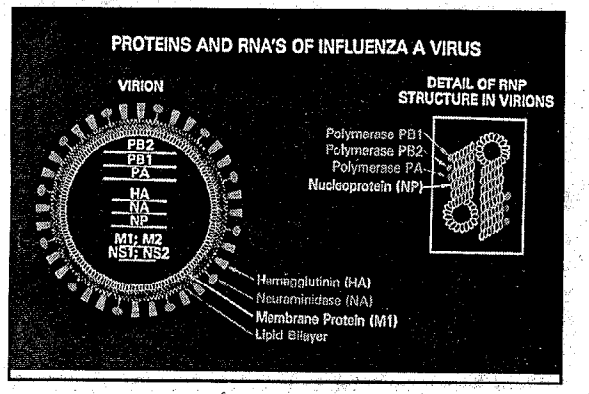
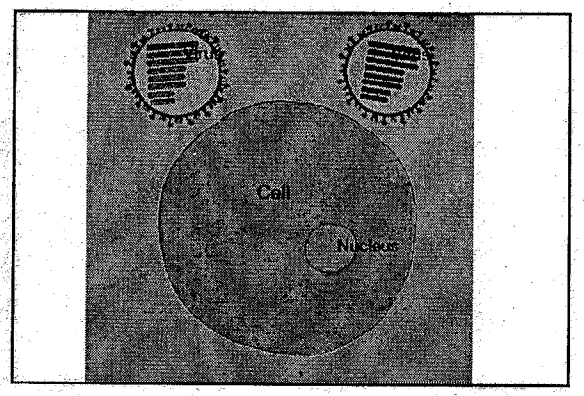
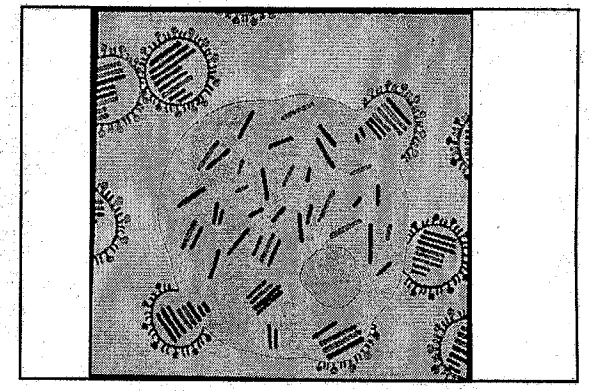
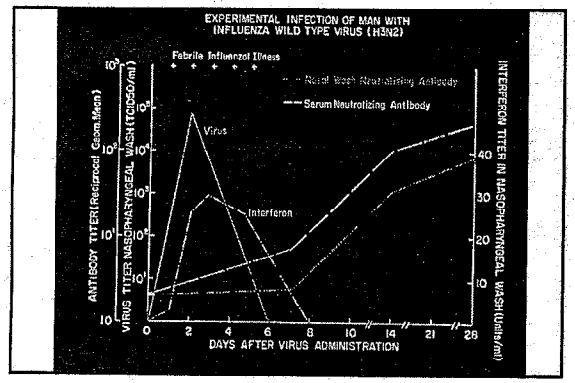
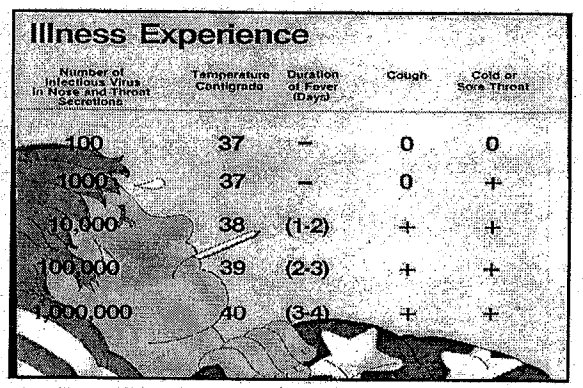




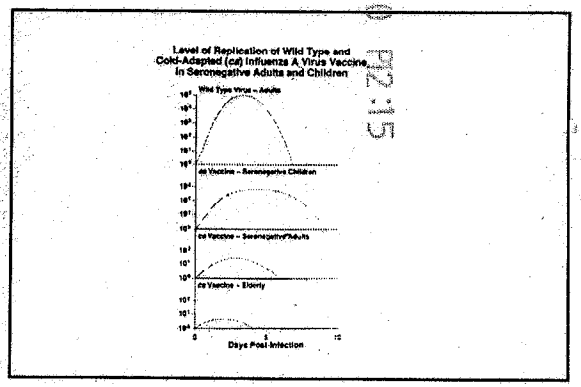






Illness Experience

Number of Infectious Virus In Nose and Throat Secretions	Temperature Centigrade	Duration of Fever (Days)	Cough	Cold or Sore Throat
100	37	–	0	0
1000	37	–	0	+
10,000	38	(1-2)	+	+
100,000	39	(2-3)	+	+
1,000,000	40	(3-4)	+	+



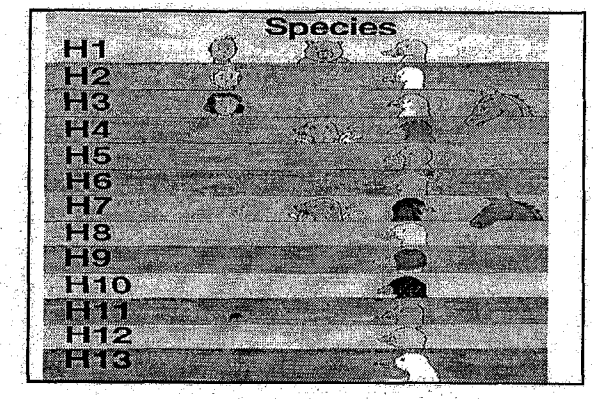
SLIDES SHOWN BY DR. BRIAN MURPHY
AT VRBPAC JULY 26, 2001

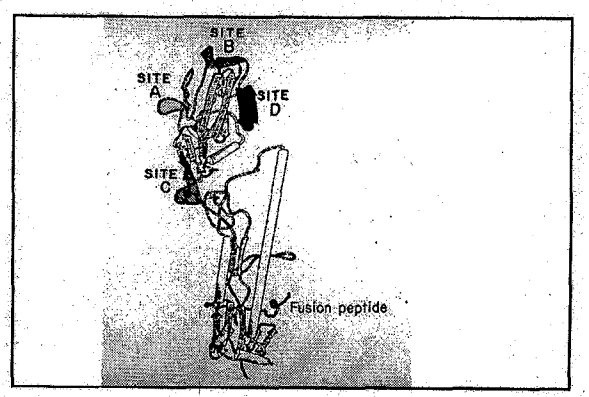
'01 AUG 10 P2:15

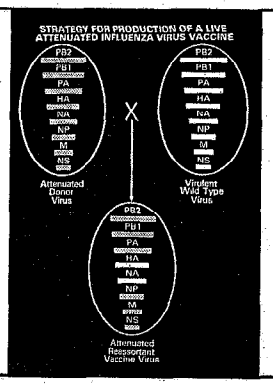
Mechanisms of Antigenic Variation in Influenza A Virus

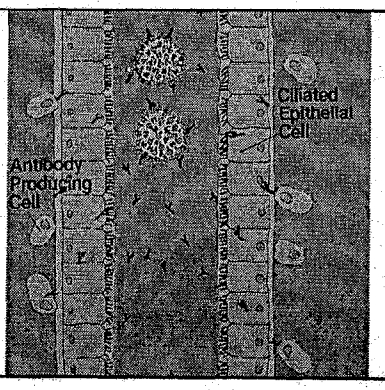
I. **Antigenic shift** – the acquisition of an antigenically distinct hemagglutinin (HA) with or without a new neuraminidase (NA) glycoprotein: observed in 1918, 1957, and 1968.

II. **Antigenic drift** – the accumulation of missence (point) mutations in the epitopes of the protective antigens, *i.e.*, the HA and NA glycoproteins – occurs continuously in humans.









Correlates of Immunity to Influenza A Virus Infection in Humans

Protective Antigen	Immunity Mediated by Antibody in Indicated Body Fluid		
	Serum	Nasal Wash	
	IgG	IgA	IgG
HA	+	+	+
NA	+	Not tested	Not tested

The Major Concepts Underlying the Development of a Live Attenuated Influenza A or B Virus Vaccine

1- Demonstration that the transfer of the 6 internal RNA segments (those not coding for the HA and NA glycoproteins) from an attenuated donor strain to each new epidemic virus as it appears in the human population reproducibly confers on the new reassortant virus a set of properties including:

a) Cold-adapted, temperature-sensitive, and attenuation phenotypes
b) A satisfactory level of attenuation in humans
c) Efficacy

2- This demonstration is sufficiently extensive to preclude the need to test each new vaccine strain as it is generated each year. This latter concept is practiced for the currently licensed non-living virus vaccine.

Derivation of the Influenza A/AA/6/60 (H2N2) Cold Adapted Donor Virus

A/AA/6/60 wild type virus (isolated in PCKC)

↓ 1 passage in PCKC at 36°C

↓ passages 3-9 in PCKC at 33°C

↓ passages 10-16 in PCKC at 30°C

↓ passages 17-23 in PCKC at 25°C

↓ passages 24-30 by plaquing at 25°C in PCKC and 3 amplifications in eggs

A/AA/6/60 cold adapted donor virus (PCKC30, E3) and its 6/2 reassortants
- *ts* phenotype
- *ca* phenotype
- *att* phenotype in ferrets

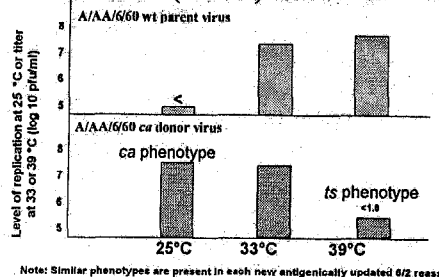
From Maassab et al. Vaccine 3: 355-368, 1985

Mutations Present in the A/AA/6/60 *ca* Donor Virus

RNA Segment	Coding Region	Other region	Mutation wt *ca*
1	PB2		N 265 S
2	PB1		K 391 E E 457 D E 581 G A 661 T
3	PA	3'NCR	K 613 E L 715 P T_{20} - C
6	NP	5' NCR	T 23 N D 34 G Insert of A at 1550
7	M2		A 86 S
8	NS1		A 153 T

From Cox et. al. Virol. 167: 554 (1988). 10 non-coding mutations not indicated.

ts and *ca* Phenotypes of the A/AA/6/60 (H2N2) *ca* Donor Virus



Note: Similar phenotypes are present in each new antigenically updated 6/2 reassortant.

Derivation of the Influenza B/AA/1/66 Cold Adapted Donor Virus

B/AA/1/66 wild type virus (isolated in PCKC)

↓ 1 passage in PCKC at 35°C

↓ passages 2-4 in PCKC at 33°C

↓ passage 5 in PCKC at 27°C

↓ passage 6 in PCKC at 25°C

↓ 7 plaque passages at 25°C in PCKC and 3 amplifications in eggs

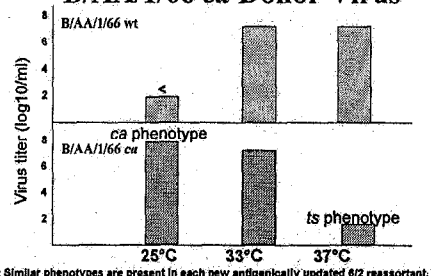
B/AA/1/66 cold adapted donor virus (PCKC13,E3)
- *ts* phenotype
- *ca* phenotype
- *att* phenotype

Unique Mutations Present in the B/AA/1/66 *ca* Donor Virus

RNA Segment	Coding Region	Other region	Mutation wt *ca*
1	PB2		S 630 N
2	PB1		I 651 V
3	PA		V 431 M (*ts* and *att*)* Y497H
6	NP	3' NCR	T55A V114A P410H A509T C 1550U
7	M1 M2		H150Q M183V V20C

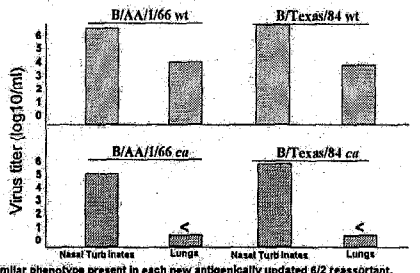
From Deborde et. al. Virol. 163: 429 (1988). 104 nt differences between ca and wt viruses.
*= From Donabedian et al Virol. 163:444-451, 1988.

ts and *ca* Phenotypes of the B/AA/1/66 *ca* Donor Virus



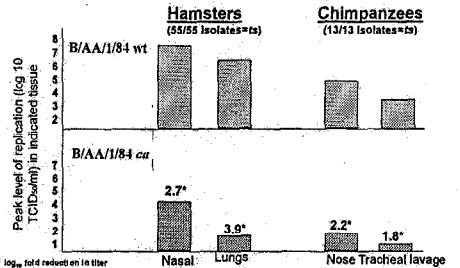
Note: Similar phenotypes are present in each new antigenically updated 6/2 reassortant.
Data from Maassab et al, Options for Control of Influenza, 1996, 271-285

Level of Attenuation of the B/AA/1/66 *ca* Donor Virus and its B/Texas/84 Reassortant Virus in Ferrets



Note: Similar phenotype present in each new antigenically updated 6/2 reassortant.
Data from Maassab et al, Options for Control of Influenza, 1996, 271-285, and Deborde et al Vaccines 86, 1986

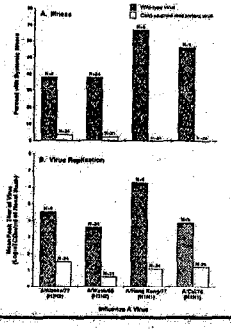
Attenuation Phenotype of the B/AA/1/84 Wt and *ca* Reassortant Virus in Hamsters and Chimpanzees



Summary of the Properties of the A/AA/6/60 *ca* Reassortant Viruses Evaluted in Humans

Property Examined	Results
1. Attenuation	Highly attenuated even for seronegatives
2. Infectivity	$10^{2.0}$ TCID50 needed for all ages
3. Genetic Stability	*ts* and *ca* phenotype stable
4. Transmissibility	Poorly transmissible
5. Efficacy or Immunogenicity	
a. Infants and young children	Very immunogenic
b. Young adults	Live = inactivated
c. Elderly adults	(a) Live – weakly immunogenic (b) Live plus inactivated is more efficacious than inactivated alone

Acquisition of the Six Transferable Genes From A/AA/6/60 *ca* Donor Virus by Epidemic Wild-Type Virus Reproducibly Attenuates the Virus for Humans



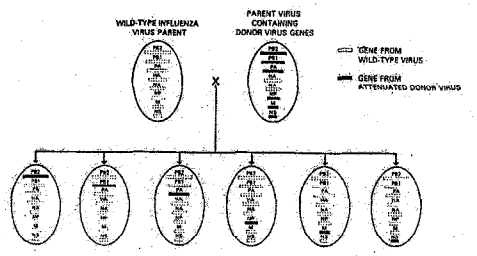
Level of Replication (Surrogate of Attenuation) of B/AA/1/66 *ca* Reassortants in Adults and Children

ca or wt reassortant virus	Mean peak titer of virus (Log_{10} $TCID_{50}$/ml)		
	Adults		Children
	wt	*ca*	
B/Texas/1/84	2.0	0.8	3.0
B/AA/1/86	4.0	0.8	3.4; 3.1*
B/Yamagata/16/88	NT	1.1	NT

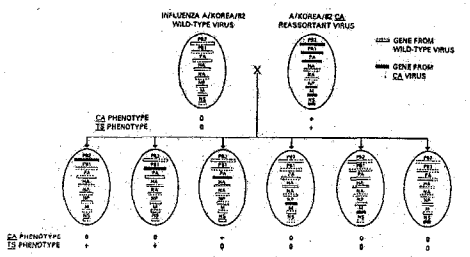
* Two separate studies. NT = not tested

Genetic Determinants of Attenuation

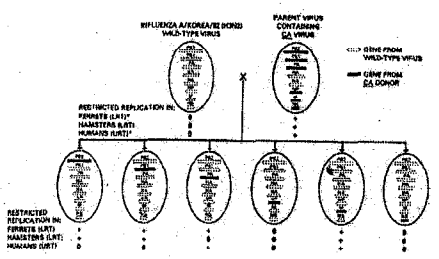
IDENTIFICATION OF GENES IN AN ATTENUATED INFLUENZA A DONOR VIRUS RESPONSIBLE FOR CHARACTERISTIC PHENOTYPES



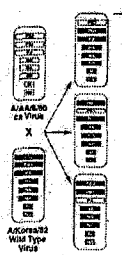
IDENTIFICATION OF INFLUENZA A/ANN ARBOR/60 CA VIRUS GENES RESPONSIBLE FOR THE COLD-ADAPTED (CA) AND TEMPERATURE SENSITIVITY (TS) PHENOTYPES



IDENTIFICATION OF INFLUENZA A/ANN ARBOR/60 CA VIRUS GENES RESPONSIBLE FOR RESTRICTED REPLICATION IN VIVO



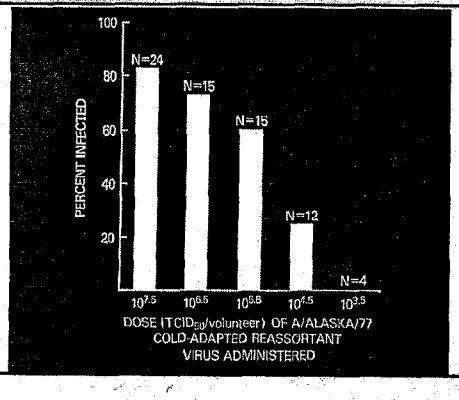
The Attenuation of the A/AA/6/60 ca Donor Virus and Its Reassortants is Determined by Mutations in Each of the Polymerase Proteins



Phenotypes of Single Gene Reassortants

Temperature Sensitive (ts)	Cold-Adapted (ca)	Attenuation (att)	Comments	Amino Acid Sequence Position	wt	ca
ts (39°C)	0	+	att-ferrets (LRT) att-hamsters (LRT)	265	Asn	Ser
ts (39°C)	0	+	att-ferrets (LRT) att-hamsters (LRT)	391	Lys	Glu
				437	Gly	Asp
				581	Glu	Gly
				601	Ala	Thr
0	ca	+	att-ferrets (LRT) att-humans (URT)	613	Lys	Glu
				715	Leu	Pro

Infectivity



Infectivity of A/AA/6/60 6-2 Gene Reassortant Viruses in Adults and Pediatric Subjects

Cold-Adapted Reassortant Virus	Surface Glycoproteins	Human Infectious Dose$_{50}$ (log_{10} $TCID_{50}$)	
		Adults[a]	Pediatric Subjects[b]
A/Hong Kong/77	H1N1	5.0	—
A/California/78	H1N1	6.1	3.5
A/Texas/85	H1N1	4.9	—
A/Kawasaki/86	H1N1	—	2.9
		Mean 5.3	*Mean* 3.2
A/Alaska/77	H3N2	5.5	—
A/Peking/79	H3N2	5.0	—
A/Washington/80	H3N2	6.0	—
A/Korea/82	H3N2	—	4.6
A/Bethesda/85	H3N2	6.4	4.4
		Mean 5.7	*Mean* 4.5

[a] Adults were selected to have serum hemagglutination-inhibiting antibody titers of ≤1:8.
[b] Pediatric subjects include infants and children 6 months to 3 years of age who were seronegative to the influenza A virus administered.

Infectivity of Influenza B/AA/1/66 *ca* Reassortant Viruses for Adults and Children

ca reassortant virus	Human infectious dose$_{50}$	
	Adults	Children
B/Texas/84	$10^{5.4}$	$10^{4.5}$
B/AA/86	$10^{6.4*}$	$10^{2.5}$

*= data did not include virus shedding

Keitel et al, JID 161:22,1990; Edwards et al, JID 163:740,1991
Clements et al, JID 161:869,1990; Anderson et al, JCMicro 30:2230,1992

Safety and Level of Virus Replication

(a) Genetic Stability
(b) Transmissibility

Methods to Evaluate the Level of Stability of the Attenuation Phenotype of a Candidate Vaccine Virus

1- Determine the genetic basis of attenuation (*att*).
2- Identify surrogate markers of attenuation, eg., *ts* phenotype.
3- Methods to examine alterations in the attenuation phenotype of virus present in original NP wash specimens or in an isolate:
   a) examine surrogate markers of attenuation phenotype,
   b) administer virus exhibiting altered phenotype to animals or adult humans to assess effect of mutation on att.
   c) determine nucleotide sequence of the modified virus,
   d) estimate frequency of occurrence of modified vaccine virus, no. of vaccinees with modified virus/no. tested and % virus in specimen with mutation.

GENETIC STABILITY OF COLD-ADAPTED REASSORTANT VIRUSES AFTER REPLICATION IN MAN

Reassortant Genotype: Wild type HA and NA genes		Reassortant Genotype: Other 6 genes: *ca*	Reassortant Genotype: Other 6 genes: wild type	Volunteers	Number of isolates tested	% with indicate phenotype: *ca*	% with indicate phenotype: *ts*
H3N2	1972	6	O	Adults	24	100	100
H3N2	1977	6	O	Adults	16	100	100
				Children*	69†	not tested	100†
H1N1	1977	6	O	Adults*	62	100	100
				Children*	78†	not tested	100
H3N2	1974	5	NS	Adults	18	61	100
H3N2	1977	5	NS	Adults	3	100	100
H3N2	1977	5	M	Adults	16	100	100
H3N2	1975	5	PI	Adults	6	83	100

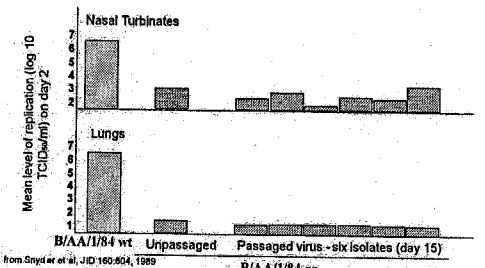
*Volunteers were not previously infected with an influenza A virus containing an immunologically related hemagglutinin.
†None of the 69 isolates plaqued at 39°C, a temperature for immunizing virus, but were at least 100-fold restricted compared to titer at permissive temperature (34°C)
‡Data from Dr. Peter Wright, Vanderbilt University, Nashville, Tennessee.

B/AA/66 *ca* 6/2 Reassortants Retain the *ts* Phenotype after Replication in Adults and Children

ca reassortant virus	No.* tested/no. with phenotype	
	ca	*ts*
B/Texas/1/84	~103/103	~103/103
B/AA/1/86	202/202	202/202
B/Yamagata/16/88	Not tested	2/2
B/AA/1/94	10/10	10/10

*= includes either an isolate or an original NP specimen. Data from 7 separate studies.

Stability of the Attenuation Phenotype of B/Texas/1/84 *ca* Reassortant Virus in Immunosuppressed Hamsters



Data from Snyder et al, JID 160:604, 1989

Conclusions on Genetic Stability

The influenza A/AA/6/60 and the influenza B/AA/1/66 *ca* reassortants are satisfactorily and reproducibly phenotypically stable in animals and humans.

FAILURE OF COLD-ADAPTED INFLUENZA A REASSORTANT VIRUS TO SPREAD FROM INFECTED VACCINEES TO SERONEGATIVE CONTACTS

COLD-ADAPTED INFLUENZA A REASSORTANT VIRUS	DOSE OF VIRUS ($TCID_{50}$)	NUMBER OF INFECTED VACCINEES	NUMBER OF CONTACTS* INFECTED/NUMBER EXPOSED
A/HONG KONG/77 (H1N1)	$10^{7.5}$	10	0/4
A/CALIFORNIA/78 (H1N1)	$10^{6.8}$	21	0/4
A/WASHINGTON/80 (H3N2)	$10^{7.0}$	25	0/7

CONTACTS HOUSED TOGETHER WITH VACCINEES IN DORMATORY-LIKE SETTING FOR NINE DAYS AFTER ADMINISTRATION OF REASSORTANT VIRUS TO THE VACCINEES.

Efficacy

(a) Adults – Wild-Type Challenge
(b) Pediatric Subjects – Attenuated Virus Challenge
(c) Seronegatives and Seropositives

Cold-Adapted Influenza A Reassortant Viruses Are Protective in Seronegative Adult Volunteers Against Experimental Challenge with Homologous Wild Type Virus

H1N1 or H3N2 *ca* Vaccine Used	Surface Antigen	No. of Volunteers	Approximate Interval Between Immunization and Challenge (y)	% Reduction in Virus Shedding	% Protected Against Systemic Illness
A/HK/77-*ca*	H1N1	22	0.2	81	82
None		16	0.2	---	---
A/Cal/76-*ca*	H1N1	14	0.6	41	78
Inact		18	0.6	8	67
None		15	0.6	---	---
A/Tex/85-*ca*	H1N1	20	0.2	63	100
Inact		16	0.2	48	100
None		28	0.2	---	---
A/Wash/80-*ca*	H3N2	16	0.2	84	100
Inact		16	0.2	24	66
None		24	0.2	---	---
A/Wash/80-*ca*	H3N2	16	0.6	15	100
Inact		16	0.6	15	91
None		27	0.6	---	---
A/Beth/85-*ca*	H3N2	11	0.2	55	100
None		10	0.2	---	---

Cold-Adapted Influenza A Reassortant Viruses Are Highly Protective In Seronegative Children Against Experimental Challenge with Homologous *ca* Influenza Virus Challenge

H1N1 or H3N2 *ca* Vaccine Used	Surface Antigen	No. of Volunteers	Approximate Interval Between Immunization and Challenge (y)	Percent Shedding Challenge Virus*	Percent Reduction In Virus Shedding
A/Cal/78-*ca*	H1N1	15	0.5	0	100
None		31	0.5	61	
A/Kaw/86	H1N1	9	0.1–0.9	0	100
None		17	0.1–0.9	41	
A/Wash/80	H3N2	13	1.0	46	54
Inact		16	1.0	84	6
Control		19	1.0	100	
A/Beth/85	H3N2	10	0.2	0	100
Control		12	0.2	67	

EFFICACY OF LIVE ATTENUATED, COLD-ADAPTED, TRIVALENT, INTRANASAL INFLUENZA VIRUS VACCINE IN CHILDREN

Virus vaccine tested	% Efficacy
Influenza A(H3N2)	96
Influenza B	91

N=849 for vaccinees and 410 for controls; data for two doses.

Data from Belshe et al, NEJM,338:1405-12,1998.

Combined Use of Live Attenuated *ca* Reassortant Vaccine Plus Inactivated Virus Vaccine Induces Greater Resistance to H3N2 Influenza A Virus in Elderly Adults Than Inactivated Virus Vaccine Alone*

Vaccine Administered	Year of Study and Subtype of Epidemic Influenza A Virus	Number of Subjects	Percent with Illness Caused by Influenza A Virus	Percent Protection In Combined Vaccine Group
Inact	1987 – H3N2	55	9.0	
Inact + Live (H3N2-85)		52	3.8	58
Inact	1988 – H3N2	39	20.5	
Inact + Live (H3N2-87)		35	8.5	59
Inact	1989 – H3N2	75	14.6	
Inact + Live (H3N2-87)		75	5.3	64

*Data from Treanor *et al*, 1992

Efficacy of Live, Cold-Adapted Reassortant Influenza Vaccines

Desired Property	Number of Reassortant Vaccine Viruses: Evaluated	Demonstrating Indicated Property
Safety	11	11
Infectivity	11	11
Immunogenicity	11	11
Protective Efficacy Against:		
Homologous Virus Challenge	4	4
Natural Infection	5	5
Genetic Stability	8	8
Transmissibility	4	4
Low Reactogenicity	10	10

Optimal Strategy for Immunoprophylaxis of Influenza Varies with Population at Risk

Target Population	Choice of Vaccine	Reason for Choice	Schedule of Immunization
Infants and children ≤4 years of age	Live	Primary immunization with live virus vaccine is more immunogenic inducing higher serum and secretory virus-specific antibodies than inactivated virus vaccine in seronegative subjects	All infants - 2 & 4 or 6 months Children - annual immunization in fall
Older children and healthy adults <65 years of age	Live	Efficacy of live virus vaccine is comparable or greater than inactivated virus vaccine, but live virus vaccine is better tolerated	Annual immunization in fall
Elderly (≥65 years of age)	Live and Inactivated	Live plus inactivated virus vaccines are more immunogenic and efficacious than inactivated virus vaccine alone	Annual immunization in fall

Introduction of Animal or Avian Influenza A Virus Genes into the Human Population

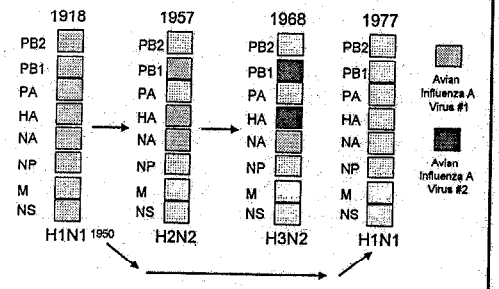
I - Pandemic Influenza

1- HINI in 1918 - Origin uncertain

2- H2N2 in 1957 - Avian-human reassortant

3- H3N2 in 1968 - Avian-human reassortant

Origin of the Genes in Pandemic Influenza A Viruses



Introduction of Animal or Avian Influenza A Virus Genes into the Human Population

II - Other Natural Introductions

1- H1N1 from Swine - 1976 and ongoing

2- H1N1 genes from birds in a human H3N2 coat (?via pigs) in 1993

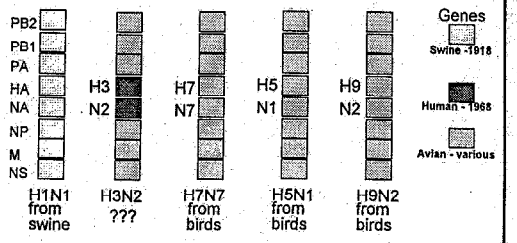
3- H7N7 avian virus from pet ducks in 1996

4- H5N1 from market birds in 1997

5- H9N2 from market birds in 1999

Introduction of Animal or Avian Influenza A Virus Genes into the Human Population

II - Other Natural Introductions



Consequences of the Introduction of Flu Viruses Bearing Novel Glycoproteins

Virus	year	Consequence to individual	Type of Epidemic
H1N1	1918-Spanish	Severe	Pandemic
H2N2	1957-Asian	Severe	Pandemic
H3N2	1968-Hong Kong	Severe	Pandemic
H1N1	1976- Swine	Severe	Abortive
H1N1	1977-Russian	Moderate	Epidemic
H7N7	1996	Moderate	Abortive
H5N1	1997-Bird	Severe	Abortive
H9N2	1999	Moderate	Abortive

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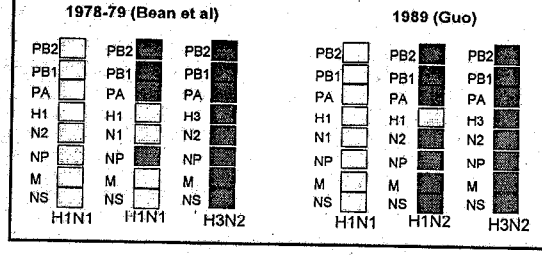
III - From Laboratory or Experimental Studies

1- H1N1 from 1950 human strain - 1977 "Russian Flu"

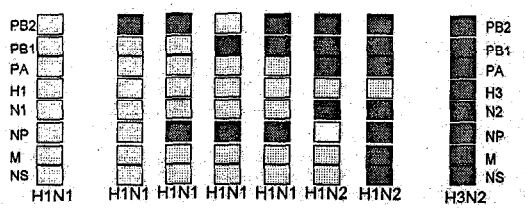
2- H7N7 avian virus from birds via seals in 1980

3- Direct Introduction
- a) Vaccine development (Snyder et al, 1986; Clements et al, 1992)
- b) Beare - infectivity and immunogenicity assessment (1991)

Reassortment between Circulating Human Influenza A Viruses



Reassortment between Circulating Human Influenza A Viruses



Consequences of the Generation of Influenza Viruses Bearing Mixed Internal Genes From H1N1 and H3N2 Human Viruses

1- Transient circulation of the viruses in the population. No apparent epidemiological advantage.

2- Illness comparable to that of either H1N1 or H3N2 parents.

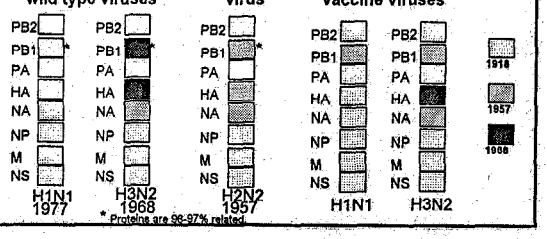
∴ No known adverse consequence despite co-circulation of H1N1 and H3N2 viruses for 24 years.

What are the Consequences of the Introduction of Genes Present in the *ca* Influenza A Virus Vaccines into the Human Population?

1- Consequences of reassortment with circulating human influenza viruses?

2- Consequences of reassortment with animal or avian influenza viruses?

Origins of Genes in Currently Circulating Wild Type Influenza A Viruses and in *ca* Vaccine Viruses



Likely Consequences of the Generation of Viruses Bearing Mixed Internal Genes From Influenza A or B *ca* and Wild Type Viruses

1- Transient circulation of the viruses in the population. Same for both A and B viruses.

2- Illness comparable to that of H1N1, H3N2, or Influenza B wild type virus .

No known adverse consequence seen during co-circulation of H1N1 and H3N2 viruses for 24 years.

Are there Unique Consequences for the Generation of Reassortant Influenza Viruses Bearing New HA or NA Genes from Avian or Animal Viruses and one or more *ca* Genes?

Answer:

Both circulating influenza A wild type viruses and *ca* vaccine viruses could generate reassortants. Nothing unique about the *ca* virus vaccines in this regard.

Chance for Generation of a Reassortant Influenza Virus Bearing New HA or NA Genes from an Avian or Animal Influenza Virus Might be Less Likely for *ca* Vaccine Viruses than for Wild Type Viruses for Several Reasons

1 - *ca* virus is poorly transmissible and unlikely to transmit to an avian or animal host. Reassortment likely would be restricted to occur in humans.

2- *ca* virus is present in lower titer and for a shorter duration in vaccinees which decreases the opportunity for reassortment to occur in humans.

3- At least 50% of reassortants will have an attenuating *ca* gene (ie., the *ca* PB2, PB1, or PA gene).

Use of *ca* Vaccine at Time of Possible Pandemic

1- In the face of a unequivocal pandemic, *ca* vaccine can be introduced into population in advance of the pandemic virus since the small risk of its reassortment with current stains of human H1 or H3 wild type virus is balanced by the significant benefit of the protection achieved by use of the vaccine.

2- Do not use *ca* vaccine bearing novel glycoproteins in context of an abortive epidemic (eg., swine influenza epidemic in 1976) since it could give rise to a pandemic virus via reassortment with circulating H1 or H3 wild type viruses (novel HA and NA from ca vaccine and wild type internal genes).

Conclusions

1- The influenza A and B *ca* reassortants are phenotypically stable following replication *in vivo*.

2- The *ca* reassortants viruses will reassort with wild type human viruses, but likely with lower frequency than that which occurs between human viruses.

3- The *ca* reassortants are less likely than wild type viruses to reassort with animal and avian viruses

4- The consequences of reassortment for *ca* and wild type viruses are similar - nothing unique about the *ca* viruses