Antigenic targets for broadly protective and universal influenza virus vaccines

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The first WHO integrated meeting on development and clinical trials of Influenza vaccines that induce broadly protective and long-lasting immune responses

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Target overview

- Internal proteins
- M2e
- Neuraminidase (NA)
- Stalk domain of the hemagglutinin (HA)

adapted from Krammer and Grabherr, Trends Mol Med 2010
Internal proteins

- Nucleoprotein (NP), M1 and polymerase subunits
  - Conserved (e.g. about 90% amino acid identity for NP between human H1 and H3 isolates)
  - Strong T-cell epitopes
  - Anti-NP antibody responses involved in protection to a lesser degree
    (LaMere et al., J Virol and J Immunol, 2011)
  - Not easily accessible for antibodies
- Various experimental T-cell based vaccines with NP and M1 in animal models
- MVA vectored vaccines
  - NP+M1 expressing MVA vaccine was able to induce strong CD8+ and CD4+ T-cell responses
    (Lillie et al., Clin Infect Dis, 2012)
- Peptide vaccines
  - Multimeric-001 (BiondVax) contains NP and M1 epitopes
    (Atsmon et al., 2012, J Clin Immunol)
M2e

- 23 N-terminal amino acids which form the ectodomain of the tetrameric M2 ion channel
- Displayed on the cell surface, low copy number on the virus
- Conserved (~80% amino acid identity)
- Early development of particle-based M2e vaccines (Neirynck et al., Nat Med, 1999 and others)
- Vaccination induces infection-permissive (not sterilizing) immunity
  - morbidity, virus replication
- Mechanism
  - Mainly antibody dependent cell-mediated cytotoxicity (El Bakkouri et al., J Immunol, 2011 and others)
- Discussed as “additive” to regular influenza virus vaccine
Neuraminidase (NA)

- Tetrameric virus surface glycoprotein
- Functions
  - Sialidase activity permits transport of the virus through mucin
  - Sialidase activity essential for release of budding virus
- Conservation:
  - Slower drift rate than HA (Sandbulte et al., PNAS, 2011; Kilbourne et al., PNAS 1990)
  - Stalk region highly variable
  - Group 1 (N1, N4, N5, N8) and group 2 NAs (N2, N3, N6, N7, N9)
    - Very low amino acid identities between groups (~40%) but some conserved patches
  - About 80-90% amino acid identity between avian and human N1s
NA vaccines

• NA content not standardized in commercial vaccines
• Immunity against NA is thought to be infection-permissive (not sterilizing)
• N1 VLP vaccines induce cross-protection against H5N1 and H1N1 (Easterbrook et al., Virology, 2012; Wu et al., Plos One, 2012; Quan et al., Virology, 2012)
  – some morbidity, no mortality
• Cross-reactive anti-NA antibody levels correlate with protection (Chen et al., Vaccine, 2012; Rockman et al., JVI, 2013)
• Mechanism of protection
  – Inhibition of NA activity
  – ADCC could play a role as well
Hemagglutinin (HA)

- Homotrimeric major surface glycoprotein and major antigen
- Mediates binding to cell receptors and fusion of viral and endosomal membranes
- Vaccines induce strain specific, hemagglutination inhibiting (HI) antibodies against the immunodominant globular head domain
  - HI active antibodies correlate with protection
  - Sterilizing/neutralizing immunity
Broadly neutralizing antibodies directed against the conserved stalk domain have been isolated recently (Throsby et al., Plos One, 2009; Wang et al., Plos Path, 2010; Ekiert et al., 2009 and 2011, Science; Sui et al., Nat Struct Mol Biol, 2009; Corti et al., Science, 2011; Tan et al., J Virol, 2011; Dreyfus et al., Science, 2012 and others)

- Neutralize (with exceptions) either group 1 or group 2 HAs
  - in vitro
  - in passive transfer studies in animals (ferrets, mice)
- Mostly conformational epitopes
- HI negative!!!
- Rare and not induced/boosted upon regular seasonal vaccination
HA subtypes are divided into two groups:

**GROUP 1**
- H1
- H2
- H5
- H6
- H8
- H12
- H9

**GROUP 2**
- H7
- H15
- H10
- H14
- H3

Shaw and Palese, 2011, Field’s Virology
Virus neutralization by head- and stalk-reactive antibodies

Head-reactive antibodies:
- HI active
- inhibit binding

Stalk reactive antibodies:
- inhibit fusion
- inhibit egress
- inhibit maturation
- ADCC

adapted from www.flutrackers.com /Cox&Kawaoka 1997
HA stalk vaccines

- Vaccines based on the long-alpha helix (LAH) and on headless HA (stalk domain only) have been reported (Wang et al., PNAS, 2010; Steel et al., mBio, 2010; Bommakanti et al., PNAS, 2010)
  - protection from mortality
  - problems with conformational epitopes/folding of the stalk domain
- Anti-stalk titer (ELISA) correlates with in vitro neutralization
- Induced by natural infection when divergent globular heads are introduced into the human population e.g. during the 2009 H1N1 pandemic (Pica et al., PNAS, 2012; Miller et al., J Infect Dis, 2012; Krammer et al., J Virol, 2012 etc)
Induction of stalk-reactive antibodies during the 2009 H1N1 pandemic

adapted from Matt Miller, Icahn School of Medicine at Mount Sinai
Chimeric hemagglutinin

Globular head domain

Conserved stalk domain

chimeric H6/1

Hai et al., 2012, JVI
Chimeric HA constructs as universal influenza virus vaccines

- cH9/1 DNA or virus vector
- cH6/1 protein
- cH5/1 protein (replaced by wild type H1 protein for H5N1 challenge)
- PR8 H1N1 FM1 H1N1 pH1N1 or H5N1 challenge
pH1N1 challenge

H5N1 challenge

% weight loss

% survival

days post challenge

Naïve
Positive control
empty VV + BSA + BSA
VV ch9/1 + ch6/1 + ch5/1 (or PR8)
VV ch9/1 + BSA + BSA

***, p = 0.0002

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Universal influenza virus vaccine based on chimeric HAs:

• **Induces neutralizing anti-stalk antibodies**
  – Serum protects in passive transfer experiments
  – CD8+ T-cell depletion does not affect protection
  – Antibodies neutralize *in vitro*
  – Correlation between ELISA reactivity and neutralizing activity

• **Induces heterosubtypic immunity**
  – Works for group 1 as well as group 2 HAs

• **Trivalent universal vaccine based on a group 1, group 2 and influenza B component**
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