IMMUNITY TO INFLUENZA INFECTION

Unit 7
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**INFLUENZA A VIRUS**

- Negative sense, segmented RNA virus
- *Orthomyxoviridae*
- Eight genes, 11 proteins (three alternate reading frames)
- Two non-structural proteins (NS1 and PB1-F2)
- Surface proteins HA and NA determine serotype

### Influenza A HA and NA Subtypes

<table>
<thead>
<tr>
<th>H1</th>
<th>N1</th>
<th>Other Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2</td>
<td>N2</td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>N3</td>
<td>Other Animals</td>
</tr>
<tr>
<td>H4</td>
<td>N4</td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>N5</td>
<td></td>
</tr>
<tr>
<td>H6</td>
<td>N6</td>
<td></td>
</tr>
<tr>
<td>H7</td>
<td>N7</td>
<td>Other Animals</td>
</tr>
<tr>
<td>H8</td>
<td>N8</td>
<td>Other Animals</td>
</tr>
<tr>
<td>H9</td>
<td>N9</td>
<td></td>
</tr>
</tbody>
</table>

[Diagram showing H1, H2, H3, H4, H5, H6, H7, H8, H9, N1, N2, N3, N4, N5, N6, N7, N8, N9 subtypes with different hosts like pigs and ducks.]
Diverse host tropism allows restriction and recombination.
INFLUENZA EVOLUTION
HUMAN INFLUENZA PANDEMICS

Diagram showing the timeline of influenza pandemics with specific years and strains marked.
All current human influenza is majority-derived from the 1918 pandemic.

Distinct reservoirs have allowed evolution to occur with varying pressures, providing diverse sources for new gene introductions into the human pool.
Swine-Origin H1N1 Incidence

New Influenza A (H1N1),
Number of laboratory confirmed cases as reported to WHO

Status as of 05 June 2009
06:00 GMT

Chinese Taipei has reported 16 confirmed case of influenza A (H1N1) with 0 deaths. Cases from Chinese Taipei are included in the cumulative totals.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization
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Map produced: 05 June 2009 08:10 GMT

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.
H7N9 is a Recombinant of H9N2 with Other Avian Viruses

- The H9N2 cassette was also the basis of the H5N1 viruses emerging in 1997 and 2002.
- However, the theoretical parent H9N2 lineage has never been observed on its own.

**Figure 3**
Schematic diagram of novel influenza A(H7N9) virus generation.

HA: haemagglutinin; NA: neuraminidase.
The novel influenza A(H7N9) viruses are likely to have acquired their HA gene from an avian H7 virus of unknown NA subtype, their NA gene from an avian H9 virus of unknown HA subtype, and their remaining six viral segments from avian H9N2 viruses circulating in poultry.
Note: Total cases include an asymptotically infected child in Beijing  

Source: Provincial CDC (China), National China CDC, WHO, and news reports
Cases of H7N9 Influenza in China by Age-Group (2/26/14)*

*Total cases = 373

Note: ages of 3 cases are unknown and identity of 52 deaths also unknown
Annual number of deaths within the U.S. attributable to influenza: 41,400 (as of 2004)
EMERGENCE OF THE 2009 PANDEMIC

Influenza Positive Tests Reported to CDC by U.S. WHO/NREVSS Collaborating Laboratories, National Summary, 2008-09
1918 (AND POSSIBLY SwORH1n1) MORTALITY CURVES SUGGEST PREVIOUS EXPOSURE

- The “U” shaped curve of regular influenza infection demonstrates the highest mortality among children (naïve) and the elderly (immunocompromised)
- The 1918 pandemic had a “W” shaped curve, with a spike in deaths among young adults—immunopathology or prior protection for ~40 year olds?
INFLUENZA LIFE CYCLE
HA is required for cell entry

- HA binding to sialic acid on the surface of cells mediates initial attachment.
- Virus is endocytosed, where the endosome is acidified.
- This triggers a conformational change in the virus, resulting in membrane fusion.
- For HA to be active, it needs to be cleaved by a protease into two pieces—this protease is generally restricted to the respiratory epithelium.
Neuraminidase acts to cleave the sialic acid receptors from the cell surface

- IAV must balance the binding and entry activity of HA with the sialic acid cleavage activity of NA so that virus efficiently enters and buds from the cell surface—thus HA and NA are often “matched” for activity
**Immune Mechanisms of Protection**

- Antibody mediated immunity exerts the most pressure on the virus, leading to seasonal antigenic drift and pandemic strains of antigenic shift.
- Internal proteins are relatively conserved allowing heterologous cellular protection.
- Mutation of dominant CD8 epitopes over time suggests that CTLs provide immunological pressure.
 IMMUNE COURSE OF INFLUENZA INFECTION

- Influenza is initially controlled by antibody and CD8+ T cells
- Secondary infection with heterologous virus is cleared with CD8+ T cell activity much more rapidly
- Homologous infection can be prevented by antibody (sterilizing immunity)
Prediction of the 2009/H1N1 Pandemic

- The 2009 H1N1 pandemic emerged as a particularly novel threat: an antigenic shift event between two swine viruses, without the “human” virus component expected to be required.
- The initial rapid spread bred fears of an equally high incidence of severe morbidity and mortality (~90,000 deaths in the US, ~1.8 million hospitalizations)
# Pre-Existing Cross-reactive Immunity to 2009/H1N1

## Table 1. Cross- Reactive Microneutralization Antibody Response against Pandemic Influenza A (H1N1) Virus in Pediatric and Adult Recipients of Seasonal Trivalent Inactivated Influenza Vaccines

<table>
<thead>
<tr>
<th>Type of Vaccine, Influenza Season, and Influenza Virus Used in Assay</th>
<th>Age Group</th>
<th>No. of Subjects</th>
<th>Increase in Antibody Titer by a Factor of ≥4</th>
<th>Microneutralization Titer of ≥40 for Children or ≥160 for Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td>Geometric Mean Titer (95% CI)</td>
<td>Before Vaccination</td>
</tr>
<tr>
<td>Trivalent inactivated influenza vaccine</td>
<td></td>
<td></td>
<td>%</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>2005–2007</td>
<td>6 mo to 9 yr</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasonal H1N1</td>
<td></td>
<td></td>
<td>67 (16–40)</td>
<td>267 (171–418)</td>
</tr>
<tr>
<td>Pandemic H1N1</td>
<td></td>
<td></td>
<td>0 (0–0)</td>
<td>6 (5–6)</td>
</tr>
<tr>
<td>2007–2008</td>
<td>5 yr to 9 yr</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasonal H1N1</td>
<td></td>
<td></td>
<td>85 (22–80)</td>
<td>575 (303–1093)</td>
</tr>
<tr>
<td>Pandemic H1N1</td>
<td></td>
<td></td>
<td>0 (0–0)</td>
<td>6 (5–6)</td>
</tr>
<tr>
<td>2008–2009</td>
<td>6 mo to 23 mo</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasonal H1N1</td>
<td></td>
<td></td>
<td>100 (4–7)</td>
<td>285 (202–402)</td>
</tr>
<tr>
<td>Pandemic H1N1</td>
<td></td>
<td></td>
<td>0 (0–0)</td>
<td>5 (0–5)</td>
</tr>
<tr>
<td>Trivalent inactivated influenza vaccine with adjuvant</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2008–2009</td>
<td>6 mo to 59 mo</td>
<td>45</td>
<td></td>
<td></td>
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<tr>
<td>Seasonal H1N1</td>
<td></td>
<td></td>
<td>96 (8–18)</td>
<td>193 (134–280)</td>
</tr>
<tr>
<td>Pandemic H1N1</td>
<td></td>
<td></td>
<td>2 (5–7)</td>
<td>8 (7–9)</td>
</tr>
<tr>
<td>Adults</td>
<td>Trivalent inactivated influenza vaccine</td>
<td>2007–2008</td>
<td>18 yr to 64 yr</td>
<td>148</td>
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<td>----------------------------</td>
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<tr>
<td></td>
<td>Seasonal H1N1</td>
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<td>75</td>
<td>48</td>
<td>598</td>
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<td></td>
<td></td>
<td>(40–58)</td>
<td>(497–720)</td>
</tr>
<tr>
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<td>Pandemic H1N1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>22</td>
<td>25</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(21–31)</td>
<td>(44–65)</td>
</tr>
<tr>
<td>Older adults</td>
<td>Trivalent inactivated influenza vaccine</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Seasonal H1N1</td>
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<td></td>
<td></td>
<td>78</td>
<td>29</td>
<td>546</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(22–38)</td>
<td>(418–713)</td>
</tr>
<tr>
<td></td>
<td>Pandemic H1N1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>12</td>
<td>11</td>
<td>21</td>
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<td></td>
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<td></td>
<td>(9–14)</td>
<td>(16–26)</td>
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</tbody>
</table>
People born prior to 1940 have “protective” levels of antibody to 2009/H1N1.
INFLUENZA MORTALITY IS ASSOCIATED WITH SECONDARY BACTERIAL INFECTIONS

- Between August 2009 and March 2010, 276 influenza-associated pediatric deaths were reported to the CDC
- 34% of the tested children had a bacterial co-infection
  - Strep-21%
  - Staph-34%
  - Rest apparently unidentified
- Several mechanisms for secondary infection susceptibility have been proposed, including action of the viral neuraminidase promoting bacterial colonization and immunological disruption
Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection.

Jamieson AM, Yu S, Annicelli CH, Medzhitov R.
2009 Pandemic H1N1

- 2009/H1N1 resulted from the recombination of two viruses (American and Eurasian Swine)
- The American Swine virus was itself a recombinant of three viruses that established itself in 1998
- These viruses are genetically distant from the human seasonal H1N1 (reference strain A/Brisbane/59/07)
Table 2. Estimates of pandemic (H1N1) 2009–related cases and rates of illness and hospitalization by age distribution of confirmed case-patients, United States, April–July 2009

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated no. case-patients</th>
<th>Estimated rate/100,000*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>90% range</td>
</tr>
<tr>
<td>Total no. case-patients by age group, y†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>3,052,768</td>
<td>1,831,115–5,720,928</td>
</tr>
<tr>
<td>5–24</td>
<td>397,033</td>
<td>238,149–744,045</td>
</tr>
<tr>
<td>25–49</td>
<td>1,820,284</td>
<td>1,091,845–3,411,237</td>
</tr>
<tr>
<td>50–64</td>
<td>612,862</td>
<td>367,608–1,148,511</td>
</tr>
<tr>
<td>&gt;65</td>
<td>42,292</td>
<td>25,368–79,256</td>
</tr>
<tr>
<td>No. hospitalized case-patients by age group, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>13,764</td>
<td>9,278–21,305</td>
</tr>
<tr>
<td>5–24</td>
<td>2,768</td>
<td>1,866–4,285</td>
</tr>
<tr>
<td>25–49</td>
<td>4,991</td>
<td>3,364–7,725</td>
</tr>
<tr>
<td>50–64</td>
<td>3,440</td>
<td>2,319–5,324</td>
</tr>
<tr>
<td>&gt;65</td>
<td>1,912</td>
<td>1,289–2,959</td>
</tr>
<tr>
<td>Multiplier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalized</td>
<td>2.7</td>
<td>1.7–4.5</td>
</tr>
<tr>
<td>Nonhospitalized</td>
<td>79</td>
<td>47–148</td>
</tr>
<tr>
<td>Through May 12</td>
<td>33</td>
<td>23–49</td>
</tr>
<tr>
<td>After May 12</td>
<td>84</td>
<td>50–163</td>
</tr>
</tbody>
</table>

†Age distributions from line list and aggregate reports of laboratory-confirmed cases and hospitalizations to the Centers for Disease Control and Prevention through July 23, 2009.
Human infections with Triple-reassortant swine viruses have occurred

- “... custom slaughter house ...”
- “... helped hold and abduct the forelimbs of one (1) freshly killed pig while his brother eviscerated it.”
- “No facial or respiratory protection was worn ...”
- “... father obtained a live chicken...”
- “...sacrificed during a ritual ceremony.”
- “... patient was never within ten (10) feet of the chicken..”

Newman et al., Emerging Infectious Diseases, 2008
**H1N1 Swine Flu Studies: Response in Human Cells**

Measures:

- Infectivity and growth of virus (TCID$_{50}$, immunofluorescence)

- Secretion of inflammatory mediators from apical and basolateral surfaces (multiplexed immunoassay)

- Transcriptional response over the first 24 hours (Exon arrays, fluidigm analysis)

- Confirm results by “swapped viruses” made by reverse genetics
In vitro cultures of human airway epithelial cells

cilia-red/green
Nucleus-blue
**Viral growth kinetics in HAE cells**

**Human primary**

**Swine primary**

All continued shedding from healthy monolayers for >3 weeks

0.01 moi
Influenza NP detection in 3D HAE cultures
viral growth kinetics in HAE cells

8 hr post infection- 0.01 moi
MORE RAPID COLONIZATION OF CULTURE BY PANDEMIC AND ESW VIRUS

By 12 hours, pandemic strains and Italy have infected ~50%-75% of the culture
Viral efficiency of spread in A549

influenza NP

DAPI (nucleus)
SUMMARY DATA OF FOCAL INFECTION STUDY

Both M and NA genes of CA/04/09 contribute to greater viral spread
**Higher NA Activity in Pandemic and ESw**

- NA activity measured as ability to convert sialic acid containing substrate.
- Results normalized to functional viral titer, so NA activity/infectious virion.
- Higher NA activity may relate to ability of virus to spread efficiently.
GROWTH SUMMARY

- The pandemic virus acquired a rapid growth phenotype in human cells similar to the Esw virus.
- This phenotype associates with both the NA and M of Esw virus.
- The Esw virus transmits more efficiently in ferrets.
- Titer and infected cell number can be de-coupled across infections/individuals.
Why wasn’t the Esw virus a pandemic?
**TRANSCRIPTOME ANALYSIS OF PANDEMIC VIRUS INFECTED HAE CULTURES**

mRNA expression in hAE cultures infected at MOI=0.01

BIC applied to k-means clustering: 2 clusters 271 upregulated in all 24 downregulated or differential
TOP 9 MOST SIGNIFICANT DIFFERENTIALLY EXPRESSED GENES 12 HOURS POST-INFECTION WITH A/BRISBANE/59/2007 (H1N1)
Does the TRIG backbone (Asw) induce a “stealthy” response?
**Fluidigm Platform for Array Verification**

- Uses microfluidics to perform multiple qPCR reactions on one plate
  - 48x48 (probes vs. targets) or 96x96 formats
  - 1 48x48 plates = 6 384 well plates
- Real time confirmation of arrays with a tiny amount of RNA
- For this project, selected 100 genes to track:
  - Most up- or down-regulated
  - Virus replication, IFN response, host response
HOST RESPONSE AS A FUNCTION OF VIRUS

Fluidigm Real Time PCR from primary human cell infections (2 donors)

Brisbane  California  Italy  North Carolina
HOST RESPONSE AS A FUNCTION OF VIRUS II

Brisbane
Califonia
Italy
North
Carolina

expression – expression_{mock}
max(expression)
M_{gene}
What's the mechanistic basis of the stealthy (or noisy) phenotype?
Average amplitude across all genes normalized to M-gene

exptName

AvgAMNorm

0 25 50 75 100 125

background
- BR
- CA
- IT
- NC
AGATHA CHRISTIE

AND THEN THERE WERE NONE

Previously published as TEN LITTLE INDIANS

Murder on the Orient Express
A HERCULE POIROT MYSTERY
Amplitude (“A”) normalized to M-gene
THE PANDEMIC STRAIN IS EFFICIENT AND STEALTHY

- Rapid + stealthy growth = Pandemic
  
  Morbidity and Mortality Weekly Report

Limited Human-to-Human Transmission of Novel Influenza A (H3N2) Virus — Iowa, November 2011

- The set of genes induced by diverse viruses is largely equivalent in the first 24 hours— “the flu program”
- The pandemic strategy is distinct from the well-adapted human seasonal virus
- Kinetic differences in the first ~18 hours of infection are critical to the quality and quantity of the later response
- The stealthy phenotype is mediated by contributions of the P-gene complex, with potential roles for NP and NS
ODE model of influenza infection

\[
\begin{align*}
\frac{dU}{dt} &= \lambda D - \frac{b}{1 + s_1 X} UV & \text{uninfected cells} \\
\frac{dE}{dt} &= \frac{b}{1 + s_1 X} UV - \frac{g}{1 + s_3 X} E & \text{latent infected cells} \\
\frac{dI}{dt} &= \frac{g}{1 + s_3 X} E - dI & \text{productively infected cells} \\
\frac{dD}{dt} &= dI - \lambda D & \text{dead cells} \\
\frac{dV}{dt} &= \frac{p}{1 + s_2 X} I - cV - \gamma \frac{b}{1 + s_1 X} VU & \text{free virus} \\
\frac{dX}{dt} &= wI - \delta X & \text{innate immune response (IFN)}
\end{align*}
\]
**AICc VALUES OF 8 DIFFERENT MODELS**

1. No IR and no cell-regrowth
2. No IR, with cell-regrowth
3. With IR reducing virus production, no cell-regrowth
4. With IR reducing infection rate, no cell-regrowth
5. With IR prolonging latency, no cell-regrowth
6. With IR reducing virus production, with cell-regrowth
7. With IR reducing infection rate, with cell-regrowth
8. With IR prolonging latency, with cell-regrowth

<table>
<thead>
<tr>
<th>Model</th>
<th>BB</th>
<th>CA</th>
<th>IT</th>
<th>NC</th>
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<tr>
<td>1</td>
<td>54.5</td>
<td>54.7</td>
<td>33.1</td>
<td>28.2</td>
</tr>
<tr>
<td>2</td>
<td>48.8</td>
<td>-22.6</td>
<td>0.8</td>
<td>28.5</td>
</tr>
<tr>
<td>3</td>
<td>52.8</td>
<td>24.8</td>
<td>17.0</td>
<td>30.3</td>
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<td>4</td>
<td>59.9</td>
<td>33.2</td>
<td>38.3</td>
<td>33.6</td>
</tr>
<tr>
<td>5</td>
<td>53.2</td>
<td>32.1</td>
<td>24.6</td>
<td>31.7</td>
</tr>
<tr>
<td>6</td>
<td>-11.6</td>
<td>-17.6</td>
<td>-11.1</td>
<td>33.2</td>
</tr>
<tr>
<td>7</td>
<td>54.5</td>
<td>-17.7</td>
<td>6.1</td>
<td>29.3</td>
</tr>
<tr>
<td>8</td>
<td>56.1</td>
<td>-17.3</td>
<td>6.2</td>
<td>34.3</td>
</tr>
</tbody>
</table>
FITS FOR MODEL 6—IR REDUCES VIRUS PRODUCTION AND CELLS REGROW
Drug Targets for Influenza Treatment

- The two approved drug families target the M2 ion channel and NA
Neuraminidase Inhibitors Prevent Viral Spread

Diagram explaining the role of neuraminidase in viral replication and how neuraminidase inhibitors prevent this process.
Neuraminidase inhibitor resistance has emerged in seasonal H1N1 influenza, amantadine resistance in H3N2

<table>
<thead>
<tr>
<th></th>
<th>Oseltamivir</th>
<th>Zanamivir</th>
<th>Adamantanes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolates tested (n)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Resistant Viruses, Number (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seasonal Influenza A (H1N1)</strong></td>
<td>825</td>
<td>820 (99.4%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Influenza A (H3N2)</strong></td>
<td>132</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Influenza B</strong></td>
<td>403</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Novel Influenza A (H1N1)</strong></td>
<td>68</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Mutations Associated with NA Inhibitor Resistance

- Group 1 (green) and group 2 (yellow) neuraminidase inhibitors and their associated mutations
  - Top panel: Group 2 mutation Arg 292 Lys (Group 1 viruses still have the Tyr347 interaction to stabilize)
  - Bottom panel: Group 1 mutation His274Tyr disrupts interaction with Tyr252
**Points for Discussion**

- How would a “cellular based vaccine” work and what types of effects would it have across a population?
- NA Inhibitor resistance mutations often need to be balanced by changes in the HA—change in NA activity requires matching change in HA activity
  - Can restrict the ability of the NA to mutate, but can also make mutations more “cryptic”—if the HA has acquired the necessary changes the NA resistance mutations may actually be favored (likely the case in the previous seasonal H1N1 situation)
- Points for modeling: how early does anti-viral treatment need to be given to stop spread? What is the appropriate use of prophylaxis given symptoms follow the contagious period?