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Broadly protective and universal influenza vaccines
Immune responses and correlates of protection

1st WHO integrated Meeting on development and clinical trials of Influenza vaccines that induce broadly protective and long-lasting immune responses.
Hong Kong, January 24-16, 2013
Heterosubtypic immunity

- Protective immunity against infection with an influenza A virus of a subtype other than that of the strain that elicited the immune response
  - Demonstrated in many different animals since 1965

- Unraveling the correlates of protection of Het-I (n.b. other than HI antibodies) may aid the development of universal influenza vaccines
  - Mimic infection-induced immunity as closely as possible
Induction of heterosubtypic immunity to influenza H5N1 -by infection with A/H3N2, not RSV-

Primary infection

% Body weight

- None (PBS)
- RSV
- A/H3N2 influenza

Proportion survival

A/Hong Kong/2/68 (H3N2)
A/Indonesia/5/05 (H5N1)

Heterosubtypic immunity in ferrets
A/Brisbane/10/07 (H3N2) – A/Indonesia/5/05 (H5N1)

Bodewes et al., 2011, J. Virol. 85(6):2695-2702
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

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

<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>
</tr>
</thead>
<tbody>
<tr>
<td>hM2</td>
<td></td>
<td>Genetic</td>
<td>Mouse</td>
<td>H2N2, H3N2</td>
<td>[46]</td>
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<tr>
<td>hM2e</td>
<td>HBC</td>
<td>Genetic</td>
<td>Mouse</td>
<td>H1N1, H3N2</td>
<td>[47]</td>
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<tr>
<td>hM2 deletion constructs</td>
<td>-/GST</td>
<td>Genetic</td>
<td>Mouse</td>
<td>H1N1, H2N2, H3N2</td>
<td>[53]</td>
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<td>hM2e</td>
<td>HBC, NP</td>
<td>Genetic</td>
<td>Pig</td>
<td>H1N1</td>
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<tr>
<td>hM2e</td>
<td>HBC</td>
<td>Genetic, chemical</td>
<td>Mouse</td>
<td>H1N1</td>
<td>[61]</td>
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<tr>
<td>hM2e</td>
<td>BSA</td>
<td>Chemical</td>
<td>H3N2 (in vitro)</td>
<td>H1N1</td>
<td>[36]</td>
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<td>Multiantigen peptide</td>
<td>Chemical</td>
<td>Mouse</td>
<td>H3N2</td>
<td>[37]</td>
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<td>GST</td>
<td>Genetic</td>
<td>Mouse, ferret, rhesus monkey</td>
<td>H1N1, H3N1, H1N1, none</td>
<td>[57]</td>
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<td>hM2e</td>
<td>KLH, OMPC</td>
<td>Chemical</td>
<td>Mouse</td>
<td>H1N1, H5N1, H6N2, H9N2</td>
<td>[56]</td>
</tr>
<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>

Table 1. Overview of published studies using M2 or M2e vaccine antigens.

Courtesy of Prof. X. Saelens, Ghent University, Belgium
Immune correlates of M2e vaccine induced protection

• Direct restriction of virus replication (Zebedee and Lamb, 1988; Hughey et al., 1995; Gabbard et al., 2009; Wang et al., 2009)

• NK cell-dependent (Jegerlehner et al., 2004)

• Not NK/NKT cell-dependent (Thompkins et al., 2007, Wang et al., 2008)

• Complement-dependent (Wang et al., 2008 but not Jegerlehner et al., 2004)

• Contribution of M2e-specific CD4⁺ T cells (Eliasson, El Bakkouri et al., 2008)

• DCs and macrophages involved (Song et al., 2011)

• FcReceptors and alveolar macrophages involved (El Bakkouri et al., 2011)

• Correlation with M2-levels in virion (Kim et al., 2012)?

Courtesy of Prof. X. Saelens, Ghent University, Belgium
FcReceptors and IgG subclasses in mice

Bruhns, Blood, 2012
Fcγ Receptors I and -III are important for protection by anti-M2e IgG

![Survival after X47 challenge graph]

* p < 0.01
** p < 0.001

Alveolar macrophages contribute to protection by anti-M2e IgG

Survival after X47 challenge

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


n = 6/group
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?
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
Non-HI/non-VN antibodies to HA

Cross-Reactive Influenza-Specific Antibody-Dependent Cellular Cytotoxicity Antibodies in the Absence of Neutralizing Antibodies

Sinthujan Jegaskanda,* Emma R. Job,* Marit Kramski,* Karen Laurie,† Gamze Isitman,* Robert de Rose,* Wendy R. Winnall,* Ivan Stratov,* Andrew G. Brooks,* Patrick C. Reading,* and Stephen J. Kent*
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

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
Innate response
Antigen-presenting cells (dendritic cells, macrophages)

\[\text{γδ-T cells} \rightarrow \text{NK cells} \rightarrow \text{Granulocytes} \]

Cytolytic cell killing

Soluble non-cytolytic factors (IFN, TNF-α, certain CC chemokines in the case of HIV-1, other molecules)

Defensin

Adaptive response
Antigen-presenting cells (dendritic cells, macrophages)

\[\text{CD8+ T cells} \rightarrow \text{CD4+ T cells} \rightarrow \text{B cells} \]

Humoral

Cellular

Cytolytic cell killing

Soluble non-cytolytic factors (IFN, TNF-α, certain CC chemokines in the case of HIV-1, other molecules)

Antibodies

CTL: a correlate of protection
-Lethal infection with heterosubtypic virus H5N1-
- H3N2-H5N1 model -

More rapid viral clearance correlates with secondary CTL responses

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.

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

Cross-recognition of NP by human CTL -FATT-CTL assay-

Group I
1. #1
2. #2
3. #3
4. #4

Group II
5. #5
6. #6
7. #7
8. #8

Group III
10. #10
11. #11
12. #12
13. #13
14. #14
15. #15

% specific lysis

E:T ratio

NP A/NL/18/94 H3N2
NP A/VN/1194/04 H5N1
Empty vector control

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
Points to consider for vaccine development

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

• CD4 and CD8+ T cells to NP, M1 or other 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