The Nature of Protective Antibodies

Robin A. Weiss

AIDS Vaccine 07
HIV positive individuals have weak but broad neutralising activity

<table>
<thead>
<tr>
<th>Virus</th>
<th>n</th>
<th>ELISA</th>
<th>IFA</th>
<th>Neutralisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTLV-1</td>
<td>9</td>
<td>1216</td>
<td>2250</td>
<td>3257</td>
</tr>
<tr>
<td>HIV-1</td>
<td>12</td>
<td>1272</td>
<td>2847</td>
<td>~50</td>
</tr>
</tbody>
</table>


Antisera raised against recombinant gp120 neutralised only the same strain of HIV

Protective and Non-Protective Antibodies

- Lessons from other infections
- Neutralizing antibodies
- Non-neutralizing antibodies
- Effector mechanisms
- Harmful antibodies?
Neutralization as a Correlate of Protection

Rolf Zinkernagel (Science 2003)

- All virus vaccines to date elicit neutralizing antibodies
- HIV elicits poor neutralizing antibodies
- Will efficacious HIV vaccines ever be realized?
- Isn’t the Global HIV Vaccine Enterprise pie in the sky?
Virus vaccines to date elicit protective antibodies

- Smallpox, Yellow fever, Rabies, MMR
- Polio: serotypes 1, 2 & 3
- Influenza: neutralization and hemagglutination inhibition (HI)
- Hepatitis B
- HPV

- WHO reference criteria for protection are based on antibody responses
- But cell-mediated immunity is seldom studied
Neutralizing Titers to SARS Coronavirus
(using an HIV pseudovirus assay)

Temperton et al, Emerg Infect Dis 11: 411-416, 2005

Studies with Xiao-Ning Xu on 128 recovered SARS patients show only 40% with CTL responses but 98% with neutralizing antibodies (Li et al, 2007)
Pseudotype Neutralisation Assay for H5N1 HA

1. Three plasmid transfection of 293T cells

2. Add neuraminidase and harvest pseudotype virus: titre 8 \( \times 10^6 \)

3. Incubate pseudotype with Ab and infect cells

4. Count fluorescent cells or rlu

(Temperton et al, 2007)
H5N1 influenza: Pseudotype neutralisation correlates with hemagglutination inhibition

N = 56
HI \( R^2 = 0.93 \)
MN \( R^2 = 0.78 \)

Nigel Temperton, Maria Zambon, Ian Brown, John Wood, M de Jong
UCL                  HPA                  VLA        NIBSC        Viet Nam
(Influenza & Other Respiratory Infections, 2007)
Combinations of HA H7 with NA N1 allow tests for “neutralisation” directed to NA of H5N1.
Findings on Neuramidase serology

Antibodies to NA are protective:

• They neutralize enzyme activity
• They act on virus maturation not virus entry
• They do not neutralize virus particles
Humoral immunity to enveloped viruses: we tend to forget complement

C1q
C3
MAC

CRPs:
CD55
CD46
CD59
Development of Specific Immunity to HIV-1 Infection

Non-neutralizing antibodies

- May help to clear primary infection
- May be important in ADCC
- Denatured spikes and gp41 stumps exceed number of functional spikes on HIV-1 virions
- May enhance infection
Antibody Enhancement

- Low affinity, poorly neutralizing Ab attach to virions
- Tethers virus to FcR Complement R
- Enhances virus infection of cells bearing FcR or CR, eg MØ
- Explains greater virulence of Dengue hemorrhagic fever upon second infection by a different serotype
- Could a similar problem arise for HIV based vaccines?
How is antibody enhancement mediated in primary HIV infection?

(Suzy Willey, Marlen Aasa-Chapman, Aine McKnight, Robin Weiss, 2007)
Neutralizing antibody:

b12 (IgG1) more potent *in vivo* if Fc is intact (Dennis Burton)

Non-neutralizing antibody:

C’ effector mechanisms may be more important
Could humoral immunity be harmful rather than protective?


Vaccinees immunized with gp120: greater enhancing titers than neutralizing titers
(Suzy Willey, thesis dissertation 2007)
HIV diversity and neutralizing epitopes

The length of each spoke indicates how far the virus envelope has mutated

(Gaschen & Korber, 2001)
Llamas: a curious route to HIV neutralization

Hans de Haard & Theo Verrips
University of Utrecht
*Camelidae* (llamas, dromedaries, camels) have classical and non-classical IgG

VHH recognising HIV may be useful as vaginal microbicides and for vaccine development:

- rapid screening of libraries
- long CDR3 loops
- small and stable 12-15kd proteins
- mapping neutralizing epitopes
Screening for VHH targeting the CD4 binding site of gp120

Selection strategy: elution with soluble CD4

Express eluted VHH
Test: binding to gp120
competition with sCD4
competition with b12
neutralization of HIV-1
VHH neutralisation of selected HIV-1 isolates of subtype B and C

<table>
<thead>
<tr>
<th>VHH</th>
<th>B</th>
<th>C</th>
<th>IC$_{90}$ (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>III B</td>
<td>SF162</td>
<td>SVPB6</td>
</tr>
<tr>
<td>A12</td>
<td>1.7</td>
<td>7.8</td>
<td>●</td>
</tr>
<tr>
<td>D7</td>
<td>3</td>
<td>9.3</td>
<td>●</td>
</tr>
<tr>
<td>C8</td>
<td>7</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>b12</td>
<td>0.8</td>
<td>1.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

● >50 µg/ml
Vaccine Targets – Protective Antibodies

- Preventing infection
  - Hepatitis B
  - Measles
  - HIV prophylactic vaccine

- Preventing virulence
  - Anti-toxins: Tetanus, Diphtheria
  - Disease symptoms: Marek's disease
  - HIV therapeutic vaccine

- Preventing systemic infection
  - Polio (non-sterilizing but effective)
  - P Debré: protective, non-neutralizing HIV Ab

Emil von Behring
Conclusions:

Neutralization is important

But there is more to protective humoral immunity than neutralizing antibodies

Beware enhancing antibodies in clinical trials
Division of Infection and Immunity

- Marlen Aasa-Chapman
- Anna Forsman
- Willie Koh
- Nigel Temperton
- Suzy Willey
- Ed Wright