Assessing Immunogenicity of Influenza Vaccines

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• Proteom: 11 proteins

• 2 surface glycoproteins:
  • **Haemagglutinin (HA)**
    • important for infection
    • bind to sialic acids that are linked to host glycoproteins
    • Type H1-H16
  
  • **Neuraminidase (NA)**
    • important for virus release from infected cells
    • Type N1-N9

Subbaro et al 2007
Seasonal Influenza Virus

- Currently circulating in humans
  - H1N1
  - H3N2
  - B type

- Pre-immunity $\Rightarrow$ low pathogenicity!

Influenza types

CDC/ Dr. Erskine. L. Palmer, Dr. M. L. Martin
Seasonal Influenza Virus

• Currently circulating in humans
  • H1N1
  • H3N2
  • B type

• **Pre-immunity ⇒ low pathogenicity!**

Pandemic Influenza Virus

• Emerges when new influenza virus
  1. jumps from animal host to humans
  2. has ability acquired to transmit **human-to-human**
  3. has different **genetic make-up ⇒ No pre-immunity ⇒ potentially highly pathogenic!**

• Example: **swine flu** - H1N1 (human/swine/avian origin)

• Next **candidates** for pandemic: H9N2, H2N2, H5N1, …
H5N1 influenza (‘bird’ flu): The Thread of a Pandemic

• >600 cases of human infection since 2003
• Mortality rate of ~60%
  ⇒ highly pathogenic
• circulating in birds
• So far animal (bird)-to-human transmission only (poultry)
  → currently only thread of a pandemic
• Adaptation of virus/reassortment ⇒ human-to-human transmission ⇒ accelerate virus spread ⇒ pandemic
Baxter’s H5N1 Pandemic-like Influenza Vaccine

Features

• based on wildtype influenza virus (strain H5N1 Vietnam/1203/2004)
• Whole virus vaccine
• Inactivated
• Grown in cell culture (Vero cells)

• Licensed in EU
Inactivated/subunit/split influenza vaccine

- Correlate of protection: Antibodies directed against influenza hemagglutinin (HA)

- Assessed by
  - Hemagglutination inhibition assay (HI)
  - Single radial hemolysis assay (SRH)

- Accepted by regulatory authorities (EMEA, CPMP/BWP/214/96), based on former challenge studies
Hemagglutinin Inhibition Assay (Palmer, 1975)

- **Principle**
  - Turkey/horse erythrocytes are clotted by hemagglutinin of H5N1 virus which can be inspected by eye
  - Anti-HA antibodies block hemagglutination
  - Titer = highest serum dilution able to block hemagglutination
  - Protective titer $\geq 1:40$
**Single Radial Hemolysis Assay (Schild, 1975)**

**Principle**

- Sheep/turkey erythrocytes are immobilized in agarose containing virus
- Serum samples diffuse radially from wells punched in the agarose
- Zones of hemolysis are developed by guinea pig complement
- Diameter of hemolysis zone correlates with antibody concentration
- Protective titer $\geq 25\text{mm}^2$
Virus Neutralization Assay

- **Principle**
  - Confluent layer of Vero cells is infected by virus
  - Infections results in cytopathogenic effect detectable by microscope
  - Need for live virus - BSL3+ lab for pandemic strains necessary
  - Functional assay
  - Works well with *avian* influenza
  - Might be more suitable for children than HI or SRH
  - Confirmatory assay

![Diagram of Virus Neutralization Assay](image)
Neuraminidase

- Important for virus release from infected cells

Role of Neuraminidase inhibiting antibodies

- NA-specific antibodies reduce the release of virus from infected cells
- NA-specific responses reduce viral spread and severity of influenza illness in animal & human challenge studies
- NA-specific responses reduced the impact of the 1968/69 H3N2 pandemic

Hence, ...

- EMA encourages applicants to measure anti-neuraminidase antibody elicited by mock-up vaccines (EMEA/CPMP/VEG/4717/03-Rev.1)
Thiobarbituric acid based neuraminidase inhibition assay (TBA) (Aymard-Henry, 1973)

**Measurement of neuraminidase activity**
- Neuraminidase (of virus) removes sialic acids from substrate fetuin
- Sialic acids oxidized by periodate
- Oxidized sialic acids reacts with thiobarbituric acid, resulting in β-formyl pyruvic acid (chromophore)
- Measure chromophore at 550nm

**Neuraminidase inhibition (NI)**
- Inhibition of neuraminidase activity by antibodies → functional assay
- Definition of NI antibody titer: serum dilution inhibiting 50% of neuraminidase activity

- **Validated** Assay (rec neuraminidase VN N1, pos. control: VN N1 specific animal serum)
Enzyme-Linked Lectin Assay (ELLA) Neuraminidase Inhibition Test (Lambre, 1990)

Measurement of neuraminidase activity

- Neuraminidase removes sialic acids from substrate fetuin
- Sugar residues remain exposed on fetuin
- Sugar residues are detected by peanut Lectin (HRP-labeled)

Neuraminidase inhibition (NI)

- Inhibition of neuraminidase activity by antibodies → functional assay
- Definition of NI antibody titer: serum dilution inhibiting 50% of neuraminidase activity

- **Validated** Assay (rec neuramindase VN N1, pos. control: VN N1 specific animal serum)
H5N1 vaccination induces NI antibodies

**Clinical study (Ehrlich et al., 2008)**

- PI/II, healthy volunteers, 18-45 yrs of age
- 7.5 and 15 µg H5N1 vaccine dose groups (n=83), Immunization days 0, 21; Sampling days 0, 21, 42

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Fritz et al, 2011
TBA NI Titer and ELLA NI Titer correlate

$r = 0.83$

$P < .0001$

Fritz et al, 2011
Correlation between Antibody Titers by different Assays

Nonparametric Spearman correlations were calculated.

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<tr>
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<th>TBA</th>
<th>ELLA</th>
<th>MN</th>
<th>SRH</th>
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<td>r = 0.52; P &lt; .0001</td>
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Abbreviations: ELLA, enzyme-linked lectin assay; MN, microneutralization; SRH, single radial hemolysis; TBA, thiobarbituric acid.

Fritz et al, 2011
• **Correlate of protection for influenza vaccine** are hemagglutinin specific antibodies (HI, SRH assay)

• **Neuraminidase inhibiting antibodies** contribute to protection

• Neuraminidase inhibiting antibodies can monitored by TBA and ELLA assay

• **Baxter’s** whole virus pandemic **H5N1 vaccine** is equally effective in
  – inducing **antibodies to hemagglutinin** (Ehrlich et al., 2008)
  – inducing **antibodies to neuraminidase** (Fritz et al., 2011)
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