Using DNA Prime to Induce High Quality Antibody Responses

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Our HIV vaccine concept

Objective
Balanced neutralizing antibody and CMI responses

Strategies
1. Multi-gene: env, gag…
2. polyvalent: primary env from major subtypes
3. DNA prime + protein boost
The DNA prime – protein boost (both in gp120 form) was able to elicit positive neutralizing antibodies against JR-FL, a relatively resistant virus (in both PBMC and target cell line systems)
Neutralization against 14 HIV-1 viruses from clades A, B, C, D, E

Wang et al., Virology, 2006, 350:34-47

DNA prime-protein boost is more effective than protein alone in eliciting broad Nab (by using a polyvalent Env formulation)
Anti-gp120 IgG in non-human primates
(Geometric means of 3 animals)

DNA priming is not dependent on the delivery method of DNA vaccines, once a protein boost is given.

Pal et al., Virology, 2006, 348:341-353
Viral load post challenge with $\text{SHIV}_{\text{Bal}}$ in non-human primates

**Immunized macaque group**

- Days post Challenge: 0, 20, 40, 60, 80, 100
- Plasma viral RNA copies: $10^3$ to $10^7$

- Lines and markers for each macaque:
  - 961L
  - 963L
  - 969L
  - 971L
  - 974L
  - 975L

**Naive macaque group**

- Days post Challenge: 0, 20, 40, 60, 80, 100
- Plasma viral RNA copies: $10^3$ to $10^7$

- Lines and markers for each macaque:
  - 184M
  - 185M
  - 186M
  - 43M
  - 44M
  - 46M
  - 48M
HIV-1 Vaccine Formulation DP6-001

• DNA vaccines (priming phase)
  – Primary HIV-1 Env (gp120): A, B, Bal, Czm, E
  – Gag antigen: Czm

• Protein vaccines (boosting phase)
  – Recombinant gp120 protein: A, B, Bal, Czm, E
    • Produced from serum free CHO cells
    • Homologous to Env DNA priming
  – Adjuvant QS-21 (in cyclodextrin excipient) with protein boost

A: 92UG037.8       B: 92US715.6
C: 96ZM651          E: 93TH976.17     Bal: Ba-L          (F. Gao and B. Hahn)

Wang et al. Vaccine, 26:1098 (2008)
• Group A: N = 11; DNA per dose = 1.2 mg - i.d. x 4 sites
• Group B: N = 11; DNA per dose = 1.2 mg - i.m. x 2 sites
• Group C: N = 10; DNA per dose = 7.2 mg - i.m x 2 sites

All groups received a total of 0.375 mg recombinant gp120 proteins at each boost by i.m. at 1 site.
**HIV-1 gp120-Specific Antibody Responses in DP6-001 Vaccinees**

**Group A (low dose DNA-ID)**

**Group B (low dose DNA-IM)**
**Broad Recognition to Heterologous Primary Env Antigens**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>5</th>
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<th>10</th>
<th>11</th>
<th>12</th>
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<tbody>
<tr>
<td><strong>DP6-001 Vaccinee sera</strong></td>
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<td>003 Pre-bleed</td>
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<td>003 Bleed 12 (after protein-2)</td>
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<td><strong>HIV infected patient sera (NIH)</strong></td>
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1. Mock  
2. CA1 (A)  
3. UG21-9 (A)  
4. JR-FL (B)  
5. ADA (B)  
6. SF162 (B)  
7. 92BR025.9 (C)  
8. CN54 (C)  
9. 92UG021 (D)  
10. CM235 (E)  
11. 93BR020.17 (F)  
12. 92UG975.10 (G)
Nab against Pseudotyped Viruses Expressing HIV-1 Env Antigens

(three sensitive viruses)

**Group A: Prot-2**

- **MN**
- **NL4-3**
- **SF162**

**Group B: Prot-2**

- **MN**
- **NL4-3**
- **SF162**
Percentage of vaccinees with NAb responses against primary isolates

<table>
<thead>
<tr>
<th># of volunteers (%)</th>
<th># of viruses neutralized</th>
<th>% of total virus neutralized</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (29%)</td>
<td>9 to 11</td>
<td>80-100%</td>
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<tr>
<td>6 (29%)</td>
<td>6-8</td>
<td>50-79%</td>
</tr>
<tr>
<td>6 (29%)</td>
<td>3-5</td>
<td>25-49%</td>
</tr>
<tr>
<td>2 (10%)</td>
<td>1 to 2</td>
<td>1-24%</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0%</td>
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</table>

Note: NAb titer > 1:20 are scored positive in Monogram’s assay.
Are DNA vaccines expressing gp120 same as the recombinant monomeric gp120 proteins?

\[ M \text{ Vaine et al., J of Virology, 82:7369-7378.} \]
Summary of antibody profile analysis

- Protein alone: V3 is more dominant
- DNA-protein: V3, CD4-bs and possible other conformational epitopes

Implication

DNA primed hosts are more capable than the recombinant Env proteins alone in eliciting antibodies against conformation epitopes.
Matched vs. unmatched prime-boost

Matched vs. unmatched prime-boost

<table>
<thead>
<tr>
<th>Prime</th>
<th>Boost</th>
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<tbody>
<tr>
<td>Vector DNA</td>
<td>JR-FL rgp120</td>
</tr>
<tr>
<td>JR-FL rgp120</td>
<td>JR-FL rgp120</td>
</tr>
<tr>
<td>JR-FL gp120 DNA</td>
<td>JR-FL gp120 DNA</td>
</tr>
<tr>
<td>JR-FL gp120 DNA</td>
<td>JR-FL rgp20</td>
</tr>
</tbody>
</table>

*5 Valent rgp20

Week 0 2 4 8 12

Anti-gp120 binding Ab titers by ELISA

JR-FL gp120

Endpoint Binding Titer

3V + 2P  5P  5D  3D + 2P  3D + 2pP
NAb against TCLA/sensitive HIV-1 isolates

SF162

p = 0.024

NL4-3

p = 0.028

NAb Titer

3V + 2P

5P

5D

3D + 2P

3D + 2pP

1

10

100

1000

10000

p = 0.024

p = 0.028

p = 0.024
Avidity of gp120 specific IgG
gp120-specific mouse ASC

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>DNA x3</th>
<th>DNA+Protein x2</th>
<th>Protein x3</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp120-specific</td>
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<tr>
<td>Total IgG</td>
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<tr>
<td>Bone Marrow</td>
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<tr>
<td>gp120-specific</td>
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<tr>
<td>Total IgG</td>
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<tr>
<td>Spleen</td>
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DNA prime-protein boost: an emerging vaccination technology platform

• Broaden antibody specificities
  – antibodies against conformation sensitive epitopes
• Improve antibody quality
  – avidity
  – overall improved B cell development (with better T help?)
• Other advantages
  – Polyvalent formulation
  – Test of designer’s antigens
  – Minimize the dosing and adjuvant requirement
# What to prime: DNA vs. viral vector

<table>
<thead>
<tr>
<th>DNA</th>
<th>Viral vector</th>
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<tbody>
<tr>
<td>Low immunogenicity</td>
<td>Low to moderate immunogenicity</td>
</tr>
<tr>
<td>Focused immune responses: inserted gene product</td>
<td>Response to both inserted and vector genes</td>
</tr>
<tr>
<td>No pre-existing anti-vector immunity</td>
<td>Possible pre-existing immunity and interference</td>
</tr>
<tr>
<td>Overall safer</td>
<td>Additional biological functions</td>
</tr>
<tr>
<td>Easy production</td>
<td>More complicated production process</td>
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</table>
Heterologous prime–boost vaccination
Shan Lu¹,²

An effective vaccine usually requires more than one time immunization in the form of prime–boost. Traditionally the same vaccines are given multiple times as homologous boosts. New findings suggested that prime–boost can be done with different types of vaccines containing the same antigens. In many cases such heterologous prime–boost can be more immunogenic than homologous prime–boost. Heterologous prime–boost represents a new way of immunization and will stimulate better understanding on the immunological basis of vaccines.

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Over the past decade, studies have shown that prime–boost immunizations can be given with unmatched vaccine delivery methods while using the same antigen, in a ‘heterologous’ prime–boost format. The most interesting and unexpected finding is that, in many cases, heterologous prime–boost is more effective than the ‘homologous’ prime–boost approach. The rapid progress of novel vaccination approaches, such as DNA vaccines and viral vector-based vaccines, has certainly further expanded the scope of heterologous prime–boost vaccination [1–3] (Table 1).

Early history of heterologous prime–boost vaccination
A 1992 landmark Science report was among the first to employ the heterologous prime–boost immunization technique in a non-human primate model [4*]. In that study, Macaca fascicularis were first immunized with recombinant vaccinia virus expressing SIVmne gp160 antigen and then boosted with gp160 protein produced in baculovirus-infected cells. Animals were protected from intravenous challenge of SIVmne viruses and this became one of the most promising protection results in the early HIV vaccine development effort.