A Self-Assembling Influenza Nanoparticle Vaccine Elicit Broad and Potent Neutralizing Antibodies

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How Can We Improve Vaccine Against Flu?

Rapid and reliable production
• Alternative production systems instead of embryonated eggs
• Avoid adaptation and replication of real virus

Improve vaccine efficacy to provide protection for longer period
• Focus immune responses to the conserved sites
• Enhance magnitude of immune responses

HA is the major target of broadly neutralizing antibodies

Hypothesis:
Re-orient HA on a new platform may increase accessibility to the conserved stem Ab epitopes.
• Increase stem-directed neutralizing Ab titer and improve breadth of neutralization

400-500 HA spikes/virion

CDC
Broadly Neutralizing Abs against Influenza

Receptor Binding Site
Mimics sialic acid ligand
Subtype specific neutralization

Conserved Stem
Prevents post-fusion conformation
Group specific neutralization
Aim

- Structure-based approach to develop a novel platform to present conserved HA epitopes to the immune system
  - Trimeric structure integrity
  - Symmetrically ordered repetitive array (multivalency)
  - Optimized space between conserved immunogenic sites
Ferritin nanoparticle

Ferritin:
- Found in most organisms as an iron storage protein
- Forms spherical particle with octahedral symmetry consisting of 24 subunits
- Made of ~20 kDa protein
- Self-assembly capability

Ferritins have been engineered as:
- Biomedical imaging agent
- Semiconductor/bionanobattery
- Potential vaccine platform
Structure-Based Design of HA-Ferritin Nanoparticle

*Helicobacter pylori* ferritin

3-fold axis

Ferritin

HA

Asp5

Outside

Inside

N=24

28 Å

Nature. 2013, 499:102-6
Self-Assembly of HA-Ferritin Nanoparticles

A/New Caledonia/20/1999

HA-Ferritin

HA1 | HA2 | Ferritin

Gel filtration FPLC

Dynamic Light Scattering

SDS-PAGE

Nature. 2013, 499:102-6
HA Spikes on HA-Ferritin Nanoparticle Visualized by EM

Nature. 2013, 499:102-6
Antigenic Characterization of HA-Ferritin Nanoparticles

Ab specificity:

Head

- HA-Ferritin np
- TIV
- Trimer

A_450

Concentration (log_{10} \mu g ml^{-1})

Stem

- HA-Ferritin np
- TIV
- Trimer

Ferritin np

Nature. 2013, 499:102-6
Enhanced Immune Responses in HA-Ferritin-Immunized Mice

Nature. 2013, 499:102-6
Protective Immunity Induced in Ferrets Immunized with HA-Ferritin Nanoparticle

Nature. 2013, 499:102-6
Elicitation of Anti-Stem Neutralizing Abs in Immune Sera

Specificity: Stem

WT

Δstem

ΔStem

HA

45Nψ

Immune sera

Absorption

Test binding to intact HA

Nature. 2013, 499:102-6
Elicitation of Anti-RBS Neutralizing Abs in Immune Sera

Specificity: RBS

WT
ΔRBS

ΔRBS HA

Immune sera
Absorption

Test binding to intact HA

Nature. 2013, 499:102-6
Trivalent HA-Ferritin Nanoparticle Vaccine

A/California/04/09 (H1)  
H1 HA-Ferritin np

A/Perth/16/09 (H3)  
H3 HA-Ferritin np

B/Florida/04/06 (B)  
B HA-Ferritin np

Immunization: Neut virus:  
Trivalent HA-Ferritin Nanoparticles

Neut virus:  
H1N1 Mexico 09
H3N2 Perth 09
B Florida 06
Summary

- We have successfully developed a self-assembled HA-ferritin nanoparticle which displays properly folded, fully glycosylated, functional trimeric HA on its surface.
- Immunization of HA-ferritin induced higher HAI and neutralization titers than licensed TIV in both mice and ferrets: symmetrically ordered, repetitive arrays of HA might contribute to this enhanced immunogenicity.
- Breadth of neutralization was improved in HA-ferritin-immune sera, and it provided better protective immune responses against an unmatched H1N1 virus challenge in ferrets.
- Neutralizing Abs directed to two distinct conserved sites were elicited by HA-ferritin: anti-stem and anti-RBS Abs.
- HA-ferritins could also be made for H3 and Type B viruses.
- The engineered HA “stem only” (HA SS) expressed satisfied expression and antigenic criteria: trimers with antigenicity comparable to full-length HA. It was engaged membrane-bound stem-directed CR6261 IgM.
- HA SS-ferritin fusion formed nanoparticles and the immunogenicity of this novel immunogen is currently being evaluated.
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Enhanced Immune Responses in HA-Ferritin-Immunized Mice

IC$_{50}$ titers

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Nature. 2013, 499:102-6
Cellular and Humoral Immune Responses against *Helicobacter pylori* and Mouse Ferritins
Elicitation of Anti-Stem Neutralizing Abs in Immune Sera

Nature. 2013, 499:102-6
Elicitation of Anti-RBS Neutralizing Abs in Immune Sera

Virus: 1934 PR8 2007 Bris

Specificity: RBS Neut virus: 2007 Bris

HAI

Absorption

Test binding to intact HA

Immune sera

ΔRBS HA

190N

ΔRBS HA

Specificity: RBS

Neutral virus: 2007 Bris

Endpoint dilution (log2)

Neut (%)

Immunogen: Control TIV HA-Ferritin np

Competitor: Control Wild type ΔRBS

100

80

60

40

20

10

2

1

1

10

10

10

10

10

10

10

10

10

10