Mapping Autologous Neutralization Targets in Newly Transmitted Clade C Primary HIV-1 Isolates

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Three desirable properties of humoral vaccine targets are:

1- Sensitive mediators of neutralization
   a- Must be exposed on the surface of native viral particles
   b- Should not be masked by other domains or by glycans
   c- Antibodies bound to these sites should inhibit some function essential for viral entry

2- Highly immunogenic

3- Broadly conserved across many isolates (and clades)

Empirical evidence suggests that for HIV, targets with all three properties may not exist
- Any Envs possessing properties 1 and 2 would be efficiently neutralized by the host, and would not spread throughout the population
Alternate approach

- Characterize targets that are immunogenic and that mediate potent neutralization, but are not widely conserved

- Once such sites are defined, it may be possible to identify additional motifs present in these regions that elicit antibodies that are more cross-reactive and have broader neutralizing activities.

- The breadth of the neutralization response elicited against such targets may be expanded by the use of multivalent immunogens that include multiple allelic forms of these epitopes.

- Such targets can be identified by studying autologous neutralizing responses
Recent studies have shown that within a year after infection, patients frequently develop potent neutralizing antibody responses specific for strains present early after infection.


Little, if anything, is known about the nature of the epitopes that mediate this autologous neutralization.
Neutralization of primary clade C HIV-1 isolates by autologous sera

<table>
<thead>
<tr>
<th>Virus</th>
<th>Month 12</th>
<th>Month 12</th>
<th>Month 24</th>
<th>Month 18</th>
<th>Month 18</th>
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<tbody>
<tr>
<td>133 MPB 3.8</td>
<td>3,008</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>41</td>
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<td>109 FPB60</td>
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<tr>
<td>106 FPB9</td>
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<td>160</td>
<td>&lt;20</td>
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<tr>
<td>55 FPB3</td>
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<td>276</td>
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<tr>
<td>53 MPB21</td>
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<td>7,874</td>
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<tr>
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<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
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<td>2,941</td>
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</tbody>
</table>

Sera from heterosexually infected African subjects were able to neutralize the autologous viruses isolated shortly after infection, but possessed limiting cross-neutralizing activities.

Data from Li et al., J. Virol. 80:5211-18, 2006
Neutralization of HIV-1 pseudotyped with SF162 Env with clade B consensus V3 sequence

- The potent neutralization of SF162 by these sera indicates that these sera do possess broadly cross-reactive antibodies against conserved neutralization targets.

- The fact that these sera do not cross-neutralize the heterologous primary isolates suggests that the conserved epitopes in those Envs are not accessible, presumably because of effective masking.

- The potent neutralization of the autologous viruses by these sera further indicates that the autologous neutralization epitopes are not sensitive to masking.

Where are the autologous neutralization targets located?
Analysis of gp120 sequence homology of clade C Env panel

Variable domains

V1/V2 domain

V3, V3' domains

V4, V5 domains

N-term heptad repeat
In order to localize the regions targeted by the autologous neutralizing activity, chimeras were prepared between two of these Envs, 53M and 133M.

Initial chimeric Envs were constructed using conserved restriction sites that separated the gp120 N-terminus (C1-V1V1-C2), gp120 C-terminus (V3-V3’-V4-C4-V5) and TM domains.

All of these chimeric Envs were infectious. These were used to study autologous targets of 53M serum.
Substituting the N-terminal region of gp120 resulted in significant reduction in the autologous neutralization titer

This is consistent with the presence of potential neutralization targets in the gp120 N-terminal region of 53M (presumably in V1/V2).

However, might be due to differential masking effects by 53M and 133M V1/V2 regions
The relative masking activities of the 53M and 133M V1/V2 domains was quantitated by exchanging these domains into a standard Env backbone, and examining their sensitivity to neutralization to anti-V3 antibodies.

The reduction in neutralizing titer of 53M serum upon replacing the N-terminal gp120 region occurred despite a corresponding reduction in the V1/V2 masking activity.
Substituting just the V1/V2 region also reduced the autologous neutralizing titer of 53M serum

These results suggest the presence of autologous neutralization targets in the V1/V2 domain of 53M Env
Substituting the gp120 C-terminal fragment (including V3-V5) significantly reduced the neutralizing titer of 53M serum.

The sites involved were further mapped by exchanging smaller fragments of this region.
Substituting the V3-V3' region reduced the autologous neutralizing titer of 53M serum
Replacing the V4 region also reduced the autologous neutralizing titer of 53M serum

53M serum neutralizations

53M serum IC50

- 53M WT: 5,400
- 53M(55F V4): 1,450
- 53M(133M V4): 860
- 53M(135M V4): 1,075
Assay of binding activity of 53M serum against soluble fusion proteins expressing isolated variable regions of 53M gp120

53M serum contains binding activity against the autologous V1/V2 and V3-V3’ regions, but not against the isolated V1, V4 or V5 regions

This suggests the absence of epitopes localized to the V1, V4 or V5 regions
Substituting the gp41 region reduced the autologous neutralizing titer of 53M serum for chimeric Envs

<table>
<thead>
<tr>
<th>gp41 exchanges</th>
<th>53M serum dilutions for 50% neut.</th>
</tr>
</thead>
<tbody>
<tr>
<td>53M V1/V2</td>
<td>16,000</td>
</tr>
<tr>
<td>53M V1/V2</td>
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<tr>
<td>p1665 V1/V2</td>
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<td>p1663 V1/V2</td>
<td>550</td>
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<td>p1662 V1/V2</td>
<td>1,650</td>
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<tr>
<td>133M V1/V2</td>
<td>&lt;&lt; 100</td>
</tr>
</tbody>
</table>
### Mapping autologous neutralization targets in 53M gp41

<table>
<thead>
<tr>
<th>53M Env</th>
<th>DIWNTTWMQWDKEVSNYGTKTIKKSQNQQEENKDLLALDSWNNLWNWF</th>
</tr>
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<tbody>
<tr>
<td>133M Env</td>
<td>DIWDNMTWMQWDKEINSNYNTTIYRLLEDSONQQEQKNEKDLLALDSWKNLWNWF</td>
</tr>
<tr>
<td>106F Env</td>
<td>DIWDNMTWMQWDKEVSNSYNTIYRLLEDSONQQEQKNEKDLLALDSWKNLWTWF</td>
</tr>
<tr>
<td>55F Env</td>
<td>DIWDNMTWEWDREISNYTNIIFGLLEDSONQQERNEKDLLALDKWNNLWNWF</td>
</tr>
<tr>
<td>135M Env</td>
<td>EIWNMTWMQWDKEISNYTDTIYKLLTESQSQDKNEKDLLALDSWKNLWNWF</td>
</tr>
<tr>
<td>109F Env</td>
<td>EIWNMTWMQWDKEVSNYTFTIYQLLEESQYSEQNEKELLALNKWNLWESWF</td>
</tr>
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</table>

53M Env contains several unique substitutions in the C-terminal heptad repeat region, N-terminal to the MPER region.

To determine the roles of these substitutions and the adjacent glycosylation site in the autologous neutralization sites present in gp41 of this Env, mutations were introduced at these positions in the chimeric p1666 Env.
Mutations in 53M gp41 HR2 region affect autologous neutralization activity

53M-specific residues K619 and K627 contribute to autologous neutralization epitopes present in 53M gp41

Removal of the adjacent glycan has no effect on sensitivity to neutralization

This maps a novel neutralization site in the HR2 region
Summary

-These studies suggest that sequences in the V1/V2, V3-V3’, V4 and gp41 domains all contribute to the potent autologous neutralizing titer of 53M serum

-Evidence supports the presence of autologous neutralization targets dependent on sequences in the V2 and gp41 domains

-For V3-V3’ and V4 it is not clear whether these effects are due to epitopes present in these regions, or to differential masking effects on distal sites

-The nature of the autologous epitopes is unknown- these may be linear, conformational or even quaternary structures
Conclusions

-It is likely that the recognition of multiple neutralization targets contributes to the exceptional autologous neutralizing activity of 53M serum

-Presumably, one or more of these specificities is also responsible for the less potent cross-neutralizing activity of this serum
Conclusions

-Further characterization of these targets, and those that mediate autologous and heterologous neutralizing activities present in other patient sera, may help identify novel regions and epitopes of HIV-1 Env that could be incorporated into new vaccine formulations.

-Identifying more conserved forms of epitopes in these regions, and combining multiple allelic forms into a multivalent formulation, may help overcome the limited cross-reactivities seen for the autologous antibodies directed against these targets.
## Collaborators

<table>
<thead>
<tr>
<th>PHRI-UMDNJ</th>
<th>Zambia-Emory HIV Research Project</th>
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<tbody>
<tr>
<td>Chavdar Krachmarov</td>
<td>Cindy Derdeyn (Emory)</td>
</tr>
<tr>
<td>Bill Honnen</td>
<td>Rebecca Lynch (Emory)</td>
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<tr>
<td>Zhong Lai</td>
<td>Susan Allen (Emory)</td>
</tr>
<tr>
<td>Xun Bu</td>
<td>Joseph Mulenga (Zambia)</td>
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<td>Aidy Strenger</td>
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</table>
This region of HR2 is relatively conserved, and related to the clade C consensus sequence

Do other sequences also contain epitopes in this region?

Would antibodies that recognize such epitopes, including those related to the consensus sequence, also possess neutralizing activities that may be broader than that of those directed against the autologous targets?