Immunological Assessment of Influenza Vaccines and Correlates of Protection

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What is a correlate of protection?

- Defined immune markers that can be used as a surrogate to predict resistance to influenza conferred by vaccination or natural infection.
- Ideally, requires demonstration that individuals with higher levels of immune marker have lower rates of infection/disease.
- Requires identification of relevant threshold of response:
  - Depends on sensitive, specific and reproducible assays
  - Clinical studies with individuals that have pre-exposure samples
  - Sufficient numbers of infected persons during study exposure period

Multiple immune effector mechanisms contribute to protection influenza

- Neutralizing anti-HA (globular head) antibody prevents infection.
- Other responses including anti-NA, anti-M2 Ab and T cell responses reduce severity and duration of disease:
  - Reduced virus load

Neutralizing antibodies against the globular head of HA are primary mediators of protection from infection

- Neutralizing Abs target epitopes in the globular head around the receptor binding site block virus binding to receptor.
- Hemagglutination-inhibition assay is a surrogate assay for the detection of neutralizing antibodies.

HI antibody is a generally accepted immune marker of protection against influenza
- An HI titer of ≥32 or 40 is associated with a 50% or more reduction in risk of influenza in populations
- 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≥40) is used as a vaccine immunogenicity criteria and standard for licensure
  - CHMP criteria:
    - HI titer of ≥40 achieved in >70% (18-60 yr) or >60% (>60 yr) of adults
    - Seroconversion rate (SCR) >40% (18-60 yr) olds or >30% (>60 yr) of adults
    - Mean GMT increase of >2.5 (18-60yr) or >2 (>60yr)
  - US FDA has similar criteria but defines the lower bounds of 95% CI should meet the GMT and SCR criteria

Is the HI titer of 40 an appropriate correlate of protection?
- Studies that established this threshold titer of protection have limitations
  - Most studies in younger adults; some used experimental challenge
  - Fewer studies evaluated effect of vaccination
    - Use of serological endpoints may underestimate infections (Petrie et al., 2011)
    - Infection observed in presence of robust "protective" titers HI titers in adults (Ohmit et al., 2011)
- T cell responses, not HI antibody a better predictor of vaccine protection in older adults (McElhaney et al., 2006)
- What is the appropriate HI titer threshold in children?
  - Black et al PIDJ 2011:
    - Higher post-TIV HI titers (=110) were associated with 50% probability of protection in children
  - Ng et al., JID 2014:
    - HI titer of 40 derived from TIV immunization was associated 55% protection against PCR-confirmed B/Victoria infection
    - HI titer of 40 derived from natural infection was associated with 48% protection against PCR-confirmed A/H1N1pdm09 infection

Other serologic assays that detect antibodies to HA
- Single Radial Hemolysis (SRH)
  - Detects antibodies that bind virus bound to RBC in the presence of C results in C'-mediated lysis
  - A ≥25mm² zone of lysis is considered a 50% protective titer
  - A ≥25mm² zone or ≥50% increase in zone size is used as a vaccine immunogenicity criteria and standard for licensure in EU
- 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
  - An MN titer of ≥40 was associated with 49% protection against PCR-confirmed H3N2 infection in a household transmission study (Tsang et al., 2014)

Measuring quality as well as quantity
- 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
NA-specific antibodies are associated with resistance against influenza

- 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., 1972; Couch et al., 1974; Kilbourne et al., 1975)
- Pre-existing serum NI antibody was an independent predictor of immunity to A(H1N1)pdm09 infection and illness (Couch et al., JID, 2013)

NI antibody is associated with resistance to challenge with wildtype virus (Clements et al J Clin Micro 1986)

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<tr>
<th>Immunity Induced by</th>
<th>Protection against</th>
<th>Antibody associated with resistance</th>
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<tbody>
<tr>
<td>Natural infection</td>
<td>Virus replication</td>
<td>Serum NI</td>
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<td></td>
<td>Illness</td>
<td>Serum HI</td>
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<tr>
<td>Inactivated vaccine</td>
<td>Virus replication</td>
<td>P &lt; 0.03</td>
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<td></td>
<td>Illness</td>
<td>P = 0.003</td>
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<td></td>
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<td>P &lt; 0.005</td>
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<tr>
<td>Live vaccine</td>
<td>Virus replication</td>
<td>NS</td>
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<tr>
<td></td>
<td>Illness</td>
<td>NS</td>
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* NS, not significant; Volunteers selected to have low HAI antibody

- Inactivated vaccine–induced NI titers >4 and HI titers >32 were significantly correlated with resistance to illness or virus replication
- However, overall an NA inhibition titer that correlates with clinical protection has not been defined

Assays to measure NA inhibition antibody titers

- **NA inhibition assays:**
  - Use large substrates (fetuin) and NA antigen that is dissociated from matched HA
- **Thiobarbituric Acid assay:**
  - measures sialic acid released by NA; uses hazardous agents
  - "Miniturized" to 96-well format
- **Enzyme-linked lectin assay (ELLA):** (Lambre et al., 1990; Cate et al., 2010)
  - Fetuin bound to plate
  - Viral NA removes sialic acid
  - HRPO-labeled peanut lectin binds to galactose
  - TMB substrate changes color
  - CONSISE international inter-laboratory study is underway

Antibody responses in the respiratory tract

- Indirect ELISA is used to measure nasal wash immunoglobulins, typically using purified viral proteins as antigens
- IgA in the respiratory tract in response to infection or vaccination with live virus is associated with resistance against subsequent infection
- Nasal wash IgG derived from plasma by transudation is associated with reduction of illness
Antibody responses to conserved proteins/epitopes: M2

- In animals, anti-M2e antibodies aid in virus clearance and protect from severe/fatal disease
  - Fc receptor-dependent ADCC or CDL
- In humans, M2 antibodies increase with age, but contribution to protection remains unknown (Zhong et al., JID 2014)
- Methods to detect:
  - M2e peptide or cell-based ELISA
  - M2 cell-based flow cytometry assay (M2-FCA) detects Ab to native M2 (Zhong et al., J Immunol Meth 2011)

Antibody responses to conserved proteins/epitopes: Stalk region Antibodies

- Broadly neutralizing mAbs that target the conserved HA stalk domain are protective in animal models
  - Inhibit membrane fusion
  - Fc receptor-dependent (ADCC)
- Chimeric HA vaccination strategy boosts stalk region antibodies
  - Protects against severe disease in animal models
- Methods to detect:
  - ELISA: using chimeric rHA as antigen
  - Plaque Reduction Assay: recombinant influenza virus expressing chimeric HA in MDCK cells
  - Pseudotype Virus (PV) Neut Assay: PV expressing chimeric HA in MDCK cells; measures ability of antibody to inhibit expression of reporter gene

Detection of Antibody Secreting Cells: Effector (or memory) B cell ELISPOT assay

1. Coat plate with antigen
2. Add mitogen-stimulated PBMC to detect memory B cells
3. Incubate overnight
4. Add enzyme-linked antibody to human Ig and develop plates
Effector B cell Responses Following Immunization with seasonal LAIV or TIV
(Sasaki et al., J Virol 2007;81:215)

- 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

Cellular immune correlates of protection: Cytotoxic T lymphocytes (CTL) responses

- CD8+ T cells recognize peptide epitopes present on infected cells and kill cell
  - release of perforin/granzyme
  - Fas/FasL mediated apoptosis
  - NP and M1 are major targets
- Protective role evident in the absence of HI, NI antibodies
  - CTL activity was inversely correlated with extent of virus replication
- Traditional assays to measure CTL responses in humans were complex; newer methods available

Detection of T cell populations: Interferon-gamma T cell ELISPOT assay

  - 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+ T IFN-γ/IL-2 responses to conserved cross-reactive viral epitopes (NP, M1, PB1) were significantly inversely correlated with a reduced disease severity
Correlation of CMI with protection against natural H3N2 influenza in young children vaccinated with LAIV

- LAIV (10^7 FFU) elicited substantial IFN-gamma T cell responses in young children (6 - <36 months)
- IFN-gamma T cell responses were a more sensitive measure of immune memory than HI antibody
- TIV elicited CMI responses in children primed by previous infection
- An IFN-gamma T cell threshold of ≥ 100 SFC/10^6 PBMC was associated with 80% probability of protection in young children

Forrest et al., 2008

Standardization of immunological assays

- Considerable inter-laboratory variation exists for serological assays used for vaccine evaluation
  - In one EU study, GMT ratios obtained by manufacturers versus a central lab differed by 0.4 to 5.8 fold.
- Adjustment of absolute titers relative to a calibrated International antibody standard (09/194) substantially reduced variability and resulted in acceptable agreement of results from different laboratories

Wagner et al., Vaccine 2012

Conclusions

- HI antibody responses remains the primary immune correlate of protection against influenza infection
- Developing next-generation influenza vaccines will demand a more sophisticated understanding of immune responses to influenza virus
  - Immune correlates will differ for each vaccine type and by age
  - More emphasis on alternate serological and B cell endpoints
    - Emphasis on quality and durability
- Recent studies provide new evidence that CD4+ and CD8+ T cell responses correlate with reduced disease severity in humans
  - Need to design clinical studies to incorporate CMI measurements
    - Different timing to antibody responses
- New assays should be qualified and standardized across labs
  - Unify endpoints/thresholds used to identify clinical responses