Immunological Basis of Current and Future Influenza Vaccines

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8th WHO meeting on development of influenza vaccines that induce broadly protective and long-lasting immune responses
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Currently Licensed Seasonal Influenza Vaccines

- **Inactivated vaccines**
  - Split, subunit, virosomal, whole virus
  - Trivalent and quadrivalent
  - Egg- or cell-culture-based
  - Standard and high dose
  - Adjuvanted
  - Intramuscular or intradermal delivery

- **Live attenuated**
  - Quadrivalent
  - Egg-based
  - Intranasal

- **Recombinant HA**
  - Trivalent
  - Baculovirus platform
  - Intramuscular
Multiple immune effector mechanisms contribute to protection from influenza infection

- Neutralizing anti-HA (globular head) antibodies prevent infection; serum and mucosal

- Other responses including anti-NA, anti-M2e and T cell responses reduce severity and duration of disease
  - Reduced virus load

Li, Rappouli and Xu. Curr Opin Immunol 2013
Current vaccines target the stimulation of neutralizing antibodies against the globular head of HA - primary mediators of protection

- Neutralizing Abs target epitopes in the globular head around the receptor binding site
  - Block virus binding to receptor

- Hemagglutination-inhibition (HI) assay is a surrogate assay for the detection of neutralizing antibodies

- Microneutralization (MN) or Virus Neutralization (VN)
  - Detects Ab that bind around globular head and block virus attachment/entry
  - More sensitive than HI for detection of low titered seroconversion

- Single Radial Hemolysis (SRH)
  - Detects antibodies that bind virus bound to RBC in the presence of C results in C-mediated lysis
Immune Correlates of Protection against Influenza

- An HI titer of $\geq 40$ is associated with a 50% or more reduction in risk of influenza infection or disease in population
  - Mostly from natural or experimental infection studies in younger adults
- Meta-analyses consistently support this threshold titer (deJong et al., 2003; Coudeville et al., 2010)
  - Higher titers are associated with 80-90% reduction
- “Seroprotective” titer (HI$\geq 40$) has been used as a vaccine immunogenicity criteria and standard for licensure (EMA and FDA)
- HI thresholds in children
  - Higher post-TIV HI titers ($=110$) were associated with 50% probability of protection in children (Black et al. PIDJ 2011)
  - HI titer of 40 derived from TIV immunization was associated with 55% protection against PCR-confirmed B/Victoria infection (Ng et al., JID 2014)
- MN and SRH titer thresholds
  - MN titer of $\geq 40$ or 160 was associated with $\sim 50\%$ protection against seasonal influenza A virus infection (Tsang et al., JID 2014; Verschoor et al., PLoS One 2015)
  - For SRH, a $\geq 25 \text{mm}^2$ zone of lysis is considered a 50% protective titer (Delem et al., JID 1978)
2014-15 Post-Vaccination HI Antibody Responses to Circulating Antigenically Drifted H3N2 3C.2a and 3C.3a Viruses Relative to A/Texas/50/2012 Vaccine Virus

Data from WHO CCs and ERLs - Vaccine Consultation, Feb 2015
Serum and Nasal Immunoglobulin Responses

- The serum anti-HA antibody response to influenza vaccination is predominantly IgG, particularly IgG1
  - Lower levels of IgM and IgA are detected
- Natural priming is a major factor that affects the magnitude of the IgG and IgA response
- Vaccine induced IgA and IgG in the respiratory tract is associated with resistance against subsequent infection
  - LAIV elicits stronger nasal wash IgA response and is associated with resistance against infection
  - IIV elicits stronger nasal wash IgG, derived from plasma by transudation, and is associated with reduction in illness

<table>
<thead>
<tr>
<th>Immunity induced by</th>
<th>Protection against</th>
<th>Antibody associated with resistance</th>
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<tbody>
<tr>
<td>Inactivated vaccine</td>
<td>Virus replication Illness</td>
<td>Nasal wash IgA</td>
</tr>
<tr>
<td>Live vaccine</td>
<td>Virus replication Illness</td>
<td>P &lt; 0.025</td>
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*NS, Not significant

Clements et al J Clin Micro 1986
NA inhibition antibodies

- Antibodies to NA inhibit virus release from infected cells

- Serum NA inhibition (NI) titers correlate with reduced virus replication and disease symptoms (e.g. Murphy et al., NEJM 1972; Couch et al., JID 1974; Kilbourne et al., J. Virol 1975)

- Vaccine-induced serum NI titers were significantly correlated with resistance to illness or virus replication (Clements et al., J Clin Micro 1986)

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<tr>
<td>Inactivated vaccine</td>
<td>Virus replication</td>
<td>Serum NI</td>
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<tr>
<td></td>
<td>Illness</td>
<td>P &lt; 0.03</td>
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<td></td>
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<td>P &lt; 0.003</td>
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<tr>
<td>Live vaccine</td>
<td>Virus replication</td>
<td>Serum HI</td>
</tr>
<tr>
<td></td>
<td>Illness</td>
<td>P &lt; 0.0005</td>
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*NS, not significant; Volunteers selected to have low HAI antibody
NI Ab is an Independent Correlate of Protection Against Infection with A(H3N2) in Vaccinated Adults

Monto et al., JID 2015

<table>
<thead>
<tr>
<th>4-fold rise in titer by</th>
<th>IIV Recipients (n=178)</th>
<th>LAIV Recipients (n=227)</th>
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<tbody>
<tr>
<td>HI assay</td>
<td>137/178 (77%)</td>
<td>48/227 (21%)</td>
</tr>
<tr>
<td>MN assay</td>
<td>35/52 (67%)</td>
<td>10/60 (17%)</td>
</tr>
<tr>
<td>NI (ELLA) assay</td>
<td>65/178 (37%)</td>
<td>14/227 (6%)</td>
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- NI antibody responses were detected in a higher proportion of IIV than LAIV recipients

- A 2-fold increase in NI titer was associated with a IIV and LAIV effectiveness of 48% and 24%, respectively, in preventing A(H3N2) infection
Generation of Antibody Secreting Cells and Memory B cells

Fig. 3 Generation of antibody-secreting and memory B cells in the germinal center. B cells are activated following encounter with professional antigen-presenting cells displaying viral antigen. Cognate interactions with T follicular helper cells promote the formation of germinal centers in which somatic hypermutation, affinity maturation, and the generation of antigen-secreting cells and memory B cells occur.

Chiu et al., 2014

ELISPOT measures ASC and memory B cells
Effector B cell Responses Following Immunization with Seasonal LAIV or TIV

- Effector IgG ASC responses are detected in a majority of adults and older children vaccinated with LAIV or TIV.
- Elevated effector IgG ASC were detected with significantly higher frequency in LAIV vaccinated adults compared with HI seroconversion.
- In adults, IIV induced a greater quantity of ASC than did LAIV.
  - LAIV induced greater relative levels of IgA ASC.

(Sasaki et al., J Virol 2007)  
(Sasaki et al., JID 2014)
CD4+ and CD8+ T Cell Immunity Against Influenza

CD8+ T cells:
- Direct lysis of virus infected cells
- Produce antiviral cytokines/chemokines

CD4+ T cells:
- Aid in activation of APC, CD8+ T cells and B cells
- Produce antiviral cytokines
- Activate innate immune cells to produce more cytokines/chemokines

ELISPOT measures IFNγ produced by T cells

*Altenburg et al., Vaccine 2015*
Cellular immune correlates of protection

  - Pre-challenge levels of CD4+ T cell IFN-γ responses correlated with reduced virus shedding and less severe disease
  - T cells predominantly recognized NP and M1 epitopes

- Sridhar et al; Nat Med 2013: Natural infection with A(H1N1)pdm09
  - Among seronegative patients, pre-existing CD8+ IFN-γ+IL-2- responses to conserved cross-reactive viral epitopes (NP, M1, PB1) were significantly inversely correlated with a reduced disease severity

![Graphs showing correlations between immune responses and disease outcomes]
**CD4 and CD8 T Cell Responses to Vaccination**

- Limited evidence of CD8+ IFN-γ T cell stimulation by IIV, particularly in adults
- Vaccination of children (5-9 yrs) with LAIV increased numbers of both CD4+ and CD8+ IFN-γ T cells
- LAIV elicited substantial IFN-γ T cell responses in young children (6 - <36 mo.)
- AT cell threshold of ≥ 100 SFC/10^6 PBMC was associated with 80% probability of protection against infection with A(H3N2)
- TIV elicited CMI responses in children primed by previous infection

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*He et al., J Virol, 2007*

*Forrest et al., Clin Vacc Immunol 2008*
A Subset of Peripheral Blood CD4 Helper T cells (ICOS+) are Correlated with Antibody Responses to Inactivated Vaccine

- T follicular helper (fh) cells in lymph nodes and tonsils provide specialized help for B cells
  - Express chemokine (CXCR5, CXCR3) and inducible costimulator (ICOS) important for migration and function
  - A subset of blood CD4+ T cells have functional characteristics of Tfh cells

- Increase in blood ICOS+ CXCR3+CXCR5+ CD4 T after seasonal IIIV correlated with rising HI and VN titers in adults and children (Bentebibel et al., Sci Trans Med, 2013)

- Increase in *virus-specific* ICOS+ CXCR5-IL-21+ CD4 T cells stimulated by one dose of adjuvanted H5N1 vaccine were correlated with increased HI titers after second dose (Spensieri et al., PNAS, 2013)
  - Surrogate for vaccine immunogenicity
### Comparison of Immune Responses to IIV and LAIV

<table>
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<tr>
<th>Immune mediator</th>
<th>Inactivated vaccine</th>
<th>Live attenuated vaccine</th>
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<tbody>
<tr>
<td>Serum HI antibody</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Serum NI antibody</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Antibody secreting cells</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Nasal IgA</td>
<td>+/-</td>
<td>+++</td>
</tr>
<tr>
<td>CD4 T cells</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>CD8 T cells</td>
<td>-/+</td>
<td>+</td>
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Other Limitations:
- Reduced antibody responses to IIV and effectiveness in older adults
- Potential for antibody waning over influenza season
- Potential for blunting of response with frequent vaccination
- Reduced LAIV effectiveness against H1N1pdm09
- Limited cross-protection
USVaccine Effectiveness (VE) Network, 2014-15: Adjusted VE against Influenza A/H3N2 by Age Group and Vaccine Type

Zimmerman et al., 2016, CID in press
**Adjuvants**

- Oil in water emulsion adjuvants (MF-59 and AS03) contain:
  - Squalene as oil phase
  - Surfactants as emulsifying agents
  - Buffer to provide H₂O phase
  - α-tocopherol (in AS03) as immunostimulant
    - Enhances cytokine production and antigen uptake by monocytes

- Temporal co-localization of adjuvant with antigen required for action

- Enhanced T cell (Th1>Th2) and antibody (IgG1>IgG2)

- Other adjuvants in clinical trials
  - Toll like receptors (TLR) agonists e.g TLR 3, 5 or 7/8
**Mode of Action**

**Injection Site**
1. MF59 recruits immune cells

- Recruitment of immune cells
- Differentiation into antigen-presenting cells (APCs)

**Lymph Node**
3. T-cell activation and B-cell expansion

- T-cell activation
- B-cell expansion
- Antibody release
- Neutralizing flu-specific antibodies

**Vaccine-specific Responses**
- Increased APC migration
- Increased antigen reuptake

**References**
- Calabro et al., Vaccine, 2011.

*Courtesy of Ethan Settembre*
Oil in water adjuvants increase affinity and breadth of antibody response

- Whole genome phage display libraries map antibody response on HA
  - Demonstrate greater breadth of neutralizing Ab elicited by adjuvanted vaccines

- Surface Plasmon Resonance (SPR) demonstrates increased IgG binding affinity

(Khurana et al., Sci Trans Med 2010)
Antigen Targets of Next Generation Influenza Vaccines

- **NA**: less variable than **HA**
- **M2e**: more conserved, Ab-mediated protection
- **NP**: highly conserved, induces CMI
- **M1**: highly conserved, induces CMI
- **PB1, PB2**: highly conserved, induce CMI
- **HA stalk**: highly conserved
- **HA head**: enhance breadth of response
Next Generation Influenza Vaccine Strategies that Target More Broadly Reactive Antibodies

- COBRA: Computationally optimized broadly reactive antigen – HA head

- HA head/stalk chimeras incorporated into IIIV and LAIV

- "Headless" HA

- M2 ectodomain

- Virus-like particles

- Broadly neutralizing Abs against HA globular head; HI/VN activity

- Broadly neutralizing Abs against HA stalk region; FcYR Ab-dependent cell cytotoxicity (ADCC)

- Broadly neutralizing Abs against HA stalk region; ADCC functionality

- Non-neutralizing, broadly cross-reactive Abs; ADCC functionality

- NA antibodies that are more cross-reactive within subtype
Next Generation Influenza Vaccine Strategies that Target Enhanced CMI and Mucosal Responses

- Recombinant protein or peptide based vaccines
  - Improved priming & CMI against targeted T (NP, M1) and B cell (HA) epitopes

- Vectored Vaccines
  - MVA and Ad5 vector-based
    - Improved T cell (NP, M1) and mucosal Ab (HA) responses; neutralizing Abs against HA
  - LAIV
    - ΔM2 and ΔNS strategies
    - Improved T cell and mucosal responses; neutralizing Abs against HA

- DNA/RNA
  - DNA prime, IIV or vectored vaccine boost
    - Improved priming, CMI and broadly neutralizing Abs
Conclusions

- Vaccination with currently licensed influenza vaccines target the stimulation of strain-specific neutralizing anti-HA antibody responses that offer limited protection against emerging variant viruses
  - Responses are highly dependent on vaccine type, age, priming and vaccination history

- Current vaccines also stimulate other desirable immune responses but to a limited degree
  - Responses differ by vaccine type and priming history

- Development of next-generation influenza vaccines should take advantage of new understanding of components of CMI, immune cell repertoire and effect of prior exposure to influenza

- This will require an understanding of immune correlates which will differ for each vaccine type
  - Require robust and standardized assays to measure immune endpoints
Thank You

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