INTRODUCTION

In a previous study, we reported that HIV-1 has become more resistant to antibody neutralization over the course of the epidemic (Bunnik et al. NatMed 2010). In that study, HIV-1 variants isolated from individuals who seroconverted in recent years showed a decreed sensitivity to polyclonal antibodies (i.e. human serum and HIV-1) and to mAbs b12, but not to mAbs 2G12, 2F5 and 4E10, as compared to viruses isolated from individuals who seroconverted early in the epidemic.

MATERIAL AND METHODS

- Viruses were isolated during primary infection from 35 individuals from the Amsterdam Cohort studies and the PRIMO-SHM studies and tested for neutralizing sensitivity from BNAbs b12, 2G12, 2F5, 4E10, PG9, PG16, VRC01 in a PBMC based neutralization assay.

<table>
<thead>
<tr>
<th>Group</th>
<th>n Subject</th>
<th>Sex</th>
<th>Year of SC</th>
<th>n clones</th>
<th>Mo after SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical SC</td>
<td>14</td>
<td>Male</td>
<td>1985-1989</td>
<td>2-4</td>
<td>0.2 – 5.6</td>
</tr>
<tr>
<td>Contemporary SC</td>
<td>21</td>
<td>Male/Female</td>
<td>2003-2006</td>
<td>1-14</td>
<td>0.2 – 5.7</td>
</tr>
</tbody>
</table>

Mutations potentially influencing sensitivity to neutralization by PG9 and/or PG16 and VRC01 in viruses from historical and contemporary seroconverters.

VRC01

- For each individual, the geometric mean (GM) IC50 value of all virus variants is indicated for PG9, PG16 and VRC01. IC50 values are color-coded using increasingly darker colors for higher neutralization sensitivity.
- There was no clear correlation with the presence or absence of certain previously described amino acids that would affect the sensitivity of VRC01, PG9 or PG16.
- However, contemporary seroconverters had more mutations in these sites than historical seroconverters, which could explain the increase in neutralization resistance.
- Longer V5 loops had a modest positive correlation with VRC01 resistance ($r = 0.037, p = 0.041$).

PG9 & PG16

- Neutralization of clonal HIV-1 variants that were isolated during primary infection from individuals who seroconverted between 1985 and 1989 or between 2003 and 2006 by monoclonal antibodies VRC01 (A), PG9 (B), and PG16 (C). Each dot represents the average IC50 of all clones per patient. The dotted horizontal line indicates the highest antibody concentration tested, SC, seroconversion.
- To better understand the potential of currently known broadly neutralizing antibodies to neutralize currently circulating recently transmitted HIV-1 variants, we compared the activity of BNAbs b12, 2G12, 2F5, 4E10, PG9, PG16 and VRC01 and of TriMab against HIV-1 variants that were isolated during primary infection from contemporary seroconverters.
- PG9 and PG16 were most potent in their neutralizing capacity, but not the broadest.
- All patients with clones resistant to PG9 or PG16 were sensitive to two or more other antibodies.
- VRC01 and 4E10 were broadest in their neutralization (see table)
- None of the viruses tested were resistant to all antibodies.
- At a concentration of 1 μg/ml 19% of the tested viruses were resistant.

CONCLUSIONS

We have shown that HIV-1 has become increasingly resistant to CD4-binding site-directed antibodies over the course of the epidemic, including the newly identified potent BNAb VRC01, and may also have evolved towards increased resistance to variable loop-directed antibody PG16. However, despite the increased neutralization resistance, currently circulating HIV-1 strains were sensitive to multiple BNAbs at low concentrations and in particular to the recently identified BNAbs. In the face of a changing HIV-1 landscape over the course of 20 years, potent antibodies with exceeding neutralizing breadth are required to target multiple sites of the viral envelope in order to achieve sterilizing immunity.

AIM OF STUDY

We here extend those findings by investigating whether this adaptation of HIV-1 to antibody neutralization also affects the neutralizing activity of the recently identified BrNAbs PG9, PG16 and VRC01. In addition, we provide a comprehensive overview of the breadth and potency of the currently known BrNAbs (b12, 2G12, 2F5, 4E10, PG9, PG16, VRC01) and TriMab (a 1:1:1 mixture of b12, 2G12, and 2F5) against recently transmitted HIV-1 variants from contemporary seroconverters.

Sensitivity of historical and contemporary HIV-1 variants to neutralization by recently identified broadly neutralizing antibodies.

<table>
<thead>
<tr>
<th>Year of SC of virus donor</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>1985-1989</td>
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<td>2003-2006</td>
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Comparison of the activity of broadly neutralizing antibodies against recently transmitted contemporary HIV-1 variants

 virus variants from particular patient were resistant ($\dagger$) or sensitive ($\ddagger$).