Influenza Virus Nomenclature

Type of Nuclear Protein

Hemagglutinin

Neuraminidase

A/USSR/90/77 (H1N1)

Virus Type
Geographic Origin
Strain Number
Year of Isolation
Virus Subtype
Proteins and RNA’s of Influenza A Virus

PB1, PB2, PA (replication)

Lipid Bilayer

NS2 (NEP)

M2 (Ion channel) (penetration)

HA (Hemagglutinin) (attachment, penetration)

NA (Neuraminidase) (release)

M1 (Matrix protein) (assembly)

NP (Nucleocapsid) (RNA transport, replication)
<table>
<thead>
<tr>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>H4</th>
<th>H5</th>
<th>H6</th>
<th>H7</th>
<th>H8</th>
<th>H9</th>
<th>H10</th>
<th>H11</th>
<th>H12</th>
<th>H13</th>
<th>H14</th>
<th>H15</th>
</tr>
</thead>
</table>

Where Do These Non-Human Viruses Come From?
## Distribution of Neuraminidases in Nature

<table>
<thead>
<tr>
<th>N1</th>
<th>Human</th>
<th>Pig</th>
<th>Duck</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>Human</td>
<td>Pig</td>
<td>Duck</td>
</tr>
<tr>
<td>N3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Influenza A viruses in humans this century

1918 Spanish Influenza
1957 Asian Influenza
1968 Hong Kong Influenza
1977

Influenza A reservoir

15 HA subtypes
9 NA subtypes
# Influenza Excess Mortality

<table>
<thead>
<tr>
<th>Influenza</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Spanish&quot; Influenza</td>
<td>500,000</td>
</tr>
<tr>
<td>September 1918–April 1919</td>
<td></td>
</tr>
<tr>
<td>&quot;Asian&quot; Influenza</td>
<td>69,800</td>
</tr>
<tr>
<td>September 1957–March 1958</td>
<td></td>
</tr>
<tr>
<td>&quot;Hong Kong&quot; Influenza</td>
<td>33,800</td>
</tr>
<tr>
<td>September 1968–March 1969</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>603,600</td>
</tr>
<tr>
<td>Interpandemic years</td>
<td></td>
</tr>
<tr>
<td>1957–1990</td>
<td>600,800</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Subtype</th>
<th>High pathogenicity?</th>
<th>Location</th>
<th>Birds killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>H5N2</td>
<td>yes</td>
<td>Pennsylvania</td>
<td>17 million</td>
</tr>
<tr>
<td>1995</td>
<td>H5N2</td>
<td>yes</td>
<td>Mexico</td>
<td>?</td>
</tr>
<tr>
<td>1997</td>
<td>H5N1</td>
<td>yes</td>
<td>Hong Kong</td>
<td>1.6 million</td>
</tr>
<tr>
<td>1999-2000</td>
<td>H7N1</td>
<td>no</td>
<td>Italy</td>
<td>13 million</td>
</tr>
<tr>
<td>2002</td>
<td>H7N2</td>
<td>no</td>
<td>Virginia</td>
<td>4.7 million</td>
</tr>
<tr>
<td>2003</td>
<td>H7N7</td>
<td>yes</td>
<td>Netherlands</td>
<td>&gt;30 million</td>
</tr>
<tr>
<td>2004</td>
<td>H5N1</td>
<td>yes</td>
<td>Asia</td>
<td>&gt;100 million</td>
</tr>
<tr>
<td>2004</td>
<td>H7N2</td>
<td>no</td>
<td>Delaware</td>
<td>?</td>
</tr>
<tr>
<td>2004</td>
<td>H5N2</td>
<td>yes</td>
<td>Texas</td>
<td>?</td>
</tr>
<tr>
<td>2004</td>
<td>H7N3</td>
<td>yes</td>
<td>BC/Canada</td>
<td>?</td>
</tr>
</tbody>
</table>
Avian Influenza Viruses Infecting Humans

- **H5**
  - Hong Kong 1997: 18 cases, 6 deaths
  - Hong Kong 2003: 3 cases, 2 deaths
  - Vietnam, Thailand 2004: 33 cases, 22 deaths

- **H7**
  - Case reports
  - Netherlands 2003: 79 cases of conjunctivitis, 13 ILI, 1 death, 3 person-person transmissions

- **H9**
  - Hong Kong and Southern China 1999: 7 cases
  - Seroprevalence in poultry workers 1999
  - Hong Kong 2003: 2 cases
A) Direct infection

B) Passage in an intermediate host without reassortment

C) Reassortment in an intermediate host

Avian influenza A virus

Human influenza A virus

Birds

Humans
Potential Social and Economic Impact of an Influenza Pandemic

- Mathematical model* estimates for first year of a pandemic in absence of effective interventions:
  - 89,000 - 207,000 in the U.S.
  - 314,000 - 734,000 hospitalizations
  - 18 - 42 million outpatient visits
  - additional 20 - 47 million illnesses
  - economic impact: $71- $166 billion

Antigenic Drift

- Gradual alteration of the influenza surface proteins (mainly HA) within a subtype resulting in the inability of antibody to previous strains to neutralize new viruses.
- Antigenic drift results from point mutations in the HA and NA genes.
- The composition of the influenza vaccine has to be updated annually as a consequence of antigenic drift.
Antibody Binding Sites of HA

Strategy for Production of Live Attenuated Influenza Virus Vaccine

Attenuated Donor Virus

Attenuated Reassortant Vaccine Virus

Virulent Wild Type Virus
**Plasmid-based Reverse Genetics**

- **vRNA expressing plasmids**:
  - PA
  - PB1
  - PB2
  - NP
  - HA
  - NA
  - M
  - NS

- **Protein expression plasmids**:
  - PB1
  - PB2
  - PA
  - NP

**Human cells**

- **OR**
  - 8 ambisense plasmids

*Fodor et al 1999; Neumann et al 1999; Hoffmann et al 2000*
Plasmid-based Reverse Genetics: 8 plasmids

PB2, PB1, PA, HA, NP, NA, M1, M2, NS1, NS2/NEP proteins

mRNAs → Poly A

translation

RNA pol II

Viral cDNA

RNA pol I

8 (-) vRNAs

PB2, PB1, PA, HA, NP, NA, M, NS vRNAs

Hoffmann et al. PNAS 2000; 97:6108-13
Molecular Determinants of Virulence and Host-range

- Specified by >1 residue in >1 gene
- Virulence: HA, NA, M, PB1 and PB2 genes
- Host-range: HA, NA, PB2 and M genes
- Determinants in the HA:
  - Receptor specificity:
    - Humans: N-acetylneuraminic acid-α 2,6,Gal
    - Birds: NeuAc-α 2,3,-Gal
  - Sequence of the connecting peptide: presence of multiple basic amino acids
  - Glycosylation sites
Strategy for the generation of a candidate reassortant H5 influenza vaccine

- Modify the cleavage site of the H5 HA gene
- N1 NA
- Influenza Vaccine Donor Strain A/AA/6/60 ca

Reassortant modified H5N1 vaccine

Ref: Li et al JID 1999; 179: 1132-38
Program to generate and evaluate pandemic influenza vaccines

- Generate 2 or 3 candidate vaccines against influenza A viruses of each subtype
- In vitro phenotypes: ts and cold adaptation
- Pathogenicity in chickens (USDA), mice or ferrets
- Efficacy of protection and cross-protection against challenge with wild-type viruses in mouse model
- Determine the importance of antigenic drift among avian influenza viruses e.g. 1997, 2003, 2004 H5N1 viruses
- Proceed to clinical trials to evaluate immunogenicity and infectivity
Replication of SARS Coronavirus in Animal Models
Our Approach

- Small animals
  - Mice
  - Hamsters
- Non-human primates
  - Rhesus monkeys
  - African Green monkeys
  - Cynomolgus monkeys
- All animal studies are conducted in an ABSL3 facility and all laboratory work in a BSL 3 lab; personnel wear positive air purifying respirators
Conclusions and Implications

- Virus replication models were established in mice, hamsters and monkeys; the level of replication in hamsters > mice > monkeys.
- Findings in mice and hamsters were more consistent and reproducible than those in African Green monkeys.
- Primary infection protects the respiratory tract from challenge in all 3 models.
- Antibody alone appears to protect mice from pulmonary virus replication.
- Vaccines that induce neutralizing antibodies against the SARS spike protein limit pulmonary viral replication and are efficacious in animal models.
- Hamster, mouse and non-human primate models have been used to assess immunogenicity and efficacy of vaccines.
- We can now compare different vaccines.