Surveillance, evolution and prospects for control of influenza viruses in our region

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Three global health challenges of influenza

- Avian Influenza
- Seasonal Human Influenza
- Pandemic Human Influenza
Three global health challenges of influenza

Avian Influenza
- 391 human deaths from H5N1 since 2003

Seasonal Human Influenza
- 250,000 – 500,000 deaths per annum

Pandemic Human Influenza
- 122 human deaths from H7N9 since 2013
- 0.3 – 50 million deaths per pandemic
Surveillance, evolution and prospects for control of influenza viruses in our region

- Seasonal, pandemic and zoonotic influenza viruses
- Global influenza surveillance
- Zoonotic influenza viruses: current threats
- Current research on influenza
- Prospects for control
Seasonal, pandemic and zoonotic influenza viruses
Seasonal influenza

Human-adapted influenza viruses (types A, B and C) circulate continuously in the world, causing outbreaks each winter in temperate climates.

Influenza viruses also circulate in the tropics.

Types A and B cause significant morbidity and mortality.
Seasonal influenza

Human-adapted influenza viruses (types A, B and C) circulate continuously in the world, causing outbreaks each winter in temperate climates.

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Types A and B cause significant morbidity and mortality.

Currently circulating seasonal influenza A and B viruses belong to:

- **subtype** A(H1N1)pdm09
- **A(H3N2)**
- **lineage** B/Victoria/2/87
- **B/Yamagata/16/88**

Current influenza vaccines contain both A subtypes and one or both B lineages.
Seasonal influenza in Australia in 2013

Notifications of laboratory-confirmed influenza
Australian Influenza Surveillance Report 2013
Surveillance of influenza in Indonesia, 2003–2007

Kosasih et al., Influenza and Other Respiratory Viruses 7:312-320 (2012)
Pandemic influenza

New influenza A viruses, not previously adapted to humans, emerge at irregular intervals and cause global epidemics (…1918, 1957, 1968, 2009…).

Most people are not protected by immunity to other influenza viruses.

Pandemic virus may infect >50% of the world’s population.

Infections may occur out of season.

Virus eventually becomes a seasonal virus.

Taubenberger & Morens, Rev Sci Tech (2009)
Pandemic influenza

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Virus eventually becomes a seasonal virus.
“Habitat” of influenza A viruses

Water birds are their natural host.

Viruses may cause sporadic infection and become adapted to new avian or mammalian hosts.

“Habitat” of influenza A viruses

Water birds are their natural host.

Viruses may cause sporadic infection and become adapted to new avian or mammalian hosts.

Influenza A viruses

Basis of nomenclature: H1N1, H3N2 etc

HA required for entry into and NA for escape from infected cells

Adapted from De Jong et al, J Infect 40:218-228 (2000)
Influenza A viruses: immunity and variation

- **8 RNA strands**
- **NA (neuraminidase)**
- **HA (haemagglutinin)**

**Major sites of variation due to immune pressure (antigenic drift)**

**10^{-4} mutation rate due to lack of proof-reading mechanism**

**Major targets of human antibodies that protect against infection**

**Segmented genome allows reassortment during co-infection (antigenic shift)**
Development of new influenza viruses

**Mutation**
- Drift: Random genetic changes, immune selected
- Altered NA and HA

**Reassortment**
- Shift: Genome shuffling when two viruses infect one cell
- New HA
Origins of new influenza viruses

• Mutation causes progressive changes in influenza A and B viruses ("antigenic drift"). This is the reason we need to update seasonal influenza vaccines every year or so.

• At least 3 of the 4 human influenza pandemics (1957, 1968 and 2009) were caused by a reassortment ("antigenic shift") between different influenza A viruses of avian, swine and/or human origin to bring together:
  - a new HA
  - sometimes a new NA
  - one or more new internal virus genes.
Specific prevention and treatment of influenza

**Vaccines** induce host to make neutralising antibodies to HA, blocking viral entry. Inactivated vaccines are largely strain-specific.

T cells are mainly directed at conserved internal viral proteins but are not strongly induced by conventional inactivated influenza vaccines.

**Antiviral drugs**

NA inhibitors block virus release from infected cell.

Adamantanes (M2 inhibitors) block viral RNA release into cytoplasm of infected cell.

NA inhibitors: Oseltamivir (*Tamiflu*) Zanamivir (*Relenza*) Peramivir Laninamivir
The challenges of influenza

• Influenza viruses cause significant morbidity and mortality worldwide with high economic impact.

• Influenza A and B viruses are highly mutable; human population immunity drives the emergence of antigenic drift variants.

• Co-infection with different influenza viruses from animal reservoirs can lead to formation of new reassorted viruses which may cause pandemics.

• Virulence and transmissibility of new influenza viruses are unpredictable.

• Vaccines require regular updating and neither vaccines nor drugs are optimal.

• There is a continuing need to monitor seasonal and zoonotic influenza viruses for public health preparedness.
Global influenza surveillance
WHO Global Influenza Surveillance and Response System

- Established in 1952
- Monitors the evolution of influenza viruses infecting humans
- Makes recommendations on vaccines, laboratory diagnostics, antiviral susceptibility and risk assessment
- Serves as a global alert mechanism for emergence of influenza viruses with pandemic potential
- Collaborates with OIE to exchange information on human and animal viruses

Six peak laboratories (WHO Collaborating Centres on Influenza):
- London (NIMR)
- Atlanta (US CDC)
- Melbourne (VIDRL) since 1992
- Tokyo (NIID) since 1994
- Beijing (CNIC at China CDC) since 2010

<table>
<thead>
<tr>
<th>Human influenza</th>
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<tr>
<td>London</td>
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<tr>
<td>Atlanta</td>
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<td>Melbourne</td>
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<td>Tokyo</td>
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<td>Beijing</td>
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</table>

<table>
<thead>
<tr>
<th>Animal influenza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memphis</td>
</tr>
</tbody>
</table>
141 WHO National Influenza Centres in 111 countries (>92% population)
5 WHO Collaborating Centres for Influenza (human), one for animal
12 H5 Reference Laboratories
4 Essential Regulatory Laboratories (FDA, TGA, NIBSC, NIID)

Coordinated by WHO Global Influenza Program in Geneva
WHO GISRS: virus sharing for surveillance, risk assessment and control of influenza

WHO National Influenza Centres (NICs):
Designated by their Ministry of Health and recognised by WHO to:
- monitor influenza in their country and report to WHO via FluNet
- diagnose and type/subtype influenza viruses
- send representative and unusual viruses to a Collaborating Centre

WHO Collaborating Centres for Reference and Research on Influenza:
Designated by WHO and funded by their host government to:
- undertake detailed antigenic and genetic analysis of submitted viruses
- monitor emergence of new antigenic variants
- monitor antiviral drug resistance
- report data back to National Influenza Centres
- isolate candidate vaccine viruses
- work with WHO to recommend virus strains for use in vaccines (twice yearly)
- supply vaccine viruses to manufacturers
- provide training, advice and subtyping kits to National Influenza Centres
- undertake research on influenza
Countries submitting viruses to WHO Collaborating Centre
A sample is submitted to a WHO CC…

- What influenza subtype or lineage is it?

- Does it differ from known circulating influenza virus strains:
  - antigens (HA, NA)?
  - genetic sequence?

- Is it detected by current diagnostic tests (PCR)?

- Would human antibodies to previous viruses or vaccines be protective?

- Is it sensitive to antiviral drugs?

- What is its geographical spread?

- If it is a new strain, can we isolate a suitable candidate virus?
Analysis of viruses at the WHO Collaborating Centre

- Virus isolate
  - MDCK cells → MDCK isolate
    - Antigenic analysis: haemagglutination inhibition microneutralisation
    - Gene sequencing: HA, NA, some full genomes
    - Antiviral drug sensitivity: phenotypic, genotypic assays
    - Other testing: eg, human serology
    - Raise ferret antiserum
    - Confirm HI & sequence

- Clinical specimen
  - PCR → Sequence
  - Eggs
    - Egg isolate
    - Vaccine candidates
Old and new technologies in influenza virus analysis

Haemagglutination inhibition
Antiviral drug resistance
Sequencing, pyrosequencing

Isolation of potential vaccine viruses in eggs
<table>
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<th>C</th>
<th>D</th>
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<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
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**Haemagglutination Inhibition Assay**

### Reference Antisera

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<thead>
<tr>
<th>Reference Antigens</th>
<th>BRIS/3</th>
<th>FLORID/4</th>
<th>WISC/1</th>
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**Test Antigens**

| Reference Antigens | BRIS/3 | FLORID/4 | WISC/1 | HBEI/158 | MAL/412 | WELL/3 | WELL/3 | BRIS/3 | MASS/2 |
|--------------------|--------|----------|--------|----------|---------|--------|--------|--------|--------|--------|
| A                  | 320    | 640      | 160    | 320      | 80      | 320    | 80     | 640    | 1280   | 320    | E4      |
| B                  | 1280   | 1280     | 320    | 640      | 320     | 320    | 2560   | 1280   | E4     |
| C                  | 1280   | 1280     | 320    | 640      | 320     | 320    | 1280   | 1280   | 1280   | 1280   | E4      |
| D                  | 1560   | 320      | 80     | 640      | 320     | 320    | 80     | 320    | 1280   | E4      |
| E                  | 1560   | 320      | 80     | 640      | 320     | 320    | 80     | 320    | 1280   | E4      |
| F                  | 1560   | 320      | 80     | 640      | 320     | 320    | 80     | 320    | 1280   | 1280   |
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| J                  | 1560   | 320      | 80     | 640      | 320     | 320    | 80     | 320    | 1280   | 1280   |

**History**

- MDCKK, MDCK3
- MDCKK, MDCK2
- MDCKK, MDCK2
- MDCKK, MDCK2
- MDCKK, MDCK2
- MDCKK, MDCK2
- MDCKK, MDCK2
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- MDCKK, MDCK2
- MDCKK, MDCK2
- MDCKK, MDCK2
- MDCKK, MDCK2
Antigenic cartography of seasonal influenza viruses: February 2014

A(H1N1)pdm09

A/California/07/2009

B/Yamagata lineage

B/Massachusetts/02/2012 (clade 2)
B/Wisconsin/1/2010 (clade 3)

Colin Russell and Derek Smith, University of Cambridge, February 2014
Phylogenetic tree of B/Yamagata viruses

Haemagglutinin

Green: Asia
Blue: Australia/New Zealand
Red: Reference virus

2014 NH Vaccine

* Indonesian viruses are antigenically similar to other clade 2 viruses

Clade 2

Clade 3
**Phylogenetic tree of B/Victoria viruses**

**Haemagglutinin**

Green: Asia
Blue: Australia/New Zealand
Red: Reference virus

*2014 NH Vaccine*

* Indonesian viruses are antigenically similar to others in region.
Phylogenetic tree of A(H1N1)pdm09 viruses

Haemagglutinin

Red: East and South-East Asia
Pink: Oceania
Purple: Middle East
Dark blue: North America
Light blue: South America
Green: Europe
Orange: Africa
Maroon: Russia
Grey: Unknown

Circled: Current vaccine virus

The great majority of tested viruses remain antigenically similar to the 2009 vaccine virus despite genetic diversification.
Basis for WHO recommendation of a vaccine strain change

Widespread and increasing circulation of viruses showing:

1. marked change in antigenic profile compared with previous vaccine strains
   AND

2. changes in sequence of HA protein, especially at antibody- or receptor-binding sites
   AND

3. poor recognition by serum antibodies from people who received the previous vaccine
   AND

4. availability of suitable candidate vaccine strains isolated in eggs

Haemagglutinin of A(H3N2)
J. McCauley & R. Daniels
WHO CC, NIMR, London
## WHO recommendations for trivalent seasonal vaccines

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>A/H1N1</th>
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<td>A/Victoria/361/2011</td>
<td>B/Wisconsin/1/2010</td>
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<td>2013</td>
<td>February</td>
<td>A/California/7/2009</td>
<td>A/Victoria/361/2011</td>
<td>B/Massachusetts/2/2012</td>
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<td>A/Texas/50/2012</td>
<td>B/Massachusetts/2/2012</td>
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<td>2014</td>
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<td>A/California/7/2009</td>
<td>A/Texas/50/2012</td>
<td>B/Massachusetts/2/2012</td>
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SH 2013
NH 2012-13
SH 2014
NH 2013-14
Zoonotic influenza viruses:
- why do we worry?
- which ones do we worry about?
Influenza in the 21st century: intense animal husbandry and trade

China has ~12 billion poultry.
Influenza in the 21st century: high population density

Influenza in the 21st century: high connectedness

Where will the next pandemic come from?

• Adaptation or reassortment of avian influenza viruses:
  o avian H5N1 in Asia, Middle East or Africa (2003 – )
  o avian H7N9 in China (2013)
  o other avian H7 or H9 viruses

• Changes to the A(H1N1)pdm09 virus due to further reassortment in pigs:
  o various swine viruses in Asia, USA and Australia (2009 – )
  o swine H3N2v in USA (2011 – )

• Something unexpected…
# Zoonotic influenza A viruses of current concern

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Source of human infection</th>
<th>Human infections*</th>
<th>Geographic spread in animals</th>
<th>Pandemic risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1N1v</td>
<td>swine</td>
<td>+, mild</td>
<td>USA</td>
<td>+/-</td>
</tr>
<tr>
<td>H3N2v</td>
<td>swine</td>
<td>++, mild</td>
<td>USA</td>
<td>+/-</td>
</tr>
<tr>
<td>HP H5N1</td>
<td>poultry</td>
<td>++++, severe</td>
<td>Asia, ME, Africa, Europe</td>
<td>++</td>
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<tr>
<td>LP/HP H7N2/N3</td>
<td>poultry</td>
<td>++, mild</td>
<td>Europe, North America, Asia</td>
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<tr>
<td>HP H7N7</td>
<td>poultry</td>
<td>+, mild</td>
<td>Europe, Australia</td>
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<td>H10N8</td>
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<td>+, severe</td>
<td>China</td>
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*Number, severity

LP, low pathogenic   HP, highly pathogenic
### Zoonotic influenza A viruses of current concern

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<td>+/-</td>
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<tr>
<td>H3N2v</td>
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*Number, severity

LP, low pathogenic   HP, highly pathogenic
Highly pathogenic avian influenza A(H5N1)

Most avian influenza viruses cause mild illness or no disease in birds. But highly pathogenic H5N1 viruses:

- cause severe, rapid-onset disease in domestic poultry
- can kill entire poultry flocks in 48 hours
- spread through droppings, contact with infected birds and carcasses, contaminated surfaces etc
- spread globally mainly through poultry trade, also migratory birds
- are transmitted to humans by close contact with infected birds
- cause average mortality in humans of ~60% (391 of 664 confirmed cases [WHO])
- have only occasionally been passed from one person to another.
A(H5N1) cases in humans reported to WHO at 24 March 2014
Phylogenetic trees of HA of highly pathogenic A (H5N1) viruses

Seven clades are no longer detected.

Clades 1, 2 and 7 continue to diversify in different countries and regions.

Many of these viruses are antigenically distinct.
Continuing threat of highly pathogenic avian influenza A(H5N1)

- H5N1 viruses continue to circulate in poultry and wild birds across Asia, the Middle East, Europe and Africa (endemic in Bangladesh, China, Egypt, Indonesia and Vietnam).

- They have diversified so they are no longer covered by one vaccine.

- H5N1 viruses continue to cause sporadic human deaths (~60% mortality).

- As few as 5 amino acid changes enable airborne transmission of H5N1 viruses between ferrets.  
  

- Two of these changes are common in H5N1 viruses in the field.  
  
A(H7N9): a novel reassortant virus of avian origin

Human Infection with a Novel Avian-Origin Influenza A (H7N9) Virus

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A(H7N9) cases in China at 30 April 2014

Cases of H7N9 Influenza in China by Week of Onset (Apr 30, 2014)
433 Total Cases: 122 Deaths
Date of Onset Missing for 15 Cases
Date of Onset for Deceased Cases Missing for 53 Cases

Source: Provincial CDC (China), National China CDC, WHO, and news reports
Location of H7N9 Influenza in China (30 April 2014)*

<table>
<thead>
<tr>
<th>Province/City</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhui</td>
<td>15</td>
</tr>
<tr>
<td>Beijing</td>
<td>5</td>
</tr>
<tr>
<td>Fujian</td>
<td>22</td>
</tr>
<tr>
<td>Guangdong</td>
<td>113</td>
</tr>
<tr>
<td>Guangxi</td>
<td>3</td>
</tr>
<tr>
<td>Hebei</td>
<td>1</td>
</tr>
<tr>
<td>Henan</td>
<td>4</td>
</tr>
<tr>
<td>Hunan</td>
<td>22</td>
</tr>
<tr>
<td>Jiangsu</td>
<td>57</td>
</tr>
<tr>
<td>Jiangxi</td>
<td>7</td>
</tr>
<tr>
<td>Jilin</td>
<td>1</td>
</tr>
<tr>
<td>Shandong</td>
<td>2</td>
</tr>
<tr>
<td>Shanghai</td>
<td>42</td>
</tr>
<tr>
<td>Zhejiang</td>
<td>139</td>
</tr>
</tbody>
</table>

*433 total cases/122 deaths
Live bird markets

Culling in Shanghai market, 6 April 2013
Impact of closure of live bird markets in three provinces in 2013

Rapid decline of epidemic in April – May 2013 was probably due to live bird market closures and change of season.

Data suggest amplification and spread through live bird market network.

Original source of the H7N9 viruses that contaminated live bird markets has not been identified.

*Murhekar et al., WPSAR 4(2) (2013)*
**Important features of human H7N9 virus sequences**

**Haemagglutinin**
- lacks multi-basic cleavage site needed for high pathogenicity in birds
- has Q226L mutation allowing strong binding to $\alpha$2,6-linked sialic acid receptors (mammalian)
- some have V186G mutation which increases $\alpha$2,6-sialic acid affinity
- has T156A mutation which causes loss of glycosylation site

**Neuraminidase**
- lacks H275Y mutation which confers Tamiflu resistance
- in treated patients can acquire R292K mutation which confers Tamiflu resistance

**PB2**
- some have E627K mutation associated with replication in mammalian respiratory tract (33°C)

**M, PB1 and NS1**
- several mutations associated with virulence in mice
- PB1 has 368V mutation associated with H5 transmission in ferrets
- M gene has S31N mutation which confers resistance to adamantane class of antiviral drugs

- Genetic signatures of adaptation to infection of mammalian hosts
## Risk assessment of human H7N9 viruses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Characteristics</th>
</tr>
</thead>
</table>
| **Source of infection**    | • domestic poultry, especially in live poultry markets  
• low pathogenicity in poultry  
• origin of virus contaminating markets still unknown |
| **Location**               | • now detected over a large area of China  
• high population and animal density  
• many uncontrolled poultry farms and traders  
• Shanghai is major travel and business hub |
| **Severity in humans**     | • severe pneumonia with high mortality  
• very few mild cases detected  
• low seropositivity in contacts  
• general population lack pre-existing antibodies |
| **Human-human transmission** | • some suspect family clusters  
• disease not generally detected in close contacts |
| **Genetic signals**        | • reassortant of avian viruses  
• several adaptations to mammalian infection |

➢ Pandemic risk difficult to assess but greater than for highly pathogenic avian H5N1
H7N9: Global preparedness for a potential pandemic

• WHO technical guidance on surveillance, laboratory procedures and biosafety

• Development and validation of PCR-based diagnostic tests:
  Sharing of protocols and primer/probe sequences (WHO CCs)
  Distribution of H7N9 RNA as reference material (WHO CCs)
  Distribution of PCR kits (CNIC, US CDC)

• Preparedness for vaccine development:
  WHO recommendation of vaccine virus
  WHO updates on availability of candidate vaccine viruses for industry
  US Government (BARDA) funding of vaccine development
  Many manufacturers taking first steps

• Commencement of vaccine development by some manufacturers, eg:
  Novartis (cell-based virus + MF59) – Phase I trial data
  Novavax (rHA/NA/M virus-like particle + Iscomatrix) – Phase 1 trial data
  Sanofi Pasteur (egg-based virus + MF59 or AS03) – trials in progress
H5N1 and H7N9: cause for continuing concern

- Elimination of avian reservoirs is difficult, perhaps impossible.
- Reduce risk through control of breeding, trade and other practices that favour virus mixing and transmission between birds, other animals and humans.
- Continuing surveillance and rapid analysis of viruses is critical to monitor spread, evaluate interventions and detect change in risk profile.
- We need cross-protective vaccines to reduce impact of new subtypes.
Current research on influenza
Peter Doherty Institute for Infection and Immunity, Melbourne

- Joint venture between University of Melbourne and Royal Melbourne Hospital
- 700 scientists, researchers, academics, clinicians and graduate students
- Undergraduate and postgraduate teaching
- Biomedical and clinical research, epidemiology
- Diagnostic and reference ID laboratories, BSL3 and BSL4 facilities, animal facility
Research program at the WHO Collaborating Centre

Many collaborative and in-house research projects, eg:

- Antiviral drug resistance in influenza viruses
- Immunity and pathogenesis of influenza viruses in ferrets
- Molecular evolution of influenza A and B viruses
- Development of new technologies for analysis of influenza viruses
- Sero-epidemiological studies of human population immunity to influenza
- Influenza vaccine effectiveness in Australia
- Influenza viruses in wild birds

and linkage to NHMRC Program Grant on influenza
Antiviral drug resistance in seasonal influenza viruses

- Two major drugs used are the neuraminidase inhibitors:
  - Oseltamivir (Tamiflu)
  - Zanamivir (Relenza)
- Active against influenza type A and B viruses
- Bind in or close to the active site of neuraminidase, preventing release of new virions from infected cells
- Mutations in the NA that interfere with drug binding cause drug resistance but normally reduce viral fitness so virus does not spread in untreated patients.
- Exception in 2007 – 2008, when A(H1N1) viruses universally became oseltamivir-resistant due to single point mutation in NA (His to Tyr at 275).
- These viruses disappeared during the A(H1N1)pdm09 pandemic.
- A(H1N1)pdm09 viruses are generally sensitive to the NA inhibitors.
Permissive mutations enable H275Y viruses to spread

Bloom, Gong & Baltimore (Science 2010) showed that other mutations in NA enabled the 2008 H275Y A(H1N1) viruses to replicate and transmit in absence of drug.
Detection of oseltamivir-resistant H1N1pdm09 the community

- May – September 2011: large community cluster of oseltamivir resistant A(H1N1)pdm09 viruses with H275Y substitution in NA gene
- Largest cluster of A(H1N1)pdm09 H275Y variants reported to date
- Of 32 cases detected, 26 were within 50 km of Newcastle on east coast of Australia
- At peak in July, 20 out of 85 H1N1pdm09 viruses tested (24%) were resistant
- Only one case had received oseltamivir treatment (not the index case)
- Appeared to be a ‘fit’ H275Y variant circulating in the community
- Not detected after end of 2011 Australian influenza season

Emergence of permissive mutations for oseltamivir resistance in A(H1N1)pdm09 viruses

Competitive mixtures experiments in ferrets using wild-type and reverse genetics viruses showed that N369K and V241I increased the fitness and NA activity of H275Y viruses.

Most A(H1N1)pdm09 viruses now carry these permissive mutations.
The fitness of A(H1N1)pdm09 H275Y variants

2009 – 2010

Low viral fitness

Oseltamivir

2011 – 2012

Moderate viral fitness

Oseltamivir

What further changes in the virus are needed?

High viral fitness

?
NHMRC Program Grant on Influenza

  National Health and Medical Research Council

• Chief investigators: Peter Doherty, David Jackson, Anne Kelso, Lorena Brown, Stephen Turner, Weisan Chen, Katherine Kedzierska

• Organisations: University of Melbourne, WHO Collaborating Centre for Influenza, La Trobe University, Australian Animal Health Laboratory, Alfred Hospital and others

• Major themes: Shaping virus-host interactions
  Determinants of cross-reactive immunity to influenza
  Regulation of effector and memory T cells
  Basis of susceptibility to severe influenza
  Optimising vaccines for cross-reactive immunity
The problem with influenza vaccines

• Seasonal influenza vaccines are mainly used in developed world.

• These vaccines are usually inactivated and must be updated regularly and re-administered because of antigenic drift. They do not protect against novel subtypes.

• A new vaccine, eg, for a pandemic virus:
  - takes months to produce
  - requires specialised facilities
  - is expensive.

• Most influenza vaccine manufacturers are commercial and located in western countries (esp. Europe).

• Developing countries cannot afford to buy or stockpile vaccines.

➤ The ultimate goal - a vaccine for all influenza
- influenza vaccines for all
Using a TLR2 ligand as a vaccine adjuvant

- Most soluble protein or subunit antigens are poorly immunogenic without an adjuvant.
- Few adjuvants are approved for human use.
- Toll-like receptors (TLRs) recognise a variety of pathogen-associated structures.
- TLR agonists can be used to deliver antigens to TLR-expressing antigen-presenting cells and trigger MyD88/NFκB-dependent gene expression.
- TLR2 is expressed on many cell types and recognises diacylated and triacylated lipids.
- Di-palmitoyl-S-glyceral-cysteine (pam2cys) is a TLR2 ligand.
- Lipopeptides constructed by covalent linkage of pam2cys to peptide epitopes are highly immunogenic in mice and other species (Jackson et al.).

- Pam2cys can also be used to improve immunogenicity of whole protein antigens:

  \[ \text{Protein antigen} \quad \text{R}_4\text{Pam}_2\text{Cys} \quad \text{Targets TLR2/6} \quad \text{Dendritic cell} \]

  *Electrostatic association between antigen and agonist*

Jackson, Chua et al.
R4Pam2cys enhances protection by split virus influenza vaccine

PR8 (H1N1)-derived split virus vaccine ± R₄Pam₂Cys

Day 30 challenge with: PR8 (H1N1) or X31(H3N2)

Day 35

Lung CD8⁺ T cells

IFN-γ producing CD8⁺ T cells (10³)

NP-specific
Virus-specific

p < 0.05

PBS
Split Virus
Split Virus+R₄Pam₂Cys

Wild-type (C57BL/6)
B-cell deficient (µMT)

Split Virus
Split Virus+R₄Pam₂Cys

Lung viral titres

Homologous (PR8)
Heterologous (X31)

Lung Viral Titre (log₁₀ p.f.u.)

PBS
Split Virus
Split Virus+R₄Pam₂Cys

Split Virus
Split Virus+R₄Pam₂Cys

Wild-type (C57BL/6)
B-cell deficient (µMT)

Lung Viral Titre (log₁₀ p.f.u.)

PBS
Split Virus
Split Virus+R₄Pam₂Cys

PBS
Split Virus
Split Virus+R₄Pam₂Cys

Jackson, Chua et al.
Pam2cys protein vaccines in mouse models

Electrostatic association of antigen (OVA, OVA-flu or split H1N1 virus) with R4Pam2cys produces a vaccine which can:

- induce maturation of dendritic cells and increase antigen uptake and processing
- reduce effective antigen dose for immunogenicity
- induce elevated titres of specific IgM, IgG, IgA and neutralising antibodies
- induce effector and memory CD8+ T cells
- protect mice against homologous and heterologous influenza virus challenge.

Cross-protection:

- is not mediated by antibody in passive transfer experiments or impaired in B cell-deficient mice
- is associated with induction of IFN-γ-producing CD8+ effector cells.

Advantages of R4Pam2cys technology:

- totally synthetic and defined, simple to synthesise
- aqueous; stable; lyophilised form does not require refrigeration

Such technologies have the potential to reduce the need for annual vaccination and provide cross-protection against new strains and subtypes.

Surveillance, evolution and prospects for control of influenza viruses in our region

- Seasonal, pandemic and zoonotic influenza viruses
- Global influenza surveillance
- Zoonotic influenza viruses: current threats
- Current research on influenza
- Prospects for control
Influenza: prospects for control

Today we have:
• Improved surveillance in many countries
• Rapid specific diagnostic tests
• Antiviral drugs
• Strain-specific vaccines
• Better understanding of influenza virology, ecology, pathogenesis and immunity
• Improved biosecurity in animal husbandry
• Rapid global communication systems.

Yet there are still many challenges:
• Limited awareness of the risks and impact of influenza on health and the economy
• Other priorities for health spending
• Unequal access to existing control measures
• Traditional farming and trade practices
• Ever increasing globalisation and connectedness of people, animals and products
• Lack of cross-protective vaccines that will protect against future strains and subtypes
• Capacity of influenza viruses to evade immunity and antiviral drugs through mutation

Further improvements in the control of influenza will depend on international cooperation in many spheres – scientific, social, political and economic.
Thank you

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