Heterosubtypic immunity:
What we know and what we need to know

Second WHO Integrated Meeting on development and clinical trials of influenza vaccines that induce broadly protective and long-lasting immune responses

5th May 2014
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Outline

Note: This talk will not cover cross-protection by antibodies to HA, head or stem - covered by others.
Will discuss:
- Heterosubtypic immunity (Het-I)
  - Vaccine candidates and their protective immune mechanisms
  - Mucosal vs. systemic administration
- Prior history: Impact of previous exposures on response to vaccination
- Transmission: Impact of conserved antigen vaccines on spread of disease
- Open questions and public health implications

Broad cross-protection to influenza A: Heterosubtypic immunity (Het-I)

Het-I: reduces viral replication, morbidity, mortality. Does not require neutralizing antibody or confer sterilizing immunity.
Live H1N1 infection provides partial protection against H2N2
Observed in mice (many investigators), chickens (Webster), ferrets (Small), pigs (Bianchi, Garcia-Sastre), cotton rats (Eichelberger), guinea pigs (Palese)

Cross-protective vaccines:
- Possible disadvantage: residual infection with its potential risks of disease and transmission
- Possible advantages: broad coverage, all flu A subtypes (broad flu B under study)
  - Priming of T cell memory with benefits later in life
  - Transient infection may induce neutralizing antibody to circulating virus

Specificity of heterosubtypic protection: no control of bystander flu B virus

<table>
<thead>
<tr>
<th>Immunize:</th>
<th>Challenge with:</th>
<th>Outcome, virus growing in lungs:</th>
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<tbody>
<tr>
<td>H1N1</td>
<td>H3N2</td>
<td>H3N2 minimal</td>
</tr>
<tr>
<td>H1N1</td>
<td>B</td>
<td>H3N2 minimal</td>
</tr>
<tr>
<td>H1N1</td>
<td>H3N2 + B</td>
<td>B</td>
</tr>
</tbody>
</table>

So flu B as specificity control
Note broad range: Het-I spans group 1 and group 2 HA subtypes
Non-specific effects / innate immunity should be considered if a vaccine is tested by challenge only at early time points.
Vaccines that induce cross-protection

Details for particular vaccines discussed by others at this meeting

Live attenuated influenza viruses
Cold-adapted, ΔNS-1, mutants in M1, M2 cytoplasmic tail, NA Tannock 1984, Subbarao, Dutton, Katz, Klimov, Garcia-Sastre, Ye, Epstein, Kawaoka

Inactivated influenza virus
γ-irradiated, Müllerbacher
Formalin-inactivated: Induce Het-I when given intranasally Takada et al. 1999, especially with various adjuvants Katz, Tumpey, Kang

Recombinant vectored vaccines
DNA alone or formulated, poxviruses (vaccinia and MVA), adenoviruses, VSV, AAV, herpes, alpha viruses (SFV and VEEV), VLPs, bacterial vectors
Antigens expressed NP, M2, M1, NA, less often polymerases or NS
(Also HA whole or stem constructs)

Correlates will differ from those for traditional vaccines

Approaches to heterosubtypic protection: Cross-protection by NA

N1 VLP vaccine. Mice given VLPs twice, 4 weeks apart. Challenge with H3N2

Universal anti-NA monoclonal antibody “HCA-2”
Prophylactic and therapeutic potential

% sequence conservation of the epitope

<table>
<thead>
<tr>
<th>NA subtype</th>
<th>HCA-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>98.75</td>
</tr>
<tr>
<td>N2</td>
<td>98.5</td>
</tr>
<tr>
<td>N3</td>
<td>96.7</td>
</tr>
<tr>
<td>N4</td>
<td>100</td>
</tr>
<tr>
<td>N5</td>
<td>100</td>
</tr>
<tr>
<td>N6</td>
<td>99.4</td>
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<tr>
<td>N7</td>
<td>99.4</td>
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<tr>
<td>N8</td>
<td>99</td>
</tr>
<tr>
<td>N9</td>
<td>95.4</td>
</tr>
<tr>
<td>N10</td>
<td>100</td>
</tr>
</tbody>
</table>

M1 also present in the vaccine, but not known to be protective in mice. (M1 is a candidate antigen in humans.)

High dose (60 mg/kg) given passively protects mice

Quan et al. Virology 430:127, 2012

Vected vaccines using combinations of antigens

Examples in animals:
Vaccinia HA, NA, M1, M2, and NP, plus IL-15 Valkenburg et al. PNAS USA 111:5676, 2014
Multiple peptides or multiple mini-genes: Plasmid encoding 20 conserved flu CD4 epitopes plus pan DR T cell epitope PADRE Alexander et al. Vaccine 28:664, 2010

Examples in humans:
DNA vaccine H5, NP, and M2 trivalent Smith et al. Vaccine 28:2565, 2010

Various prime-boost combinations

Cross-protection by NP

Protection by immunity to NP, a prominent T cell target:


Immune mechanisms:

Various investigators have shown reduction in protection by T cell depletion in vivo
Also T cells transfer protection
Non-neutralizing antibody to NP can transfer protection
Protection by NP: CD4+ and CD8+ T cells can each contribute to survival and recovery

Example: BALB/c mice immunized with NP DNA, and challenged with A/HK/68 (H3N2)


Adoptive transfer of immune T cells

CD4+ CD8+

Adoptive transfer of CD8+ T cell clones promotes recovery even in absence of antibody


Non-neutralizing antibodies to NP also contribute to protection

B cell-deficient µMT mice given passive antibody, 1 day before challenge.

NP-specific IgG has anti-viral activity

Mechanism:
Less effective in FcR γc−/− mice
Less effective in CD8-depleted mice


Cross-protection by M2

Protection by M2 immunity: many forms (fusion proteins, M2 multiple antigenic peptide, peptide conjugates, vectored expression)

Lamb, Fiers, Katz, Gerhard, Shiver, Chen, Tompkins, Bachmann

Immune mechanisms:

- Serum antibodies transfer protection
- T cells play a role under some conditions

Protective immunity induced by M2: antibodies and T cells contribute to protection

Antibodies to M2 passively transfer protection, T cells play a role as well. Depends upon the experimental conditions used.

Table 2: Survival of mice after passive immunization followed by potentially lethal heterologous influenza A challenge

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Immunizing antigen</th>
<th>Surviving mice</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>IM2H8c</td>
<td>11/12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>1/12</td>
<td></td>
</tr>
<tr>
<td>Passive</td>
<td>IM2H8c</td>
<td>12/12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>control (mock)</td>
<td>1/12</td>
<td></td>
</tr>
</tbody>
</table>


Tompkins et al. EID 13:426, 2007
**M2 as a supplement enhances protection by split vaccine**

Split vaccine: A/California/07/2009 (H1N1) i.m., alone or with M2 VLPs added
Mice challenged 12 months later with H3N2, 5 LD50

Kim et al. Mol Ther doi:10.1038/mt.2014.33, 2014

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**Limitations in identifying protective immune mechanisms**

With *in vivo* depletion of T cell subsets, adoptive transfer, or use of knockout mice, the results can depend on the details:

- Vaccine and route of administration
- Challenge virus strain and dose
- Anatomical sites tested (periphery versus lungs versus lymph nodes)

*Note* that in knockout mice, the immune system has made compensatory changes over the lifetime of the animal.

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**Immune mechanisms are complex and redundant: Examples**

- **Example:** β2-microglobulin gene knockout affects class I MHC, but is also a component of an Fc receptor, shortens IgG half-life
  

- **Example:** μMT mice are often termed B cell-deficient, but are also deficient in APC function that B cells provide, and thus can have a T-cell defect.

- **Example:** Protection by NP can be seen by transfer of only NP-specific CD8 cells or only anti-NP antibody

Keep the limitations in mind when interpreting these studies

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**Additional aspects, mechanisms of immune protection**

Observations, but not necessarily generalizable

**ADCC (Ab-dependent cellular cytotoxicity)**

- **Example:** M2
  

**γδ T cells contribute to flu protection**

Mucosal immunity: Potent protection against influenza virus recognized by 1950

Power of mucosal immunization long known
Mice immunized with influenza virus by nasal vs parenteral routes, then homologous challenge

- Relative protection against influenza challenge was millions-fold better for the nasal exposure
- Protection did not correlate with serum antibody titers to HA.
  Suggests local immunity

Mechanisms? Multiple. One classic example for heterosubtypic challenge:
- CD4, CD8 depletion abrogates protection in the nose, but leaves some protection in the lungs (interpreted as double negative T cells or B cell-mediated)

Superiority of mucosal administration: inactivated influenza virus

Intranasal route shown to promote cross-protection (Takada et al. 1999)
Recent example: Vaccination with γ-ray inactivated H3N2 flu virus

H1N1 challenge 3 weeks after vaccination

Routes:

Alisharifi et al. PLoS ONE e5336, 2009

Mucosal rAd-NP+M2: Greater local immune responses and protection with i.n. than i.m.

10 months post-vaccination, pre-challenge

ELISA

Serum

IgA

CD8+ Lung T-cell responses

Challenge – 10 LD50 of A/VN1203 (H5N1), 10 months post-immunization

Human data for mucosally versus systemically administered vaccine: T cell responses

Study in young children of LAIV and TIV
(There are of course other differences between the vaccines besides route)

ELISPOT with conserved influenza peptide pools
PP1: HLA class I peptide pool 1
PP2: HLA class II peptide pool 2

LAIV induces T-cell responses to class I and class II peptide pools. TIV does not.


Clinically, LAIV is more effective

Belshe, NEJM 356:685, 2007

Price et al. PLoS ONE 5: e13162, 2010

With Terrence Tumpey, CDC

Clinically, LAIV is more effective

Belshe, NEJM 356:685, 2007

Clinically, LAIV is more effective

Belshe, NEJM 356:685, 2007
In animals, immune mechanisms are redundant, overlapping, idiosyncratic depending on details. Thus, not surprising if studies in humans don’t give one simple answer.

**Evidence** for cross-protection:
- 1957 pandemic suggestive Slepushkin, 1959; Epstein, 2006

**Studies with T cell analysis:**

**Caveat:** PBL usually tested. CD8 memory can be underrepresented in periphery, as shown by comparison of human PBL and lung surgical samples. de Bree et al. JEM 2005; Piet et al. J Clin Invest 2011

**Prior history example:** Prior immunity to rAd5 blocks protection by rAd5- but not PanAd3-vaccine

Ad5: one solution, flu vaccine based on adenovirus PanAd3 isolated from a bonobo


Challenge at 6 weeks with 100 LD₅₀ of FM (H1N1)

<table>
<thead>
<tr>
<th>Pre-Ad5</th>
<th>Vaccine (i.n.)</th>
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<tbody>
<tr>
<td>Yes</td>
<td>PanNPM1</td>
</tr>
<tr>
<td>No</td>
<td>PanNPM1</td>
</tr>
<tr>
<td>Yes</td>
<td>Ad5-NP</td>
</tr>
<tr>
<td>No</td>
<td>Ad5-NP</td>
</tr>
<tr>
<td>No</td>
<td>PanAd3-RSV</td>
</tr>
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Unpublished, Misplon, Lo, and Epstein

Many studies are done in naïve, pathogen-free animals

New vaccines are introduced into a human population with a varied history of virus infections and vaccinations

**How does the prior history of recipients influence effectiveness of cross-protective vaccines?**

**Effects of prior history vary with exposures, genetics, due Het-I and other factors**

- Priming can increase or decrease subsequent response. Besides anti-vector immunity:
  - Original antigenic sin for some sequences of strains, not all Delayed/reduced Ab to HA of 2nd infection Nayak et al. J Imm 191:1001, 2013

**Prior history an active area of investigation**
Cross-protective vaccines and transmission

- Some have expressed concern that non-sterilizing immunity induced by cross-protective vaccines is not good enough, because vaccinees still get infected and can spread disease to others.
- Modeling shows large impact on the size of epidemics and pandemics if transmission is reduced. Arinaminpathy et al, PNAS 109:3173, 2012
- Do cross-protective vaccines reduce transmission?

Mouse model for influenza virus transmission

Difficult to achieve statistically significant results with ferrets (outbred, costly)

Mouse model originally developed by Schulman and Kilbourne in 1960’s
Adapted for studies of conserved antigen vaccines by Dr. Graeme Price in my lab

Vaccination to NP + M2 greatly reduces transmission by combined airborne and direct contact

<table>
<thead>
<tr>
<th>Donors</th>
<th>Contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination Lung Virus Positive</td>
<td>Lung Virus Positive</td>
</tr>
<tr>
<td>Lung Virus Positive</td>
<td>Total Positive (% infected)</td>
</tr>
<tr>
<td>A/NP+M2-rAd</td>
<td>22/22 12/22 6/36 2/36 6/36 (16.7%) 66.7% (p=0.006)</td>
</tr>
<tr>
<td>B/NP-rAd Control</td>
<td>15/15 15/15 14/36 7/36 18/36 (50.0%)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>A/NP+M2-rAd</td>
<td>28/29 2/29 2/44 1/44 3/44 (6.8%) 83.3% (p&lt;0.001)</td>
</tr>
<tr>
<td>B/NP-rAd Control</td>
<td>23/23 20/23 17/45 6/45 18/45 (40.0%)</td>
</tr>
</tbody>
</table>

Summary, heterosubtypic immunity

Cross-protection:
In animals, conserved influenza antigens delivered by various vectors can induce protection against disease caused by highly diverse influenza A subtypes, and lasting up to a year or more.

Some evidence for cross-protection in humans.

Promising features of mucosal vaccination:
Both magnitude and distribution of immune responses differ from systemic
- Antibody and T cell responses in the respiratory tract enhanced
- Antibody isotype distribution changed
- Breadth of cross-reactivity increased in some cases
- Superior protection seen in animal models

NP+M2 vaccination in mice can reduce transmission to contacts
Influenza vaccines inducing heterosubtypic immunity: What we need to understand better

- Roles and breadth of reactivity for IgG subclasses, IgA
- Mechanisms and magnitude of protection by non-neutralizing antibodies
- What can we use as correlates of protection in humans? (not lung T cells)
- Is there damage by too-potent T cell responses?
- Vaccine effectiveness in people with different prior histories
  - Includes differences between the young and the elderly
  - Priming in one's youth establishes memory that can later be boosted

Regarding the public health potential of cross-protective vaccines in control of epidemics and pandemics:

- Will immune pressure on new targets drive selection of escape mutants in previously conserved antigens (including HA stem)?
- Head-on comparisons would be useful in choosing among the many candidate vaccines. How do they differ:
  - For effectiveness in animals? In humans?
  - For durability and breadth of protection?
  - For affordability and ease of distribution, including in resource-poor areas?

Vaccine strategies and public health implications

Broadly protective vaccines could be used off the shelf as a first line of defense during a rapidly spreading outbreak when traditional vaccines are not yet available, perhaps someday instead of strain-matched vaccines.

- Reduce illness, death, viral titers.
- May protect not only the vaccinee but also the community by reducing transmission
- The mild or asymptomatic infection they permit may induce neutralizing antibodies to the circulating strain

ACKNOWLEDGMENTS

FDA/CBER/OCTGT
Graeme Price
Julia Misplon
Chia-Yun Lo
Mark Soboleski
Mayra Garcia

CDC
Terrence Tumpey, Claudia Pappas
Katherine Houser, Melissa Pearce

Univ. Georgia
S. Mark Tompkins

Viruses
Brian Murphy, NIAID
Earl Brown, Univ. Ottawa
Gary Nabel, NIH VRC
Daniel Perez, Univ. MD

Funding
CBER/FDA pandemic influenza funding, NIAID Trans-NIH/FDA Intramural Biodefense Program, FDA Medical CounterMeasures Initiative (MCMi)