Development of HA-based “Universal” Immunogens against HPAI H5N1 Viruses and Beyond

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Paul Zhou
Institut Pasteur of shanghai, Chinese Academy of Sciences
Variability of epitopes versus protective immunogenicity
Broadly neutralizing antibodies and their conserved epitopes

In stem region

- CR8020 – group 2 (Science 2011)
- FI6 – group 1 and 2 (Science 2011)
- CR9114 - Influenza A and B; in influenza A groups 1 and 2 - (Science 2012)
- 12D1 - H3 (PLoS Path. 2009)

In head region

- CH65 - within RBS; 30 of 36 H1 strains tested (PNAS 2011)
- C05 - within RBS; some strains in H1, H2, H3, H9 and H12 (Nature 2012)
- 65C6 and 100F4 - outside RBS; all H5 clades and subclades except for subclade 7.2 (JVI 2012; JVI 2013)
Approaches for viral envelope protein-based vaccine design

- Reverse vaccinology – Analysis of protein sequence diversity; then design ancestral, consensus, mosaic and multivalent immunogens

- Analytic vaccinology - Analysis of the human immune response, the isolation of neutralizing antibodies and characterization of neutralization epitopes; then design immunogens

- Serologic vaccinology – Comprehensive analysis of cross-reactivity of neutralizing antibody responses among subtypes and clades of a given virus and then design immunogens based on antigenic clusters. (Zhou et al. *JVI* 2012)
Vaccine-induced broadly antibody responses against influenza A viruses

- Wang *et al.* demonstrated that a conserved peptide of HA2 elicited binding antibodies against H1, H2, H3, H5 and H7 HA and partially protected heterosubtypic influenza challenge (*PNAS* 2010).

- Wei *et al.* showed that priming with plasmid encoding H1 HA and boosting with seasonal influenza vaccine or replication-defective ad5 vector induced cross-neutralizing antibody responses and conferred protection against divergent H1N1 viruses in mice and ferrets (*Science* 2010).

- We showed that broadly neutralizing antibody responses against all clades and subclades of HPAI H5N1 viruses could be elicited with a tri-clade DNA vaccine (Zhou *et al.* *JVI* 2012).
1. Select a vaccine strain closest to the consensus sequence

2. DNA-DNA-VLP prime-boost vaccination (TH-D-D-V)
Cross-divergent H5 neutralizing antibody responses elicited with TH D-D-V prime-boost
Elicitation of HA peptide-specific CD8 T cell responses
Immune protection against heterologous HPAI H5N1 challenge by active and passive immunization

![Graphs showing body weight and survival rates over days after challenge.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Virus</th>
<th>HI Titer</th>
<th></th>
<th>MN Titer</th>
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<th>PN Titer</th>
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Cross-H5 neutralizing antibody responses predominantly against head region
Immune sera bound to HA from all 16 HA subtypes

$P = 0.027$ in end-point values between group 1 and group 2 HA;
$P = 0.022$ in end-point values between group 1 minus 2 H5 HA and group 2 HA
Heterosubtypic protection against H1N1, H3N2 and H9N2 by active immunization
Cross-subtypic protection against H1N1, H3N2 and H9N2 by passive immunization

### HI Titer

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### Graphs

- **WSN/33**
  - %Body Weight
  - %Survival
  - Days After Challenge

- **Sw/GD/05**
  - %Body Weight
  - %Survival
  - Days After Challenge

- **Ck/JS/02**
  - %Body Weight
  - %Survival
  - Days After Challenge
Binding regions of HA by immune sera

(a) A/VietNam/1194/2004

(b) A/California/04/2009

Fragments selected by the immune sera

Frequency of nucleotides among the selected fragments

(a) A/VietNam/1194/2004

(b) A/California/04/2009

Residue position along HA protein

(f) 1 2 3 4 5 6

HA0

NA

HA1

HA2

95 72 55 43 34 26
Heterosubtypic antibody responses block low pH, HA-mediated hemolysis
Summary and Conclusion

• The TH DDV prime-boost elicited antibody responses that cross-neutralize all H5 clades and subclades of HPAI H5N1 viruses, bind to HA from all 16 HA subtypes and protect mice from divergent H5N1, H1N1, H3N2 and H9N2 challenge by both active and passive immunizations.

• The cross-H5 neutralization is mainly directed to the head region and blocks both virus attachment and post-attachment; whereas cross-subtypic antibodies binds to the stem region and blocks virus-cell membrane fusion.

Thus, the demonstration that TH DDV elicits broad antibody responses that confer protection against lethal challenge of heterosubtypic viruses in mice supports further development of this prime-boost regimen in ferrets and in humans.
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Pasteur Institute in Cambodia

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