Direct Antibody Access to the HIV-1 MPER Positively Correlates with Neutralization Sensitivity

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MPER Model in the Context of the Functional Spike

Liu J et al., 2008, Nature
Liu J et al., 2009, Biochemistry

Javier Guenaga, unpublished, Poster # P04.18
Aims

• To determine if MPER-directed neutralizing antibodies bind to “static” Env spikes or do they bind only after Env:receptor engagement on target cells?

• To understand the mechanism of neutralization by gp41-directed neutralizing antibodies to inform immunogen design
Differential Binding of Antibodies to JR-FL Env on Cell-Surface

Differential Binding of Antibodies to JR-FL Env on Cell-Surface

gp120-directed Antibodies

- b12 (neutralizing)
- F105 (non-neutralizing)
- 2G12

Dilutions of Antibodies (µg/ml)

Mean Fluorescence Intensity

Phenomena:
- b12 (neutralizing) binds more intensely to gp120.
- F105 (non-neutralizing) binds at a lower intensity.
- 2G12 exhibits a moderate binding intensity.

gp41-directed Antibodies

- 2F5
- 4E10
- 7B2
- 22B

Dilutions of Antibodies (µg/ml)

Mean Fluorescence Intensity

Phenomena:
- 2F5 shows a moderate fluorescence intensity.
- 4E10 displays a low binding intensity.
- 7B2 has a similar binding intensity to 2G12.
- 22B binds with a high intensity.

Pancera et al, 2005; Chakrabarti et al, 2011
Antibody-Virus Washout Assay
to Assess Accessibility on Static Spike
MPER-directed Antibodies Cannot Directly Access the JR-FL Env Spike Prior to Target Cell Engagement

**HXBc2**
- **b12**
- **2F5**
- **4E10**

**JR-FL**
- **b12**
- **2F5**
- **4E10**

**Antibody concentration (μg/ml)**

**% Neutralization**

**No wash** vs **Wash**

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Graphs show the neutralization of viruses by antibodies of different concentrations.
MPER-directed Antibodies are Accessible to Neutralization-Sensitive Primary Isolates

\[ \text{Antibody concentration (µg/ml)} \]

\[ \text{% Neutralization} \]

**ADA**

- No wash
- Wash

**MW965.26**

- No wash
- Wash

Graphs showing the relationship between antibody concentration and neutralization percentage for ADA and MW965.26 with and without wash.
Mutant JRCSF Virus is More Sensitive to Neutralization than WT

<table>
<thead>
<tr>
<th>gp120 domain</th>
<th>Mutation</th>
<th>CD4</th>
<th>b12</th>
<th>VRC01</th>
<th>VRC03</th>
<th>b6</th>
<th>F425</th>
<th>X5</th>
<th>2F5</th>
</tr>
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<tbody>
<tr>
<td>V2</td>
<td>Y177A</td>
<td>&gt;10000</td>
<td>2416</td>
<td>89</td>
<td>61</td>
<td>&gt;21008</td>
<td>41100</td>
<td>&gt;100000</td>
<td>683</td>
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<tr>
<td></td>
<td>L179A</td>
<td>1526</td>
<td>19</td>
<td>13</td>
<td>25</td>
<td>&gt;10</td>
<td>5</td>
<td>2</td>
<td>3</td>
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<tr>
<td>V3</td>
<td>I307A</td>
<td>&gt;10000</td>
<td>7399</td>
<td>119</td>
<td>&lt;1</td>
<td>&gt;120</td>
<td>&gt;2000</td>
<td>&gt;200</td>
<td>17</td>
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<tr>
<td></td>
<td>I309A</td>
<td>&gt;10000</td>
<td>3493</td>
<td>82</td>
<td>89</td>
<td>&gt;27933</td>
<td>6850</td>
<td>&gt;156250</td>
<td>1984</td>
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</tbody>
</table>
Accessibility of MPER-directed Antibodies is Related to Variable Loop Element

![Graphs showing the accessibility of MPER-directed Antibodies in different conditions](image)

- **b12**
  - JRC SF-WT
  - JRC SF-mut I309A
  - JRC SF-mut Y177A

- **2F5**

- **4E10**

**No wash**

**Wash**

**Antibody concentration (µg/ml)**

**% Neutralization**

Mutant JRCSF Env is not in a Receptor Triggered State

**Dependence on CD4 for entry**

- WT JRCSF virus
- I309A- JRCSF virus
- Y177A- JRCSF virus
- I675A- JR-FL virus

**JRCFS-I309A**

- No wash
- Wash

**% Neutralization**

- T-20 Peptide concentration (μg/ml)
Sensitivity to Neutralization correlates with Resistance to Washing

<table>
<thead>
<tr>
<th>Virus Tested</th>
<th>Antibody (2F5) No wash (IC50) [μg/ml]</th>
<th>Resistance to wash-IC50 [μg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN</td>
<td>0.246</td>
<td>Yes (4.45)</td>
</tr>
<tr>
<td>HIV2-C3</td>
<td>0.246</td>
<td>Yes (1.28)</td>
</tr>
<tr>
<td>HXB</td>
<td>0.38</td>
<td>Yes (1.15)</td>
</tr>
<tr>
<td>JR-FL-mut41</td>
<td>0.44</td>
<td>Yes (6.35)</td>
</tr>
<tr>
<td>JRCsSF-mut V3</td>
<td>0.55</td>
<td>Yes (4.95)</td>
</tr>
<tr>
<td>JR-FL-delICT(+)</td>
<td>0.736</td>
<td>Yes (ND)</td>
</tr>
<tr>
<td>JRCsSF-mut V1/V2</td>
<td>0.8</td>
<td>Yes (0.8)</td>
</tr>
<tr>
<td>ADA</td>
<td>1.18</td>
<td>Yes (2.27)</td>
</tr>
<tr>
<td>SF162</td>
<td>1.29</td>
<td>Yes (20.64)</td>
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<tr>
<td>JR-FL</td>
<td>3.21</td>
<td>No (&gt;25)</td>
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<tr>
<td>REJO4541.67</td>
<td>3.50</td>
<td>No (&gt;25)</td>
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<td>SC422681.8</td>
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<tr>
<td>BaL</td>
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<tr>
<td>CAAN</td>
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<td>JRCsF</td>
<td>8.8</td>
<td>No (&gt;25)</td>
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<tr>
<td>TRO</td>
<td>&gt;25</td>
<td>No (&gt;25)</td>
</tr>
<tr>
<td>PVO</td>
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<td>No (&gt;25)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Virus Tested</th>
<th>Antibody (4E10) No wash (IC50) [μg/ml]</th>
<th>Resistance to wash-IC50 [μg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW965.28</td>
<td>0.1092</td>
<td>Yes (0.96)</td>
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<tr>
<td>HXB</td>
<td>0.24</td>
<td>Yes (1.75)</td>
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<tr>
<td>MN</td>
<td>0.32</td>
<td>Yes (4.5)</td>
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<tr>
<td>JRCsSF-mut V1/V2</td>
<td>0.49</td>
<td>Yes (ND)</td>
</tr>
<tr>
<td>JRCsSF-mut V3</td>
<td>0.75</td>
<td>Yes (4.45)</td>
</tr>
<tr>
<td>JR-FL-mut41</td>
<td>1.53</td>
<td>Yes (ND)</td>
</tr>
<tr>
<td>ADA</td>
<td>2.39</td>
<td>Yes (5.5)</td>
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<tr>
<td>CAAN</td>
<td>2.68</td>
<td>No (&gt;25)</td>
</tr>
<tr>
<td>SF162</td>
<td>4.18</td>
<td>No (&gt;25)</td>
</tr>
<tr>
<td>JRCsF</td>
<td>4.53</td>
<td>No (&gt;25)</td>
</tr>
<tr>
<td>TRO</td>
<td>4.7</td>
<td>No (&gt;25)</td>
</tr>
<tr>
<td>JR-FL</td>
<td>7.475</td>
<td>No (&gt;25)</td>
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<tr>
<td>JR-FL delICT(+)</td>
<td>8.7</td>
<td>No (&gt;25)</td>
</tr>
<tr>
<td>BaL</td>
<td>ND</td>
<td>No (&gt;25)</td>
</tr>
<tr>
<td>PVO</td>
<td>&gt;25</td>
<td>No (&gt;25)</td>
</tr>
</tbody>
</table>
Model for Accessibility of Antibodies to MPER Epitopes

Primary Virus Env

Side View (Closed)

- V3 loop
- V1 / V2 loops
- CD4 binding site
- 2F5, 4E10 (MPER)

Lab-adapted Virus Env

Side View (Open)

- V3 loop
- V1 / V2 loops
- CD4 binding site
- 2F5, 4E10 (MPER)

Receptor binding/lab-adapted
Summary

- The MPER epitopes are directly accessible to neutralizing antibodies in lab-adapted isolates.

- MPER epitopes in the pre-receptor engaged spikes of most primary isolates are inaccessible.

- Point mutations in either in variable loop (or in the gp41 region) spontaneously exposes the MPER region in primary isolates.

- There is a quantitative IC_{50} “threshold” value that correlates with antibody access to the MPER.

- MPER epitopes are exposed on the static Env spike (perhaps pre-formed). For primary isolates, receptor-mediated triggering likely exposes the site, which may not require the putative fusion intermediate state for formation.
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