/group, ×3 dose groups: 1.8, 3.6, and 7.2 g cMDLf/d).</u></p>	<ul style="list-style-type: none"> Biochemical response was observed in two patients (cMDLf of 1.8 g/d and 3.6 g/d) but no patient achieved ALT normalization. Duration of responses was 5 and 6 mo. Virological response was observed in four patients (cMDLf of 1.8 g/d) at the end of treatment, but had persistent, detectable serum HCV RNA levels. Duration of responses ranged from 6-10 mo. Age was associated with the response to cMDLf treatment: patients 60-yr or older had higher response rates (p<0.01). All patients who responded to cMDLf relapsed during the follow-up period. 	<ul style="list-style-type: none"> Adverse events occurred in four patients and included diarrhea (n=1; 3.6 g cMDLf/d); skin eruption (n=1; 7.2 g/d); anorexia and fatigue (n=1; 7.2 g/d); chills and constipation (n=1; 7.2 g/d). There were no clinically significant abnormalities in laboratory values during or after treatment.
Di Mario et al., 2003	<p>To test the efficacy of triple antibiotic therapy plus cMDLf in the eradication of <i>Helicobacter pylori</i> infection.</p> <p><i>Study type:</i> Open label, multi-arm, randomized, single-center</p> <p><i>Source:</i> cMDLf was from Dicofarm-Rome, Italy; tablets contained 100 mg/each.</p>	<p><u>150 <i>Helicobacter pylori</i>-positive patients (76 M/74F) were enrolled in the study.</u> Patients had dyspeptic symptoms, gastritis and peptic ulcer disease. Infection was assessed at baseline by either histology plus a ¹³C-urea breath test or histology plus <i>H. pylori</i> stool antigen test. Patients were randomized to receive one of the following treatments: 1) rabeprazole (20 mg 2×/d), clarithromycin (500 mg 2×/d), tinidazole (500 mg 2×/d), and cMDLf (200 mg 2×/d; 400 mg/d total) for 7 d (n=51; 25M/26F; 50.1 ± 11.1 yr); 2) the antibiotic regimen alone for 7d(n=52; 26M/26F; 50.5 ± 15.3 yr); or 3) the antibiotic regimen alone for 10d(n=47; 23M/24F; 52.3 ± 15.8 yr). Subjects were considered noncompliant if < 90% of study medication was taken.</p>	<ul style="list-style-type: none"> All but 27 patients had a negative ¹³C-urea breath test or <i>Helicobacter pylori</i> stool antigen test by 2 mo after the end of the therapy. Eradication rates in the groups were as follows, based on intent-to-treat analysis: Group 1: 92.2% (p=0.01 versus groups 2 and 3) Group 2: 71.2% Group 3: 70.2% Eradication rates in the groups were as follows, based on per-protocol analysis: Group 1: 95.9% (p=0.005 versus groups 2 and 3) Group 2: 72.5% Group 3: 75.0% 	<ul style="list-style-type: none"> Major side effects led to treatment discontinuation in: Group 1 (2 patients; dizziness, headache, fatigue, nausea) Group 2 (1 patient; nausea, hypotension, taste disturbance) Group 3 (3 patients; fatigue, nausea, diarrhea) All but 6 patients completed treatment with good compliance.

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Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Ishii et al., 2003	<p>To study the effects of long-term oral administration of cMDLf on serum parameters in patients with chronic hepatitis C.</p> <p><i>Study design:</i> Randomized, controlled</p> <p><i>Source:</i> "cMDLf active R" was from Morinaga Milk Industry Co., Ltd, Japan and contained 600 mg cMDLf plus 600 mg lactulose and 3 billion active <i>Bifidobacterium longum</i></p>	<p>63 patients with chronic hepatitis C (38M/25F) aged 24-77 yr were enrolled. All patients were serum-positive for anti-HCV antibodies and HCV-RNA., with an HCV genotype of 1b. Concurrent hepatitis B infection was not present. Patients were randomly assigned to one of two groups: 1) 600 mg cMDLf + 600 mg lactulose + 3 billion active <i>Bifidobacterium longum</i>/day for 12-36 mo (n=36) or 2) no cMDLf (unclear whether a placebo was given) (n=27) for 6 mo. Serum levels of alanine aminotransferase (ALT), HCV-RNA, IL-18, IL-10, CD4+ Th1 cells, and CD4+ Th2 cells were followed over time.</p>	<ul style="list-style-type: none"> • There was a significant (p<0.01) increase in serum IL-18 at 3 mo, versus 0 mo, in the treatment group, but this effect was transient. • Treatment had no effect on serum ALT, HCV-RNA, IL-10, CD4+ Th1 cells or CD4+ Th2 cells, at any measured time point during the 12 mo treatment time. 	<ul style="list-style-type: none"> • None
Okuda et al., 2005	<p>To study the effect of oral supplementation of cMDLf on <i>Helicobacter pylori</i> colonization in humans.</p> <p><i>Study type:</i> Randomized, double-blind, placebo-controlled</p> <p><i>Source:</i> cMDLf tablets were from Morinaga Milk Industry, Tokyo, Japan</p>	<p>25 healthy children and 34 healthy adults having <i>H. pylori</i> infection either without upper gastrointestinal symptoms or with minimal upper gastrointestinal symptoms who were not being treated were enrolled. <i>H. pylori</i> infection was diagnosed when both the ¹³C-urea breath test (UBT) and serum- or urine-based enzyme-linked immunosorbent assay (ELISA) were positive. [Note: It was not stated what a cutoff for a "positive" value in the UBT test might be.] Subjects who were milk intolerant were excluded. The four treatment groups were: 1) adults consuming two 100 mg cMDLf tablets twice/day (400 mg cMDLf/d) (n=17), 2) adults consuming placebo tablets (n=17), 3) children consuming two 100 mg cMDLf tablets twice/day (400 mg cMDLf/d) (n=14), and 4) children consuming placebo tablets (n=11) for 12 wk.</p>	<ul style="list-style-type: none"> • The mean UBT values were significantly different at wk 0 between the two child groups (p<0.01), which may have introduced a greater tendency for a change in UBT to be observed, versus the adult groups. • After 12 wk supplementation, 10 of 31 (32.3%) subjects in the combined cMDLf groups had a >50% decrease of their UBT value, versus baseline. One of 28 (4%) combined control subjects had a >50% decrease in their UBT value, versus baseline. [Note: Baseline UBT values appeared to vary widely across all groups, and it was unclear what constituted a positive result.] 	<ul style="list-style-type: none"> • None

000122

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
			<ul style="list-style-type: none"> In most of the responders in the cMDLf groups, the UBT values returned to baseline levels by 4 wk after the end of the study. 	
Zullo et al., 2005	<p>To test the efficacy of triple antibiotic therapy plus cMDLf in the eradication of <i>Helicobacter pylori</i> infection.</p> <p><i>Study type:</i> Prospective, open-label, randomized, multicenter</p> <p><i>Source:</i> cMDLf was from Dicofarm (Rome, Italy)</p>	<p>133 patients (74M/59F) with dyspepsia who were referred by primary care physicians for upper endoscopy were enrolled in the study. All patients underwent endoscopy with biopsies for histology and a rapid urease test. Infection was confirmed if both tests were positive. Patients were randomized to one of two groups: 1) esomeprazole (20 mg 2×/d), clarithromycin (500 mg 2×/d), amoxicillin (1 g 2×/d) (n=68; 39M/29F) for 7d or 2) the antibiotic regimen plus 200 mg cMDLf (2×/d; 400 mg/d total) (n=65) for 7 d. Bacterial eradication was checked 4-6 wk after treatment by using a ¹³C-urea breath test. Compliance was defined as consumption of > 90% of the treatment as ascertained by personal interview.</p>	<ul style="list-style-type: none"> There was no significant difference in eradication rate of <i>Helicobacter pylori</i> infection among the groups. 	<ul style="list-style-type: none"> All but 3 patients completed the study. Dropouts were due to: <ul style="list-style-type: none"> Group 1: 2 patients, lost to follow-up Group 2: 1 patient, severe epigastric pain and vomiting Side effects reported: <ul style="list-style-type: none"> Group 1: 7 patients; diarrhea, abdominal pain, taste disturbance, pruritis, vomiting Group 2: 6 patients; diarrhea, abdominal pain, glossitis All side effects were self-limiting after cessation of therapy, and compliance to the therapies was good.
Di Mario et al., 2006	<p>To test the efficacy of triple antibiotic therapy after administration of cMDLf in the eradication of <i>Helicobacter pylori</i> infection.</p> <p><i>Study type:</i> Prospective, open-label, randomized, multicenter</p> <p><i>Source:</i> cMDLf was from Dicofarm (Rome, Italy)</p>	<p>402 <i>Helicobacter pylori</i>-positive patients (mean age 52.4 yr; range 19-84 yr) were randomized into one of three groups: 1) esomeprazole (20 mg 2×/d), clarithromycin (500 mg 2×/d), and tinidazole (500 mg 2×/d) (n=136) for 7d, 2) cMDLf (200 mg 2×/d; 400 mg total/d) (n=132) for 7d, followed by the triple antibiotic therapy regimen for 7d, and 3) concurrent treatment with both the triple antibiotic therapy plus cMDLf (200 mg 2×/d; 400 mg total/d) (n=134) for 7d. Infection was assessed at baseline by histology and the ¹³C-urea breath test or histology and the <i>H. pylori</i> stool antigen-test.</p>	<ul style="list-style-type: none"> The eradication rate was significantly higher in group 3 (concurrent administration of cMDLf with triple antibiotic therapy), versus groups 1 and 2 (p = 0.01, intent-to-treat analysis; P = 0.001, per-protocol analysis). 	<ul style="list-style-type: none"> Of the 402 patients enrolled, 389 were fully compliant with the therapy, defined as > 95% of study drugs taken. Major side effects leading to treatment discontinuation by 6 patients included diarrhea and rash (? Possible spelling error in paper) (two patients/group) One patient in group 2 died because of a street incident. Six patients were lost to follow up (3 in group 1, 2 in group 2 and one in group 3).

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Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Konishi et al., 2006	<p>To evaluate the effect of cMDLf on lipid peroxidation, hepatic inflammation and iron metabolism in patients with chronic hepatitis C.</p> <p><i>Study design:</i> Randomized, controlled</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industry, Japan</p>	<p>Ninety Japanese patients having chronic hepatitis C (58M/32F) were enrolled. Patients had high serum HCV RNA (> 100 kIU/mL), HCV genotype 1b, consumed < 20 g alcohol/day, and did not have other causes of liver dysfunction. Of the 90 chronic hepatitis C patients, 47 (31M/16F) received 3.6 g cMDLf/d for 8 wk. The remaining 43 chronic hepatitis C patients (27M/16F) did not receive cMDLf and were the control group. Thirty-eight HCV-negative healthy volunteers (15M/23F) served as additional controls. Blood samples were collected for analysis.</p>	<ul style="list-style-type: none"> By 8 wk, the cMDLf-treated group had significantly reduced (p<0.05) serum alanine aminotransferase (ALT) levels, versus baseline levels at 0 wk. The authors considered this to be an indicator of reduced levels of lipid peroxidation in the body. Plasma levels of 8-isoprostane were significantly reduced (p<0.05), versus baseline levels, by 8 wk in the cMDLf-treated group, but not in the control group. The authors report that 8-isoprostane is a marker of lipid peroxidation. 	<ul style="list-style-type: none"> None
Paesano et al., 2006	<p>To study the effect of oral supplementation of cMDLf on iron parameters in pregnant women.</p> <p><i>Study design:</i> Not specified</p> <p><i>Source:</i> cMDLf was Lf100 from Dicofarm, Rome, Italy; 30% iron saturation</p>	<p>Three hundred pregnant women were included in the study. Women were offered treatment with oral iron supplementation or cMDLf; the control group was composed of women who refused treatment of any kind. Of the 259 women who participated, 54 refused oral iron supplementation (control group), 98 were given 520 mg ferrous sulfate once/day, and 107 were given 100 mg cMDLf twice/day (200 mg cMDLf total/day). Treatment was for 30 d. 520 mg ferrous sulfate delivered 156 mg elemental iron; 200 mg cMDLf delivered 8.8 mg ferric ions. Blood samples were taken and analyzed for hemoglobin and total serum iron at 0 and 30 d.</p> <p><i>Note:</i> Inclusion and exclusion criteria were not defined; nor was a detailed analysis plan provided (e.g., intent to treat, etc.)</p>	<ul style="list-style-type: none"> The delta mean value was increased for hemoglobin levels in the ferrous sulfate group, versus the control group, at 30 d (p<0.01). The increase in delta mean value for hemoglobin levels in the cMDLf group, versus the control group, at 30d was considered non-significant by the authors (p=0.02). The delta mean value was increased for serum iron levels in both the ferrous sulfate group and the cMDLf group, versus the control group, at 30 d (p<0.01 for both). Additionally, the increase in delta mean value for total serum iron was significantly increased in the cMDLf group, versus the ferrous sulfate group, at 30 d (p<0.01). 	<ul style="list-style-type: none"> Thirty-one subjects were excluded or lost to analysis (reason not specified), 4 moved or were lost to follow-up after 30d of treatment, 3 had miscarriages, 3 were excluded for other reasons (not specified). The authors report that, of the 98 women receiving ferrous sulfate, 95% had stomach pain, cramps and constipation; 2% had at least one episode of diarrhea. Authors report no side effects in the 107 women taking cMDLf.

000124

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Ueno et al., 2006	<p>To evaluate the virologic response to cMDLf in patients having chronic hepatitis C.</p> <p><i>Study type:</i> Randomized, double-blind, placebo-controlled, phase III</p> <p><i>Source:</i> Morinaga Milk Industries, Tokyo, Japan</p>	<p>Patients 20-74 yr-old who met a detailed <u>criteria indicating established hepatitis C virus (HCV) infection with adequate bone marrow and renal function</u> were assigned randomly to one of two treatment groups in equal proportions using permutation blocks stratified by centers. One hundred ninety-nine patients were enrolled; one refused to participate. Treatments consisting of either 1) <u>cMDLf at a dose of 1.8 g cMDLf/d (as 450 mg/tablets) (n=97)</u> or 2) placebo (n=101) were administered orally twice daily for <u>12 wk</u>. Serum HCV RNA level and serum alanine aminotransferase (ALT) were measured before treatment, at 4, 8, and 12 wk during treatment, and at 4 wk after treatment. IL-18 and lymphocyte phenotypes were also measured.</p>	<ul style="list-style-type: none"> • cMDLf treatment did not significantly affect the virologic or biochemical response rates, or significantly alter serum IL-18 levels or CD4+, CD8+, CD16+ and CD56+ peripheral blood lymphocyte populations. 	<ul style="list-style-type: none"> • Three participants in the cMDLf group withdrew due to reasons besides adverse events (not specified). • cMDLf was well tolerated and no serious complications occurred during treatment. • Minor adverse events occurred with similar frequency across both groups and included: neutropenia, γ-GTP elevation and hyperglycemia.
Tursi et al., 2007	<p>To investigate the efficacy of cMDLf in combination with quadruple antibiotic therapy in <i>Helicobacter pylori</i> infected patients who experienced failure of a first antibiotic therapy.</p> <p><i>Study type:</i> Prospective, randomized</p> <p><i>Source:</i> cMDLf was Elleffe 100® from Dicofarm S.p.A., Rome, Italy</p>	<p><u>70 patients with persistent <i>Helicobacter pylori</i> infection after failure of a first standard treatment schedule were enrolled in the study.</u> Infection was confirmed by gastroscopy and biopsy. Patients were randomized into one of two groups: 1) ranitidine bismuth citrate (400 mg 2×/d), esomeprazole (40 mg/d), amoxicillin (1 g, 3×/d), tinidazole (500 mg 2×/d) (n=35) and 2) the regimen listed in 1), <u>plus cMDLf (200 mg 2×/d; 400 mg total/d) (n=35).</u> [<i>*Note:</i> The length of treatment was not specified.] Endoscopy was carried out at one month post-therapy in those patients “for whom the examination was clinically relevant.” In these patients, <i>H. pylori</i> presence was ascertained by the rapid urease test and by Giemsa stain. Remaining patients were checked by the ¹³C-urea breath test.</p>	<ul style="list-style-type: none"> • Eradication of <i>Helicobacter pylori</i> infection was obtained in 88.57% of patients in group 1 and 94.28% of group 2 patients, based on intent-to-treat analysis. The difference between groups was not statistically significant. 	<ul style="list-style-type: none"> • One patient in group 1 experienced severe diarrhea, vomiting and abdominal pain such that treatment was discontinued. • Nine other patients in group 1 experienced mild side effects that did not require discontinuation of treatment: self-limiting diarrhea, candidosis, abdominal pain, black feces, and nausea. • In group 2, six patients experienced mild or slight side effects that did not require discontinuation of treatment: self-limiting diarrhea, abdominal pain, black feces, nausea, and gastric fullness.

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Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Koikawa et al., 2008	<p>To determine whether cMDLf supplementation would improve or prevent anemia in female long distance runners at high risk of iron-deficiency anemia.</p> <p><i>Study type:</i> Double-blind, randomized</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industry, Tokyo; 17% iron saturation, 0.23 mg Fe/g protein</p>	<p>16 female long distance runners approximately 20 yr-old were randomized to one of two groups: 1) 1.8 g cMDLf + 6 mg Fe (as ferric pyrophosphate)/day (n=8) and 2) 6 mg Fe (as ferric pyrophosphate)/day (n=8). cMDLf and Fe were consumed as tablets; the cMDLf tablets contained 0.45 g cMDLf + 5 mg ferric pyrophosphate (1.5 mg as Fe) with 1.05 g carbohydrate; iron tablets contained 5 mg of ferric pyrophosphate (1.5 mg as Fe) plus 1.5 g carbohydrate/tablet. <u>Four tablets were taken daily in each group for 8 wk.</u> Additionally, all subjects were given 30 g of hydrolyzed milk protein in 700 mL water/day.</p>	<ul style="list-style-type: none"> • cMDLf-supplemented runners did not have the significant decrease in serum iron and ferritin levels, as was observed in the control group. • Compared with the control group, cMDLf-supplemented runners had significantly increased RBC count (p<0.01) by 8 wk. • Compared with baseline levels, at 8 wk, the cMDLf-supplemented runners had significantly elevated MCV (p<0.01) and MCH (p<0.05) values, although final values did not differ significantly from those observed in the control group. • Blood lactate levels after a 3000 m run were significantly lower in the cMDLf group versus the control group at 8 wk (p<0.05). Additionally, the increase in lactic acid at 8 wk versus 0 wk was less for the cMDLf group (non-significant) versus the increase observed for the control group, which was statistically significant (p<0.01). 	<ul style="list-style-type: none"> • cMDLf supplementation preserved iron status of female long distance runners at-risk of iron-deficiency anemia.

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Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
<p>Kondo et al., 2008 [Abstract only available]</p> <p style="text-align: center;">000127</p>	<p>To study the effects of oral cMDLf administration in periodontitis patients.</p> <p><i>Study design:</i> Randomized, double-blind, placebo-controlled</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industries, Japan</p>	<p>18 patients with chronic periodontitis were randomized to one of two groups: 1) cMDLf tablets (n=8); 1.8 g cMDLf/d, information provided by Morinaga) or 2) placebo tablets (n=10) for three mo. Two tablets were administered orally three times a day. Clinical disease indications, total bacterial content (qPCR) of subgingival plaque and saliva and levels of human and cMDLf (ELISA) in the gingival crevicular fluid and saliva were measured. Endotoxin levels in gingival crevicular fluid and saliva were also determined using a Limulus test.</p>	<ul style="list-style-type: none"> • At 1 wk, <i>Porphyromonas intermedia</i> was significantly reduced in the subgingival plaque in the cMDLf group, versus control (p<0.05). • A significant reduction in the total number of bacteria was observed in the subgingival plaque after 1 mo in the treatment group, versus control (p<0.01). • <i>Porphyromonas gingivalis</i> was significantly reduced at 1 and 3 mo in the treatment group, versus control (p<0.05). • Levels of cMDLf, but not human cMDLf, were significantly increased in the gingival crevicular fluid and saliva in the treatment group, versus control, at 1 wk and 1 mo (p<0.001 for both measurements, at each of the two time points). • Clinical measurements and saliva levels of total bacteria were unchanged by the cMDLf treatment. • There was no change in the levels of endotoxin in the gingival crevicular fluid and saliva of the cMDLf group, versus controls. 	<ul style="list-style-type: none"> • None

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Mulder et al., 2008	<p>To investigate the immune modulation and antioxidant activity of an oral cMDLf supplement in human males.</p> <p><i>Study design:</i> Intraindividual repeated measure; nonblinded</p> <p><i>Source:</i> Not stated</p>	<p>8 healthy males aged 30-55 yr consumed one placebo capsule daily for 7 d, then 100 mg cMDLf/day for 7d, followed by 200 mg cMDLf/day for 7 d. Immune cell populations and function, cytokines and serum antioxidant capacity were measured before and after each supplementation. Medications and supplement use was prohibited 14 prior to and throughout the study.</p>	<ul style="list-style-type: none"> • T cells were activated during the 200 mg cMDLf/d phase of the study. Statistically significant increases in total (CD3+), helper (CD4+), and cytotoxic (CD8+) T cells were observed on d 16 and 21 (p<0.001 for all), versus d0 and d7. • The hydrophilic, but not lipophilic, antioxidant capacity of serum was statistically significantly increased on d 16 and 21 (200 mg cMDLf/d), versus d0 (p<0.05). 	<ul style="list-style-type: none"> • None
Bharadwaj et al., 2009	<p>To measure the effect of milk ribonuclease-enriched cMDLf on bone turnover markers in postmenopausal women.</p> <p><i>Study design:</i> Randomized, placebo-controlled</p> <p><i>Source:</i> Ribonuclease (angiogenin)-enriched cMDLf was either co-isolated from milk (1:1 ratio, w/w) or separately admixed to this ratio. [Assume purity of cMDLf is ≤ 50%.]</p>	<p>35 healthy, ambulatory postmenopausal women, 45- to 60-yr-old, with no menses for at least 12 mo were randomized to one of two groups: 1) 250 mg ribonuclease-enriched cMDLf/day (n=20; estimate 125 mg cMDLf/d) or 2) control capsules (n=15) for 180 d. Each group also received an oral supplement containing 100% of the RDA for calcium. Blood and urine samples were collected on d 0, 15, 30, 60, 90, and 180. Analyses were completed on 19 subjects in the cMDLf group and 12 subjects in the control group.</p>	<ul style="list-style-type: none"> • Bone resorption markers (serum N-telopeptides, NT_x, and urine deoxypyridinoline crosslinks, Dpd) were significantly decreased (p<0.001 and 0.01, respectively) and bone formation markers (serum bone-specific alkaline phosphatase, BAP, and serum osteocalcin, OC) were significantly increased (p<0.001 for both) in the treatment group versus baseline levels. 	<ul style="list-style-type: none"> • None

000128

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
<p>Kozu et al., 2009</p> <p style="writing-mode: vertical-rl; transform: rotate(180deg);">000129</p>	<p>To determine whether oral cMDLf could inhibit the growth of adenomatous colorectal polyps in human patients.</p> <p><i>Study design:</i> Randomized, double-blind, controlled</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industry Co. Ltd.; 10-20% iron-saturated.</p>	<p>215 patients underwent scheduled colonoscopic examinations and were qualified for enrollment based on results and prior medical history. 108 patients aged 40-75 yr and having ≤ 5 mm-diameter polyps showing a pit pattern III (Kudo's classification) were enrolled in the study. Subjects were randomized and assigned to one of three treatment groups: 1) placebo (n=35), 2) 1.5 g cMDLf (cMDLf)/d (n=37), and 3) 3.0 g cMDLf/d (n=34) for 12 mo. Tablets contained 0, 250, or 500 mg cMDLf/tablet. Subjects took six tablets daily. Intake of products containing cMDLf was prohibited during the study; however, no meals were recorded. Peripheral blood samples were collected at 0, 3, 6, 9, and 12 mo and analyzed for lymphocyte subsets and natural killer (nK) cell activity. Colon polyps were removed at the end of the study and histologically evaluated.</p>	<ul style="list-style-type: none"> • Polyp growth was significantly inhibited by 3.0 g cMDLf/d in subjects aged ≤ 63 yr-old (n=16) (p=0.006) and in females taking 3.0 g cMDLf/d (n=5) (p=0.019), versus placebo. • Serum levels of human cMDLf were significantly increased in the 3.0 g cMDLf/d group, versus control (p<0.001). • Natural killer cell activity was significantly increased in the 1.5 g cMDLf/d group, versus control (p=0.048). This parameter was also increased in the 3.0 g cMDLf/d group but was not statistically significant versus control (p=0.058). • Higher serum levels of human cMDLf correlated with reduced invasion of polymorphonuclear leukocytes into the stroma surrounding the target polyps (p=0.021). 	<ul style="list-style-type: none"> • Two subjects were excluded from the trial: one participant assigned to placebo did not have a target polyp; another assigned to the 3.0g cMDLf/d group was found to have used statins. • Two participants from the placebo group withdrew from the study (reason not stated). • Two subjects were excluded from the full analysis set due to use of non-steroidal anti-inflammatory drugs. • In the 3.0 g cMDLf/d group, lung metastases from colorectal cancer were observed in one patient and liver metastases were observed in another patient. Both subjects had a history of colon cancer. • One subject in the 1.5 g cMDLf/d group had a mild increase in alkaline phosphatase levels, and a subject in the 3.0 g cMDLf/d group had a moderate increase in total bilirubin levels during the trial, after an initial mild increase upon commencement of the trial. Both biochemical parameters returned to normal levels at the end of the study. • No other serious adverse events occurred.

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Nappi et al., 2009	<p>To study the effect of cMDLf on iron status and tolerability in pregnant women with iron deficiency anemia.</p> <p><i>Study type:</i> Prospective, randomized, controlled, double-blind</p> <p><i>Source:</i> cMDLf was Elleffe 100®; Dicofarm, Rome, Italy</p>	<p>139 <u>pregnant women with iron deficiency anemia who met the following inclusion criteria</u> were evaluated: physiological course of pregnancy, singleton pregnancy, gestational age > 12 wk and < 36 wk, hemoglobin (Hb) values < 11 mg/dL, serum iron (Fe) < 30 µg/dL, serum ferritin < 12 µg/dL and total iron binding capacity (TIBC) > 450 µg/dL. Exclusion criteria included: gestational or persistent disease and fetal abnormalities. 100 women were enrolled and randomized into two groups: 1) <u>100 mg cMDLf/d (n=50) and 2) 520 mg ferrous sulfate (100 mg diferric Fe) (n=50) for 30 d.</u> Hb, serum ferritin, serum Fe and TIBC were measured at 0 and 30 d.</p>	<ul style="list-style-type: none"> • Both cMDLf and ferric sulfate supplementation caused a significant increase in Hb, serum ferritin and serum iron at 30 d, versus baseline levels (p<0.01). • cMDLf supplementation was as effective as ferric sulfate on the above parameters, based on the magnitude of improvement observed for each measurement. However, the cMDLf group reported fewer side effects versus the ferric sulfate group. 	<ul style="list-style-type: none"> • One patient in the cMDLf group was excluded due to miscarriage. • Two patients in the ferrous sulfate group discontinued treatment due to severe gastrointestinal symptoms. • Median scores of abdominal pain and constipation were significantly higher in the ferrous sulfate group versus the cMDLf group. • cMDLf was well tolerated.
Paesano et al., 2009 (Data reported within a review paper)	<p>Data on pregnant women supplemented with cMDLf or ferrous sulfate were included within the review.</p> <p><i>Study type:</i> Not specified</p> <p><i>Source:</i> Unknown</p>	<p><i>Note:</i> No details were included regarding the inclusion or exclusion criteria, randomization scheme, treatments (besides doses), or statistical plan, etc. The study description within the review mentions the pregnant women are either iron deficient or have iron deficiency anemia, but the criteria which were used to establish this were not reported.</p> <p>143 <u>pregnant women</u> were offered iron supplementation. The <u>33 who refused any treatment were designated as a control group.</u> <u>60 women were given cMDLf</u> (dose not specified, but previous study administered 200 mg total cMDLf/day, in 2 divided doses), and 50 women received ferrous sulfate (dose not stated). <u>Treatment was for 30d</u> and hematological and iron-related parameters were measured at 0 and 30 d.</p>	<ul style="list-style-type: none"> • Hemoglobin levels were significantly increased in both cMDLf and ferrous sulfate groups (p<0.0001 for both), versus baseline levels. • Serum ferritin, total serum iron, and total red blood cells were all significantly increased in the cMDLf group (p<0.0001 for all), but not the ferrous sulfate group, versus baseline levels. • Hemoglobin, total serum iron and serum ferritin all declined in the control group, versus baseline levels (p=0.0042, 0.0017 and 0.0066, respectively). 	<ul style="list-style-type: none"> • None

000130

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
<p>Bharadwaj et al., 2010</p> <p style="writing-mode: vertical-rl; transform: rotate(180deg);">00034</p>	<p>To measure the effect of milk ribonuclease-enriched cMDLf on inflammatory responses in postmenopausal women.</p> <p>[Same study as reported on in Bharadwaj et al. 2009?]</p> <p><i>Study design:</i> Randomized, placebo-controlled</p> <p><i>Source:</i> Ribonuclease (angiogenin)-enriched cMDLf was either co-isolated from milk (1:1 ratio, w/w) or separately admixed to this ratio. [Assume purity of cMDLf is ≤ 50%.]</p>	<p>38 <u>healthy postmenopausal women, aged 45-60 yr, with no menses for at least 12 mo</u> were enrolled; three were excluded based on history of treatment for bone health or hypothyroidism. Women were randomized to one of two groups: 1) <u>250 mg ribonuclease-enriched cMDLf/day (n=20; estimate 125 mg cMDLf/d)</u> or 2) control capsules (n=15) for <u>180 d</u>. Each group also received an oral supplement containing 100% of the RDA for calcium. Blood samples were collected on d 0, 30, 90 and 180. Cytokine levels were measured.</p>	<ul style="list-style-type: none"> • IFN-γ levels were reduced (no statistics) at 30, 90 and 180 d in the treatment group, versus placebo. • In general, TNF-α, IL-6, and CRP levels were decreased in both groups at 30 and 90 d, but the magnitude of the decrease was increased in the treatment group, versus placebo. [It should be noted that the initial levels of TNF-α were greater in the treatment group (p=0.07) versus control group.] • IL-10 levels were increased in the treatment group at all time points, relative to the control group. • A small decrease in TGF-β was noted in the treatment group at all time points but it is not known if this is statistically significant or clinically meaningful. The authors report that TGF-β levels vary widely in postmenopausal women. • Effects of treatment on IL-1β, IL-12, and RANKL were either equivocal or not consistent over time. 	<ul style="list-style-type: none"> • There were three drop-outs from the control group before day 15 and one subject from the treatment group was dropped due to non-compliance. • All subjects had > 95% compliance with supplements. • No adverse events were reported during the 6 mo study or 3 mo follow-up period.

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
<p>Ishikado et al., 2010</p>	<p>To study the effect of orally administered cMDLf in subjects with periodontal disease.</p> <p><i>Study design:</i> Open intervention</p> <p><i>Source:</i> cMDLf was from Morinaga Milk Industry Co., Ltd., Japan</p>	<p>14 volunteers (11M/3F) aged 37-59 having periodontal disease were given a total of 180 mg cMDLf/d as tablets (4/d) formulated as liposomes with soy phosphatidylcholine for 4 wk. Compliance was assessed via pill count and intake record. Probing depth, bleeding on probing, gingival crevicular fluid volume and levels of cytokines and monocyte chemoattractant protein-1 (MCP-1) in gingival crevicular fluid was evaluated over time, and blood samples were taken.</p>	<ul style="list-style-type: none"> • Levels of MCP-1 in gingival crevicular fluid were significantly decreased at 2 (p<0.01) and 4 wk (p<0.001), versus wk 0. TNF-α and IL-1β levels were not affected. • At 4 wk, TNF-α and IL-6 (p<0.05) and IL-1β and MCP-1 (p<0.01) levels in culture supernatants of <i>Porphyromonas gingivalis</i> LPS-stimulated peripheral blood monocytes from subjects were significantly decreased, versus 0 wk. No effect of treatment on mRNA expression of Toll-like receptor-2 (TLR2) or -4 (TLR4) was observed. 	<ul style="list-style-type: none"> • Two subjects were dropped from the study due to lack of compliance and extenuating circumstances. • No clinically relevant effects on total protein, aspartate aminotransferase (AST), alanine transaminase (ALT), total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides, alkaline phosphatase, albumin, A/G ratio, gamma-glutamyltranspeptidase (γ-GT), amylase, urea, nitrogen, ureic acid and creatine, glucose, HbA1c, erythrocytes, hemoglobin, hematocrit, leukocytes, platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC) were found. Some significant changes were observed in total protein, A/G ratio, ureic acid, HbA1c, MCV, MCHC and monocyte count but all variations were within established normal variation ranges.

000132

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
Ono et al., 2010	<p>To determine whether enteric-coated cMDLf would improve visceral fat-type obesity.</p> <p><i>Study type:</i> Double-blind, placebo-controlled, randomized</p> <p><i>Source:</i> Not stated.</p>	<p>30 <u>healthy Japanese men and women over 20 yr-old, with a BMI > 25 kg/m² and a visceral fat area > 100 cm²</u> volunteered for the study. Two subjects did not meet inclusion criteria; the remaining 28 were randomized to one of two groups: 1) control (lactose tablets; n=14) or 2) <u>enteric-coated cMDLf tablets (300 mg cMDLf/d, taken as 3×100 mg cMDLf/tablets; n=14)</u>. After a 2 wk run-in period, subjects received three enteric-coated tablets/day for 8 wk. Energy and fat intake were not limited but authors state that supplemental foods or medications known to influence lipid or carbohydrate metabolism were prohibited. Subjects were instructed to maintain their usual dietary intake and physical activity. Subjects fasted overnight for visits at 4 wk intervals at which time anthropometric, circulatory, biochemical and hematological measurements were taken. Interviews were carried out at -2, 0, 4, and 8 wk and computed tomography (CT) was performed at 0 and 8 wk to measure abdominal fat area. One subject was discontinued from each group due to job relocation or work pressure.</p>	<ul style="list-style-type: none"> • Subjects in the cMDLf group had a statistically significant reduction in weight and BMI at wk 4 (p<0.05 for both) and wk 8 (p<0.01 for both), versus baseline measurements, and a statistically significant reduction in waist circumference and hip circumference at wk 8 (p<0.01 and P<0.05, respectively), versus baseline measurements. Similar effects were not observed in the control group. • Subjects taking cMDLf experienced a statistically significant decrease in visceral and total fat area at 8 wk (p<0.01 for both), versus baseline measurements. Similar effects were not observed for the control group. 	<ul style="list-style-type: none"> • No effect of cMDLf was observed for systolic or diastolic blood pressure, or pulse rate, versus control. • cMDLf did not affect blood lipids, including total cholesterol, HDL, LDL, triacylglycerides, total lipid, non-HDL cholesterol, and non-esterified fatty acids (nEFA). • No adverse events were observed with regard to safety parameters.
Paesano et al., 2010	<p>To study the effect of orally supplemented cMDLf on iron parameters in pregnant and non-pregnant women.</p> <p><i>Note:</i> Two separate clinical trials are reported on.</p>	<p>Subjects for both studies were enrolled when at least one of the four following hematological parameters was deemed indicative of iron deficiency by the study authors: red blood cell count (< 4 × 10⁶/mL), hemoglobin level (≤ 11 g/dL), total serum iron concentration (≤ 30 mg/dL), and serum ferritin level (≤ 12 ng/mL). Exclusion criteria included women in their first or second trimester of pregnancy (study #1 only), non-pregnant women (study #1 only), pregnant</p>	<p><i>Study #1:</i></p> <ul style="list-style-type: none"> • After 30 d, RBC, hemoglobin, total serum iron, serum ferritin and hematocrit levels increased significantly in the cMDLf group (n=30), versus baseline levels (p<0.0001 for all parameters). • Only hemoglobin was increased (p=0.03) in the ferrous sulfate group (n=30), versus baseline levels. • None of the measured 	<p><i>Study #1:</i></p> <ul style="list-style-type: none"> • The authors report that there were no dropouts in the cMDLf group. • Three subjects taking ferrous sulfate dropped out due to side effects. • One subject in the control group dropped out due to severe anemia requiring iron treatment.

000133

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
	<p><i>Study type:</i> Open-label, prospective, randomized</p> <p><i>Source:</i> cMDLf was from Lattoglobina, Grunenthal, Italy; iron saturation was ~30%.</p>	<p>women (study #2 only), prior treatment with iron supplements, concomitant diseases, recent blood transfusion and allergy to milk or iron products.</p> <p><i>Study #1:</i> 12 pregnant women refusing iron therapy constituted the control group. 75 pregnant women consenting to iron therapy were randomized into one of two groups: 1) 33 women receiving 520 mg ferrous sulfate once daily with food or 2) 30 women receiving 100 mg cMDLf twice daily before meals (200 mg cMDLf total). Treatments were administered for 30 d. Hematological parameters were measured.</p> <p><i>Study #2:</i> 9 women refusing therapy constituted the control group. One hundred eighty-nine women consenting to iron therapy were randomized to one of two groups: 1) 90 subjects receiving 520 mg ferrous sulfate once a day with food or 2) 90 subjects receiving 100 mg cMDLf twice daily (200 mg cMDLf total). Treatments were administered to subsets of subjects for 30, 60, or 90 d. Hematological parameters were measured.</p>	<p>hematological parameters changed significantly by 30d in the control group (n=11).</p> <p><i>Study #2:</i></p> <ul style="list-style-type: none"> All measured parameters (RBC, hemoglobin, total serum iron, serum ferritin, and hematocrit) were significantly increased (p<0.0001 for all) at 30 (n=34), 60 (n=22), and 90 d(n=34) after cMDLf treatment, versus baseline levels/group. None of the measured hematological parameters changed significantly in the ferrous sulfate groups at any time point, versus baseline levels. 	<p><i>Study #2:</i></p> <ul style="list-style-type: none"> The authors report there were no study dropouts in the cMDLf group due to side effects. Fourteen of ninety subjects taking ferrous sulfate dropped out due to side effects.
<p>Shin et al., 2010</p> <p style="writing-mode: vertical-rl; transform: rotate(180deg);">000134</p>	<p>To test the effects of oral cMDLf and lactoperoxidase on oral malodor and salivary bacteria.</p> <p><i>Study type:</i> Randomized, double-blind, placebo-controlled, cross-over</p>	<p>15 healthy volunteers aged 26-54 yr (11M/4F) were randomly assigned to receive either 1) cMDLf (cMDLf; 100 mg cMDLf + 1.8 mg lactoperoxidase + 24.0 mg glucose oxidase/tablet; n=8) tablets or 2) placebo tablets (maltitol + cornstarch; n=7) daily for 1 day, then underwent a 1 wk washout period before crossing over to the alternate therapy for 1 day. Two tablets were ingested in the morning with a 1 h interval in between doses</p>	<ul style="list-style-type: none"> Concentrations of CH₃SH and total volatile sulfur compounds in mouth air were significantly reduced (p<0.05 for both) after 10 min treatment with the first tablet of cMDLf, versus control tablets. Versus baseline levels of H₂S, CH₃SH, and total volatile sulfur compounds, both cMDLf and control tablets caused an overall decrease (no 	<ul style="list-style-type: none"> None

Table 17. Clinical Studies of cMDLf in Adults

Reference	Objective/Test Article	Study Design	Outcome	Safety-Related Observations Reported
000135	<p><i>Source:</i> cMDLf tablets were from Morinaga Milk Industry, Tokyo, Japan</p>	<p>(total of 200 mg cMDLf/d). Tablets were sucked for 10 min and then chewed and swallowed if still remaining. Volatile sulfur compounds and saliva samples were taken just prior to administration and 10, 60 and 120 min after the tablet treatments.</p>	<p>statistics) in these parameters [suggests an inhibitory substance may be present in the control tablets or that a placebo effect exists].</p> <ul style="list-style-type: none"> • Culture methods and quantitative PCR did not indicate any differences in salivary bacteria populations for the two treatments. • However, terminal restriction fragment length polymorphism analysis (T-RFLP) on the DNA extracted from saliva indicated statistically significant ($p < 0.05$ between 0 and 2 h and/or $P < 0.05$ between cMDLf and placebo) inhibition of specific bacterial species by the cMDLf tablets at the 2 h time point, versus baseline. [Refer to report for list of organisms.] 	
	<p>Mueller et al., 2011</p> <p>To study the effect of oral supplementation of cMDLf in subjects with mild to moderate facial acne vulgaris.</p> <p><i>Study type:</i> Open-label, prospective, single-center, single-arm (treatment-only)</p> <p><i>Source:</i> cMDLf was supplied as Dermaplus™ chewable tablets (also contained whey milk protein) (manufactured and supplied by Mepha LLC, Aesch, Switzerland; now marketed by Biotan Ltd, Switzerland)</p>	<p>43 healthy subjects (18F/21M), aged 17.5 ± 3.8 yr, having dermatologist-confirmed mild to moderate acne vulgaris of the face were enrolled. Subjects with known milk intolerance or allergy were excluded. Subjects took one chewable tablet containing 100 mg cMDLf/tablet twice/day for 8 wk (200 mg cMDLf/d). Based on tablet counts, > 95% of the cMDLf formulation was ingested. Acne lesions were counted at regular intervals during the intervention.</p>	<ul style="list-style-type: none"> • At 2, 4 and 8 wk treatment, the total count of acne lesions was significantly decreased from baseline levels ($p = 0.005$, 0.007 and $p < 0.001$, respectively). • The number of inflammatory lesions was significantly reduced only at 2 wk treatment ($p = 0.002$). • The number of non-inflammatory lesions was significantly reduced at 4 and 8 wk treatment ($p < 0.001$ for both time points). 	<ul style="list-style-type: none"> • Three subjects were lost to follow-up. One subject was excluded due to protocol violation (non-compliance with use of cosmetics). • No serious adverse events and no adverse events leading to discontinuation of test product were noted. • None of the subjects experienced an adverse event considered to be cMDLf-related by the investigator during the study.

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000150

Appendix 1 – Raw Material Product Specification

000151

Raw material specification

2006 Sweet Whey

Criteria	Method	Dimension	Target	Toleranz	
				min	max
Taste, Odor			normal		
Temperatur		°C	8		
Scorched Particles	ADPI	----	A		
Sediment	ADPI	ml	0,00		0,20
pH	VDLUFA C 8.2	----	6,3	6,00	6,70
Dry matter	3 h / 102 +/- 2 °C	%	6,20		
Fat	Gerber	%	0,00	0,00	0,05
Protein in d.m.	Kjeldahl (Nx6.38)	%	14,00	13,00	
Nitrate/Nitrite	Merckoquant	ppm	neg/neg		10/neg
aerob. mesoph. Spores	PC+MM 30°C/48h	/ml	0		10
aerob. thermoph. Spores	PC+MM 55°C/48h	/ml	0		10
Bc. cereus	Bc. cer. - Selekt Agar	/ml	0		2
Sulfite Red. Clostridia (SR)	PCM 30°C/96h	/ml	0		0,3
Staph. aureus	Baird Parker 37°C/48h	/ml	neg		
Salmonella	§ 35 LMBG L00.00	/25ml	n.n.		

The product has to be conform to the hygienic guidelines of European Union and the German Law of food and commodity (LMBG) in the respective legal version, as well as all updates

Date, Signature MILEI - GmbH (b) (6)	
	24.05.2011

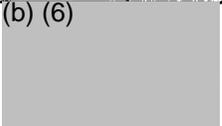
Date, Signature Supplier

Raw material specification

2200 Skim milk

Criteria	Method	Dimension	Target	Toleranz	
				min	max
Taste, Odor			normal		
Temperatur		°C	8		
pH	VDLUFA C 8.2	----	6,60	6,40	6,90
Dry matter	3 h / 102 +/- 2 °C	%	9,4		
Fat	Gerber	%	0	0	0,05
Protein in d.m.	Kjeldahl (Nx6.38)	%	38	35	39
Nitrate/Nitrite	Merckoquant	ppm	neg/neg		neg/neg
Total Plate Count	PC 30°C/48h	/ml	0		100000
aerob. mesoph. Spores	PC+MM 30°C/48h	/ml	0		100
aerob. thermoph. Spores	PC+MM 55°C/48h	/ml	0		100
Bc. cereus	Bc. cer. - Selekt. Agar	/ml	0		5
sulfred. anaerobe Spores	DRCM 30°C/96h	/ml	0		0,1
Staph. aureus	Baird Parker 37°C/48h	/ml	neg		
Salmonella	§ 35 LMBG L00.00	/25ml	n.n.		

The product has to be conform to the hygienic guidelines of European Union and the German Law of food and commodity (LMBG) in the respective legal version, as well as all updates

Date, Signature M I L E I - GmbH
(b) (6)  24.05.2011

Date, Signature Supplier

Appendix 2A – HPLC Method

000154

Dec, 28, 2010

In-house validation of the measuring method of purity and / or quantity of bovine lactoferrin of a production lot (measuring a purified form of the analyte)

1. Linearity

Measured 250mg of standard bovine lactoferrin in 50ml measuring flask, and dissolved the 250mg of the standard bovine lactoferrin with sodium chloride solution (3%) to a specific volume. Then, the solution was diluted so that its concentration range would be "0.5 ~ 3.0 mg/ml" and "Concentration level : 6" to prepare a linearity testing solution.

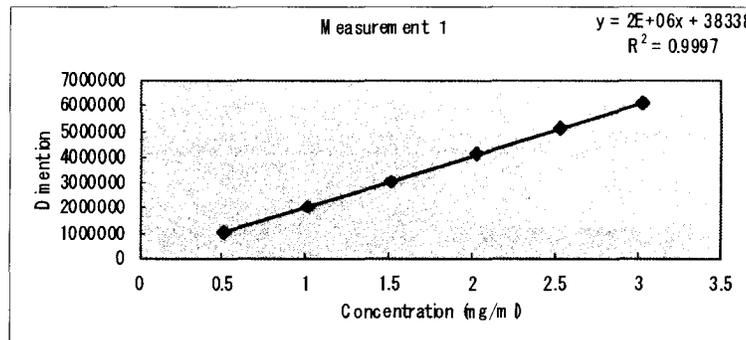
This test was conducted on another day by different person.

Concentration of the linearity testing solution : 0.5, 1.0, 1.5, 2.0, 2.5, 3.0mg/ml

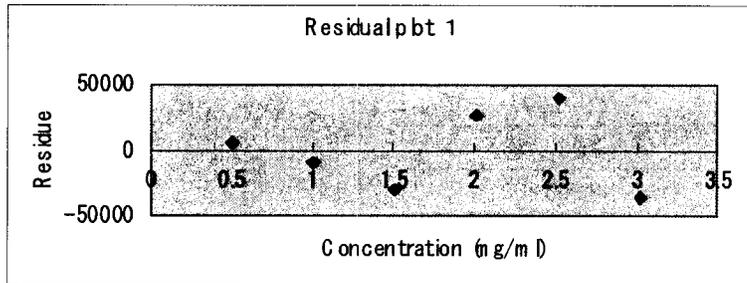
Result : Obtained HPLC dimension and residual plot at each concentration.

Measurement 1

Concentration (mg/ml)	Dimension value of the peak
0.504	1,057,939
1.008	2,056,366
1.512	3,049,160
2.016	4,120,039
2.520	5,146,618
3.024	6,084,465

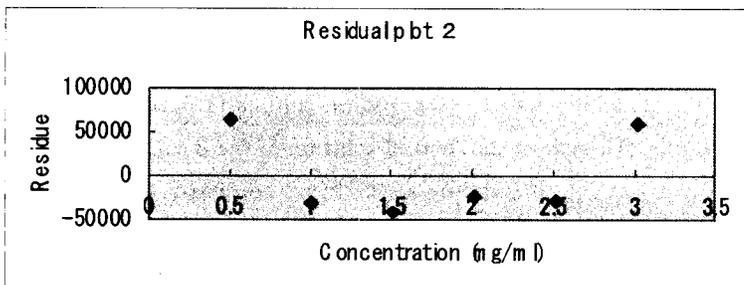
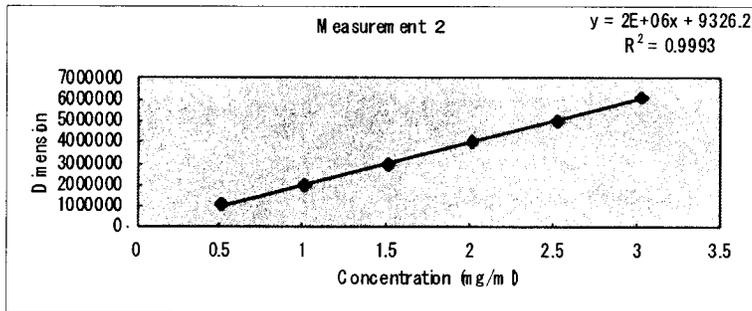


000155



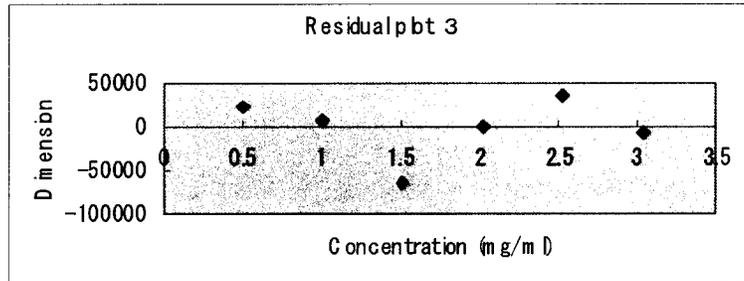
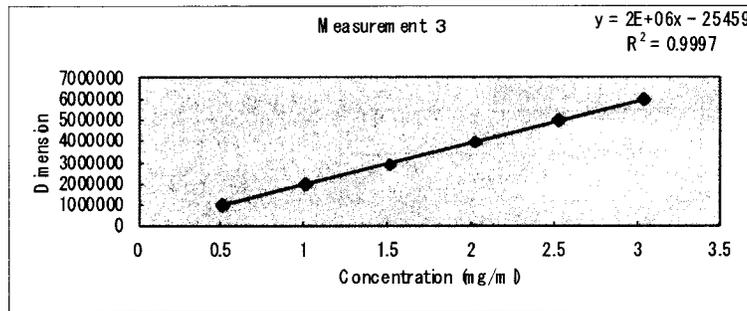
Measurement 2

Concentration (mg/ml)	Dimension
0.504	1,081,569
1.008	1,993,516
1.511	2,989,010
2.015	4,014,259
2.519	5,016,267
3.023	6,112,795



Measurement 3

Concentration (mg/ml)	Dimension
0.504	995,937
1.009	1,980,458
1.513	2,906,608
2.018	3,970,917
2.522	5,003,346
3.036	5,979,276



2. Precision

2. 1 Repeatability

Measured 15, 20 & 25mg of sample (MLF-2) in 10ml measuring flask and dissolved the samples with sodium chloride solution (3%) to a specific volume to make them the Repeatability testing solutions". Measured the testing solution of the 3 concentrations 3 times respectively, obtained the quantitative values from standard curve, then calculated the repeatability of the 3 concentrations respectively.

Result

Concentration of testing solution (mg/ml)	Measurement 1	Measurement 2	Measurement 3	MEAN	SD	RSD(%)
1.5	1.48	1.45	1.45	1.46	0.01732	1.19
2.0	1.96	2.02	2.00	1.99	0.03055	1.53
2.5	2.43	2.42	2.46	2.44	0.02082	0.85

000157

2. Intermediate Precision

Measured 20mg of sample (MLF-2) in measuring flask and dissolved the sample with sodium chloride solution (3%) to a specific volume to make it the intermediate precision testing solution. Conducted the repeatability test with the sample solution and obtained quantitative results from standard curve.

3 persons (A,B & C) conducted the repeatability tests on different days, obtained total 6 quantitative results and calculated the intermediate precision.

Result

	Quantitative result(mg/ml)
A-1	1.89
A-2	1.89
B-1	1.89
B-2	1.89
C-1	1.87
C-2	1.86
MEAN	1.881
SD	0.01329
RSD(%)	0.71

3. Specificity

Compared the peak time of HPLC with lactoferrin and whey proteins (bovine β Lactoglobulin, bovine serum albumin, bovine IgG and bovine LPO) (HPLC peak chart attached)

Result

Bovine protein	Time (min) of the peak
lactoferrin	10.216
IgG	4.668
bovine serum albumin	9.047
β Lactoglobulin	14.143, 14.572
LPO	15.161, 15.763

- End -

000158

Appendix 2B – HPLC Method

Determination of Lactoferrin (HPLC Method)

1. Preparation

Accurately weigh 20mg of the MLF (Morinaga Lactoferrin) into 10ml volumetric flask.
Dissolve in 0.5mol/l NaCl solution and dilute to 10ml using 0.5mol/NaCl solution.

2. Chromatography conditions

- Column : SHODEX ASAHIPAK C4P50 4D
- Temperature : 30°C
- Flow rate : 0.8ml/min.
- Detection wavelength : 280nm
- Injection volume : 25 μ l
- Stop time of chromatogram : 25min.
- Gradient condition (linear gradient)
 - Mobile phase A : Acetonitrile 0.5mol/l NaCl (1:9)
 - Mobile phase B : Acetonitrile 0.5mol/l NaCl (5:5)
 - TFA is added to mobile phase A, B (conc. 0.03%)

Time	A %	B%
0.0	50	50
25.0	0	100
25.1	50	50
35.0	50	50

3. Calculation

$$\text{Lactoferrin (\%)} = \text{ALF} / \text{APK} \times 100$$

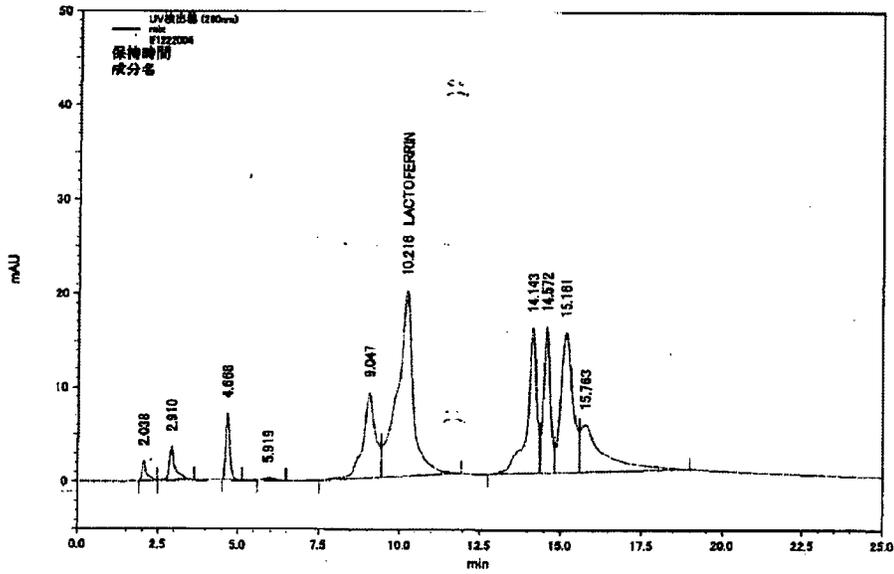
where ALF : Peak area of lactoferrin

APK: Total peak area of peaks detected in chromatogram

000160

Appendix 2C – HPLC Method

ファイル名 : D:\CLASS-VP\2010\大塚\LF\Fif-mix.met
 データ名 : D:\CLASS-VP\2010\大塚\LF\data\Fif1222004
 ユーザー : System
 サンプルID: mix
 注入量 : 25 ul
 分析日時 : 10/12/22 午後 12:02:20
 印刷日時 : 10/12/22 午後 1:37:40
 サンプル量 : 1
 希釈率 : 1
 ファイル名 : D:\CLASS-VP\2010\大塚\LF\batch\Fif1222.seq
 コメント : MDX



UV Detector (280nm)	Retention	Constituent	Dimension	Dimension	Height	ESTD concentra	Waveform processing
1	2.038		20986	0.93	2127	0.000	BB
2	2.910		48649	2.15	3587	0.000	BB
3	4.688		84176	2.83	7196	0.000	BB
4	5.919		6077	0.27	244	0.000	BB
5	9.047		252709	11.14	9065	0.000	BV
6	10.218	LACTOFERRIN	694864	30.64	19770	0.449	VB
7	14.143		317705	14.01	15494	0.000	BV
8	14.572		222463	9.81	15511	0.000	VV
9	15.161		380773	16.79	14876	0.000	VV
10	15.783		259149	11.43	5015	0.000	VB

TOTAL			226795	100.00	32764	0.449	
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000162

Appendix 3 – Stability of cMDLf

000163



MORINAGA MILK INDUSTRY CO., LTD.
 33-1, SHIBA 5-CHOME, MINATO-KU, TOKYO 108 8384, JAPAN

TEL: 81-3-3798-0152
 FAX: 81-3-3798-0107
 E-mail: intrentl@morinagamilk.co.jp

Data on Stability of Lactoferrin in MLF-1, MLF-2

Item		MLF-1			MLF-2	MLF-1	
Lot		970728	980427	10124	311002	040209	
Manufacturing date		28-Jul-97	27-Apr-98	24-Jan-01	31-Oct-02	4-Feb-09	
Storage period		102 months	96 months	60 months	37 months	2 month	
Storage condition		Room temperature in aluminum bag	Room temperature in aluminum bag	Room temperature in aluminum bag	Room temperature in polyethylene bag	Room temperature in polyethylene bag	
Result		No abnormality found	No abnormality found	No abnormality found	No abnormality found	No abnormality found	Specification
Appearance		Good	Good	Good	Good	Good	Pink and odorless powder
Flavor		Good	Good	Good	Good	Good	-
Solubility		Good	Good	Good	Good	Good	-
Loss on drying	%	1.6	1.2	2.4	0.8	0.3	≤ 4.2%
pH		6.0	5.9	5.6	5.9	5.8	5.2 - 7.2
Lactoferrin Purity (HPLC) on protein	%	96.8	96.7	96.3	97.4	96.1	≥ 96.0% (by HPLC)
Total Bacteria	/g	0	10	10	0	0	≤ 1,000 cfu /g
Coliform bacteria	/0.2g	Negative	Negative	Negative	Negative	Negative	Negative / 0.1g
Coagulase positive staphylococci	0.01g	Negative	Negative	Negative	Negative	Negative	Negative / 0.01g
Yeast	/g	0	0	0	0	0	≤ 30 cfu /g
Mold	/g	0	0	0	0	0	≤ 30 cfu /g

000164



MORINAGA MILK INDUSTRY CO., LTD.
33-1, SHIBA 5-CHOME, MINATO-KU, TOKYO 108-8384, JAPAN

TEL: 81-3-3798-0152
FAX: 81-3-3798-0107
E-mail: infant@morinagomilk.co.jp

Stability of Lactoferrin in Infant Formula products

Product name : ① Infant Formula "Hagukumi"
② Follow Up Formula "Chil Mil"

Shelf life : ① 18 months at room temperature
② 18 months at room temperature

Comparison of lactoferrin analysis results assuming the result at production is "100%".

Product	Lot	At production	24months	36 months
Infnt formula "Hagukumi"	7J03	100.0%	97.3%	n.a.
Infnt formula "Hagukumi"	7F05	100.0%	n.a.	117.3%
Infnt formula "Hagukumi"	7E21	100.0%	n.a.	113.3%
Follow up formula "Chil mil"	7L25	100.0%	93.6%	n.a.
Follow up formula "Chil mil"	7I20	100.0%	90.0%	n.a.
Follow up formula "Chil mil"	7B02	100.0%	n.a.	118.2%

* "n.a." = not available



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Stability of Lactoferrin in Yogurt product

Product

name : Lactoferrin & Bifidobacterium Yogurt

Shelf 16 days at refrigerated condition (less than

life : 10°C)

* Shelf life of yogurt products in Japan is normally around
15 days.

Comparison of lactoferrin analysis results assuming the result at "3 days
after production" is "100%".

Lot	3 days after production	17 days after production
10.01.04	100.0%	90.8%
10.01.05	100.0%	102.8%
10.01.06	100.0%	97.5%

000166



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FAX: 81-3-3798-0107
E-mail: internit@morinogamilk.co.jp

Stability of Lactoferrin in Skimmed milk powder

Product

name : Lactoferrin skim (Skim milk powder for adult)

Shelf life: 12 months at room temperature

Comparison of lactoferrin analysis results assuming the result at production is "100%".

Lot	at production	46 months after production
60215	100.0%	100.8%

000167

**Appendix 4 – Grant Certificate by the
Japaneses Ministry of Health**

LF Use-Level, History in Infant Formula Products

1) Current actual LF use-level in our infant & follow-up formula products sold in Japan

	Lactoferrin used Product	Producer	Lactoferrin Use-Level	Target population	Market place	Launch time	Current sales volume	Post market Monitoring	Morinaga Lactoferrin
1	Infant Formula "Hagukumi" (はぐくみ)	Morinaga	80mgLF / 100g powder 10mg LF/100ml milk 15 mg / kg body (assuming takig 150ml per 1kg body weight)	0 - 9 months	Japan	Since 1986 (*1) LF contents has varied from 50 mg/100g to the current contents	Approx. 3,200 metric ton / year	No significant health problem or allergy reaction has been reported so far	○ MLF-1 or MLF-2
2	Follow up Formula "Chil-mil" (チルミル)	Morinaga	45mgLF/ 100g powder 6.3mg LF/100ml milk 6 mg / kg body (assuming takig 100ml per 1kg body weight)	9months - 3 years	Japan	Since 1989	3,500 metric ton / year	No significant health problem or allergy reaction has been reported so far	○ MLF-1 or MLF-2

(*1) In 1986, the product name was originally "BF-L", instead of "Hagukumi". LF use-level at that time was *

2) Other infant formula products using lactoferrin

	Product	Producer	Lactoferrin Use-Level	Target population	Market place	Launch time	Current sales volume	Post market Monitoring	Morinaga Lactoferrin
1	Infant formula "Pure"	Snow Brand (Japan)	55mg LF / 100g	0 - 9 months	Japan	-	-	-	-
2	Follow-up formula "Tacchi"	Snow Brand (Japan)	50mg LF / 100g	9 months - 3 years	Japan	-	-	-	-
3	Infant formula "Hai-Hai"	Wakodo (Japan)	100mg LF / 100g	0 - 9 months	Japan	-	-	-	-
4	Infant Formula "BF-1"	Morinaga	50mg LF / 100g	0-6months	Pakistan	2001	Approx. 700 ton / year	No significant health problem or allergy reaction has been	○ MLF-1
5	Follow-up formula BF-2 (Morinaga)	Morinaga	50mg LF / 100g	6 months - 3 years	Pakistan	2001	Approx. 500 ton / year	No significant health problem or allergy reaction has been	○ MLF-1
6	Infant formula "BMT"	Morinaga (Indonesia)	50mg LF / 100g	0-6months	Indonesia	-	-	No significant health problem or allergy reaction has been	○ MLF-2
7	Follow up formula Chil-mil (Morinaga)	Morinaga (Indonesia)	50mg LF / 100g	6 months - 3 years	Indonesia	-	-	No significant health problem or allergy reaction has been	○ MLF-2
8	Infant Formula New CHIL-MIL ①	Morinaga (China)	50mg LF / 100g	0-6 months	China	-	-	No significant health problem or allergy reaction has been	○ MLF-2
9	Follow up Formula New CHIL-MIL ②	Morinaga (China)	40mg LF / 100g	6 months - 3 years	China	-	-	No significant health problem or allergy reaction has been	○ MLF-2
10	Infant Formula "Imperial"	Namyang (Korea)	80 mg LF / 100g	0-6 months	Korea	-	-	-	-
11	Infant Formula "Regenti"	Namyang (Korea)	75 mg LF / 100g	0-6 months	Korea	-	-	-	-
12	Infant Formula "Omega"	Maeil (Korea)	75 mg LF / 100g	0-6 months	Korea	-	-	-	-
13	Infant Formula YILI	YILI	30 mg LF /100g	0-6 months	China	-	-	-	-
14	Infant Formula BEINGMATE	BEINGMATE	30 - 100 mg / 100g	0-6 months	China	-	-	-	-
15	Infant Formula Anticong	Wandashan	30-100 mg LF / 100g	0-6 months	China	-	-	-	-
16	Infant Formula YASHILI	YASHILI	38 mg LF /100g	0-6 months	China	-	-	-	-
17	Infant Formula SCIENT	SCIENT	30 - 80 mg LF /100g	0-6 months	China	-	-	-	-
18	Infant Formula AUSNUTRIA	AUSNUTRIA	80 mg LF / 100g	0-6 months	China	-	-	-	-

000169

Grant certificate by Japanese Ministry of Health to Morinaga
on the use of "Special Nutritious Foods description (Infant Formula)" (Free translation)

厚生省生衛第542号

特殊栄養食品標示許可書

申請者 森永乳業株式会社

昭和61年6月3日付けで申請のあった「森永BF-Lドライミルク」
について栄養改善法（昭和27年法律第248号）第12条第1項の
規定により、下記のとおり特殊栄養食品の標示をすることを許可
する。

昭和61年9月1日

厚生大臣 齋藤十朗

記

許可番号 第12892号

許可の有効期限 昭和61年9月1日から2年

標示内容 赤ちゃんにとっては、健康なお母さんの
母乳が最良です
母乳が不足したり与えられない時、
心掛けてお与えください。

その他

Ministry of Health, No. 542

Grant of Special Nutritious Foods Description

Applicant : Morinaga Milk Industry Co., Ltd.

With regard to "Morinaga BF-L Dry Milk (Infant Formula)" which applied on 13th June 1986,
we grant the description of the Special Nutritious Foods as follows according to
the Nutrition Improvement Law.

September 1st 1986

Juro Saito / Minister of Health & Welfare

Grant No. 12892

Valid until 2 years from September 1st 1986 (We revised the grant each 2 year)

Contents of description Mother's breast milk is the best food for infants

Please feed this product (BF-L) when mother's breast milk is not available sufficiently.

Others

000171

Grant certificate by Japanese Ministry of Health to Morinaga
on the use of "Special Use Food description (Infant Formula)" (Free translation)

厚生労働省発食安第0127001-1号

特別用途食品表示許可書

申請者 森永乳業株式会社

平成20年12月19日付けで申請のあった「森永ドライミルク はぐくみ」
について、健康増進法（平成14年法律第103号）第26条第1項の規定に
基づき、下記のとおり特別用途食品の表示をすることを許可する。

平成21年1月27日

厚生労働大臣 舛 添 要 一

記

許可番号 第20019号

表示内容 乳児用調製粉乳
赤ちゃんにとって、健康なお母さんの母乳が最良
です。
母乳が足りない赤ちゃんに、安心してお使いい
ただけます。
赤ちゃんの体質や健康状態によって、医師、管理
栄養士等にご相談ください。

Issue of Ministry of Health, Labour & Welfare, Food Safety No. 0127001-1

Grant of Special Use Food Description

Applicant : Morinaga Milk Industry Co., Ltd.

With regard to "Morinaga Dry Milk (Infant Formula) HAGUKUMI (はぐくみ)" which was applied on December 19th, 2008,
we grant the description of Special Use Food as following according to the Health Promotion Law

January 27rd 2008

Ministor of Health, Labour & Welfare
Yoichi Masuzoe

Grant No. 20019
Description "Infant Formula"

"Mother's breast milk is the best food for infants
Please feed this product to baby in case mother's breast milk is not available.
Please consult doctor or nutritionist according to the health condition of your baby"

000173

**Appendix 5A-1 – Specification monograph
for cMDLf produced by Morinaga Milk
Industry**

第 三 版

既存添加物 自主規格

平成14年11月

■日本食品添加物協会■

000175

ラクトフェリン濃縮物

Lactoferrin concentrates

定義 本品は、ほ乳類の乳から得られた、ラクトフェリンを主成分とするものである。

含量 本品を乾燥物換算したものは、窒素 (N=14.01) 14.0~16.5% 含み、たん白質中にラクトフェリン 85.0% 以上を含む。

性状 本品は、淡赤だいたい~濃赤褐色の粉末で、においが無い。

確認試験 (1) 本品の水溶液 (1→100) 10ml に水酸化ナトリウム溶液 (1→10) 1 ml を加え、更に硫酸銅溶液 (1→8) 1 滴を加えて振り混ぜるとき、青色の沈殿を生じ、液は紫色を呈する。

(2) 本品の水溶液 (1→20) 10ml に希塩酸を 1 ml 加えるとき、溶液の赤色は消える。

純度試験 (1) 液性 pH5.2~7.2 (1.0g, 水50ml)

(2) 鉄 Fe として 0.050% 以下

本品 0.5g を量り、水を加えて溶かし、塩酸 1 ml 及び水を加え 100ml とし、検液とする。別に鉄標準液 25ml を正確に量り、塩酸 1 ml 及び水を加えて正確に 100ml とし、比較液とする。検液及び比較液につき、次の操作条件で原子吸光度を測定するとき、検液の吸光度は、比較液の吸光度以下である。

操作条件

光源ランプ 鉄中空陰極ランプ

分析線波長 248.3nm

支燃性ガス 空気

可燃性ガス アセチレン

(3) 重金属 Pb として 20 μ g/g 以下 (1.0g, 第 2 法, 比較液 鉛標準液 2.0ml)

(4) ヒ素 As₂O₃ として 4.0 μ g/g 以下 (0.50g, 第 2 法, 装置 B)

乾燥減量 6.0% 以下 (105℃, 5 時間)

強熱残分 2.5% 以下

定量法 (1) 窒素 本品約 20mg を精密に量り、窒素定量法中のセミマイクロケルダール法により窒素を定量し、更に乾燥物換算を行う。

(2) たん白質中のラクトフェリン 本品約 0.1g を量り、塩化ナトリウム溶液 (3→100) を加えて溶かして正確に 50ml とし、検液とする。検液 25 μ l を量り、次の操作条件で液体クロマトグラフィーを行い、ピーク面積を自動積分法により測定する。主ピークをラクトフェリンのピークとし、主ピークの保持時間の 2 倍の範囲までに溶出するすべてのピーク面積を測定し、全ピーク面積に対する主ピーク面積の比をラクトフェリン含量とする。

$$\text{ラクトフェリンの含量} = \frac{\text{主ピーク的面積}}{\text{総ピーク面積}} \times 100 (\%)$$

操作条件

検出器 紫外外部吸収検出器 (測定波長 280nm)

カラム充てん剤 5 μ m のブチル基を化学結合したポリビニルアルコールゲル

カラム管 内径 4.6mm, 長さ 15cm のステンレス管

カラム温度 30~40℃ の一定温度

移動相 A トリフルオロ酢酸を 0.03% 含む アセトニトリル/塩化ナトリウム溶液 (3→100) 混液 (1:9)

B トリフルオロ酢酸を 0.03% 含む アセトニトリル/塩化ナトリウム溶液 (3→100) 混液 (1:1)

濃度勾配 A:B (50:50) から (0:100) までの直線濃度勾配を 30 分間行う。

流速 主ピークの保持時間が約 10 分となるよう調整する。

**Appendix 5A-2 – Specification monograph
for cMDLf produced by Morinaga Milk
Industry (English Translation)**

000177



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Free translation (summary) of specification for "Lactoferrin Concentration"
in the existing food additives list in Japan

Definition :

Substance whose major content is lactoferrin derived from mammal milk.

Contents :

On dry matter basis, it should contain 14.0 – 16.5% of nitrogen (N=14.01). And in protein, more than 85% of lactoferrin should be contained.

Appearance :

Pink salmon color powder, no odor.

Confirmation test :

- (1) When 1ml of sodium hydroxide solution and a drop of copper sulfate solution are added into 10 ml of lactoferrin solution and shaken, it brings about blue precipitation and color of solution turns to purple.
- (2) When 1 ml of diluted hydrochloric acid is added into lactoferrin solution, the red color in the solution disappears.

Purity test :

- (1) pH : 5.2 – 7.2 (1.0g, water 50ml)
- (2) Iron content : not more than 0.050% as Fe. (Atomic absorption analysis)
- (3) Heavy metals : not more than 20 μ g / g as Pb.
- (4) Arsenic : not more than 4.0 μ g / g as As₂O₃

Loss on drying : not more than 6.0% (105°C, 5 hours)

Residue on ignition : not more than 2.5%

Quantitative determination method :

- (1) Nitrogen : Determines quantity of nitrogen Semimicro Kjeldahl method
- (2) Lactoferrin in protein : HPLC

Make 50ml of test solution by dissolving 0.1g of lactoferrin into sodium chloride solution.

Measure 25 μ l test solution and do the HPLC test and determine lactoferrin contents by the following formula.

000178



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- Lactoferrin (%) = $ALF / APK \times 100$

- ALF : Main peak area (lactoferrin)
- APK : Total peak area

- Operating condition

- Detector : Ultra-Violet Absorbance Detector (Detection wavelength : 280nm)
- Column packing material : Polyvinyl alcohol gel made by chemical binding of 5 μ g of butyl group.
- Column : Stainless column of 4.6mm inner diameter and 15cm length.
- Column temperature : 30 – 40 °C
- Mobile phase A : Acetonitrile / NaCl solution (1:9)
- Mobile phase B : Acetonitrile / NaCl solution (1:1)
- Concentration gradient : 30 minutes of linear gradient from A:B (50:50) to A:B (0:100)
- Flow rate : Adjust so that the retention time of main peak would be about 10 minutes.

000179

**Appendix 5B-1 – List of Food Additives
Other Than Chemically-Sythesized
Compounds in Japan**

第 一 版

化学的合成品以外の食品添加物リスト

1989

厚生省生活衛生局食品化学課

神奈川県座間市東原5-1-83
森永乳業株式会社
分析センター

000181

品名		簡略名又は類別名	基原・製法・本質	備考
慣用名	別名			
木炭			カバノキ科シラカバ(<i>Betula platyphylla</i> SUKA T. var. <i>japonica</i> HARA)等の木材を炭化して得られたものである。	Carbon Charcoal
木灰			ブナ科ブナノキ(<i>Fagus crenata</i> BLUME)等の木材を灰化して得られたものである。	Timber ash
モミガラ抽出物			イネ科イネ(<i>Oryza sativa</i> L.)の種皮(もみがら)にアルカリ性の水を加えて水蒸気蒸溜して得られたものである。	Rice hull extract
ラクトフェリン濃縮物		ラクトフェリン	母乳、牛乳を脱脂分離精製後、濃縮して得られたものである。成分はラクトフェリン、乳清たんぱく質等である。	Lactoferrin concentrates
流動パラフィン	ミネラルオイルホワイト	パラフィン	石油の軽質留分を留去した残りを分留し、精製して得られたものである。	Liquid Paraffin
リントーセルロース		セルロース	アオイ科ワタ(<i>Gossypium hirsutum</i> L.)の実の単毛を精製して得られたものである。成分はセルロースである。	Linter cellulose
ルテニウム			元素 (^{96}Ru 、 ^{98}Ru 、 ^{99}Ru 、 ^{100}Ru 、 ^{101}Ru 、 ^{102}Ru 、 ^{104}Ru)	Ruthenium
ワサビ抽出物			アブラ科ワサビ(<i>Wasabi japonica</i> MATSUM.)の根茎、葉よりエタノールで抽出して得られたものである。	Wasabi extract

000182

**Appendix 5B-2 – List of Food Additives
Other Than Chemically-Synthesized
Compounds in Japan (English Translation)**

000184

(Free Translation)

The 1st Version

List of Food Additives other than chemically-synthesized compounds

1989

Food Chemistry Division, Environmental Health Bureau, Ministry of Health and Welfare

Name of additive		Short name or Category name	Base, Production method, nature	Note
Trivial name	Other name			
Carbon Charcoal		Lactoferrin	Obtained by removing fat from breast milk or bovine milk and refining and concentrating. Its component is lactoferrin and whey proteins.	Carbon charcoal
Timber ash				Timber ash
Rice hull extract				Rice hull extract
Lactoferrin concentrate				Lactoferrin concentrate
Liquid Paraffin	Mineral oil white			Liquid paraffin
Linter cellulose				Linter cellulose
Ruthenium				Ruthenium
Wasabi extract				Wasabi extract

000185

**Appendix 5B-3 – List of Existing Food
Additives in Japan for the Japan Food
Chemical Research Foundation**



The Japan Food Chemical Research Foundation



List of Existing Food Additives

Last update: 2011/05/09

List of Existing Food Additives

This list of food additives from natural origin is compiled and published by the Ministry of Health and Welfare on April 16, 1996.

These additives are listed here in alphabetic order. The number preceding the name of each additive is the sequence number given to the corresponding additive in the original Japanese list.

Effective from May 6, 2011

No.	Name	Note
236	Absinth extract	A substance composed mainly of sesquiterpenes obtained from the whole absinth grass.
10	α -Acetolactate decarboxylase	-
146	Acid clay	-
147	Acid phosphatase	-
3	Actinidine	-
56	Activated acid clay	-
55	Active carbon	A substance obtained by carbonizing and activating carbon-containing substances.
5	Acylase	-
11	5'-Adenylic acid	-
2	Agarase	-
4	Agrobacterium succinoglycan	A substance composed mainly of succinoglycan obtained from the cultured solution of bacteria belonging to Agrobacterium.
17	L-Alanine	-
23	Alginate lyase	-
22	Alginic acid	-
24	Aluminium	-
196	Amino acid-sugar reaction product	A substance obtained by heating the mixture of amino acids and monosaccharides.
14	Aminopeptidase	-
15	α -Amylase	-
16	β -Amylase	-
12	Annatto extract	A substance composed mainly of norbixin and bixin obtained from the seed coats of annatto.
25	Anthocyanase	-
19	Arabino galactan	-

000187

20	L-Arabinose	-
21	L-Arginine	-
145	Artemisia sphaerocephala seed gum	A substance composed mainly of polysaccharides obtained from the seed coats of SABAKU-YOMOGI (<i>Artemisia sphaerocephala</i> KRASCH).
6	Ascorbate oxidase	-
7	L-Asparagine	-
8	L-Aspartic acid	-
9	Aspergillus terreus glycoprotein	A substance composed mainly of glycoprotein obtained from the cultured solution of mould belonging to <i>Aspergillus terreus</i> .
1	Aureobasidium cultured solution	A substance composed mainly of beta-1, 3-1, 6-glucan obtained from the cultured solution of yeast belonging to <i>Aureobasidium</i> .
230	Bacillus natto gum	A substance composed mainly of polyglutamic acid obtained from the cultured solution of bacteria belonging to <i>Bacillus natto</i> .
320	Bees wax	A substance composed mainly of myricyl palmitate obtained from honeycomb.
253	Beet red	A substance composed mainly of betanin and isobetanin obtained from beet roots.
303	Bentonite	-
290	Betaine	-
135	Bone carbon black	A substance composed mainly of carbon obtained by carbonizing bones.
134	Bone charcoal	A substance composed mainly of carbon powder and calcium phosphate obtained from bovine bones.
270	Brazilian licorice extract	A substance composed mainly of peliandorin obtained from Brazilian licorice roots.
277	Bromelain	-
184	Buckwheat ash extract	A substance obtained by extraction from the ashes of buckwheat stems or leaves.
266	Butane	-
50	Cacao colour	A substance composed mainly of polymers of anthocyanins obtained from cacao beans.
60	Caffeine (extract)	A substance composed mainly of caffeine obtained from coffee beans or tea leaves.
163	Calcinated calcium	A substance composed mainly of calcium compounds obtained by calcinating sea urchinshells, shells, coral, whey, bones or eggshells.
77	Candelilla wax	A substance composed mainly of hentriacontane obtained from candelilla stems.
144	Cane wax	A substance composed mainly of myricyl palmitate obtained from cane stems.
216	Capsicum water-soluble extract	A substance composed mainly of water-soluble substances obtained by extraction from capsicum fruits.

000188

65	Caramel I (plain)	A substance obtained by heat-treating food-grade carbohydrates including starch hydrolysates, molasses or saccharides, excluding "Caramel II", "Caramel III" and "Caramel IV".
66	Caramel II (caustic sulfite process)	A substance obtained by adding sulfite compounds to, and heat-treating, food-grade carbohydrates including starch-hydrolysates, molasses or saccharides, excluding "Caramel IV".
67	Caramel III (ammonia process)	A substance obtained by adding ammonium compounds to, and heat-treating, food-grade carbohydrates including starch-hydrolysates, molasses or saccharides, excluding "Caramel IV".
68	Caramel IV (sulfite ammonia process)	A substance obtained by adding sulfite compounds and ammonium compounds to, and heat-treating, food-grade carbohydrates including starch-hydrolysates, molasses or saccharides.
71	Carboxypeptidase	-
70	Carnauba wax	A substance composed mainly of ceryl hydroxycerotate obtained from the leaves of carnauba trees.
73	Carob bean gum	A substance obtained by grinding, or dissolving and precipitating the seed albumens of locust beans.
72	Carob germ colour	A substance obtained by grinding the seed germs of locust beans.
61	Carrageenan	A substance composed mainly of ι-carrageenan, κ-carrageenan and λ-carrageenan obtained from the whole algae of IBARA-NORI (<i>Hypneaceae hypnea</i>), KIRINSAI (<i>Solieriaceae eucheuma</i>), GINNAN-SOU (<i>Gingartinaceae iridaea</i>), SUGI-NORI (<i>Gingartinaceae gigartina</i>), or TSUNOMATA (<i>Gingartinaceae chondrus</i>).
238	Carrot carotene	A substance composed mainly of carotene obtained from carrot roots.
293	Carthamus red	A substance composed mainly of carthamin obtained from safflower flowers.
294	Carthamus yellow	A substance composed mainly of safflor yellow obtained from safflower flowers.
53	Cassia gum	A substance composed mainly of polysaccharides obtained by grinding the seeds of EBISU-GUSA-MODOKI (<i>Cassia tora</i> LINN.).
54	Catalase	-
58	Catechin	-
181	Cellulase	-
332	Charcoal	A substance obtained by carbonizing bamboo or wood.
199	Chicle	A substance composed mainly of amyryl acetate and polyisoprenes obtained from the secretion of sapodilla trees.
203	Chilte	A substance composed mainly of amyryl acetate and polyisoprenes obtained from the secretion of chilte trees (<i>Chidoscolus elasticus</i> LUNDELL).
337	Chinese bayberry extract	A substance obtained by extraction from the fruits, bark, or leaves of Chinese bayberry.

000189

82	Chitin	-
81	Chitinase	-
84	Chitosan	-
83	Chitosanase	-
117	Chlorophyll	-
116	Chlorophylline	-
217	Cholesterol	A substance composed mainly of cholesterol obtained from fish oil or "lanolin".
115	Clove extract	A substance composed mainly of eugenol obtained from the buds, leaves or flowers of clove.
133	Cochineal extract	A substance composed mainly of carminic acid obtained from cochineal insects.
232	Coffee bean extract	A substance composed mainly of chlorogenic acid and polyphenols obtained from coffee beans.
214	Copper	-
101	Cristobalite	-
183	Crude magnesium chloride (sea water)	A substance composed mainly of magnesium chloride obtained by separating potassium chloride and sodium chloride from sea water.
182	Crude potassium chloride (sea water)	A substance composed mainly of potassium chloride obtained by separating sodium chloride from sea water.
59	Curdlan	A substance composed mainly of β -1,3-glucans obtained from the cultured solution of bacteria belonging to <i>Agrobacterium</i> or <i>Alcaligenes</i> .
150	Cyanocobalamin	-
155	Cyclodextrin	-
156	Cyclodextrin glucanotransferase	-
157	L-Cystine	-
160	5'-Cytidylic acid	-
207	5'-Deaminase	-
208	Depolymerized natural rubber	A substance composed mainly of polyisoprenes obtained by decomposing the secretion of para rubber trees.
211	Dextran	-
210	Dextranase	-
119	Diatomaceous earth	-
213	Dunaliella carotene	A substance composed mainly of b-carotene obtained from the whole alga of dunaliella.
40	Elemi resin	A substance composed mainly of β -amyryn obtained from the secretion of elemi trees.

129	Enzymatically decomposed apple extract	A substance composed mainly of catechins and chlorogenic acid obtained by enzymatically decomposing apple fruits
130	Enzymatically decomposed lecithin	A substance composed mainly of phosphatidic acid and lysolecithin obtained from vegetable "lecithin" or

000190

		"yolk lecithin".
141	Enzymatically decomposed rice bran	A substance composed mainly of phytic acid and peptides obtained from dewaxed rice bran.
354	Enzymatically decomposed rutin	A substance composed mainly of isoquercitrin obtained from "rutin (extract)".
92	Enzymatically hydrolyzed guar gum	A substance composed mainly of polysaccharides obtained by grinding and hydrolyzing guar seeds.
128	Enzymatically hydrolyzed licorice extract	A substance composed mainly of glycyrrhetic acid-3-glucuronide obtained by enzymatically hydrolyzing a "licorice extract".
125	Enzymatically modified hesperidin	A substance obtained by adding glucose to "hesperidin" using cyclodextrin glucosyl transferase.
123	Enzymatically modified isoquercitrin	A substance composed mainly of alpha-glucosylquercetin obtained from "enzymatically decomposed rutin".
127	Enzymatically modified lecithin	A substance composed mainly of phosphatidylglycerol obtained from "vegetable lecithin" or "yolk lecithin".
124	Enzymatically modified naringin	A substance composed mainly of α -glucosyl naringin obtained from "naringin".
126	Enzymatically modified rutin (extract)	A substance composed mainly of α -glucosyl rutin obtained from "rutin (extract)".
174	Essential oil-removed fennel extract	A substance composed mainly of glucosyl sinapiralcohol obtained from fennel seeds.
39	Esterase	-
38	Exomaltotetrahydrolase	-
263	Ferritin	-
264	Ferulic acid	-
259	Ficin	-
87	Fish scale foil	A substance obtained by extraction from the epithelium of fish.
279	Fractionated lecithin	A substance composed mainly of sphingomyelin, phosphatidyl inositol, phosphatidyl ethanolamine and phosphatidyl choline obtained from "vegetable lecithin" or "yolk lecithin".
271	Fructosyl transferase	-
265	Fukuronori extract	A substance composed mainly of polysaccharides obtained from FUKURO-NORI (<i>Gloiopeltis furcata</i> POSTEL et RUPR).
257	Furcellaran	A substance composed mainly of polysaccharides obtained from the whole algae of furcellaria (<i>Furcellaria fastigiata</i> HUD).
62	α -Galactosidase	-
63	β -Galactosidase (Lactase)	-
306	Gallic acid	-
96	Gardenia blue	A substance obtained by adding β -glucosidase to the mixture of iridoid glycosides obtained from gardenia fruits and protein-decomposed substances.

000191

97	Gardenia red	A substance obtained by adding β -glucosidase to the mixture of ester-hydrolysates of iridoid glycosides obtained from gardenia fruits and protein-decomposed substances.
98	Gardenia yellow	A substance composed mainly of crocin and crocetin obtained from gardenia fruits.
153	Gellan gum	A substance composed mainly of polysaccharides obtained from the cultured solution of bacteria belonging to <i>Pseudomonas elodea</i> .
120	Gentian root extract	A substance composed mainly of amarogentin and gentiopicroside obtained from gentian roots or rhizomes (<i>Gentiana lutea</i> LINNE).
162	Ginger extract	A substance composed mainly of shogaol and zingerol obtained from ginger rhizomes.
102	Glucanase	-
103	Glucoamylase	-
104	Glucosamine	-
109	Glucose isomerase	-
110	Glucose oxidase	-
105	α -Glucosidase	-
106	β -Glucosidase	-
107	α -Glucosyltransferase	-
108	α -Glucosyltransferase-treated stevia	A substance composed mainly of α -glucosylsteviosides obtained from a "stevia extract".
111	Glutaminase	-
112	L-Glutamine	-
89	Gold	-
52	Granite porphyry	-
269	Grape seed extract	A substance composed mainly of proanthocyanidins obtained from the seeds of American grapes or grapes
267	Grape skin colour	A substance composed mainly of anthocyanins obtained from the pericarps of American grapes or grapes.
268	Grape skin-derived substance	A substance composed mainly of polyphenols obtained from the pericarps of American grapes or grapes.
113	Grapefruit seed extract	A substance composed mainly of fatty acids and flavonoids obtained from grapefruit seeds.
93	Guaiac resin (Guajac resin)	A substance composed mainly of guaiacetic acid, guaiaretic acid, and β -resin obtained from the trunks/branches of guaiacum trees.
94	Guajac resin (extract)	A substance composed mainly of α - and β -guaiacetic acids obtained from the secretion of guaiacum trees.
91	Guar gum	A substance composed mainly of polysaccharides obtained from guar seeds, excluding "Enzymatically hydrolyzed guar gum".
18	Gum Arabic	A substance composed mainly of polysaccharides

000192

		obtained from the secretion of acacia trees.
57	Gum ghatti	A substance composed mainly of polysaccharides obtained from the secretion of ghatti trees (<i>Anogeissus latifolia</i> WALL.).
99	Gutta hang kang	A substance composed mainly of amyirin acetate and polyisoprenes obtained from the secretion of gutta hang kang trees (<i>Palaquium leiocarpum</i> BOERL.).
100	Gutta percha	A substance composed mainly of polyisoprenes obtained from the secretion of gutta percha trees (<i>Palaquiurn gutta</i> BURCK.).
299	Haematococcus algae colour	A substance composed mainly of astaxanthin obtained from the whole alga of haematococcus (<i>Haematococcus</i> C.A.AGARCH).
287	Hego-ginkgo leaf extract	A substance obtained by extraction from the leaves of HEGO (<i>Cyathea fauriei</i> COPEL) and ginkgo (<i>Ginkgo biloba</i> LINNE).
302	Helium	-
301	Heme iron	-
300	Hemicellulase	-
297	Heptane	-
289	Hesperidin	-
288	Hesperidinase	-
283	Hexane	-
121	Higher fatty acid	A substance obtained by hydrolyzing animal or vegetable fats/oils or their hardened fats/oils.
252	L- Histidine	
175	Horseradish extract	A substance composed mainly of isothiocyanates obtained from horseradish roots.
249	Hyaluronic acid	-
168	Hydrogen	-
254	L-Hydroxyproline	-
32	Inositol	-
31	Inulinase	-
33	Invertase	-
212	Iron	-
27	Iso- α -bitter acid	A substance composed mainly of isohumulones obtained from hop flowers.
26	Isoamylase	-
28	Isomaltodextranase	-
29	Itaconic acid	-
161	Jamaica quassia extract	A substance composed mainly of quassin and neoquassin obtained from the trunks/branches or bark of Jamaica quassia trees.
333	Japan wax	A substance composed mainly of glycerol palmitate obtained from the fruits of Japanese wax trees (<i>Rhus</i>

000193

		<i>succedanea</i> LINNE).
51	Japanese persimmon colour	A substance composed mainly of flavonoids obtained from Japanese persimmon fruits.
154	Jelutong	A substance composed mainly of amyirin acetate and polyisoprenes obtained from the secretion of jelutong trees.
307	Jjoba wax	A substance composed mainly of icosenyl icosenate obtained from jjoba fruits.
132	Kaoliang colour	A substance composed mainly of apigeninidin and luteolinidin obtained from kaoliang seeds.
49	Kaolin	-
69	Karaya gum	A substance composed mainly of polysaccharides obtained from the secretion of KARAYA trees (<i>Sterculia urens</i> ROXB.) or silk cotton trees (<i>Cochlospermum gossypium</i> A.P.DeCandolle).
114	Kooroo colour [Matsudai colour]	A substance obtained by extraction from the roots of SOMEMONO-IMO (<i>Dioscorea matsudai</i> HAYATA).
342	Lac colour	A substance composed mainly of laccaic acids obtained from the secretion of lac scale insects (<i>Laccifer lacca</i> KERR).
341	Lactoferrin concentrates	A substance composed mainly of lactoferrin obtained from mammals' milk.
340	Lactoperoxidase	-
343	Lanolin	A substance composed mainly of esters of higher alcohols and α -hydroxylic acids obtained from waxy substances bearing the surface of sheep wool.
358	Leche de vaca	A substance composed mainly of esters of amyirin obtained from the secretion of leche de vaca trees (<i>Brosimum utile</i> (H.B.K.) PITT.).
361	L-Leucine	-
359	Levan	A substance composed mainly of polysaccharides obtained from the cultured solution of bacteria belonging to <i>Bacillus subtilis</i> .
75	Licorice extract	A substance composed mainly of glycyrrhizic acid obtained from the roots or rhizomes of Chinese licorice, Xinjiang licorice or licorice.
76	Licorice oli extract	A substance composed mainly of flavonoids obtained from the roots or rhizomes of Chinese licorice, Xinjiang licorice or licorice.
13	Linseed gum	A substance composed mainly of polysaccharides obtained from linseeds.
353	Lintier cellulose	A substance composed mainly of cellulose obtained from cotton single pilus.
349	Lipase	-
350	Lipoxygenase	-
352	Liquid paraffin	-
362	Logwood colour	A substance composed mainly of haematoxylin obtained from the heart wood of logwood.

000194

347	L-Lysine	-
348	Lysozyme	-
311	Macrophomopsis gum	A substance composed mainly of polysaccharides obtained from the cultured solution of microorganism belonging to <i>Macrophomopsis</i> .
316	Maltose phosphorylase	-
317	Maltotriohydrolase	-
357	Mannentake extract	A substance obtained by extraction from the mycelium or fruit body of MANNENTAKE (<i>Ganoderma lucidum</i> KARST.) or its cultured solution.
315	Marigold colour	A substance composed mainly of xanthophylls obtained from marigold flowers.
314	Massaranduba balata	A substance composed mainly of amyirin acetate and polyisoprenes obtained from the secretion of massaranduba balata trees.
313	Massaranduba chocolate	A substance composed mainly of amyirin acetate and polyisoprenes obtained from the secretion of massaranduba chocolate trees.
312	Mastic gum	A substance composed mainly of masticdienonic acid obtained from the secretion of mastic trees.
328	Melaleuca oil	A substance composed mainly of essential oil obtained from melaleuca leaves.
326	Menaquinone (extract)	A substance composed mainly of menaquinone-4 obtained from the cultured solution of bacteria belonging to <i>Arthrobacter</i> .
327	Mevalonic acid	-
310	Microcrystallin wax	-
250	Microcrystalline cellulose	A substance composed mainly of crystalline cellulose obtained from pulp.
251	Microfibrillated cellulose	A substance composed mainly of cellulose obtained by microfibrillating pulp or cotton.
167	Milt protein	A substance composed mainly of basic proteins obtained from fish testes.
319	Mixed tocopherols	A substance composed mainly of d- α -, d- β -, d- γ - and d- δ -tocopherols obtained from vegetable oils.
292	Monascus colour	A substance composed mainly of ankaflavin and monascolubrin obtained from the cultured solution of mould belonging to <i>Monascus</i> .
291	Monascus yellow	A substance composed mainly of xanthomonacins obtained from the cultured solution of mould belonging to <i>Monascus</i> .
329	Mousouchiku dry distillate	A substance obtained by dry distillation from the stems of MOUSOU-CHIKU bamboo (<i>Phyllostachys heterocycla</i> MITF.).
330	Mousouchiku extract	A substance composed mainly of 2,6-dimethoxy-1,4-bezoquinone obtained from the stem skins of MOUSOU-CHIKU bamboo (<i>Phyllostachys heterocycla</i> MITF.).

000195

325	Muramidase	-
64	Mustard extract	A substance composed mainly of allyl isothiocyanate obtained from Indian mustard seeds.
321	Myrrh	A substance obtained by extraction from the secretion of myrrh trees.
234	Naringin	-
233	Naringinase	-
237	Nickel	-
235	Niger gutta	A substance composed mainly of amyriin acetate and polyisoprenes obtained from the secretion of niger gutta trees (<i>Ficus platyphylla</i> DELILE.).
200	Nitrogen	-
318	Non-calcinated calcium	A substance composed mainly of calcium salts obtained by drying shells, pearl layers, coral, bones or eggshells.
44	Oligogalacturonic acid	-
190	Onion colour	A substance composed mainly of quercetin obtained from onion bulbs.
47	Orange colour	A substance composed mainly of carotene and xanthophylls obtained from the fruits or peels of AMA-DAIDAI (<i>Citrus sinensis</i> OSBECK).
46	Oregano extract	A substance composed mainly of carvacrol and thymol obtained from oregano leaves.
45	γ -Oryzanol	A substance composed mainly of both esters consisting of each combination of sterols and ferulic acid, and triterpene alcohols and ferulic acid obtained from rice bran or germ oil.
148	Oxygen	-
42	Ozokerite	-
43	Ozone	-
246	Palladium	-
244	Palm oil carotene	A substance composed mainly of carotene obtained from oil palm fruits.
248	Pancreatin	-
243	Papain	-
215	Paprika colour	A substance composed mainly of capsanthins obtained from capsicum fruits.
247	Paraffin wax	-
336	Peach gum	A substance composed mainly of polysaccharides obtained from the secretion of peach trees.
282	Pecan nut colour	A substance composed mainly of flavonoids obtained from the pericarps or astringent skins of pecan nuts.
285	Pectin	-
286	Pectin digests	A substance composed mainly of galacturonic acid obtained from "pectin".
284	Pectinase	-

296	Pepsin	-
298	Peptidase	-
158	Perilla extract	A substance composed mainly of terpenoids obtained from perilla seeds or leaves.
245	Perlite	-
241	Peroxidase	-
231	Petroleum naphtha	-
258	Phaffia colour	A substance composed mainly of astaxanthins obtained from the cultured solution of yeast belonging to <i>Phaffia</i> .
86	Phellodendron bark extract	A substance composed mainly of berberine obtained from the bark of phellodendron trees (<i>Phellodendron amurense</i> RUPR.).
304	Phosphodiesterase	-
305	Phospholipase	-
260	Phytase	-
261	Phytic acid	A substance composed mainly of inositol hexaphosphate obtained from rice bran or corn seeds.
262	Phytin (extract)	A substance composed mainly of magnesium inositol hexaphosphate obtained from rice bran or corn seeds.
242	Platinum	-
309	ϵ -Polylysine	-
308	Polyphenol oxidase	-
195	Powdered bile	A substance composed mainly of cholic acid and desoxycholic acid obtained from bile.
280	Powdered cellulose	A substance composed mainly of cellulose obtained by decomposing pulp, excluding "Microcrystalline cellulose".
281	Powdered rice hulls	A substance composed mainly of cellulose obtained from rice hulls.
170	Powdered stevia	A substance composed mainly of steviol glycosides obtained by grinding stevia leaves.
278	L-Proline	-
275	Propane	-
276	Propolis extract	A substance composed mainly of flavonoids obtained from honeycomb.
274	Protease	-
143	Psyllium seed gum	A substance composed mainly of polysaccharides obtained from the seed coats of blond psyllium.
273	Pullulan	-
272	Pullulanase	-
323	Purple corn colour	A substance composed mainly of cyanidine-3-glucoside obtained from corn seeds.
322	Purple sweet potato colour	A substance composed mainly of cyanidine acylglucosides and peonidin acylglucosides obtained from the tuberous roots of sweet potatoes.

000197

324	Purple yam colour	A substance composed mainly of cyanidine acylglucosides obtained from yam tuberous roots.
95	Quercetin	-
173	Quicklime	-
88	Quillaia extract (Quillaja extract)	A substance composed mainly of saponins obtained from the bark of quillaia trees.
339	Rakanka extract	A substance composed mainly of mogulosides obtained from rakanka fruits (<i>Momordica grosvenori</i> SWINGLE).
85	Redbark cinchona extract	A substance composed mainly of quinidine, quinine and cinchonine obtained from the bark of redbark cinchona trees.
360	Rennet	-
139	Resin of depolymerized natural rubber	A substance composed mainly of diterpenes, triterpenes and tetraterpenes obtained from "rubber".
345	L-Rhamnose	-
344	Rhamsan gum	A substance composed mainly of polysaccharides obtained from the cultured solution of bacteria belonging to <i>Alcaligenes</i> .
351	D-Ribose	-
140	Rice bran oil extract	A substance composed mainly of ferulic acid obtained from rice bran oil.
142	Rice bran wax	A substance composed mainly of myricyl lignocerate obtained from rice bran oil.
30	Rice straw ash extract	A substance obtained from the ashes of rice stems or leaves.
239	Roasted rice bran extract	A substance composed mainly of maltol obtained from roasted rice bran.
240	Roasted soybean extract	A substance composed mainly of maltol obtained from roasted soybean seeds.
365	Rosemary extract	A substance composed mainly of carnosic acid, carnosol and rosemanol obtained from rosemary leaves or flowers.
363	Rosidinha	A substance composed mainly of amyriin acetate and polyisoprenes obtained from the secretion of rosidinha trees (<i>Sideroxylon</i>).
364	Rosin	A substance composed mainly of abietic acid obtained from the secretion of pine trees.
138	Rubber	A substance composed mainly of polyisoprenes obtained from the secretion of Pararubber trees, excluding "Depolymerized natural rubber".
74	Rumput roman extract	A substance composed mainly of capillin obtained from the whole grass of rumput roman.
356	Ruthenium	-
355	Rutin (extract)	A substance composed mainly of rutin obtained from the whole grass of AZUKI (<i>Azuki angularis</i> OHWI), the buds or flowers of Japanese pagoda trees or the whole buckwheat grass.

000198

178	Sage extract	A substance composed mainly of carnosic acid and phenolic diterpenes obtained from salvia leaves.
159	Sandalwood red	A substance composed mainly of santalin obtained from the trunks/branches of red sandalwood trees.
48	Seaweed ash extract	A substance composed mainly of potassium iodide obtained from the ashes of brown algae.
179	Sepiolite	-
180	L-Serine	-
136	Sesame seed oil unsaponified matter	A substance composed mainly of sesamol obtained from sesame seeds.
137	Sesami straw ash extract	A substance obtained by extraction from the ashes of sesami stems or leaves.
149	Shea nut colour	A substance obtained from the fruits or seed coats of shea.
151	Shellac	A substance composed mainly of esters of aleuritic acid and shellolic acid or jalaric acid, obtained from the secretion of scale insects.
152	Shellac wax	A substance composed mainly of wax obtained from the secretion of scale insects.
90	Silver	-
118	Smoke flavourings	A substance obtained by capturing the gas generated by burning sugar canes, bamboo, corn stalks or wood, or a substance obtained by dry distillation from such materials.
41	Sodium chloride-decreased brine (saline lake)	A substance composed mainly of salts of alkaline metals or alkaline earth metals obtained by separating sodium chloride from saline lake water.
185	Sorva	A substance composed mainly of amyris acetate and polyisoprenes obtained from the secretion of sorva trees.
186	Sorvinha	A substance composed mainly of amyris acetate and polyisoprenes obtained from the secretion of sorvinha trees (<i>Couma utilis</i> MUELL.).
187	Soybean saponin	A substance composed mainly of saponins obtained from soybeans.
172	Sphingolipid	A substance composed mainly of sphingosine derivatives obtained from bovine brains or rice bran.
122	Spice extracts	Substances obtained by extraction or steam-distillation from hemp seeds, asafetida, ajowan, anise, angelica, fennel, turmeric, allspice, oregano, orange peel, Chinese pepper, cassia, chamomil, mustard, cardamon, curry leaves, licorice, caraway, gardenia, cumin, cress, clove, poppy seeds, caper, pepper, sesame seeds, coriander, sassafras, saffron, savory, salvia, Japanese pepper, perilla, cinnamon, shallot, juniperberry, ginger, star anise, spearmint, horseradish, celery, sorrel, thyme, onion, tamarind, tarragon, chive, chervil, dill, capsicum, nutmeg, wormwood, nigella, carrot, garlic, basil, parsley, mint, vanilla, paprika, hyssop,

000199

		fenugreek, peppermint, horsemint, marjoram, MYOGA (Zingiber Mioga ROSC.), lavender, linden, lemongrass, lemonbalm, rose, rosemary, laurel or WASABI (Japanese horseradish), excluding "Turmeric oleoresin", "Oregano extract", "Orange colour", "Mustard extract", "Licorice extract", "Licorice oil extract", "Gardenia yellow", "Clove extract", "Sesame seed oil unsaponified matter", "Perilia extract", "Ginger extract", "Essential oil-removed fennel extract", "Horseradish extract", "Sage extract", "Onion colour", "Tamarind colour", "Tamarind seed gum", "Tannin (extract)", "Paprika colour", "Capsicum water-soluble extract", "Absinth extract", "Carrot carotene", and "Rosemary extract".
171	Spirulina colour	A substance composed mainly of phycocyanin obtained from the whole alga of spirulina.
169	Stevia extract	A substance composed mainly of steviol glycosides obtained by extraction from stevia leaves.
255	Sunflower seed extract	A substance composed mainly of isochlorogenic acid and chlorogenic acid obtained from sunflower seeds.
194	Talc	-
191	Tamarind colour	A substance composed mainly of flavonoids obtained from tamarind seeds.
192	Tamarind seed gum	A substance composed mainly of polysaccharides obtained tamarind seeds.
197	Tannase	-
198	Tannin (extract)	A substance composed mainly of tannin and tannic acid obtained from Japanese persimmon fruits, Japanese gall, angelica powder, nutgall or silver wattle bark.
193	Tara gum	A substance composed mainly of polysaccharides obtained from the seeds of tara trees (<i>Caesalpinia spinosa</i> (MOL.)(O.KUNTZE)).
189	Taurine (extract)	A substance composed mainly of taurine obtained from the viscera or meat of fish or mammals.
201	Tea dry distillate	A substance obtained by dry distillation from tea leaves.
202	Tea extract	A substance composed mainly of catechins obtained from tea leaves.
188	Thaumatococin	A substance composed mainly of thaumatococin obtained from the seeds of <i>Thaumatococcus daniellii</i> .
209	Theobromine	-
206	Thujaplicin (extract)	A substance composed mainly of thujaplicins obtained from the trunks/branches or roots of HIBA trees (<i>Thujopsis dolabrata</i> SIEB. et ZUCC.).
334	Timber ash	A substance obtained by ashing bamboo or wood.
335	Timber ash extract	A substance obtained by extraction from "timber ashes".
219	d- α -Tocopherol	-
220	d- γ -Tocopherol	-

221	d- δ -Tocopherol	-
218	Tocotrienol	-
222	Tomato colour	A substance composed mainly of lycopene obtained from tomato fruits.
229	Tororoaoi	A substance composed mainly of polysaccharides obtained from the roots of TORORO-AOI (<i>Abelmoschus manihot</i> MED.).
223	Tragacanth gum	A substance composed mainly of polysaccharides obtained from the secretion of tragacanth trees.
224	Transglucosidase	-
225	Transglutaminase	-
227	Trehalose	-
228	Trehalose phosphorylase	-
226	Trypsin	-
205	Tunu	A substance composed mainly of amyryne acetate and polyisoprenes obtained from the secretion of tunu trees (<i>Castilla fallax</i> COOK).
35	Turmeric oleoresin [Curcumin]	A substance composed mainly of curcumin obtained from turmeric rhizomes.
204	L-Tyrosine	-
37	Urease	-
36	Urushi wax	A substance composed mainly of glycerol palmitate obtained from the fruits of Japanese lacquer trees (<i>Rhus verniciflua</i> STOKES).
165	Vegetable carbon black	A substance composed mainly of carbon obtained by carbonizing plants.
166	Vegetable lecithin	A substance composed mainly of lecithin obtained from rape seeds or soybeans.
164	Vegetable sterol	A substance composed mainly of phytosterols obtained from oil seeds.
295	Venezuelan chicle	A substance composed mainly of amyryne acetate and polyisoprenes obtained from the secretion of Venezuelan chicle trees.
256	Vermiculite	-
34	Welan gum	A substance composed mainly of polysaccharides obtained from the cultured solution of bacteria belonging to <i>Alcaligenes</i> .
331	Wood chip	A substance obtained by grinding the trunk/branches of Siberian filbert or BUNA (<i>Fagus crenata</i> BLUME).
78	Xanthan gum	A substance composed mainly of polysaccharides obtained from the cultured solution of bacteria belonging to <i>Xanthomonas</i> .
79	Xylanase	-
80	D-Xylose	-
131	Yeast cell wal	A substance composed mainly of polysaccharides obtained from the cell walls of yeast belonging to

000201

		<i>Saccharomyces.</i>
346	Yolk lecithin	A substance composed mainly of lecithin obtained from egg yolk.
338	Yucca foam extract	A substance composed mainly of saponins obtained from the whole grass of yucca joshua (<i>Yucca arborescens</i> TREL.) or yucca schidigera (<i>Yucca schidigera</i> ROEYL ex ORLGIES).
176	Zein	A substance composed mainly of vegetable proteins obtained from corn seeds.
177	Zeolite	-

Note: The additives listed in No.1 to No.365 do not include substances obtained by causing chemical reactions, excluding decomposition to elements or compounds using chemical means.

000202

Appendix 5C-1 – cMDLf Notification in China

ICS 67.040

C 54



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Hygienic standards for uses of food additives

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000204

目录

前言	0
正文	1
附录 A 食品添加剂的使用规定	4
表 A.1 食品添加剂的使用范围和使用量	5
表 A.2 食品中允许使用的添加剂及使用量	89
表 A.3 可在各类食品中按生产需要适量使用的添加剂名单	168
表 A.4 按生产需要适量使用的添加剂所例外的食品类别名单	171
附录 B 食品用香料名单	172
表 B.1 允许使用的食品用天然香料名单	172
表 B.2 允许使用的食品用天然等同香料名单	185
表 B.3 允许使用的食品用人造香料名单	211
附录 C 食品工业用加工助剂使用名单	217
表 C.1 食品工业用加工助剂使用名单	217
表 C.2 食品用酶制剂及其来源名单	220
附录 D 胶基糖果中基础剂物质及其配料名单	226
附录 E 食品添加剂功能类别	228
附录 F 食品分类系统	230

16.05.01 油炸小食品（仅限油炸薯片） 1.0

乳酸链球菌素

nisin

CNS号 17.019

INS号 234

功能 防腐剂

食品分类号	食品名称/分类	最大使用量g/kg	备注
01.0	乳及乳制品（除外01.01.01、13.0涉及品种）	0.5	
04.03.02.04	食用菌和藻类罐头	0.2	
06.04.02.01	八宝粥罐头	0.2	
08.02	预制肉制品	0.5	
08.03	熟肉制品	0.5	
14.0	饮料类（除外14.01包装饮用水类）	0.2	固体饮料按冲调倍数增加使用量

乳酸钠

sodium lactate

CNS号 15.012

INS号 325

功能 水分保持剂、酸度调节剂、抗氧化剂、膨松剂、增稠剂、稳定剂

食品分类号	食品名称/分类	最大使用量g/kg	备注
06.03.02.01	生湿面制品（如面条、饺子皮、馄饨皮、烧麦皮）	2.4	

乳糖醇

lactitol

CNS号 19.014

INS号 966

功能 乳化剂、稳定剂、甜味剂、增稠剂

食品分类号	食品名称/分类	最大使用量g/kg	备注
01.02.01	原味发酵乳（全脂、部分脱脂、脱脂）	30.0	
01.05.01	稀奶油	按生产需要适量使用	
12.09	香辛料类	按生产需要适量使用	

乳铁蛋白

lactoferrin

CNS号 00.019

INS号

功能 其他（铁促进吸收剂）

食品分类号	食品名称/分类	最大使用量g/kg	备注
-------	---------	-----------	----

13.01 婴儿配方食品、较大婴儿和 0.3-1.0
 幼儿配方食品

噻苯咪唑 thiabendazole (TBZ)

CNS号 17.018 INS号 233

功能 防腐剂

食品分类号	食品名称/分类	最大使用量g/kg	备注
04.01.01.02	经表面处理的鲜水果	0.02	
04.02.01	新鲜蔬菜（仅限蒜苔和青椒）	0.01	残留量≤2.0mg/kg

三聚甘油单硬脂酸酯 tripolyglyceryl monostearate

CNS号 10.021 INS号

功能 乳化剂、消泡剂

食品分类号	食品名称/分类	最大使用量g/kg	备注
03.01	冰淇淋类	3.0	
07.01	面包	0.1	
07.02	糕点	0.1	

三聚磷酸钠 sodium tripolyphosphate

CNS号 15.003 INS号 451i

功能 水分保持剂

食品分类号	食品名称/分类	最大使用量g/kg	备注
01.0	乳及乳制品（除外 01.01.01、13.0涉及品种）	5.0	
03.01	冰淇淋类	5.0	
06.04.02.01	八宝粥罐头	1.0	
06.07	方便米面制品	5.0	
08.02	预制肉制品	5.0	
08.03	熟肉制品	5.0	
08.03.08	肉罐头类	1.0	
14.02.03	果蔬汁（肉）饮料	1.0	
14.03	蛋白饮料类	1.0	
14.05.01	茶饮料类	1.0	

Appendix 5C-2 – cMDLf Notification in China - 2

Notification

公告

2004年第6号

根据《中华人民共和国食品卫生法》和《食品添加剂卫生管理办法》的规定，批准以下食品添加剂的新品种、扩大使用范围和使用量的品种以及食品用香精名单，并予以公告，自公告之日起实施。

二〇〇四年四月十二日

April 12th, 2004

1. 新品种

种类 Category (代码)	Name 名称	Range of use 使用范围	Maximum dosage 最大使用量 (g/kg)
抗氧化剂 (04)	竹叶抗氧化物	食用油脂、肉制品、水产品、膨化食品	0.5
着色剂 (08)	喹啉黄	预调酒	100 mg/L
增稠剂 (20)	海藻胶	胶基糖果	10
Others 其他 (00)	Lactoferrin 乳铁蛋白	Infant Formula, 婴儿配方奶粉、较大婴儿 Follow-up Formula, 和幼儿配方奶粉 Grow-up Formula	30-100mg/100g

2. 扩大使用范围、使用量的品种

类别	名称	使用范围	最大使用量 (g/kg)
着色剂 (08)	苋菜红 (08.001)	果酱	0.5
		水果调味糖浆 (fruit toppings)	0.5
	苋菜红及其铝色淀 (08.001)	饼干夹心	0.05 (以苋菜红计)
		胭脂红 (08.002)	果酱
	水果调味糖浆 (fruit toppings)		0.5
胭脂红及其铝色淀 (08.002)	饼干夹心	0.05 (以胭脂红计)	
	柠檬黄 (08.005)	果酱	0.5
水果调味糖浆 (fruit toppings)		0.5	

000209

**Appendix 5C-3 - cMDLf Notification in
China (English Translation)**



MORINAGA MILK INDUSTRY CO., LTD.

33-1. SHIBA 5-CHOME, MINATO-KU, TOKYO 106-8364, JAPAN

TEL: 81-3-3798-0152

FAX: 81-3-3798-0107

E-mail: interntf@morinagamilk.co.jp

(Free translation of the Copy of "Notification" by Chinese government)

Notification

No 6, Year 2004

According to "Food Sanitation Law of People's Republic of China" and "Sanitary Management Law for Food Additives", we hereby notify that the new food additives and its scope of usage and the flavor name whose use level is increased as the following. These are effective from the notified date.

April 12, 2004

1. New item

Category (Code)	Name	Scope of use	Maximum use level (g /kg)
Antioxidant (04)	Bamboo leave antioxidant	Edible oil, meat, fishery product, puffed food	0.5
Coloring agent (08)	Quinoline Yellow	Alcopops	100mg / L
Thickening agent (20)	Funoran	Chewing gum	10
Others (00)	Lactoferrin	Infant Formula Follow-up Formula	30 – 100mg / 100g

2 Items whose scope of use is extended and use level is increased

Category	Name	Scope of use	Maximum use level
Coloring agent (08)	Amaranth (08.001)	- Jam(Jelly) - Fruits flavored topping syrup	0.3 0.3
	Amaranth and its aluminum lake (08.001)	Biscuit sandwich	0.05 (amaranth)
	Carmine	- Jam(Jelly) - Fruits flavored topping syrup	0.5 0.5
	Carmine ad its aluminum lake (08.002)	Biscuit sandwich	0.05 (Carmine)
	Tartrazine (08.005)	-Jam(Jelly) -Fruits flavored topping syrup	0.5 0.5

000211

**Appendix 5D-1 – Certificate of Registration
of cMFLf at the Ministry of Health in Taiwan**

審署證字第006553號
檢附文件號碼：0110.09.5550.7

行政院衛生署食品添加物許可證

添加物名稱：A 亞精片
ASPIRIN A. B. V. (ASAC) TABLETS

劑型：片劑

用途：鎮痛解熱劑

製造廠名稱：M. C. S. AMER

製造廠地址：1562 - 1, LEIPZIGER STR. 1, 10001 LM ALINGAU, GERMANY

申請商號：尹太一有限公司

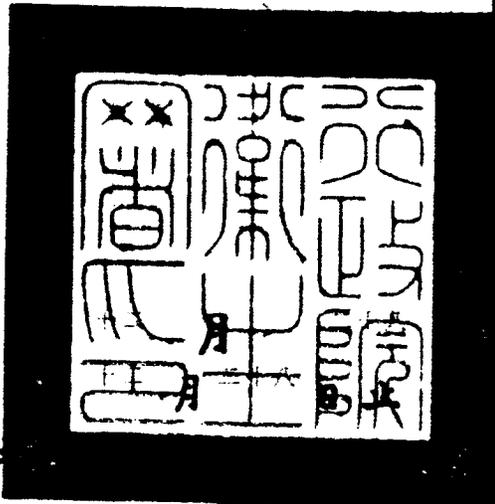
負責人：陳添柱

地址：台北市吉林路166巷2號一樓

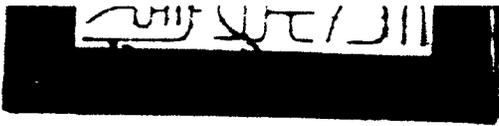
包裝：
 **10,000公斤內塑膠袋 外紙桶 裝
 **1,000公斤內裝 外紙箱裝

前項添加物經本署審核與食品衛生管理法之規定相符應發給許可證以資證明

署長 李 明 亮



中華民國 八十九 年
本證有效期間至 九十四



核准變更事項之記載	變更事項	核准變更情形	核准日期			備考
			年	月	日	
	包裝調整	10公斤內塑膠袋外紙箱裝。 1公斤內手，外紙箱袋裝。	09	20	03	037010
核准展延至						

extended until

Year 99, Dec. 28 (Taiwanese calendar year)

Dec. 28th, 2010

000214

**Appendix 5D-2 – Certificate of Registration
of cMFLf at the Ministry of Health in Taiwan
(English Translation)**



MORINAGA MILK INDUSTRY CO., LTD.

33-1, SHIBA 5-CHOME, MINATO-KU, TOKYO 108-8364, JAPAN

TEL: 81-3-3798-0152

FAX: 81-3-3798-0107

E-mail: internl@morinogemilk.co.jp

(Free translation of Certificate of Registration at Ministry of Health in Taiwan)

(1st page)

Ministry of Health Food Additive Registration Certificate

Name of food additive : Morinaga Bovine Lactoferrin
 Type : Powder
 Purpose : Nutritional Supplement
 Manufacture name : Milei GmbH
 Place of manufacture : 88299 Leutkirch Adrazhofen, Algu, Germany
 Applicant : 尹太有限公司
 Person in charge of application : 陳 添桂
 Address : 186 - 2 - 1, Chin , Taipei
 Packing : *** 10kg inner / plastic bag, outer / carton box
 *** 1kg inner / none, outer / carton box

This is to certify that the above mentioned food additive meets the regulation of Food sanitation management according to our (Ministry of Health) check.

Director : 李 明亮

Republic of China, December 28th, 2000 (ROC's year 89)

This certificate is valid until December 28th, 2005 (ROC's year 94)

(2nd page)

Approved Changes	Changed Matters	Details of approved changes	Approved date			Remark
			Year	Month	date	
	Change of package	10kg inner plastic bag, outer carton bag. 1kg inner bag, outer carton bag.				
Approved to be extended until :	December 28 th , 2010 Year Month Date	 Year Month Date	 Year Month Date	 Year Month Date	 Year Month Date	

900216

Appendix 5E – Specification Monograph for cMDLf for Korea

Standard and Specification > Natural Additives > Lactoferrin Concentrates

Lactoferrin Concentrates

Definition This is obtained by concentrating milk that is previously defatted and purified by separation. The major component is lactoferrin. It also contains whey protein.

[Compositional Specifications of Lactoferrin Concentrates]

Content Lactoferrin Concentrates should contain not less than 90.0% of lactoferrin.

Description Lactoferrin Concentrates is scentless pale orange red ~ pale reddish brown powder.

Identification When Lactoferrin Concentrates is quantitatively analyzed, a lactoferrin peak is observed at 280 nm.

(1) Arsenic : 0.5 g of Lactoferrin Concentrates is placed in a platinum, quartz, or porcelain crucible. 10 ml of magnesium nitrate in ethyl alcohol (1→50) is added to the crucible and then alcohol is ignited. It is then reduced to ash by heating at 450 ~ 550°. If carbonaceous substance persists, it is wetted with minute amount of nitric acid, which is further heat treated at 450 ~ 550°. After cooling, 3 ml of hydrochloric acid is added to the residue, which is then dissolved by heating in a water bath. When test for arsenic is carried out with this test solution, it should not be more than 2ppm.

(2) Heavy Metals : 2 g of Lactoferrin Concentrates are carbonized by heating mildly in a quartz or porcelain crucible. After cooling, add 2 ml of nitric acid and 5 drops of sulfuric acid, it is heated until white smoke disappears, which is then reduced to ash by further heating at 450 ~ 550°. After cooling, 2 ml of hydrochloric acid is added, which is then evaporated to dryness in a water bath. 3 drops of hydrochloric acid and 10 ml of hot water are added to the resulting residue, which is then heated for 2 minutes. After cooling, 1 drop of phenolphthalein indicator solution is added, then ammonia solution is added until the color of the solution becomes pale red. The resulting solution is transferred into a Nestler cylinder by rinsing with water. 50 ml of test solution is prepared by adding 2 ml of diluted acetic acid (1→20) and water. When this solution tested for heavy metals, the content should not be more than 10ppm. Color standard solution is prepared by the following procedure. 2 ml of nitric acid, 5 drops of sulfuric acid, and 2 ml of hydrochloric acid are added and evaporated to dryness in a crucible that is made of the same material used for test solution preparation. 3 drops of hydrochloric acid are added to the residue, which is then transferred into another Nestler cylinder as described above. Finally, 2 ml of lead standard solution, 2 ml of diluted acetic acid (1→20), and water are added to bring the total volume to 50 ml.

Purity

(3) pH : pH of this solution (2→100) should be 5.2 ~ 7.2.

(4) Coliform Group : Lactoferrin Concentrates is tested by Microbe Test Methods for [Coliform Group] in General Test Methods in Food Code. It should contain 30 or less per 1 g of this product.

Residue on Ignition When thermogravimetric analysis is done with 1 g of Lactoferrin Concentrates, the amount of residue should not be more than 1.3%.

Approximately 20 mg of Lactoferrin Concentrates is accurately weighed and dissolved in 0.5 M of sodium chloride solution (total volume 10 ml). The solution is filtered through a 0.45 μm Millipore filter (Test Solution). Separately, a Standard Solution is prepared with 20 mg of lactoferrin standard following the same procedure. 20 μl each of Standard Solution and Test Solution is injected into liquid chromatograph and the content of lactoferrin is obtained from the following equation.

$$\text{Content (\%)} = \frac{\text{Au} \times \text{Ws}}{\text{As} \times \text{Wu}} \times 100$$

Assay

- Au : Peak area of Test Solution
- As : Peak area of Standard Solution
- Ws : amount of standard material (mg)
- Wu : amount of sample (mg)

[Operation Conditions]

- Detector : UV 280 nm
- Column : Ashaipak C4P 50(4.6 mm × 150 mm) or its equivalent

000218

- Column Temperature : Room temperature
- Mobile Phase : Solution A : Solution B (30 : 70)
 - Solution A : acetonitrile : 0.5M sodium chloride solution (1 : 9)
 - Solution B : acetonitrile : 0.5M sodium chloride solution (5 : 5)
 - Solutions A, B contains 0.03% of Trifluoroacetic acid.
- Flow rate : 0.8 ml/min

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000219

SUBMISSION END

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