TOWARDS A UNIVERSAL INFLUENZA VIRUS VACCINE

Peter Palese

Icahn School of Medicine at Mount Sinai
New York
Mount Sinai has submitted patent applications for a universal influenza virus vaccine.

Work has been supported by the NIH, The Bill & Melinda Gates Foundation, GSK.

My presentation does not include discussion of off-label or investigational use.
Surface glycoprotein diversity of different viruses

Influenza virus HA diversity
- Group 1
- Influenza A
- Group 2
- Influenza B

HIV-1 env diversity
- Group M
- Group O
- Group N

HCV E protein diversity

Similar variation for influenza, HIV and HCV
EIGHTEEN SUBTYPES OF INFLUENZA A VIRUS HEMAGGLUTININS
Influenza viruses circulating in the human population

A

H1N1 (Group1)
H2N2 (Group1)
1960 1918 1940 2000 1980

B

H3N2 (Group2)

pH1N1

H2N2 (Group1)

H1N1 (Group1)

1918 1940 1960 1980 2000
# AVIAN INFLUENZA VIRUSES INFECTING HUMANS

<table>
<thead>
<tr>
<th>H5N6</th>
<th>China</th>
<th>2016</th>
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<tr>
<td>H10N8</td>
<td>China</td>
<td>2013</td>
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<tr>
<td>H6N1</td>
<td>Taiwan</td>
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<tr>
<td>H10N7</td>
<td>Australia, Egypt</td>
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<td>H7N3</td>
<td>Mexico, UK, Canada, Italy</td>
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<td>H7N2</td>
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<td>H7N7</td>
<td>Netherlands, UK, USA, Austr., USA</td>
<td>2003, 96, 80, 77, 59</td>
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INFLUENZA VIRUS VACCINES

INACTIVATED
LIFE ATTENUATED
RECOMBINANT
INFLUENZA VIRUS VACCINE STRAINS 2016-2017

A/California/7/2009 (H1N1)pdm09
A/Hong Kong/4801/2014 (H3N2)

B/Phuket/3073/2013
B/Brisbane/60/2008
• INFLUENZA VIRUS VACCINES ARE UNIQUE.
• THEY HAVE TO BE GIVEN ANNUALLY, BECAUSE NOVEL VACCINE FORMULATIONS HAVE TO BE PREPARED REFLECTING THE RAPID ANTIGENIC CHANGE OF THE VIRUS.
Antigenic diversity: analysis of the flexible influenza A virus and rigid measles virus glycoproteins

Nicholas Heaton, PhD
Ben Fulton

Palese Lab
Icahn School of Medicine at Mount Sinai
INSERTION MUTATIONS ARE TOLERATED IN THE HEAD OF THE HEMAGGLUTININ

NICK HEATON
The measles virus glycoproteins (and the polymerase) are resistant to insertions.
TOLERANCE OF THE INFLUENZA A VIRUS AND OF MEASLES VIRUS GENOMES

**Influenza A Virus**

**Measles Virus**

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<tr>
<td>HA</td>
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<tr>
<td>PA</td>
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<td>L</td>
<td>0.00</td>
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<tr>
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HOW CAN WE DO BETTER?

UNIVERSAL INFLUENZA VIRUS VACCINES
Vision for a human universal influenza virus vaccine

pre-existing immunity against e.g. H1

full length H1

boost with cH5/1 construct

boost with cH6/1 construct
APPROACHES

• ADJUVANTS
• MVA-VECTORED
• M2e-BASED
• EPITOPES/PEPTIDES
• NEURAMINIDASE
• COBRA (computationally optimized broadly reactive antigens)
• STALK ONLY, HEADLESS HEMAGGLUTININ
• CHIMERIC HEMAGGLUTININ
Induction of protective levels of stalk-reactive antibodies using chimeric HA constructs in mice

Control groups:
ch9/1 DNA + BSA + BSA
matched vaccine (pos. contr.)
Induction of protective levels of stalk-reactive antibodies using chimeric HA constructs in mice

Control groups:
cH9/1 DNA + BSA + BSA
matched vaccine (pos. contr.)

PR8 H1N1
FM1 H1N1
pH1N1
H5N1
H6N1 challenge
Induction of protective levels of stalk-reactive antibodies using chimeric HA constructs in mice

Control groups:
ch9/1 DNA + BSA + BSA
matched vaccine (pos. contr.)
Induction of protective levels of stalk-reactive antibodies using chimeric HA constructs in mice

**PRIME**
- cH9/1 DNA
- cH6/1 protein

**BOOST**
- cH5/1 protein

**BOOST**
- PR8 H1N1 \(^{(1934)}\)
- FM1 H1N1 \(^{(1947)}\)
- pH1N1 \(^{(2009)}\)
- H5N1
- H6N1

**CHALLENGE**

Control groups:
- cH9/1 DNA + BSA + BSA
- matched vaccine (pos. contr.)
Vaccination with cHA constructs protects from pH1N1 (A/Netherlands/602/09) challenge

Similar results for A/PR/8/34 H1N1 and A/FM/1/47 challenges

Krammer et al. JVI, 87, 6542, 2013
cHA constructs protect mice from heterosubtypic challenge

**H5N1 challenge**

- **positive control (matched inactivated)**
- **cH9/1 DNA + H1 protein/cH6/1 protein + cH5/1 protein/H1 protein**
- **cH9/1 DNA + BSA + BSA**

**H6N1 challenge**

- **positive control (matched inactivated)**
- **cH9/1 DNA + H1 protein/cH6/1 protein + cH5/1 protein/H1 protein**
- **cH9/1 DNA + BSA + BSA**

cH5/1 (H5 challenge) or cH6/1 (H6 challenge) protein was replaced by full length H1 protein to exclude head-based protection.
cHA constructs protect ferrets from pH1N1 challenge

Krammer et al., JVI Jan. 8, 2014
Protection is antibody mediated

ELISA reactivity to Cal09 (pH1N1) protein

Passive transfer of serum protects from viral challenge

![Graph showing ELISA reactivity and percent survival](image)

- **OD 490 nm**
  - cH9/1 + cH6/1 + cH5/1
  - cH9/1 + BSA + BSA
  - naïve serum

- **Percent survival**
  - Naïve
  - Positive control
  - vector +BSA+BSA
  - cH9/1 + cH6/1 + cH5/1

**Graph Details**
- **Days post challenge**
  - Days 0 to 14
  - **** p = 0.0036
Targeting group 2 HA viruses
Protection against group 2 HA expressing viruses in the mouse model

PRIME

BOOST

BOOST

CHALLENGE

Control groups:

- cH4/3 DNA + BSA + BSA
- naïve (neg. contr.)
- matched vaccine (pos. contr.)

Margine et al., JVI, 87,10435, 2013
Group 2 cHA vaccine protects against challenge with novel H7N9*virus

*CH7/3 protein was replaced by full length H3 protein for the H7N1 challenge group

Krammer et al. JVI, 88, 2340, 2014
WHAT IS THE MECHANISM BY WHICH THESE BROADLY PROTECTIVE STALK-SPECIFIC ANTIBODIES MEDIATE THEIR ANTIVIRAL ACTIVITY?
Broadly neutralizing hemagglutinin stalk–specific antibodies require FcγR interactions for protection against influenza virus *in vivo*
Antibody-dependent Cell-mediated Cytotoxicity (ADCC) can be induced by stalk-specific, but not head-specific antibodies. Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

A

NO

Infected cell

Fab

Fab

Antibody

Fc

B

YES

Infected cell

Viral hemagglutinin
Can we elucidate the role epitope location plays in the induction of ADCC by broadly cross-reactive hemagglutinin antibodies?

Yes, by introducing FLAG epitopes into different locations in the viral hemagglutinin
A stalk-based FLAG epitope can induce FcγR-mediated effector function

Target Cell – Transfected HEK 293T cells (16 hpt)
Effector Cells – Jurkat cells expressing murine FcγRIV
Fold Induction = \frac{(RLU_{\text{Induced}} - RLU_{\text{Background}})}{(RLU_{\text{No mAb}} - RLU_{\text{Background}})}
Why do antibodies targeting the hemagglutinin head domain lack the ability to optimally induce ADCC activity?
Disruption of sialic acid engagement

• Blocking with Head-specific F(ab)_2

• Blocking with 6’ Sialyllactose

• Mutating Y108F in Receptor Binding Site
Head-specific F(ab)$_2$ prevents ADCC induction of stalk-specific 6F12 mAb

WT HA (PR/8)

PY102 (Head-specific)
6F12 (Stalk-specific)
Disruption of sialic acid engagement

- Blocking with Head-specific F(\(ab\))\(_2\)

- Blocking with 6’-sialyllactose

- Mutating Y108F in Receptor Binding Site
10 mM of 6’-sialyllactose decreases ADCC induction of stalk-specific antibodies
Disruption of sialic acid engagement

• Blocking with Head-specific F(\text{ab})_2

• Blocking with 6’ Sialyllactose

• Mutating Y108F in Receptor Binding Site
Y108F mutation lowers RLU values when compared to WT Cal09

Y108F plasmid was generated and provided by Madhu
Two-contacts model for optimal induction of ADCC by influenza virus-specific mAbs

A

Effector cell

No effector function induced

1

Infected cell

B

Effector cell

Effector function induced

1

Infected cell

Sialic acid

(host receptor for binding of viral hemagglutinin)

Fcy-receptor

Fab

Antibody

Fc

Viral hemagglutinin

Sialic acid binding site
SUMMARY

Towards a universal influenza virus vaccine by reducing the immunodominance of the hemagglutinin head and thereby increasing the immunogenicity of the hemagglutinin stalk and of the neuraminidase
Vision for a human universal influenza virus vaccine

Trivalent vaccine with group 1, group 2 and influenza B stalk component necessary

FLORIAN KRAMMER   ADOLFO GARCÍA-SASTRE   PETER PALESE
SUMMARY (cont.)

MECHANISM OF ADCC INDUCTION
(TWO-CONTACTS MODEL)

• The location of a FLAG-Tag epitope plays a critical role in determining the level of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) induction

• The ability of the hemagglutinin to bind to effector cells via its sialic acid receptor is required for optimal ADCC induction

• By blocking/mutating the sialic acid receptor binding site with F(\text{ab})_2, \text{6’-sialyllactose} or a Y108F mutation, ADCC induction can be lowered substantially
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