Strategies for control of influenza by targeting broadly conserved viral features

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Targeting conserved components

Advantages in the vaccine arena:

♦ Do not need to know which virus strain is coming

♦ Vaccines made in advance, available off the shelf

♦ Could prime to establish immunity, then boost later with matched vaccine when available.

Therapies also target conserved components.
Influenza virus components

Highly variable, targets of neutralizing antibodies:

HA  hemagglutinin
NA  neuraminidase

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

Image reproduced with permission from Dr. Hervé Zender, SNM.
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Roadmap of topics

Common theme of conserved sites

♦ Broad cross-protection to influenza A in animals

♦ DNA vaccines:
  • Cross-protection
  • Potential against pandemic subtypes

♦ Human influenza in the pandemic of 1957

♦ RNA inhibition of influenza *in vivo*
Heterosubtypic immunity to influenza A (Het-I)

- Immunity induced by virus of one influenza A subtype that protects against virus of another subtype. (Broad cross-protection.)
- Reduces morbidity and mortality.
- Reduces but does not eliminate viral replication.
- Flu B too distant, no cross-protection of this type.
Heterosubtypic immunity
to influenza A (Het-I)

Induced by live virus:

• Studied in animals since mid-1960’s.
  J.L.Schulman and E.D.Kilbourne, Yetter, et. al. (P.A.Small lab)

• Specific at the effector stage, no control of bystander virus. Role of T cells.
  Liang, et. al. (Gerhard lab)

• Induced by infection of the total respiratory tract but not the upper respiratory tract only.
  Nguyen, et. al. (Mestecky, McGhee)
Heterosubtypic immunity to influenza A

Induced by inactivated virus:
• Antibody mechanism suggested by vaccination during pregnancy. Mbauike, et. al. (Couch lab)
• Inactivated H3N1 virus given mucosally protected against H5N1. Takada, et. al. (Kida lab)
• Inactivated H3N2 i.n. protected against H5N1. Evidence is antibody-mediated. Tumpey, et. al. (Katz lab)

Live attenuated virus:
Replicates mainly in URT. Potential unclear.
DNA vaccination to flu

• NP DNA shown to induce antibodies, CTL, and to protect. Later NP+M used. Rhodes, et. al. (Vical), Ulmer, et. al. (Merck)

• HA DNA given by various routes protected mice, chickens; NP inadequate. Fynan, et. al. (Robinson lab), Kodihalli, et. al., (Webster lab)

• T cell depletion at the effector stage abrogated protection by NP+M DNA vaccine. Ulmer, et. al. (Merck), and our work
Why study DNA vaccines?
Possible advantages

The polio vaccine campaign and the cold chain:

http://www.endofpolio.org/home.html

DNA vaccines relatively heat stable, relatively safe.
Not produced in eggs or mammalian cells.
May eventually be cheaper or easier to mass-produce.
Even if DNA vaccines do not turn out to be practical, useful tool for investigation of individual components.
Immune protection against mismatched challenge

DNA given i.m. x 3

H3N2 challenge i.n. 8 months later
PA, another conserved antigen with vaccine potential

With Zhiping Ye, OVRR. CBER
Can immunity to conserved antigens have an impact on a potential pandemic subtype? H5N1

• Not sterilizing; transient infection occurs, is cleared more quickly than in naïve hosts.

• For infection with the virulence and rapid kinetics of H5N1, immune response could lose the race.

• Test: NP and M genes from A/PR/8/34 (H1N1), challenge with H5N1 viruses from human cases in the 1997 outbreak in Hong Kong

With Terrence Tumpey, Jackie Katz, CDC
DNA vaccination partially controls replication of A/HK/156 (H5N1)

500-fold reduction by A/NP+A/M

Immunization:
- A/NP, A/M DNA
- B/NP, empty DNA
- live H1N1

DNA vaccination protects against lethal challenge with A/HK/156 (H5N1)

Partial protection against HK/483 (even more virulent)
Vaccination against pandemic subtypes

- Vaccine based on conserved antigens could be useful as a first line of defense, while antigenically-matched vaccines were being prepared.

- Live attenuated vaccines (H5N1, H9N2) derived, but would only be used under extreme circumstances due to risk of reassortment with coinfecting wild type virus.
Various strategies to improve the potency of DNA vaccination

• Example: boost with recombinant adenovirus expressing the vaccine antigen.

• More suitable for human use than poxviruses.

• Possible role in pandemic preparedness or response:
  Advance DNA priming, rAdeno boosting to encircle areas of outbreak.

With Wing-Pui Kong, Gary Nabel, NIH Vaccine Research Center
Terrence Tumpey, CDC
Broad cross-protection in humans?

Most human immunity to influenza is subtype-specific.

♦ Can humans be protected against new flu subtypes by prior exposures?

♦ Could such immunity have an impact in a pandemic?

♦ Can vaccines based on conserved components of influenza A make a public health contribution?
The Cleveland Family Study

- Carried out 1947-1957, ~ 60 families monitored for illness

- Pandemic of 1957: major subtype shift (H1N1 → H2N2)
  Slepushkin data based on fevers, no virus testing

- Data not analyzed at the time for cross-protection
  (William Jordan)

- Archival case records for culture-proven influenza examined
Effect of prior infection on incidence of Asian influenza in 1957: children and adults

Adults differ from children in the effect of prior influenza infection in 1950, 1951, or 1953 on susceptibility in 1957.

(p<.001 by Chi-square)
Why do adults differ from children in the effect of prior infection?

Differences in behavior or hygiene? In that case, incidence in children and adults would differ to this extent every year, but the pandemic year was different.

Antibodies from the last time H2N2 virus circulated in humans? No, ruled out by age of participants and also serology results.

Immunity generated by accumulated exposures to influenza virus?

Possibly. Further investigation of Het-I in humans warranted.
RNA interference to control viral infection

- Prior work by many on viral systems *in vitro*, a few *in vivo*.
- Highly specific.
- Approach: Use short interfering RNA’s (siRNA) to inhibit expression of proteins needed for viral replication.
Can RNA interference control influenza virus infection *in vivo*?

- By targeting highly conserved viral sequences, hope to inhibit broad range of flu strains.
- Used flu sequences identified as most effective by Ge *et al*, *Proc Natl Acad Sci* 2003.
  
  NP-1496 and PA-2087 vs GFP-949 control, given before infection

*With Terrence Tumpey, CDC*
Influenza-specific RNAi is broadly protective

A/PR/8/34 (H1N1)  A/HK/156/97 (H5N1)  A/NL/219/03 (H7N7)

% body weight

Day post-challenge

% survival

P<0.002, all 3 viruses

Additional studies of influenza siRNA *in vivo* by Chen group, MIT

- si RNA given after infection reduced viral replication in mice.
- Plasmid expressing small hairpin RNA given prior to challenge reduced viral replication.
- Plasmid could be given mucosally with surfactant.

*Ge, et. al., PNAS, June 2004.*
Conclusions, control of influenza based on conserved viral features

- Conserved antigens can induce immunity that, while not sterilizing, greatly reduces morbidity and mortality upon heterosubtypic infection.

- Whole virus, DNA vaccines, and DNA prime-viral boost regimens can protect against some mismatched challenges, including some H5N1.

- The potential for broad cross-protection in humans should be investigated more fully.

- Conserved sequences can be targeted by siRNA for prevention and therapy.
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The “Critical Path”: path of products from prototype design or discovery through preclinical and clinical testing and on to FDA approval and marketing. Critical Path research can identify issues and bottlenecks, help find solutions to problems, even try to avoid problems before they occur.

Read more on FDA's Critical Path website at http://www.fda.gov/oc/initiatives/criticalpath/

At the bottom of the web page is a link (“Provide Your Input: Creating a National Critical Path Opportunities List”) to a list of specific questions for stakeholders. You can submit comments to the docket. The closing date for the docket is July 30.

Comments will be publicly available at http://www.fda.gov/ohrms/dockets/dockets/dockets.htm/
Results for everyone monitored in all study years

Blue: No flu in 1950, ’51, ’53
Green, Flu+ in 1950, ’51, or ’53

Suggestive, worth further investigation

% of individuals culture+ for influenza in 1957

Kids

Adults

n=39/75 16/29

11/66 1/18