Immunity and vaccination against influenza in children

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Influenza virus

- Orthomyxoviridae
- Enveloped virus
- Three types, A, B and C
- Type A:
  - 18 subtypes of HA
  - 10 subtypes of NA
- 5-120 nm, spiked particles
- Negative strand RNA genome
- Eight genome segments
Influenza epidemics
- evasion of host immunity -
  - Antigenic drift (within subtype)
    - Gradual change of the antigenic epitopes in HA or NA
    - Annual epidemics, attack rate 5-10%

Caused by drift variants
- A/H1N1
- A/H3N2
- B

source: NIVEL
Influenza pandemics
- evasion of host immunity -
  - Antigenic shift
    - Introduction of new subtypes of the glycoproteins
    - From animal reservoir, zoonotic event
    - No pre-existing antibodies in the population at large
    - Excess morbidity and mortality
    - After genetic reassortment or interspecies transmission of complete virus

Reservoir of influenza A viruses:
Aquatic birds
## Most recent pandemics

<table>
<thead>
<tr>
<th>Year</th>
<th>Subtype</th>
<th>Name</th>
<th>Estimated number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1918</td>
<td>H1N1</td>
<td>Spanish Flu</td>
<td>20-40x10^6</td>
</tr>
<tr>
<td>1957</td>
<td>H2N2</td>
<td>Asian Flu</td>
<td>1x10^6</td>
</tr>
<tr>
<td>1968</td>
<td>H3N2</td>
<td>Hong Kong Flu</td>
<td>7x10^5</td>
</tr>
<tr>
<td>2009</td>
<td>H1N1</td>
<td>Mexican Flu</td>
<td>2x10^4 – 3x10^5</td>
</tr>
</tbody>
</table>

Spanish Flu 1918
Continuous pandemic threat:
- Need for universal influenza vaccines -

- Subtypes of influenza A virus with pandemic potential
  - Subtypes **H1, H2 and H3**
    - Have caused pandemics in the past
    - In US: transmission of human-like swine H3N2 viruses!
  - Subtypes **H5, H7, H9 and H10**?
    - Avian viruses that infected humans
      - H5N1 >650 cases since 2003, +/- 60% CFR
      - For transmission of H5 viruses between mammals:
        - Few amino acid substitutions are required
  - For 2013 H7N9 viruses
    - 335 cases since 2013, +/- 20% CFR
    - Partially adapted to mammals
Prevention

Vaccines against seasonal influenza

- inactivated
- trivalent (influenza A/H3, A/H1 en B )
- > 15 µg HA per strain

Effectiveness

- 70-90% in young adults
- 30-70% in elderly

More than 60 years the method to prevent influenza

Recommended for high risk patients

NB: Vaccine strain need to match epidemic/pandemic strains
Heterosubtypic immunity - animal models -

Induction of Partial Specific Heterotypic Immunity in Mice by a Single Infection with Influenza A Virus

JEROME L. SCHULMAN AND EDWIN D. KILBOURNE

Heterologous protection against lethal A/HongKong (H5N1) influenza virus infection in C57BL/6 mice

Eduardo O’Neill,1 Scott L. Krauss,1 Janice M. Riberdy,2 Robert G. Webster1 and David 3

Heterotypic Immunity to Influenza in Ferrets

ROBERT A. YETTER, W. HENRY BARBER, AND PARKER A. SMALL, JR.

Respiratory and systemic humoral and cellular immune responses of pigs to a heterosubtypic influenza A virus infection

Paul P. Heinen, Els A. de Boer-Luitze and Andre T. J. Bianchi
Induction of heterosubtypic immunity to influenza H5N1 - by infection with A/H3N2, not RSV -

Primary infection

% Body weight

- None (PBS)
- RSV
- A/H3N2 influenza

Proportion survival

A/Hong Kong/2/68 (H3N2)
A/Indonesia/5/05 (H5N1)

CTL: a correlate of protection
-Lethal infection with heterosubtypic virus H5N1-
- H3N2-H5N1 model -

More rapid viral clearance correlates with secondary CTL responses

Heterosubtypic immunity in ferrets
A/Brisbane/10/07 (H3N2) – A/Indonesia/5/05 (H5N1)

Bodewes et al., 2011, J. Virol. 85(6):2695-2702
The frequencies of pre-existing crossreactive T cells are inversely associated with illness severity in infected individuals.

Seroprevalence of antibodies against seasonal influenza A and B viruses in children in the Netherlands

Proportion of children lack Het I

Pandemic influenza
- Children <5 years of age most at risk -

Age distribution
H5N1 cases in Egypt
Source: WHO

Age distribution
2009 H1N1 cases in The Netherlands
- Hospitalizations –

-Source: Osiris/Pandora/Cib
Annual influenza vaccination and heterosubtypic immunity

- Thus, infection with influenza virus induces heterosubtypic immunity
  - Associated with cross-reactive virus-specific CD8+ T cell responses

- Hypothesis: Could vaccination of children against seasonal influenza by preventing infection, prevent induction of heterosubtypic immunity?

- Vaccinated subjects more susceptible to infection with pandemic virus of a novel subtype?

- Relevant regarding the recommendation to vaccinate all healthy children 6-59 months of age against seasonal influenza
  - implemented in some countries (e.g. USA, Canada, Finland)
Effectiveness of inactivated influenza vaccine in children aged 9 months to 3 years


<table>
<thead>
<tr>
<th></th>
<th>Number of influenza positive (%)</th>
<th>VE (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unvaccinated</td>
<td>Fully vaccinated</td>
<td></td>
</tr>
<tr>
<td>All children</td>
<td>N=631</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A</td>
<td>36 (8%)</td>
<td>2 (1%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Influenza B</td>
<td>27 (6%)</td>
<td>5 (3%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Any influenza</td>
<td>61 (13%)</td>
<td>7 (5%)</td>
<td>0.003</td>
</tr>
<tr>
<td>9 mo to &lt;2 yrs</td>
<td>N=278</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A</td>
<td>17 (10%)</td>
<td>2 (2%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Influenza B</td>
<td>5 (3%)</td>
<td>2 (2%)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Any influenza</td>
<td>21 (12%)</td>
<td>4 (4%)</td>
<td>0.03</td>
</tr>
<tr>
<td>2 to 3 yrs</td>
<td>N=353</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A</td>
<td>19 (7%)</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>Influenza B</td>
<td>22 (8%)</td>
<td>3 (5%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Any influenza</td>
<td>40 (14%)</td>
<td>3 (5%)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Does vaccination against seasonal H3N2 virus prevent induction of heterosubtypic immunity?

**Experimental groups and study design**

<table>
<thead>
<tr>
<th>Time</th>
<th>Sub-lethal infection</th>
<th>Lethal infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A/HK/68 (H3N2)</td>
<td>A/IND/05 (H5N1)</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>88</td>
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**C57BL/6**

**H3N2 vaccination**

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</table>
Antibody responses after subunit vaccination

A

HI Antibody Titer (GMT)

<table>
<thead>
<tr>
<th>Prime</th>
<th>Boost</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
</tr>
</tbody>
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B

VN Antibody Titer (GMT)

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<tr>
<th>Prime</th>
<th>Boost</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
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</table>

Subunit + adjuvant
Subunit
Adjuvant
PBS
Outcome of infection with A/HK/68 (H3N2) - histopathology, virus specific CD8+ T cell responses 28 days p.i.

**C**
- Group 2 (Vaccinated)
- Group 3 (Unvaccinated)

**D**
- % CD8+ TmHK+ T-cells
- p=0.030

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Subunit</th>
<th>Adjuvant</th>
<th>Infection</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>7</td>
</tr>
</tbody>
</table>

Bodewes et al, PLOS one 4(5):e5538
Outcome of infection with A/HK/68 (H3N2)
- weight loss, lungs virus titers day 4 p.i.-

**Group 1**  No vaccination, no infection
**Group 2**  Vaccination with subunit/adjuvant, infection with A/HK/68 (H3N2)
**Group 3**  No vaccination, infection with A/HK/68 (H3N2)
**Group 4**  No vaccination, no infection
**Group 5**  Vaccination with subunit/adjuvant, no infection
**Group 6**  Vaccination subunit only, infection with A/HK/68 (H3N2)
**Group 7**  Vaccination adjuvant only, infection with A/HK/68 (H3N2)
Outcome of infection with A/IND/05 (H5N1) - weight loss, mortality -

- **Group 1**: No vaccination, no infection
- **Group 2**: Vaccination with subunit/adjuvant, infection with A/HK/68 (H3N2)
- **Group 3**: No vaccination, infection with A/HK/68 (H3N2)
- **Group 4**: No vaccination, no infection
- **Group 5**: Vaccination with subunit/adjuvant, no infection
- **Group 6**: Vaccination subunit only, infection with A/HK/68 (H3N2)
- **Group 7**: Vaccination adjuvant only, infection with A/HK/68 (H3N2)

Bodewes et al, PLOS one 4(5):e5538
Outcome of infection with A/IND/05 (H5N1) - lungs virus titers day 4 p.i., virus specific CD8+ T cell responses

C

D

Bodewes et al, PLOS one 2009, 4(5):e5538
Development of virus-specific T cell immunity in children - Effect of annual vaccination?

- Vaccinated children
  - Children with cystic fibrosis receiving flu-shot annually < 4 years of age
  - N=14
  - 3.1-9.0 years of age

- Unvaccinated children
  - Healthy children undergoing correctional surgery
  - N=27
  - 2.0-8.8 years of age

Both groups:
- No clinical signs of acute disease,
- No immunosuppressive medications
- Similar Ab titers to antigens used in National immunization program
- PBMC response to SEB is similar

Development of virus-specific T cells immunity in children
- Effect of annual vaccination?

- In unvaccinated children: Age-depending increase of the frequency of virus-specific CD8+ T cells
  - not present in vaccinated children with CF (p<0.05)

Heterosubtypic immunity - Protection against pandemic influenza? –
- Theoretical scenarios -
Inactivated seasonal vaccines annually
  e.g. LAIV

Seasonal influenza A virus (H3N2/H1N1)

Naive

Pandemic flu

A

Partial protection

B

Protection

CD4+ T cells

CD8+ T cells

No protection?

C
LAIV induces CD4 and CD8 T cell responses in children.

He et al. J. Virol. 2006, 80(23):11756-11766

- Or: other vaccine candidates:
  - Live viral vectors e.g.
    - Rec poxvirus (MVA)
    - Rec adenovirus
  - DNA
  - Adjuvants
    - ISCOMs
  - Delivery systems
    - Virosomes
Pandemic scenario
- What to do with (vaccinated) children? -

• Vaccinate pregnant mothers
  • In last trimester
    • To protect pregnant women (high risk)
    • To protect new born through maternal antibodies
• Vaccinate children >6 months of age with priority
• Vaccinate household contacts when children are <6 mo of age

• Develop universal vaccine
  • Live attenuated vaccines?
  • Induction of cross protective CMI
  • Induction of cross protective antibodies to
    • M2 protein
    • HA stem region
    • Neuraminidase
Conclusions

- VN antibodies directed to HA that match the strain causing the infection remain the primary correlate of protection.

- However, it is largely unpredictable which strains will cause future epidemics or pandemics. Ideally seasonal influenza vaccines are used that not only induce antibodies that match the seasonal strains but that also induce cross-protective immune responses.

- The use of universal vaccines may buy time in pandemic scenarios and prevent morbidity and mortality in selected groups of patients at risk.

- T cells specific for conserved proteins (NP, M1) are cross-reactive and correlate with protection.

- Especially immunologically naïve children may benefit from the use of vaccines that induce heterosubtypic immunity.
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