Vaccines in Modern Era: New Paradigms to Address Unmet Needs

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A Biomarker for Successfully Licensed Vaccines: Serotypes

Three poliovirus strains found in Nature: three serotypes are required for a protective vaccine.
28 Licensed Vaccines to 24 Infectious Diseases

- Anthrax
- Diphtheria
- *Haemophilus influenzae* type b
- Hepatitis A
- Hepatitis B
- Herpes Zoster (shingles)
- Human papillomavirus
- Influenza A, B
- Pertussis
- Pneumococcal disease
- Polio
- Rabies
- Rotavirus
- Rubella
- Smallpox
- Tetanus
- (BCG)
- Japanese Encephalitis
- Tuberculosis
# The Burden of Infectious Diseases without Vaccines

<table>
<thead>
<tr>
<th>Disease</th>
<th>Prevalence</th>
<th>Deaths</th>
</tr>
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<tbody>
<tr>
<td>HIV/AIDS</td>
<td>33.4 million infected</td>
<td>2.0 million</td>
</tr>
</tbody>
</table>
| Tuberculosis| ~ 2 billion infected
9.4 million active cases | 1.8 million |
| Malaria     | 243 million cases                 | 863,000   |

Sources: UNAIDS, WHO
Can HIV-1 Be Serotyped? Contrast with Polio

Infinite number of viruses

? Role of Abs in immunity

Evolving neutralization profiles
A Site of Vulnerability to Antibody
Strategy for Isolation of New Monoclonal Antibodies Based On HIV Protein Structure

Designer Envelopes

Core → Stabilized Core → Resurfaced Stabilized Cores (RSC)

- Stabilizing inner domain and bridging sheet
- Stabilizing the inner/outer domains
Resurfaced Stabilized Cores: Probes for Human Abs and Templates for Immunogens

1. Probe to isolate B cells and clone broadly neutralizing abs
2. Prototype immunogens to elicit antibodies to the highly conserved CD4 binding site
Strategy for Isolation of New Monoclonal Antibodies Based On HIV Protein Structure: Rescue of Antigen-Specific B Cells

1. Select special subjects with broadly neutralizing, potent antiserum

2. Incubate B cells with wild type and CD4 binding site mutant resurfaced cores

3. Select for CD4 binding site-specific B cells by flow cytometry with positive selection on wild type and negative selection on mutant resurfaced cores

4. PCR amplify and express IgG of HIV-1 specific neutralizing antibodies

Three mAbs bind to the RSC protein

- Two closely related somatic variants (VRC01, VRC02)
  - bind to CD4bs region of gp120
  - Neutralize ~90% viruses, often < 1ug/ml

- 1 additional mAb (VRC03)
  - CD4bs directed
  - Neutralizes ~ 60% viruses

Pan-Reactive Antibody VRC01

Mimicry of CD4 Receptor by Antibody VRC01

CD4 and VRC01 in highly similar positions
Why does VRC01 Work So Well?

1. Partial mimicry of CD4 binding to gp120

2. Binding focused on the conformationally invariant site of initial CD4 attachment.
454 pyrosequencing to Identify Additional VRC01-like Antibodies

- Known mAbs (VRC01 – 03): Use knowledge of specific gene usage and structural motifs to identify and study the family of related antibodies in a specific donor

- cDNA library from donor B cells; isolate antibody heavy chain sequences; analyze sequence and predicted structural motifs – to find VRC01-like antibodies

- Understand lineage and evolution of affinity maturation of antibody responses
Evaluation of 454 sequences

Distribution of IGHV1-2*02 divergence

Sequence similarity to VRC01

57203 heavy chain

- Only 59% aa sequence homology to VRC01
- Only 9% divergence from germline

J Zhu, L Shapiro, T Zhou (Kwong lab)
Eliciting VRC01-like Antibodies...

Elicitation depends on three stages of antibody development: recombination, deletion of autoreactive antibodies, and affinity maturation.

Engaging the B Cell Receptors
Affinity Maturation and VRC01 Affinity

Affinity Matured AA’s Needed for Env Binding

VRC01 germ line

Mature VRC01
Design of Immunogens to Elicit Broadly Neutralizing Abs to the CD4 Binding Site

Structure-based design:

1. Trimers
2. Monomers
3. Outer Domains
Induction of CD4 BS Antibodies by Glycan Modified RSC3: Y5
Induction of CD4 BS Antibodies by Glycan Modified RSC3: Y5
Summary

1. An understanding of HIV-1 “serotypes” has presented a major conceptual challenge to the AIDS vaccine scientific community. A solution to this problem is developing through increased success of the field in identifying broadly neutralizing human monoclonal antibodies.

2. Definition of the specificities and targets of broadly neutralizing antisera and monoclonal antibodies have facilitate the identification of “structural” serotypes.

3. It is now possible to elicit CD4 BS neutralizing abs through structure-based vaccine design with trimeric Env proteins, modified core protein (RSCs), and possibly with arrayed ODs. Further modifications of these prototypes are in progress that may improve their breadth of neutralization.
Scope of Clinical Applications of Anti-HIV Neutralizing Antibodies

Scope

• Prevention

• Therapy

• Eradication of reservoir
Influenza Vaccines-The Yearly Cost

New vaccine every year

120-150 million doses per year

2.8-4.0 billion dollars total expenditure
Can We Make a Better Vaccine?

Improve potency

Increase breadth

Can we make a universal influenza vaccine that is administered during childhood and lasts a lifetime?
Influenza: Broadly Neutralizing Antibodies

Heterosubtypic Neutralizing Antibodies are Produced by Individuals Immunized with a Seasonal Influenza Vaccine
Davide Corti, et al. JCI 120, 2010

Antibody Recognition of a Highly Conserved Influenza Virus Epitope.
Damian C. Ekiert, Gira Bhabha, Marc-André Ebliger, Robert H. E. Friesen, Mandy Jongeneelen, Mark Throsby, Jaap Goudsmit, Iain A. Wilson

A Common Neutralizing Epitope Conserved Between the Hemagglutinins of Influenza A Virus H1 and H2 Strains.
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Combinatorial Antibody Libraries from Survivors of the Turkish H5N1 Avian Influenza Outbreak Reveal Virus Neutralization Strategies.

Structural and Functional Bases for Broad-Spectrum Neutralization of Avian and Human Influenza A Viruses.

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Interaction of a Broadly Neutralizing Influenza Antibody with Hemagglutinin

Structural Basis for Broad Recognition of HA

>700 human H1N1 strains; Cyan, 100% conservation; Red, 98% conservation

Jeffrey Boyington and Gary Nabel
Questions

• Can we elicit broadly neutralizing HA antibodies through immunization?
  -DNA/Seasonal vaccine or DNA/rAd

• Can this prime-boost regimen increase the breadth of neutralizing antibodies against other H1 HAs?
### Increased Breadth of Neutralization by Prime-Boost Immunization

#### Immune Mouse

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#### Immune Ferret

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#### Immune NHP

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<th>Virus</th>
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<th>1995 Bei</th>
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<tr>
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<tr>
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Pseudotyped IC<sub>50</sub> titers
1999 NC HA DNA/Vaccine Prime-Boost Immunization Protected Mice against 1934 PR8 Challenge

Mouse Virus: 1934 PR8

- PR8
- DNA/PR8 rAd
- DNA/Vaccine
- Control
- Vaccine Only
- DNA only

% Survival vs Day after challenge
Anti-Stem mAb C179 Binds to Wild-type 1999 NC Trimer but Does Not React with Stem Mutant (ΔStem)

Site of stem antibody binding

H1N1 Target: mAb: 1934 PR8 C179

Neutralization Inhibition (%)

Trimer Competitor: HIV WT ΔStem

IP: Control C179
WT ΔStem WT ΔStem

HA
Cell Absorption and mAb Competition Assay

Human sera

Cell absorption

Anti-stem Ab

Anti-head Ab

293 Cells expressing △Stem HA

Competition with anti-stem or anti-head mAbs

ELISA
Evidence of Stem-Directed Antibodies Elicited by DNA/Vaccine Immunization in Humans

H5 HA Binding (OD490) vs. Dilution (log2)

- Pre
- Post (DNA/Vac, 6 mo.)

- Control
- Anti-stem
- Anti-head
1. Vaccination with plasmid DNA encoding H1N1 influenza HA and boosting with seasonal vaccine or rAd stimulated the production of broadly neutralizing influenza antibodies in mice, ferrets, and NHPs.

2. This vaccine protected mice against lethal challenge by a seasonal strain dating back to 1934, and also conferred protection against divergent H1N1 viruses from 1934 and 2007 in ferrets.

3. These broadly neutralizing antibodies were directed to the conserved stem region of the HA and were also elicited in monkeys and humans and provide the basis for a first-generation universal flu vaccine.
The Product Development Cycle for Challenging Vaccines

- Basic Science
- Translational Science
- Clinical Science
- Efficacy Trials and Licensure
- Production and Assay Technology
Vaccine Development at the VRC

Development Cycle at the VRC

Basic Research

Immune Assessment

Clinical Trials

cGMP Production