Adenoviral Vector-based H5N1 Influenza Vaccines

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Need for H5N1 influenza vaccine?

- H5N1 viruses detected in birds in 62 countries
- 12 countries reported over 300 human cases with approx. 60% fatality due to H5N1 virus infection
- Virus has drifted into 3 different clades
  - A/VN/1203/04 = Clade 1
  - A/Indo/05/05 = Clade 2.1
- Potential for anti-viral resistance
- Currently licensed trivalent seasonal vaccines consisting of H1N1, H3N2, and B components do not provide protection against H5N1 viruses
Vaccine strategies

Traditional Approaches (split, or whole-virus inactivated vaccines)
- Need strong adjuvants for dose sparing

Alternative Approaches
- Reassortant virus vaccines
- Cold-adapted vaccines
- Viral vectored vaccines
- Virus-like particles (VLPs)
- Plant or insect cell-based subunit vaccines
- DNA vaccines
Advantages of adenoviral vectors as a vaccine delivery system

- Non-pathogenic
- Grown to high titers
- Availability of certified cell lines
- Availability of technology for large scale purification
- No integration into the host genome
- Targets macrophages and DCs
- Induce both humoral and CMI responses
- Effectively delivered by mucosal or parental route
- Egg-independent approach
- No need for high containment
Protection of HAd-H5HA immunized mice against antigenically distinct H5N1 virus strains

Groups
(HAd-H5HA (10^8 pfu i.m.))
(HAd-H5HA (10^8 pfu i.n.))
(HAd-ΔE1E3 (10^8 pfu i.m.))
rH5HA + Alum (i.m.)

Humoral response
Microneutralization Assay
Hemagglutination Inhibition

Cell-based response
HA-epitope pentamer staining
T cell ELIspots

Virus-neutralizing antibody response against homologous and heterologous H5N1 strains in mice immunized with HAd-H5HA vaccine
### Conservation of immunodominant epitope (HA 518) in HA among influenza viruses

<table>
<thead>
<tr>
<th>Strain of influenza virus</th>
<th>HA and NA type</th>
<th>Immunodominant Epitope (K&lt;sub&gt;d&lt;/sub&gt;)</th>
<th>Binding score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/PR/8/34</td>
<td>H1N1</td>
<td>IYSTVASSL</td>
<td>30</td>
</tr>
<tr>
<td>A/HK/156/97</td>
<td>H5N1</td>
<td>IYSTVASSL</td>
<td>30</td>
</tr>
<tr>
<td>A/HK/213/03</td>
<td>H5N1</td>
<td>IYSTVASSL</td>
<td>30</td>
</tr>
<tr>
<td>A/VN/1203/04</td>
<td>H5N1</td>
<td>IYSTVASSL</td>
<td>30</td>
</tr>
<tr>
<td>A/HK/1073/99</td>
<td>H9N2</td>
<td>IYSTVASSL</td>
<td>30</td>
</tr>
<tr>
<td>A/NL/219/03</td>
<td>H7N7</td>
<td>WFSFGASCFF</td>
<td>8</td>
</tr>
</tbody>
</table>
Frequency of HA 518 epitope-specific CD8 T cells
Induction of MHC class I-restricted cellular immune response as determined by IFN-γ secretion in HAd-H5HA-immunized mice
Morbidity and mortality against lethal challenge in mice immunized with an HAd-H5HA vaccine

[Graphs showing data for different categories and time points]
Virus titers in lungs following challenge of mice immunized with an HAd-H5HA vaccine

![Graph showing lung viral titers EID (log_{10}/mL)](image)
“Our findings highlight the potential of an Ad-vector-based delivery system, which is both egg-independent and adjuvant-independent and offers stockpiling options for the development of a pandemic influenza vaccine.”

See Articles page 475
Longevity of immune responses in mice immunized with HAd-H5HA vaccine

**Groups**
- HAd-H5HA (10^8 pfu i.m.)
- HAd-H5HA (10^8 pfu i.n.)
- HAd-ΔE1E3 (10^8 pfu i.m.)

**Humoral response**
- Microneutralization Assay
- Hemagglutination Inhibition

**Cell-based response**
- HA-epitope pentamer staining
- T cell ELIspots
Longevity of humoral immune responses induced by HAdH5HA vaccine

HI titers

Neutralizing Antibody titers

Weeks post-boost

i.m.
i.n.
empty vector

empty vector

4 wks
55 wks
Longevity of HA 518 epitope-specific CD8+ T-cells at 4 weeks and 55 weeks post-boost with HAd-H5HA vaccine

% epitope-specific CD8+ T cells per total lymphocytes

* p<.001
Morbidity of mice vaccinated with HAd-H5HA (4 weeks and 55 weeks post-boost) and challenged with 50 LD$_{50}$ A/HK/483/97.
Conclusion

- The humoral and cell-mediated immune responses induced by HAd-H5HA vaccine lasts at least for 1 year.
- Immunized animals were protected from morbidity and mortality following challenge.
Development of broadly protective pre-pandemic influenza vaccine

- Adenoviral Vectors constructs
  - HA = VN/1203
  - HA = Indo/05
  - NP = VN/1203
  - HA = VN/1203 + Indo/05
  - HA (Indo/05) + NP
- HAd-ΔE1E3
- Viral controls (i.p.)
  - Clade 1 = ΔHAVN/1203xA/PR/8
  - Clade 2 = ΔHAIIndo05xA/PR/8

Humoral response
- Microneutralization Assay
- Hemagglutination Inhibition

Cell-based response
- HA & NP-epitope pentamer staining
- T cell ELIspots

Prime 4 wks 4 wks Boost
Bleed
Challenge Spleen collection
Neutralizing antibodies (pooled sera) against VN/1203 and Indo/05 in immunized mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Route</th>
<th>VN/1203</th>
<th>Indo/05</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAd 1203 HA</td>
<td>i.m.</td>
<td>320</td>
<td>10</td>
</tr>
<tr>
<td>HAd Indo 05 HA</td>
<td>i.m.</td>
<td>10</td>
<td>320</td>
</tr>
<tr>
<td>HAd 1203 HA + Indo 05 HA</td>
<td>i.m.</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>HAd Indo 05 HA + NP</td>
<td>i.m.</td>
<td>10</td>
<td>640</td>
</tr>
<tr>
<td>HAd NP</td>
<td>i.m.</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Empty Vector</td>
<td>i.m.</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>△HAVN/1203xA/PR/8(Clade 1)</td>
<td>i.p.</td>
<td>320</td>
<td>80</td>
</tr>
<tr>
<td>△HAIndo05xA/PR/8(Clade 2)</td>
<td>i.p.</td>
<td>10</td>
<td>640</td>
</tr>
</tbody>
</table>
Induction of HA 518 and NP 147 epitope-specific CD8+ T cells in HAd-H5HA immunized mice
Viral lung titers of mice challenged with Clade 1 and Clade 2 reassortant virus (100 MID$_{50}$) after HAd-H5HA immunization

<table>
<thead>
<tr>
<th>Group</th>
<th>VN/1203</th>
<th>Indo/05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log$_{10}$</td>
<td>S.D.</td>
</tr>
<tr>
<td>HAd 1203 HA</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>HAd Indo 05 HA</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>HAd 1203 HA + Indo 05 HA</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>HAd Indo 05 HA + NP</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>HAd NP</td>
<td>3.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Empty Vector</td>
<td>5.3*</td>
<td>1.4</td>
</tr>
<tr>
<td>ΔHAVN/1203xA/PR/8(Clade 1)</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>ΔHAVIndo05xA/PR/8(Clade 2)</td>
<td>1.5</td>
<td>0</td>
</tr>
</tbody>
</table>

* p<0.05
Conclusions

- Inclusion of recent H5HA in HAd constructs elicits immune responses against currently circulating H5N1 viruses.
- Addition of NP in the HAd vaccine broadens the immune response compared to HAd-H5HA alone.
Concerns about human adenoviral vector system?

- Is it fair to apply drawbacks of adenoviral vector-based gene therapy approaches directly to vaccine applications?

- Impact of pre-existing vector immunity on efficacy of adenoviral vector-based vaccines.
Circumvention of exceptionally high levels of vector immunity by BAd vectored vaccine

**Naïve Groups**
- BAd-H5HA (10^8 pfu i.m.)
- HAd-H5HA (10^8 pfu i.m.)
- HAd-ΔE1E3 (10^8 pfu i.m.)

**HAd5-primed Group**
- BAd-H5HA (10^8 pfu i.m.)

**Humoral response**
- Hemagglutination Inhibition

**Cell-based response**
- HA-epitope pentamer staining
- T cell ELIspots
HAd5 neutralizing antibody titers in naïve or HAd5-primed (2 X) mice before immunization.
Induction of HA-specific humoral immune response in naïve or HAd5-primed mice immunized with BAd-H5HA vaccine
Frequency of HA 518 epitope-specific CD8 T cells in naïve or HAd5-primed mice immunized with BAd-H5HA vaccine
Morbidity and mortality against lethal challenge with homologous H5N1 virus (HK/483/97) in mice immunized with HAd-H5HA vaccine

(A) 

(B)
Conclusion

Exceptionally high levels of vector immunity do not adversely affect protective immune responses induced by BAd vector-based H5N1 vaccine
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