Universal influenza vaccines: Future prospects

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Influenza virus: **NOT** a “sitting duck”
2009(H1N1) pandemic vaccine preparation - response time -

VACCINES ARRIVED TOO LATE

The 6-month production delay for flu vaccines meant that they were not deployed in time to have any impact on the burst of pandemic flu cases. In Australia, for example, the number of confirmed H1N1 cases had fallen dramatically before vaccination began.

Vaccination began

It may take too long to produce “tailor made” vaccines!
Universal influenza vaccines

What is a universal influenza vaccine?
- Vaccine that induces protective immunity to:
  - Various intrasupotypic variants (antigenic drift variants)
  - Various subtypes of influenza A virus

Issues to be addressed
- Which arms of the immune system?
- Which viral proteins need to be targeted?

“Heterosubtypic immunity” induced by infection with influenza A virus
- Affords protection against viruses of other subtypes
  - correlates of protection other than HI or VN antibodies
Basis for universal influenza vaccines
- Conserved proteins or regions thereof -

Viral targets for cross-reactive antibodies
- M2 protein
- Stalk region of HA
- NA
- NP?

Viral targets for cross-reactive T cell responses
- All structural proteins in particular
  - NP
  - M1
- The non-structural proteins
  - NS1/NS2
  - PB1-F2, PA-X
- Polymerase proteins
  - PB1/PB2/PA
M2 has a conserved ectodomain: M2e

Courtesy of Prof. X. Saelens, Ghent University, Belgium
M2e fused to different carriers affords protection against influenza A

<table>
<thead>
<tr>
<th>M2 antigen</th>
<th>Carrier</th>
<th>Type of fusion</th>
<th>Animal model</th>
<th>Virus</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>hM2</td>
<td>-</td>
<td>-</td>
<td>Mouse</td>
<td>H2N2, H3N2</td>
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<tr>
<td>hM2e</td>
<td>Hbc</td>
<td>Genetic</td>
<td>Mouse</td>
<td>H1N1, H3N2</td>
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<td>hM2 deletion constructs</td>
<td>-/GST</td>
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<td>hM2e</td>
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<td>Pig</td>
<td>H1N1</td>
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<td>Mouse</td>
<td>H1N1</td>
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<td>hM2e</td>
<td>BSA</td>
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<td>H3N2 (in vitro)</td>
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<td>hM2e</td>
<td>Multiantigen peptide</td>
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<td>Mouse</td>
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<td>hM2e</td>
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<td>hM2e</td>
<td>KLH, OMPC</td>
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<td>Mouse</td>
<td>H1N1, H5N1, H6N2, H9N2</td>
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<tr>
<td>hM2e, avM2e, M2-DNA vaccine, M2-adenovirus</td>
<td>Hydrophobic domain</td>
<td>Genetic</td>
<td>Mouse</td>
<td>H1N1, H5N1</td>
<td>[58]</td>
</tr>
</tbody>
</table>

Protective effect has been demonstrated in animal models
- After hyper-immunization or
- Passive administration of M2-specific (monoclonal) antibodies
  - Probably mediated by antibody dependent cytotoxicity
  - NK cells
  - Complement system

Courtesy of Prof. X. Saelens, Ghent University, Belgium
**Fcγ Receptors I and -III are important for protection by anti-M2e IgG**

Survival after X47 challenge

- wt
- FcγRII/−
- FcγRI−/−
- (FcγRI, FcγRIII)−/−
- FcRγ−/− **

* p < 0.01  
** p < 0.001

Alveolar macrophages contribute to protection by anti-M2e IgG

Protection is restored in Fcγ Receptors-I and -III ko mice by alveolar macrophages of wt mice

Conclusions mode of action of M2e-specific antibodies

- Infected cells are primary target
- ADCC by NK and possibly neutrophils dependent on FcReceptors
- ADPC by alveolar and possibly exudate macrophages of opsonized cells

Challenges:
- Confirm FcReceptor contributions in human
- Develop robust in vitro assay mimicking this mechanism for anti-M2e IgG
Correlates of protection:
- Antibodies other than HI antibodies -

**NP specific antibodies**

**Evidence in mouse models**
- Rangel-Moreno et al., J. Immunol 2007
- Carragher et al., J. Immunol. 2008
- LaMere et al., J. Immunol. 2011

**Mechanism?**
- Non-VN antibodies promote rapid expansion of X-reactive memory T cells
  - FcRs
  - CD8+ T cells
  - Formation of NP-immune complexes?
Human NP-specific antibodies
- Lack of immunological relevant activity in vitro -

Bodewes et al, CVI 2013
Correlates of protection:
- Antibodies other than HI antibodies -

**HA-stem specific antibodies**
- Relatively conserved
- Protective effect demonstrated
  - after hyperimmunization or passive administration

Bommakanti et al., 2010, PNAS 107(31):13701-13706
Wei et al., 2010, Science 329:1060-1064
Kashyap et al., 2010, PLoS Pathogens 6(7):e1000990
Wang et al., 2010, PNAS 107(44):18979-18984
Wang et al., 2010, PLoS Pathogens 6(2):e1000796
Steel et al., 2010, mBio 1(1):e00018-10
Sui et al., 2009, Nat Struct Mol Biol 16(3):265-273
Kashyap et al., 2008, PNAS 105(16):5985-5991
Ekiert et al., 2009, Science 324:246-251
Corti et al., 2010, J Clin Invest. 120(5):1663-1673

Ekiert et al., 2011, Science 333:843-850
Corti et al., 2011, Science 333:850-856

Achilles heel ??
The structure of the influenza virus hemagglutinin (HA).

Krammer and Palese
Classical HI active antibodies neutralize by inhibiting attachment of the viral HA to sialylated host cell receptors and block entry at an early stage.

Stalk-reactive antibodies (green) bind to HA on the virus surface and may be taken up with the virus into the endosome. During acidification of the endosome they may prevent conformational change of the HA and inhibit release of the viral genome into the cytosol.

Broadly neutralizing antibodies may also inhibit viral egress.

Stalk-reactive antibodies may inhibit HA maturation by sterical hindrance of the interaction of host proteases with the HA cleavage site.

Stalk-reactive antibodies may also work through ADCC, infected cells as well as viruses are killed/cleared by macrophages and natural killer (NK) cells.

Stalk-reactive antibodies trigger complement mediated lysis of infected cells and may also help to clear influenza virus particles.

Non-HI/non-VN antibodies to HA
- Role for ADCC -

Cross-reactive influenza-specific antibody-dependent cellular cytotoxicity antibodies in the absence of neutralizing antibodies

Antibody-dependent cellular cytotoxicity is associated with control of pandemic H1N1 influenza virus infection of macaques.

Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo.
Design of stalk-based immunogens

(a) **HA2 subunit** only
(b) **Headless HA** constructs
Whole stalk domain including HA1 and HA2 parts. The globular head domain located between cysteines 52 and 277 (H3 numbering) was replaced by a glycine linker.
(c) The **long alpha helix** of the HA2 (amino acid 404–458) contains epitope of broadly neutralizing antibody 12D1
(d) **Chimeric HA** molecules consist of H1 or H3 stalk domain combined with ‘exotic’ globular head domains.

Vaccination strategies based on chimeric HAs

Correlates of protection:
- Antibodies other than HI antibodies -

• **NA specific antibodies**
  • Also subtype specific
  • less likely to contribute to heterosubtypic immunity
  • Protective effect demonstrated in vitro and mouse models predominantly

• Kilbourne and Schulman, 1965
• Kilbourne et al., 1968
• Schulman et al., 1968
• Couch et al., 1974
• Beutner et al., 1979
• Johansson et al., 1989

• Johansson et al., 1993
• Johansson et al., 1993
• Johansson et al., 1998
• Kilbourne et al., 2004
• Sandbulte et al., 2007
• Bosch et al., 2010
• Marcelin et al., 2011
Influenza virus NA: under selective pressure

Active site (yellow)

Colored: surface exposed positively selected:
(43, 46, 52 in stalk)

NA for the induction of protective immunity

Evasion from recognition by antibodies in nature: antigenic drift and shift

Functionally important in the virus replication cycle

Can confer a degree of cross-protection in the absence of matching HA (e.g. H5N1/H1N1)

Challenges:

• NA-specific antibodies with NAI activity protect
  • sensitive and specific assays needed

• NA-content in vaccines: standardize and stabilize

• Role of pre-existing NA-specific T cells: could contribute to improved vaccine responses
CTL: a correlate of protection
-Lethal infection with heterosubtypic virus H5N1-
- H3N2-H5N1 model -

More rapid viral clearance correlates with secondary CTL responses

Adoptive transfer of post H3N2-infection T cells affords clinical protection against infection with H1N1pdm09 virus - But not of serum or B cells -

Cross-reactive T cells are involved in rapid clearance of 2009 Pandemic H1N1 influenza virus in nonhuman primates - Primary infection with seasonal H1N1 virus -

Magnitude and kinetics of secondary T cell response......

correlate with reduction of virus shedding and more rapid clearance of infection.

Human CD8+ T cells to seasonal influenza viruses cross-react with viruses of other subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Reference</th>
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<tbody>
<tr>
<td>vH3N2</td>
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<tr>
<td>H7N9</td>
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</table>
Kinetics of T cell responses during acute A(H1N1)pdm09 influenza virus infection

- In adults with history of previous infections
- Very rapid recall response
  - Peaked < 1 week post infection
  - Recruitment and expansion of virus-specific CTL responses surprisingly fast

Hillaire et al. 2011, J. Virol. 85(22):12057-61
Analysis of the T cell response during acute A(H1N1)pdm09 influenza virus infection

Hillaire et al. 2011, J. Virol. 85(22):12057-61
The frequencies of pre-existing crossreactive T cells are inversely associated with illness severity in infected individuals.  
- Sridhar et al., Nature Med 2013 19:1305–1312 -
Points to consider for vaccine development

• Antibodies to stem HA / M2 protein/NA
  • special delivery/antigen presentation systems/vaccination regimens
  • use of adjuvants
    • to guarantee induction of antibody levels sufficiently high for protection

• CD4 and CD8+ T cells to NP, M1 or other conserved proteins
  • Induction requires specialized antigen delivery
    • endogenous antigen processing and MHC class I presentation
      • live vaccines
      • vectors (e.g. rec adenovirus, poxvirus)
      • DNA vaccines
      • special adjuvants (e.g. virosomes, ISCOMs)
    • Needs to be balanced

• For all these correlates of protection:
  • Minimal requirements of protection need to be established (surrogates)
    • assays?
  • Pre-clinical and clinical testing of candidate vaccines

• Local Immunity
  • Mucosal IgA antibodies
  • Resident virus-specific T cells
InFLuenza virus UNIversal VACcine development program

**Academic partners**
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