Hemagglutinin-stalk specific antibodies: How to induce them and how to measure them

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May 5th 2014

2nd WHO Integrated Meeting on development and clinical trials of Influenza vaccines that induce broadly protective and long-lasting immune responses

Antibodies against the influenza virus HA stalk domain

Antibodies against the stalk domain:

- Rare and not induced/boosted upon regular seasonal vaccination
- Have been isolated from humans and mice
- Cross-reactive between HAs of different subtypes
- Broad neutralizing activity
  - in vitro
  - in passive transfer studies in animals (ferrets, mice)
- Alternative mechanisms of neutralization +ADCC +CDC

Influenza virus hemagglutinin

Globular head domain: mediates binding to host receptors
Stalk domain: mediates fusion of viral and endosomal membranes

The HA stalk is conserved among group 1, among group 2 HAs and among influenza B HAs
Can protective levels of broadly neutralizing antibodies be induced by vaccination?

Chimeric hemagglutinins (cHAs)

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.)
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Vaccination with cHA constructs protects from pH1N1 (A/Netherlands/602/09) challenge

Group 1-Group 2 cross-reactivity is not sufficient to protect from virus challenge

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

Krammer and Pica et al., JVI, 2013
Conclusions from the animal studies

• A broadly protective immune response to the stalk domain can be induced by vaccine constructs in mice and ferrets
• Chimeric HA constructs protect mice and ferrets from challenge with heterologous and/or heterosubtypic virus strains
• Chimeric HA vaccination impacts on transmission (unpublished)
• The observed protection is antibody mediated
• Good protection from H7 challenge and strong reactivity to H7 HA of H7N9 origin, reduction in lung titers for H10 and good protection from challenge with H6N1
• A trivalent vaccine with an group 1, group 2 and influenza B stalk component will be needed

How can stalk-reactive antibodies be measured?

• Quantitative endpoint titer ELISAs using cHA e.g. ch6/1
  – If cHAs were used for vaccination head domain of ELISA substrate has to be different from head domain in the vaccine
• Neutralization assays using cHA viruses
  – Irrelevant NA (e.g. N3) needed
  – Microneutralization assay
  – Plaque reduction assay
  – Pseudotyped particle entry assay
• Passive transfer into mice and challenge with cHA viruses
  – Catches in vivo relevance
  – FcR-humanized mice can be used to optimally measure ADCC

Support for a cHA based universal influenza virus vaccine from H5N1 clinical trials

Induction of stalk-reactive antibodies by H5N1 vaccination – endpoint titer ELISA

In collaboration with Rebecca J. Cox, UiB
Passive transfer experiments in mice with day 0 and day 42 H5N1 sera and cH9/1N3 challenge

** p=0.0036
*, p=0.0243
*, p=0.0144

Conclusions

• Stalk-based vaccine constructs protect from heterologous and heterosubtypic challenge in animal models
• Assays to measure induction of stalk-reactive antibodies in sera are readily available
• H5N1 vaccination induces high levels of stalk-reactive antibodies in humans providing support for a cHA based vaccine

Acknowledgements

• Peter Palese
• Raffael Nachbagauer
• Teddy John Wohlbold
• Irina Margine
• Natalie Pica
• Rong Hai
• Ariana Hirsh

• Adolfo García-Sastre
• Randy Albrecht
• Rebecca Cox/UiB

Austrian Science Fund