Heterosubtypic Immunity to Influenza A

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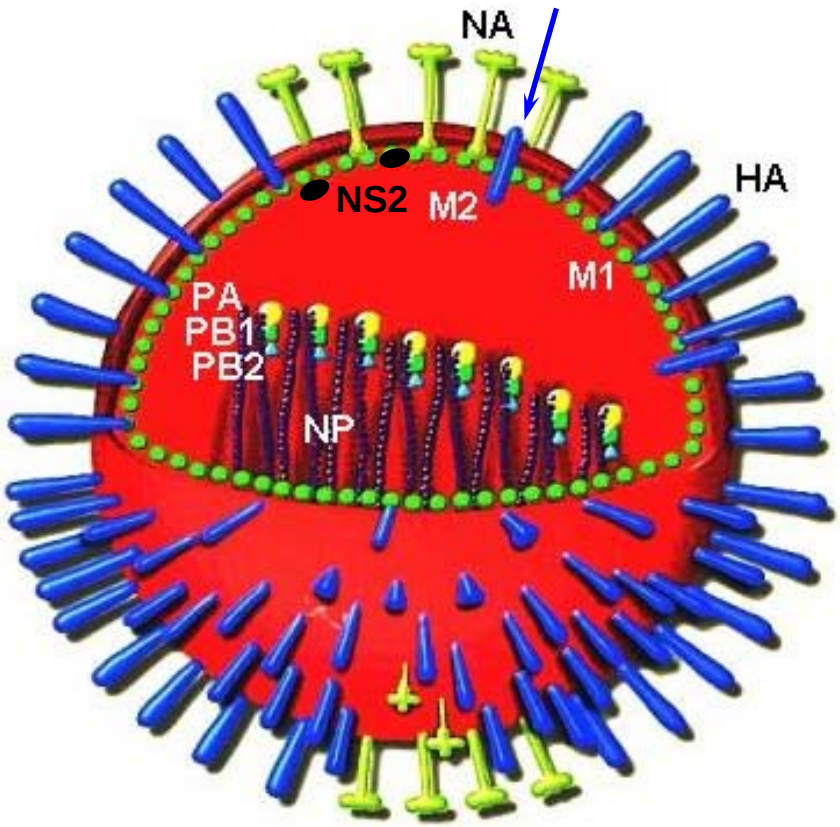
Workshop, Immune Correlates of Protection, Influenza A

Suzanne Epstein, Ph.D.
Division of Cellular and Gene Therapies
FDA, CBER, OCTGT, Bethesda, MD

Heterosubtypic immunity (Het-I), topics to be covered

- **Background on Het-I and its characteristics, questions of potency, duration**
  (Thanks to Robert Couch, Brian Murphy, Jack Bennink for covering some background)
- **Induction by natural infection, by various vaccines**
- **Implications about immune correlates or surrogates relevant to this protection**

Influenza virus components



Highly variable, targets of protective antibodies:

HA hemagglutinin H1-16
NA neuraminidase N1-9

Relatively conserved:

M matrix encodes M1, M2
NP nucleoprotein
PA acidic polymerase
PB1 basic polymerase 1, PB1-F2
PB2 basic polymerase 2
NS "nonstructural" - NS1, NS2

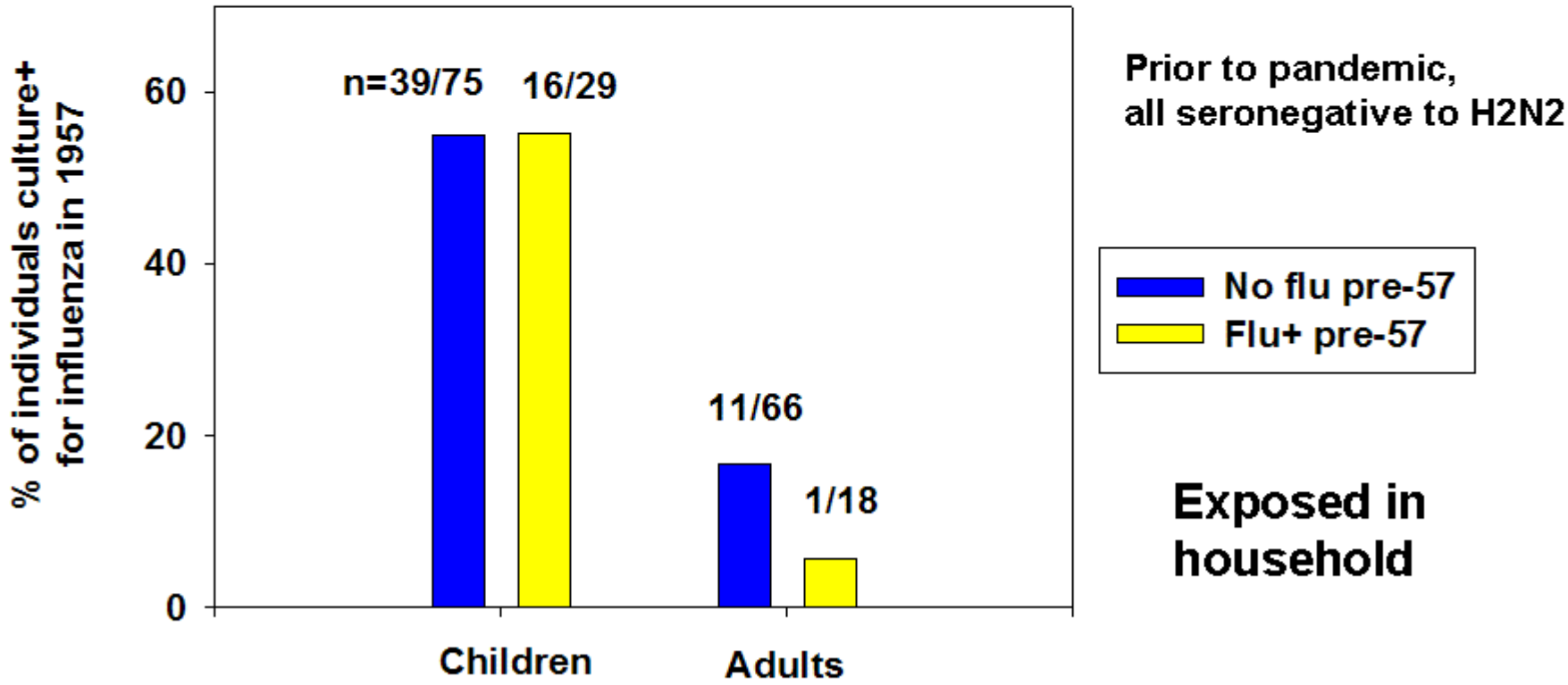
Heterosubtypic immunity: Broad protection

Immunity induced by virus of one influenza A subtype or its antigens that protects* against virus of another subtype

- **Long studied in animals.** Schulman and Kilbourne, 1965
- ***Not sterilizing immunity, permits some viral replication, but accelerates clearance, reduces morbidity and mortality.**
- **Conserved antigens recognized, protection against various influenza A subtypes. Does not require cross-reactive serum antibody.**
- **Specific to flu A at effector stage (no control of bystander flu B virus), not just local interferon or cytokines.** Gerhard lab

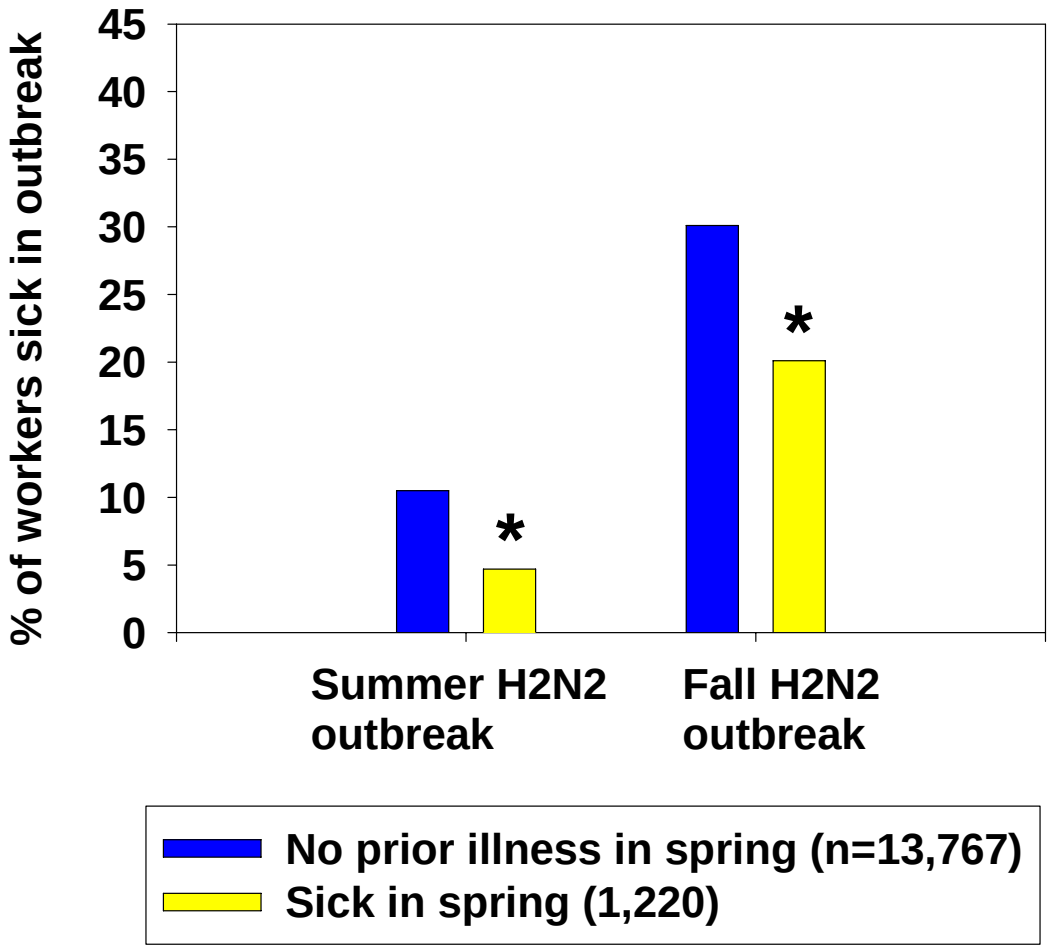
Natural infection in humans: symptomatic influenza during the 1957 pandemic (H1N1→H2N2)

Cleveland Family Study participants monitored in 1950, 1951, 1953 and 1957. Culture-confirmed influenza A



Epstein, J. Infect. Dis, 2006

Natural infection in humans: Effect of prior illness, 1957 pandemic



USSR, 15,072 workers.
Flu-like illness,
not virus testing.

Influenza in spring was H1N1.

Significant difference, despite dilution of signal by other viruses in the spring.

•p<.001, Chi square

A.N. Slepushkin, Bull.Wld.Hlth.Org. 20:297, 1959

Heterosubtypic immunity in mice: multiple immune mechanisms

Multifactorial protection: roles of antibodies (IgG, IgA) and/or T cells, depends on antigens used, immunization.

Importance of anatomical site: mucosal, URT vs LRT

(de st. Groth, 1950), Lamm, Gerhard, Mestecky, Mbawuike, Couch

Mediators: Studied by adoptive transfer of T cells, passive transfer of antibodies or in vitro treatment of virus before use in challenge, foster nursing, depletion of T cells or NK cells, use of knockout mice

- **T cells (incl NP)** — Doherty, Gerhard, Braciale, Couch, Woodland, Topham, Small, Rimmelzwaan
- **Antibodies** — Couch, Fiers, Katz, Tumpey, Mestecky, Kang
- **ADCC (M2)** — Bachmann

Heterosubtypic immunity in animal models

Het-I observed in mice, chickens Webster**, ferrets** Small**,**
pigs Bianchi, Garcia-Sastre**, cotton rats** Eichelberger

Depending on the system, reduced viral replication, shedding, morbidity, mortality, histopathology

Examples

Ferrets: Earlier clearance shown by virus shedding, protection persisted at 18 months Small

Chickens: H9N2 protection against H5N1, T cell-mediated Webster

Vaccines that induce Het-I in animals

Inactivated virus

Induce Het-I when given intranasally Takada
especially with various adjuvants Katz,Tumpey, Kang

Cold-adapted influenza viruses

Induce Het-I protective against challenge with various subtypes
Tannock, Subbarao, Dutton, Katz, Klimov, this talk

Experimental vaccines: protein, peptide, conjugate, DNA, viral vectors

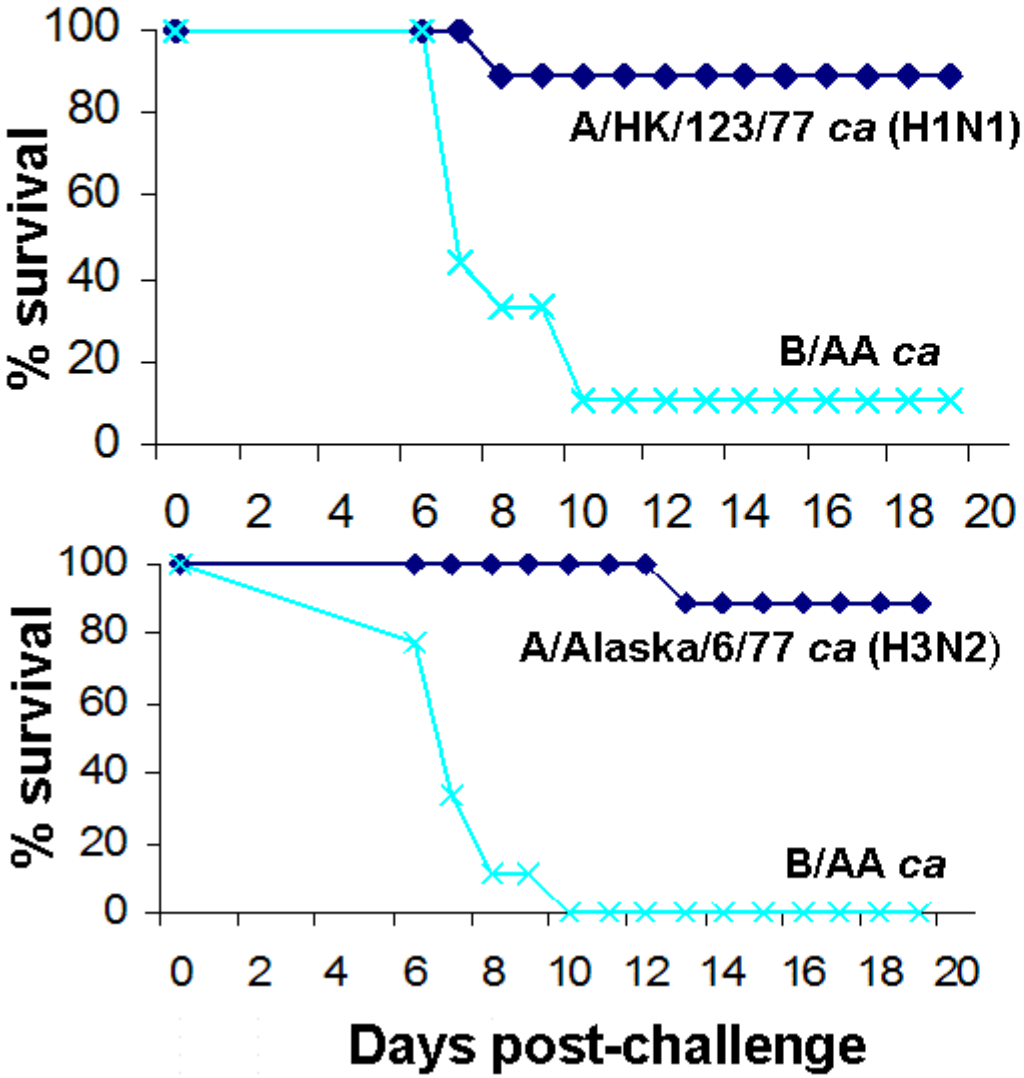
NP immunity Askonas, Ada, Braciale, Rhodes, Ulmer, Liu, Epstein, Couch

Antibodies do not transfer protection, T cells do, CD8 more efficient than CD4

M2 immunity Lamb, Cox, Katz, Treanor, Gerhard, Okuda, Chen, Epstein

Protection by M2 fusion protein Fiers**, M2 multiple antigenic peptide** Gerhard**, peptide conjugates** Shiver
Serum antibodies transfer protection

Cold-adapted influenza virus can protect against heterosubtypic challenge



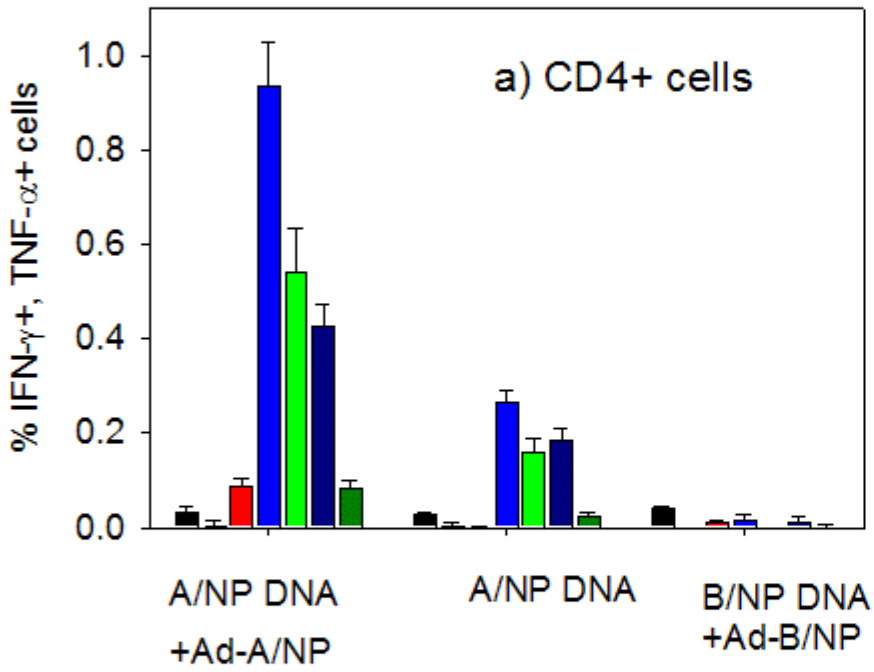
2 doses of vaccine given i.n.

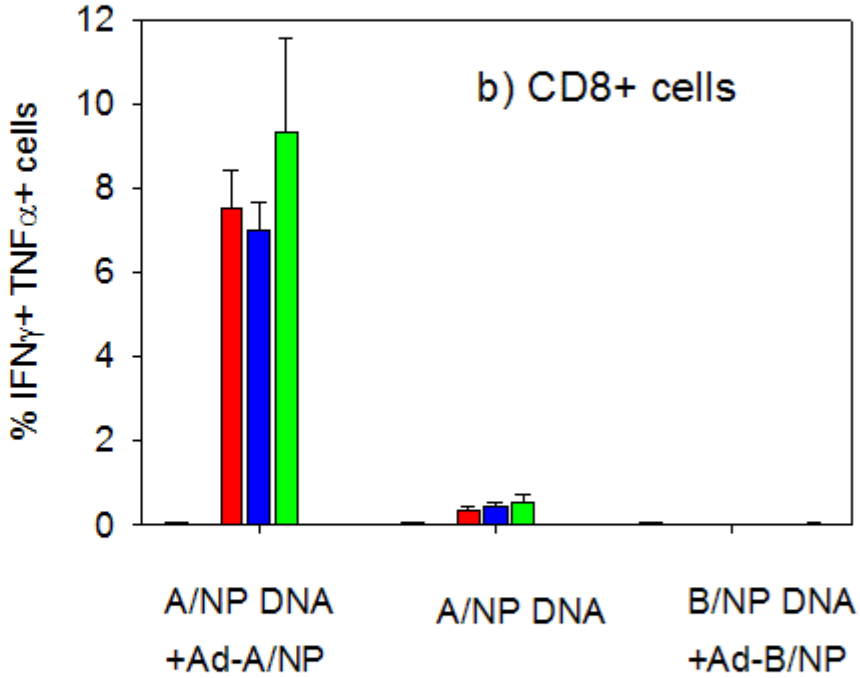
Challenge: X-79 (H3N2)

Challenge: A/PR/8 (H1N1)

ca viruses from Brian Murphy, NIAID. X-79 from Roland Levandowski, CBER

NP: rAd boosting enhances T cell responses to DNA vaccination

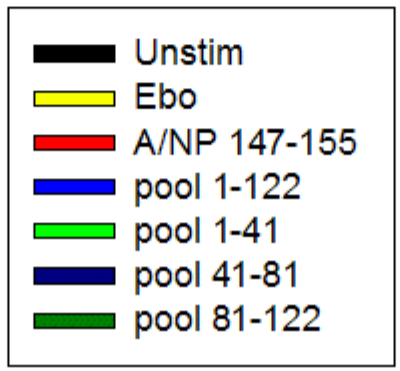




NP sequence of A/PR/8 (H1N1)

Spleen, 12 days after rAd

VR-1012 from Vical under MTA

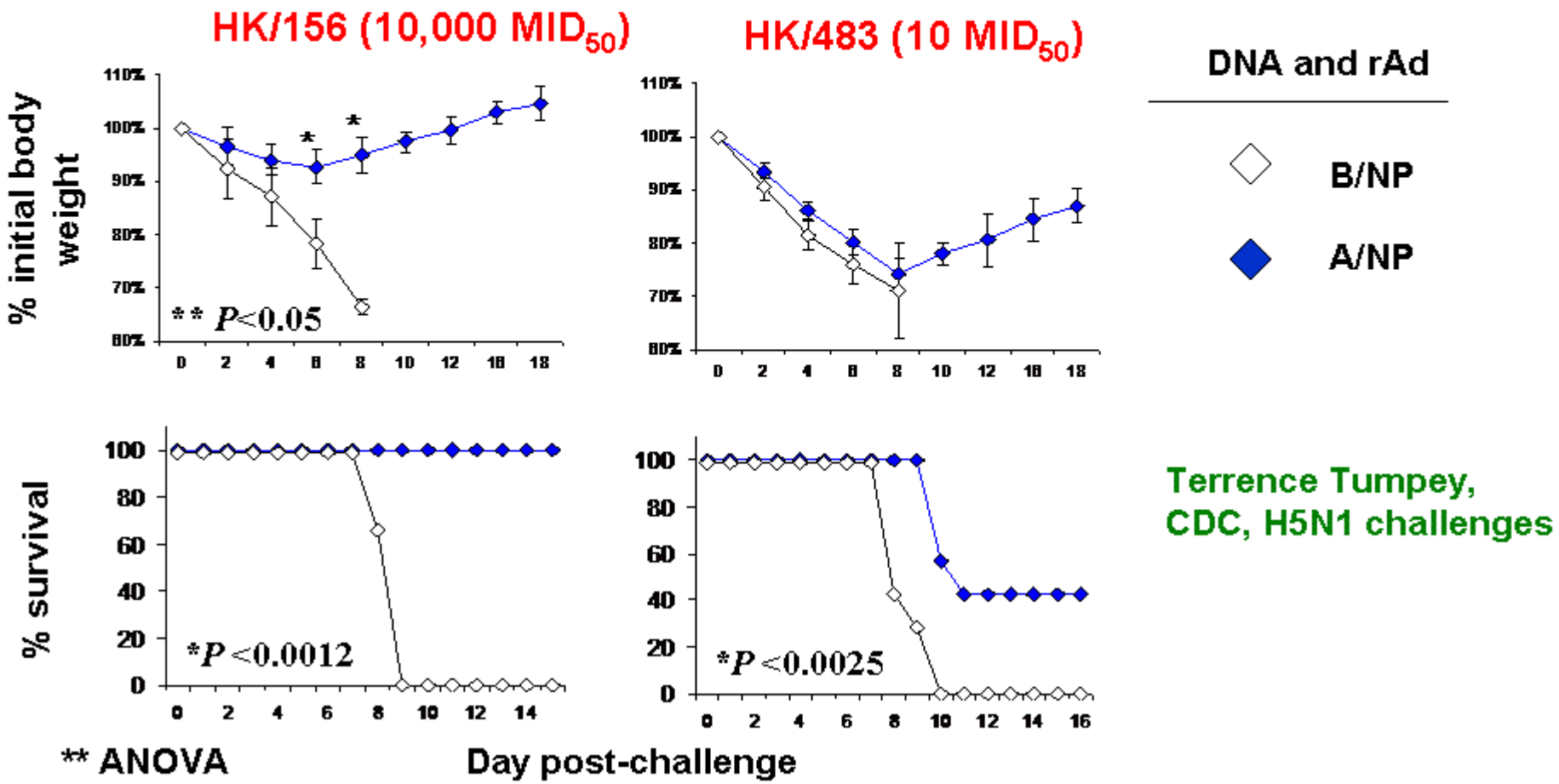


Antibody response also enhanced

Epstein, et al, Vaccine, 2005
w. Gary Nabel, Wing Kong, VRC

NP DNA prime-Ad boost protects against highly pathogenic H5N1 virus

Challenge 5 months after adeno boost



Terrence Tumpey, CDC, H5N1 challenges

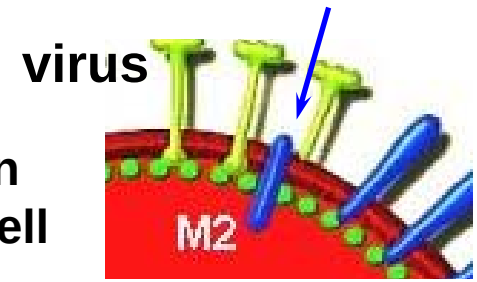
** ANOVA
* Log-Rank

M2 immunity: How broad?

"Consensus" sequence		MSLLTEVETPIRNEWGCRCNDSSD
PR/8	H1N1	MSLLTEVETPIRNEWGCRCNGSSD
HK/483, HK/156	H5N1	MSLLTEVETLTRNGWGCRCSDSSD
FM-MA	H1N1	MSLLTEVETPTKNEWECRCNDSSD
SP-83	H5N1	MSLLTEVETPTRNEWECRCSDSSD

Consensus shared by most viruses of circulating human subtypes H1, H2, H3

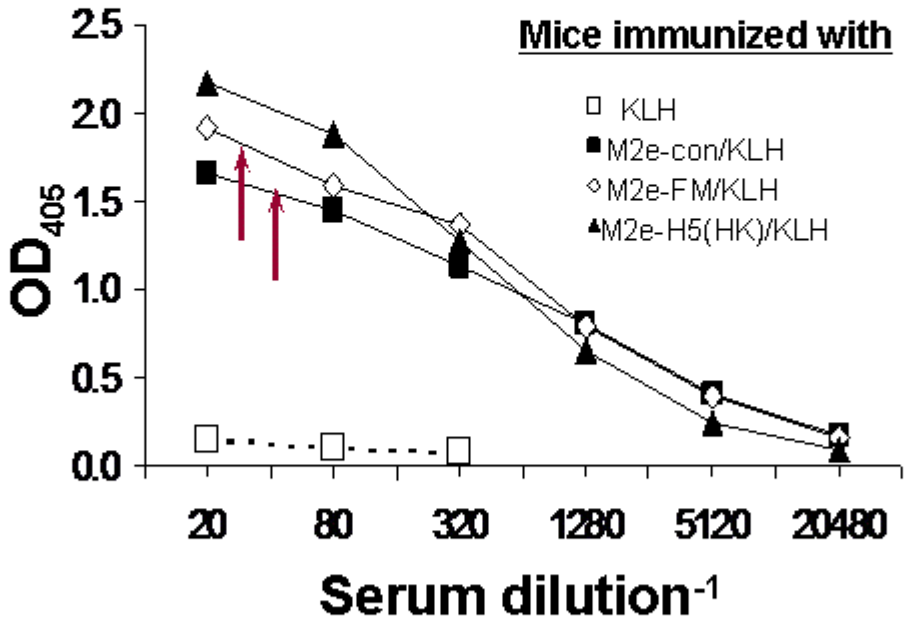
Mark Tompkins,
now at Univ. Georgia



M2 also on infected cell

Immunization with M2e-peptide/KLH primes subtype-crossreactive antibody responses

ELISA on plates coated with M2e-H5(SP-83)

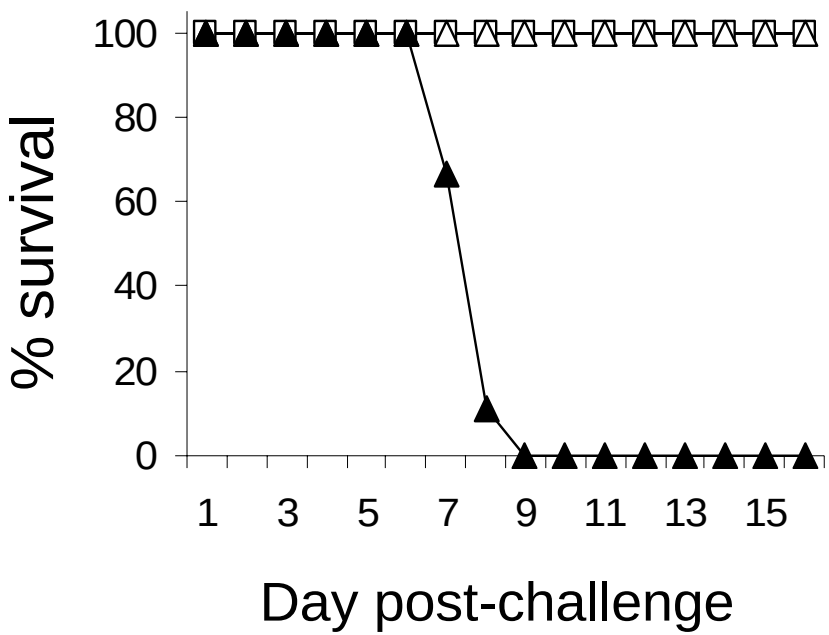


Next, DNA-rAd with whole M2 gene

DNA prime-rAd boost to M2 protects against mismatched challenge

M2 consensus sequence in DNA and rAd
Challenge 3 weeks after rAd boost

A/FM challenge, 10 LD_{50}
(3 amino acid differences)



A/PR/8 challenge:

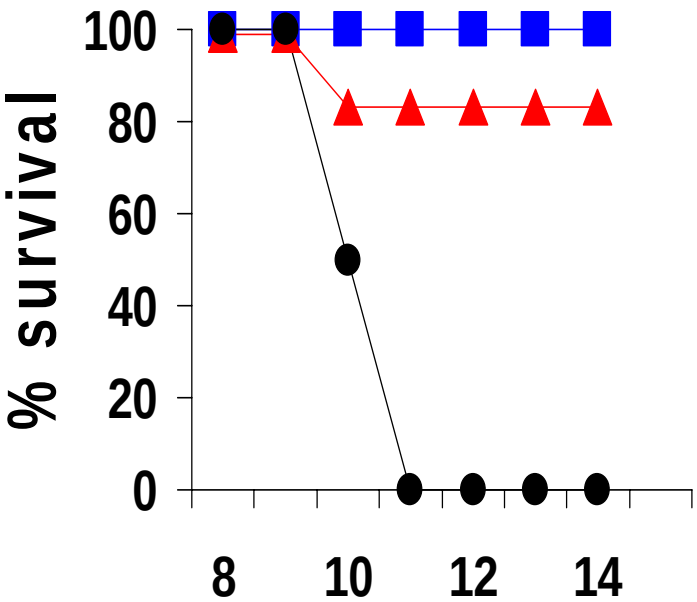
- **Passive serum transfer, protects against challenge the next day**
- **T cell depletion during challenge period abrogates some protection**

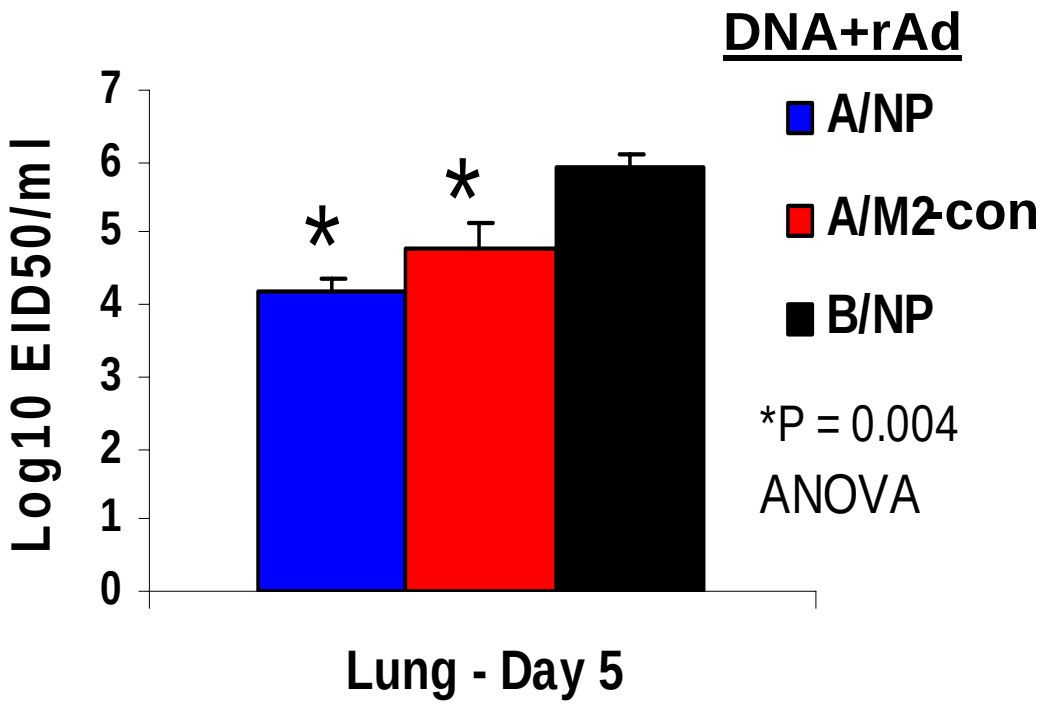
M2 DNA, Zhiping Ye, Teresa Liu, OVRR

Mouse-adapted A/FM virus from Earl Brown, U. Ottawa

M2 can protect against a lethal H5N1 challenge

Challenge with A/Thailand/SP83/04
17 days after boost





Morbidity also reduced (body weight)

Tompkins, et al., Emerg. Infect. Dis., 2007
w. Terrence Tumpey

Multiple antigens (NP, M2) can induce Het-I

Advantages of multi-antigen cocktail as vaccine:

- **Induce wider variety of immune effector mechanisms**
- **Reduce probability of escape mutations**
- **People of different HLA types less likely to be non-responders overall**

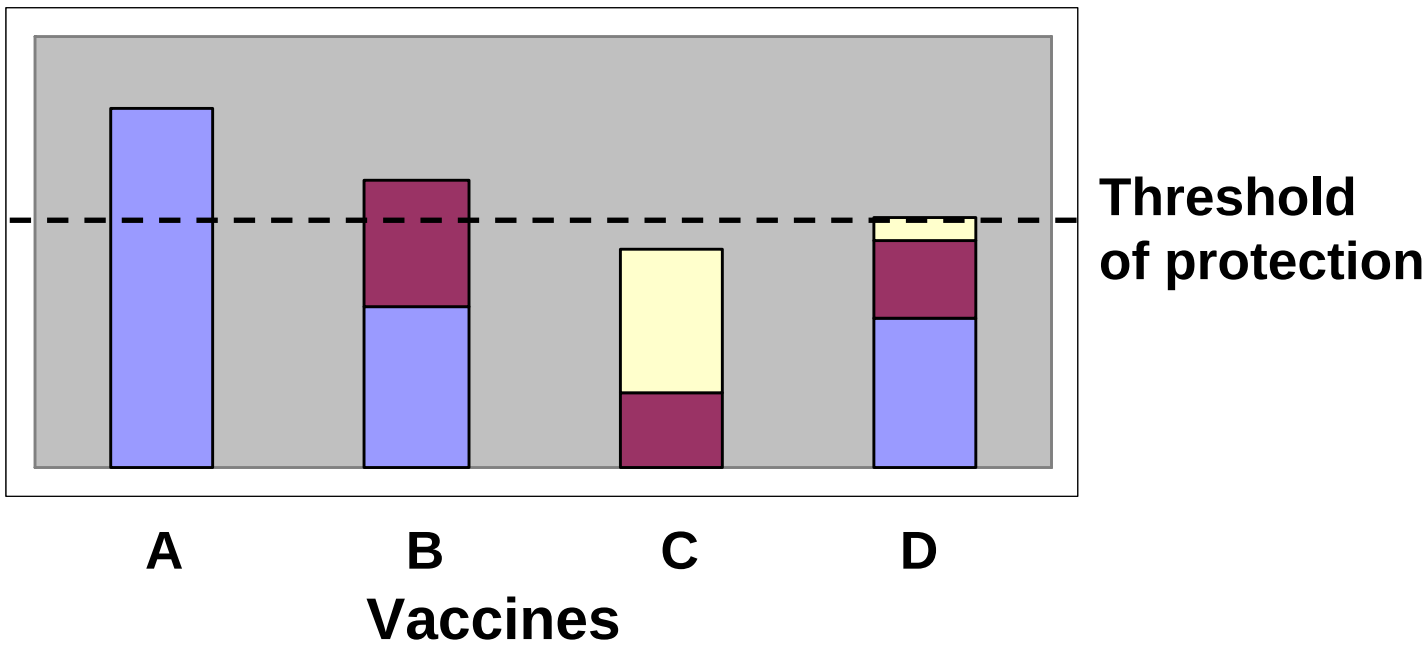
Possible disadvantage:

- **Interference/antigenic competition**

Vaccine comparisons ongoing. Most potent Het-I not necessarily achieved by giving all antigens.

Immune correlates: multiple responses may contribute to protection

- Serum HAI or VN antibodies
- Respiratory tract IgA
- Local cytokines
- CD4+ and CD8+ T cells (memory subsets, cytokine producers)
- ADCC
- Combinations of multiple responses



For practical reasons, could measure in priority order.

Testing algorithm will depend on vaccine type

If HA, is level of HAI or VN antibody protective?

Yes → Done

No → Next test, choice depends on nature of vaccine

Is level of anti-M2 antibody protective alone or in combination with the above?

Yes → Done

No → Next test, choice depends on nature of vaccine

Is level of certain T cell response protective alone or in combination with the above?

etc.

Summary, heterosubtypic immunity to influenza A

- **Various vaccines induce Het-I in animals that can be long-lived, reduces morbidity and mortality, can involve T cells and/or antibodies.**
- **Protection is effective against challenge with strains of various subtypes, including some H5N1 strains.**
- **Historical data suggest Het-I in humans may alter susceptibility during a pandemic.**
- **Findings call for comparison of vaccines in preclinical models, for clinical trials, and for surveillance of at-risk populations.**

Public health implications for control of influenza

- **Het-I may provide a first line of defense, reduce morbidity and mortality despite imperfect protective immunity.**
- **Prime in advance. In the event of an outbreak of an unexpected strain or a pandemic, boost in high risk areas.**
- **Het-I to be augmented by antigenically-matched vaccines (inactivated and/or live attenuated) when sufficient supplies become available.**

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