Advances in the development of influenza virus vaccines

Interdisciplinary Technical Journal Club:
special series on Laboratory Animal Science

16.01.2018

Regina Reimann
Influenza affects us all

USZ influenza campaign 2017/2018:

**Fluarix® Tetra (egg based split vaccine):**

- A/Michigan/20015(H1N1) pdm09
- A/Hong Kong/2014 (H3N2)
- B/Brisbane/2008 (Victoria line)
- B/Phuket/2013 (Yamagata-line)

- 5-15% of the human population are infected by the seasonal influenza each year
- Resulting in around 500‘000 death/year worldwide
- <10% of the world population is routinely vaccinated
- Global seasonal influenza virus vaccine production: 500 million doses
Primary target of the anti-influenza antibody response

Hemagglutinin (HA) = Major antigen
• Homotrimeric viral surface glycoprotein
• Binds to sialic acid on glycan structures of cellular receptors (attachment to cells)
• Mediation of fusion of viral and endosomal membrane (release of the viral genome)

Neuraminidase (NA)
• Homotetrameric viral surface glycoprotein
• Sialidase activity (transport, release)

▶ Subtypes of influenza A based on combination of HA (18) and NA subtypes (11)
▶ A virus with circulation in humans: A/H1N1, A/H2N2 and A/H3N2
▶ Subtypes of influenza B: B/Victoria and B/Yamagata circulating in humans without animal reservoir

Orthomyxoviridae, negative-stranded RNA genome
Three genera are susceptible to infecting humans: A, B, C
Eight segment of viral DANN (A&B) encoding for: PB1, PB2, PA, M1, M2, NS NA, HA and NP

S. Sridhar, K. A. Brokstad and R. J. Cox, Vaccines, 2015
Established influenza vaccines

**Inactivated**: formalin and phenylmercuric nitrate

**Split**: Detergent and/or ether

**Subunit**: Split + Neuraminidase and Haemagglutinin + purification and enrichment

**Live attenuated influenza vaccines (LAIVs)**: Temperature-sensitive and cold-adapted / replicate efficiently in the upper respiratory tract / but not in the lower respiratory tract (nasal spray)

<table>
<thead>
<tr>
<th>Product name</th>
<th>Commercial name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>Afluria</td>
<td>CSL Limited</td>
</tr>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>FluLaval</td>
<td>ID Biomedical Corporation of Quebec (a division of GlaxoSmithKline)</td>
</tr>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>Fluarix</td>
<td>GlaxoSmithKline Biologicals</td>
</tr>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>Fluvirin</td>
<td>Novartis Vaccines and Diagnostics Limited</td>
</tr>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>Agriflu</td>
<td>Novartis Vaccines and Diagnostics Limited</td>
</tr>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>Fluzone, Fluzone High-Dose, and Fluzone Intradermal</td>
<td>Sanofi Pasteur</td>
</tr>
<tr>
<td>Influenza virus vaccine (quadrivalent)</td>
<td>Fluarix Quadivalent</td>
<td>GlaxoSmithKline Biologicals</td>
</tr>
<tr>
<td>Influenza virus vaccine (quadrivalent)</td>
<td>Fluzone Quadrivalent</td>
<td>Sanofi Pasteur</td>
</tr>
<tr>
<td>Influenza virus vaccine (quadrivalent)</td>
<td>FluLaval Quadrivalent</td>
<td>ID Biomedical Corporation of Quebec</td>
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<table>
<thead>
<tr>
<th>Egg-based live attenuated influenza vaccines (LAIV)</th>
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<tr>
<td>No trade name</td>
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<tr>
<td>FLuMist</td>
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**Trivalent**: Antigens from three circulating strains: H1 and H3 for influenza A and one strain influenza B

**Trivalent inactivated vaccine (TIV)**: Most common formulation of influenza vaccine, 15 μg of each component

Seasonal influenza vaccine production timetable

Goal: Production of safe and effective vaccines

C. Gerdil, Vaccine, 2003

Colaborating center for onfluenza reference and research:
London, Atlanta, Melbourne, Tokyo, Memphis and Beijing
Generation of multivalent seeded strain

Genetic reassortment:
- Seasonal influenza H1N1 and H3N2
- H1N1/A/PR/8/34 strain with a high yield grow in embryonated egg
- Organisation of genome in eight negative sense, single-stranded viral RNA segments (vRNA)
- Co-infection in the allantoic cavity of embryonated eggs
- OR plasmid based reverse genetic (co-infection, cell culture)

→ **Generation of seed precursor virus: must be generated in eggs (regulations)**
Problematic: Isolates from cell culture might be reisolated from eggs and manufacturer may be de novo readapt to grow in mammalian cells

Nogales, Martinez-Sobrido, Int J Mol Sci 2016
Reverse genetic approaches to generate influenza vaccines

- vRNAs are cloned into eight bi-directional plasmids
- Co-transfection into FDA-approved cell lines

→ Rescue of the recombinant influenza viruses

Bi-directional rescue plasmids (required for negative sense RNA viruses):
- Human Pol-I: Eight negative sense vRNAs
- CMV Pol-II: Eight viral mRNAs translated into the influenza proteins
- \textit{vRNP}: \textit{viral ribonucleoprotein complex} (vRNAs, NP, PA, PB1, PB2); transcription from viral promoters $\rightarrow$ mRNA $\rightarrow$ protein; vRNA $\rightarrow$ cRNA (amplification of vRNA)
- vRNAs + structural proteins $\rightarrow$ \textit{new influenza virus}

Nogales, Martinez-Sobrido, Int J Mol Sci 2016
Very low vaccine effectiveness during the 2014–2015 Influenza season

Estimation of vaccine effectiveness in UK (week 40 2014 – week 3 2015)

<table>
<thead>
<tr>
<th>Influenza virus</th>
<th>Change in reactivity with A/Texas/50/2012 antiserum</th>
<th>4-fold</th>
<th>&gt;4-fold</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>n (%)</td>
<td>N (%)</td>
<td>n (%)</td>
<td>N (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>All influenza (A and B)</td>
<td>65 (51.2)</td>
<td>35 (27.6)</td>
<td>27 (21.3)</td>
<td>127</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All influenza A</td>
<td>64 (51.1)</td>
<td>35 (27.6)</td>
<td>27 (21.3)</td>
<td>127</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A(H3N2) only</td>
<td>61 (51.2)</td>
<td>35 (27.6)</td>
<td>27 (21.3)</td>
<td>127</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Crude VE (95% CI) | Adjusted VE (95% CI)

-26.7% (-74.0 to 7.8) | 3.4% (-44.8 to 35.5)
-32.2% (-82.2 to 4.0) | -0.7% (-52.0 to 33.2)
-39.8% (-94.1 to -0.7) | -2.3% (-56.2 to 33.0)

Cl: confidence interval; VE: vaccine effectiveness.

\[ VE = \frac{ARU - ARV}{ARU} \times 100, \]

VE = Vaccine efficacy, ARU = Attack rate of unvaccinated people, ARV = Attack rate of vaccinated people.

- Hemagglutination inhibition (HI) assay
- Fold difference in comparison with A/Texas/50/2012 strain (2014-15 norhern hemispheric vaccine, H3C.1)
- Influenza A (H3N2) virus isolates 2014-2015 from infected patients (n=127)
- A/Texas/antiserum: post infection anti-ferret sera

4-fold difference in HI assay is a significant antigenic drift (mutations)
Antigenic mismatch between the circulating strain and vaccine strain

R G Pebody, Eurosurveillance 2015
HA mutations in 2014-2015 Viruses

Percentage of viruses That belong to each HA Clade; Sequence deposit form GISAID* database

HA Mutations in 2014-2015 Viruses/ New clades: 3C.2a, 3C.3 and 3C.3a

H3 component of 2014-2015: Texas/50/2012 strain (H3C.1)

H3 component of 2015-2016: A/Switzerland/9715293/2013 (3C.3a)

*BISAID: Global Initiative on Sharrning all Influenza data
Mutant viral Texas/50/2012 panel

**Goal:** Determination which HA residues are responsible for the observed antigenic drift of 2014–2015 H3N2 strains

Significance of data: Proper selection of viral strains in future vaccine formulation

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Sera</th>
<th>Ferret α-A/Texas/50/12</th>
<th>Sheep α-A/Texas/50/12</th>
<th>Sheep α-A/Switzerland/9715293/13</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Texas/50/12-WT</td>
<td>960</td>
<td>10,240</td>
<td>2,560</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/12-N128A</td>
<td>1,280</td>
<td>10,240</td>
<td>2,560</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/12-A138S</td>
<td>480</td>
<td>5,120</td>
<td>1,280</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/12-R142G</td>
<td>640</td>
<td>7,680</td>
<td>1,920</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/12-N144S+N145S</td>
<td>1,280</td>
<td>10,240</td>
<td>3,840</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/12-N145S</td>
<td>480</td>
<td>5,120</td>
<td>1,920</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/12-F159S</td>
<td>240</td>
<td>3,840</td>
<td>3,840</td>
<td></td>
</tr>
<tr>
<td>A/Texas/50/12-N225D</td>
<td>640</td>
<td>7,680</td>
<td>2,560</td>
<td></td>
</tr>
<tr>
<td>A/Switzerland/9715293/13</td>
<td>60</td>
<td>1,280</td>
<td>2,560</td>
<td></td>
</tr>
</tbody>
</table>

HAI assays were completed using antisera isolated from ferrets 19 days post-infection or sheep 28 days post-infection. Data are representative of three independent assays.

- Reverse genetic derived viruses: HA plasmids + plasmids from A/Puerto Rico/8/1934; transfection of 293T/MDCK co-culture;
- propagation in 10 day-old fertilized eggs
- HAI titer: invers of the highest dilution

⇒ The F159S HA mutation result in an **asymmetrical** antigenic change

B. Chambers et al, Cell Reports, 2015
Relevance of F159S HA mutation

- ELISA plates coated with VLP A/TX/12-WT or Mutant
- Control: A/Port Chalmers/1/1973 HA (A/PC/73); 1973 H3 virus
- Anti-sera from ferrets infected with A/TX/12
- F49 mAb: recognize the conserved stalk region of the HA

Conclusion

- Demonstration that a mutation in the antigenic B side is primarily responsible for the mismatch
- Support of the WHO decision to update the H3N2 component

B. Chambers et al, Cell Reports, 2015
Relatively low vaccine effectiveness during the 2016-2017 influenza season

- Vaccine from 2016-2017: 3C.2a H3N2
- Majority of viruses during influenza season: 3C.2a H3N2
- Relatively low VE: 43%

**Egg-adapted 3C.2a vaccine strain lacks the B glycosylation site; present on circulating 3C.2a H3N2 strains** (egg adapted strain posses a T160K reversion mutation; K160T strain grow poorly in egg)

**Goal:** Determination weather the difference in glycosylation of HA antigenic site B of H3N2 vaccine strains and circulating strain contributes to vaccine mismatch

**Appearance of K160T HA mutation**

**GISAID database**

**Western blot of homogenates from viruses generated with reverse genetic**

**Blue: New putative glycosylation sides introduced by the K160T mutation**

**Black: Other putative glycosylation sides**

S. Zost, PNAS, 2017
H3N2 viruses with T160 HA are antigenically distinct

- ELISA plates coated with viral particle
- Human monoclonal antibodies isolated from donor peripheral blood mononucleat cells following vaccination with the 2010-2011 influenza vaccine

→ 2016-2017 circulating influenza virus possess a new glycosylation site that affects antigenicity, this site is not present in 2016-2017 vaccine strain

S. Zost, PNAS, 2017
Different antibody response in ferrets

- Ferrets (n=3) infected with viruses, sera collected 28 d later
- FRNT assay: Inverse dilution of sera that reduced foci by 90%, pre-incubation of reverse-genetic transfection supernatant of each virus + diluted sera; Added to confluent monolayer of MDCK-SIAT1 cells; staining

➤ The new glycosylation site effectively “shields” antigenic site B
Disadvantages of egg based influenza vaccine production

- Long vaccine production process (window for new virus variants to emerge)
- Human vaccine grown in eggs often possess adaptive and unintended mutations (increase viral attachment to chicken cells)
- High-level bio-containment facilities
- Antigenic proteins in eggs (allergic reaction)
- Scale is dependent on a large egg production
Allternatively established influenza vaccine production

<table>
<thead>
<tr>
<th>Product name</th>
<th>Commercial name</th>
<th>Manufacturer</th>
<th>Cell platform</th>
<th>Commercially available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>Flucelvax</td>
<td>Novartis Vaccines and Diagnostics Limited</td>
<td>MDCK</td>
<td>EU/FDA</td>
</tr>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>FluBlok</td>
<td>Protein Sciences Corporation</td>
<td>Insect cells</td>
<td>FDA</td>
</tr>
<tr>
<td>Influenza virus vaccine (trivalent)</td>
<td>PrefluCel</td>
<td>Baxter</td>
<td>Vero</td>
<td>EU</td>
</tr>
<tr>
<td>Influenza virus vaccine (H5N1)</td>
<td>Celvapan</td>
<td>Baxter</td>
<td>Vero</td>
<td>EU</td>
</tr>
<tr>
<td>Influenza A (H1N1) 2009 monovalent</td>
<td>Celvapan</td>
<td>Baxter</td>
<td>Vero</td>
<td>EU</td>
</tr>
</tbody>
</table>

Table 2: Licensed influenza vaccines produced using cell culture technology.
Cell-based inactivated influenza vaccine production

Cells: Madin-Darby canine kidney cells (MDCK); human embryonic retinal cells (PER.C6); monkey kidney cells (Vero); human embryo kidney cells (HEK293)

Optaflu/Flucelvax (Novartis): Trivalent subunit vaccine produced in MDCK cells form egg-adapted influenza viral seeds.

Perflucel and Celvapan: Production in Vero cell

Recombinant trivalent hemagglutinin (rHA) vaccine


<table>
<thead>
<tr>
<th>Insect cell line</th>
<th>Source</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sf9 and Sf21</td>
<td><em>Spodoptera frugiperda</em></td>
<td>All general types of recombinant protein expression</td>
</tr>
<tr>
<td>Mimic™ Sf9</td>
<td><em>Spodoptera frugiperda</em></td>
<td>Expression of mammalian glycoproteins.</td>
</tr>
<tr>
<td>High Five™</td>
<td><em>Trichoplusia ni</em></td>
<td>Secretion of recombinant proteins. Shorter culture period.</td>
</tr>
</tbody>
</table>

FlubBlok: trivalent recombinant hemagglutinin vaccine

Produced in insect cell culture using the baclovirus expression system
Production in insect cell with the baclovirus expression system

- Capacity to produce many recombinant proteins at high levels
- Provide eukaryotic protein processing capabilities
- Baclovirus system is well suited to avoid adoptive mutations

https://www.creativebiomart.net/baculovirus-insect-cell-expression-systems.htm
Increase in antibody response against T160 HA following vaccination with Flublok

Donors were vaccinated with seasonal influenza vaccines, sera were collected before and 28d after vaccination. FRNT (neutralization assay) with the use of viruses.

 ⇒ Flublok induced higher fold change to T160 HA than did Flucelvax and Fluzone (Flublok vaccine possesses the T160 HA)
The hemagglutinin-stem is highly conserved

Five classical antigenic sides

Immune escape Substitutions A/H3N2

→ Generation of a hemagglutinin-stem only immunogen

Petrova and Russell, Nature Reviews 2018
Structure-based removal of the HA head

Goal: Structure based development of an H1 HA stem only immunogen

- Source: H1N1 A/New Caledonia/20/1999; crystal structure, foldon trimerization domain as design templates
- Evaluation of the truncated mutant: Expression as soluble trimers (gel filtration), reactivity to stem-specific monoclonal antibodies
- **Crystal structure:** stem epitope conformation was preserved, stem trimer subunit were splayed apart

Overlapping PCR and site directed mutagenesis; freestyle HEK 293 or HEK 293 MGAT1-/-

Yassine et al, nature medicine 2015
Ferritin nanoparticle displaying of HA stem

Kanekiyo et al, nature 2013

Yassine et al, nature medicine 2015
Immune response of immunized mice

- Sera from BALB/c mice immunized with SAS-adjuvanted H1-SS-np
- ELISA with HA protein
- Neutralization IC$_{50}$: Reciprocal dilution required to inhibit 50% of pseudotyped lentiviral reporter (viral-vectors, recombinant HA-NA lentiviral vectors expressing a luciferase reporter, preincubation with antiserum)

Yassine et al, nature medicine 2015
H1-SS np versus two other immunization regimes

- Sera from ferrets immunized with three regimens
- H5 HA DNA 1 x MIV: H5 DNA priming + monovalent inactivated vaccine (MIV); H5N1

Yassine et al, nature medicine 2015
Protection against lethal H5 2004 VN in mice

BALB/c mice (n: 10) vaccinated three times; challenged 4 weeks post final vaccination with a lethal H5 2004 VN dose

Passive immunization with 10 mg Ig from either naive or immune animals 24h before challenge with a lethal H5 2004 VN dose

H5N1 neutralization activity was negligible, however heterosubtype protection observed; other effector mechanism than in vitro neutralization

Yassine et al, nature medicine 2015
Protection against lethal H5 2004 VN in ferrets

Ferrets were vaccinated three times, 6 weeks post final vaccination challenge with a lethal dose of H5 2004 VN

Yassine et al, nature medicine 2015
Stalk antibodies as broadly reactive antibodies

Summary Yassine et al, nature medicine 2015:
- Proof of concept
- Successful generation of HA stem-only nanoparticle vaccine immunogen
- Antibody mediated heterosubtype protection immunity against H5N1 disease in mice and ferrets
  - High preservation of stalk
  - Antigenic escape from antigens that target the stalk has not been widely reported
  - However, this could be caused by a lack of selection pressure
  - In in vitro experiments can be achieved

Substitutions to facilitate escape from broadly neutralizing anti-HA antibodies

Petrova and Russel, Nature Reviews 2018
Thank you for your attention!

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