Abstract presented at XVIII Intnl AIDS Conference 2010, July 18-23, Vienna, Austria

Prototype covalent HIV vaccine for inducing antibodies that neutralize genetically divergent virus strains
S. Planque1, Y. Mitsuda1, D. Ghosh1, Y. Nishiyama1, C.V. Hanson2, S. Paul1
1University of Texas Health Science Center at Houston, Medical School, Chemical Immunology Research Center, Department of Pathology, Houston, United States, 2Viral and Rickettsial Disease Laboratory, California Department of Public Health, Richmond, United States

Background: The conserved CD4 binding site (CD4BS) of HIV is vulnerable to host immunity as it is essential for virus propagation. However, HIV infection and previous vaccine candidates do not induce neutralizing anti-CD4BS antibodies, presumably because the superantigen character of the CD4BS downregulates B cell adaptive immunity. Rare monoclonal antibodies to the exposed CD4BS residues 421-433 were detected by immunization with gp120 containing electrophiles that bind covalently to B lymphocytes (JBC 2009, 284:30627). This suggested covalent immunization as a strategy to overcome the poor CD4BS immunogenicity.

Methods: BALB/cJ mice and New Zealand White rabbits were immunized with synthetic E-416-433, a mimetic of CD4BS residues 421-433 containing electrophilic phosphonates at Lys residues. HIV neutralization was assayed using human peripheral blood mononuclear cells and primary virus isolates. Antibody binding was measured by ELISA. IgG was purified using immobilized protein G. Epitope-specific antibodies were purified using immobilized E-416-433.

Results: E-416-433 displayed specific binding to soluble CD4, suggesting that it adopts the native CD4BS conformation found on HIV surface. Immunization of mice and rabbits with KLH-conjugated E-416-433 induced polyclonal IgG and IgA responses with E-416-433 binding activity in blood and vaginal lavage fluid. Apparent affinity constants for antibody binding to E-416-433 were 36-41 nM. The sera and purified antibody fractions neutralized genetically diverse HIV strains from subtypes A, B, C and D. Epitope-specific antibodies purified using immobilized E-416-433 displayed enriched neutralizing and E-416-433 binding activities. Soluble CD4 inhibited antibody binding to E-416-433, confirming CD4BS specificity.

Conclusions: E-416-433 is the first HIV vaccine candidate capable of inducing polyclonal anti-CD4BS antibodies that neutralizes diverse HIV strains in experimental animals. Properties underlying success of the E-416-433 immunogen are mimicry of the native CD4BS conformation and ability to bind B cells covalently, a highly energetic reaction that increases CD4BS immunogenicity.
Prototype covalent HIV vaccine for inducing antibodies that neutralize genetically divergent virus strains

Planque S\textsuperscript{1}, Mitsuda Y\textsuperscript{1}, Ghosh D\textsuperscript{1}, Nishiyama Y\textsuperscript{1}, Hanson CV\textsuperscript{2}, Paul S\textsuperscript{1}

\textsuperscript{1}Chemical Immunology Research Center, Depts of Pathology and Pediatrics, Univ Texas–Houston Med School; \textsuperscript{2}Viral and Rickettsial Disease Laboratory, California Dept of Public Health

\textit{Disclosures}
NIH: Grants R01 AI067020, R01 AI085565
Covalent Immunology Products Inc: Planque and Nishiyama are consultants; Paul is Board Member
Central Problems in HIV Vaccination

- No or marginal protection against infection by previous test vaccines
- Mutability of immunodominant viral epitopes
- Poor adaptive response to conserved epitopes essential for viral life cycle

Hypothesis

Focusing the immune response at a structurally conserved epitope is necessary for effective HIV vaccination
Very few broadly gp120 neutralizing epitopes

<table>
<thead>
<tr>
<th>Abs</th>
<th>Epitope location</th>
<th>Function</th>
<th>Vaccine Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Epitope accessibility</strong></td>
</tr>
<tr>
<td>IgG b12, IgG VRC01</td>
<td>Conformational CD4BS epitope in exposed gp120 outer domain</td>
<td>Initial CD4 contact site</td>
<td>No evidence for limiting epitope exposure</td>
</tr>
<tr>
<td>IgG YZ23, scFv JL413, scFv JL427, IgA LTS</td>
<td>Linear CD4BScore epitope, residues 421-433; exposed bridging sheet</td>
<td>Peptide mimetics bind CD4</td>
<td>No evidence for limiting epitope exposure</td>
</tr>
<tr>
<td>IgG 2G12</td>
<td>Glycan substituents, e.g., at residues 332, 392</td>
<td>Not known</td>
<td>No evidence for limiting epitope exposure</td>
</tr>
<tr>
<td>IgG X5, IgG 17b, IgG 48d</td>
<td>CD4-induced epitope; β2, β3, β20-β21 regions</td>
<td>Coreceptor binding</td>
<td>Exposure requires prior CD4 binding</td>
</tr>
<tr>
<td>IgG 447-52D</td>
<td>V3 domain apex, residues GPXR</td>
<td>Coreceptor binding</td>
<td>Full exposure may requires CD4 binding</td>
</tr>
<tr>
<td>IgG PG9, IgG PG16</td>
<td>Conformational epitope; V2, V3, C4 domain residues</td>
<td>Not known</td>
<td>No evidence for limiting epitope exposure</td>
</tr>
<tr>
<td>Polyclonal Abs to CD4-independent gp140</td>
<td>Epitope not identified</td>
<td>Not known</td>
<td>CD4-independent but not CD4-dependent epitope may be exposed</td>
</tr>
</tbody>
</table>
The vulnerable but challenging CD4BS\textsuperscript{core}: E-416-433 mimetic

Solving conformational flexibility problems: E-416-433 binds CD4 120-fold better than previous CD4BS\textsuperscript{core} peptides (K\textsubscript{d}=110 nM). E-416-433 is combined probe for reversibly-binding and catalytic CD4BS\textsuperscript{core} reagents.
The vulnerable but challenging CD4BS\textsuperscript{core} - superantigenicity

*Left,* Modest preimmune human Ab recognition of CD4BS\textsuperscript{core} (neutralization+catalysis); Mouse/rabbit preimmune Abs recognize gp120 similarly; Others reported CD4BS\textsuperscript{core} SAg character from binding studies

*Bottom,* Deficient IgM→IgG class switching

[Abstract MOPE0045]
**CD4BS\textsuperscript{core} vulnerability in long-term survivors**

416-433 epitope:
- Autologous, clade B ..........................
- Heterologous, clade C 97ZA009  .............R.

296-331 V3 loop:
- Autologous, clade B CIRPNNTRRSVHIGPGSAFYTTGEIIGNIRQAHC
- Heterologous, clade C 97ZA009 ..G........MR....QV..A.NG.........

---

**Left, Total IgA**

**Right, CD4BS\textsuperscript{core}-specific IgA**

*Planque et al AIDS 2010*
Polyclonal Abs raised by E-416-433 covalent immunization

Covalent immunization of mice with full-length E-gp120 induced broadly neutralizing anti-CD4BS\textsuperscript{core} IgG MAbs (Nishiyama JBC 2009) and rectified the problem of deficient IgG class switching (poster at this meeting). To focus the Ab response at the CD4BS\textsuperscript{core}, E-416-433 was employed as immunogen. Class-switched Ab responses were evident.
Mouse and rabbit neutralizing polyclonal Abs raised by E-416-433 covalent immunization

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Coreceptor</th>
<th>Strain</th>
<th>Titer</th>
<th>416-433 epitope sequence</th>
<th>V domain divergence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rabbit</td>
<td>Mouse</td>
<td>V1</td>
</tr>
<tr>
<td>A</td>
<td>R5</td>
<td>97USSN54</td>
<td>296</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>92BR021</td>
<td>125</td>
<td>170</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>SHIV&lt;sub&gt;SF162P3&lt;/sub&gt;</td>
<td>NA</td>
<td>101</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>X4</td>
<td>92HT599</td>
<td>50</td>
<td>109</td>
<td>91</td>
</tr>
<tr>
<td>B</td>
<td>R5</td>
<td>97ZA009</td>
<td>1714</td>
<td>1238</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>98TZ017</td>
<td>61</td>
<td>NA</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>R5</td>
<td>98TZ013</td>
<td>NA</td>
<td>351</td>
<td>80</td>
</tr>
<tr>
<td>C</td>
<td>R5</td>
<td>94UG114</td>
<td>160</td>
<td>61</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>X4</td>
<td>92UG001</td>
<td>50</td>
<td>NA</td>
<td>90</td>
</tr>
</tbody>
</table>

Serum dilution, fold

Mouse anti-E-416-433 serum (group 1)
Mouse anti-E-416-433 serum (group 2)
Nonimmune serum

Rabbit anti-E-416-433 serum
Rabbit nonimmune serum
HIV neutralization by the induced Abs is due to E-416-433 recognition
CD4BScore-specificity of E-416-433 induced polyclonal IgG

Mouse anti-416-433 IgG, Kd: 41 nM
Rabbit anti-416-433 IgG, Kd: 36 nM

Anti-E-416-433 IgG, IC50: 7 µg/mL
Pre-immune IgG, IC50: 44 µg/mL
ovalbumin
Molecular determinants of vaccine efficacy

- Rigid, correct conformation of E-vaccine needed for adaptive neutralizing Ab synthesis

![Diagram showing the relationship between conformation and antibody production](image)

- Highly energetic B cell stimulation to bypass physiologically forbidden adaptive B cell response

![Diagram showing B cell stimulation and antibody production](image)

- Bonus: An E-vaccine induced Ab subset catalyzes gp120 degradation rapidly, increasing vaccine efficacy

![Graph showing gp120 hydrolysis](image)
Conclusions

- E-416-433 a conformationally correct CD4BS mimetic that binds CD4 autonomously.

- Unlike traditional immunogens, E-416-433 stimulates B cells covalently, bypassing the problem of B cell downregulation due to its superantigenicity, and inducing IgM $\rightarrow$ IgG/IgA class-switching required for effective protection.

- E-416-433 induced polyclonal neutralizing Abs in two animal species with the ability to neutralize genetically divergent strains.

- E-416-433 is the first prototype vaccine holding out the hope that a broadly neutralizing polyclonal Ab response to a conserved epitope can be induced for globally effective HIV vaccination.

Future direction: Improve neutralizing titers by optimizing the vaccination protocol (route, adjuvant, carrier protein).
## Acknowledgments

<table>
<thead>
<tr>
<th>Yukie Mitsuda</th>
<th>Sudhir Paul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert Dannenbring</td>
<td>Yasuhiro Nishiyama</td>
</tr>
<tr>
<td>Dipanjan Ghosh</td>
<td>Carl Hanson</td>
</tr>
<tr>
<td>Maria Salas</td>
<td></td>
</tr>
<tr>
<td>Mary-Kate Morris</td>
<td><strong>David Montefiori</strong></td>
</tr>
<tr>
<td>Mylene Volk</td>
<td><strong>Christina Ochsenbauer</strong></td>
</tr>
</tbody>
</table>