Cross-reactive neutralizing humoral immunity in HIV-1 disease: dynamics of host-pathogen interactions
van Gils, M.J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Evolution of human immunodeficiency virus type 1 in a patient with cross-reactive neutralizing activity in serum
Chapter 10

ABSTRACT
Analysis of longitudinally obtained HIV-1 env sequences from an individual with reported cross-reactive neutralizing activity revealed that the majority of viral variants obtained from serum between 4 and 7 years after seroconversion were unable to persist in peripheral blood. Here we show that these viral variants were more sensitive to autologous serum neutralization, had shorter envelopes with fewer potential N-linked glycosylation sites, and showed lower replication kinetics than successfully evolving HIV-1 variants. These data reflect the host selection pressures on phenotypic characteristics of HIV-1 and illustrate in detail the dynamic interaction between HIV-1 and its hosts’ humoral immune responses.
MAIN TEXT

The high mutation rate of HIV-1, which is the result of rapid replication dynamics in combination with an error-prone HIV-1 reverse transcriptase and a lack of proofreading, contributes to the high genetic variability of the virus and results in the continuous emergence of new viral variants. The generation of a genetically diverse viral population allows HIV-1 to adapt to the host environment by facilitating the escape from the host immune responses and the selection of viral biological properties such as co-receptor use and replication capacity. The envelope glycoprotein (Env) of HIV-1 is highly variable, as reflected by a sequence variability which may be as high as 10% within the viral population in a single individual. The random generation of single point mutations in the viral envelope gene, together with insertions and/or deletions, facilitates escape from neutralizing antibodies by altering or shielding the antibody epitope. Viral escape variants are rapidly selected due to the humoral immune pressure eliminating the neutralization sensitive virus variants, and thereby changing the genetic composition of the viral population.

Recently, we reported on the comparison of longitudinally obtained HIV-1 envelope glycoprotein sequences from viral RNA in serum (serum-RNA), replication competent clonal HIV-1 variants (CV) isolated from Peripheral Blood Mononuclear Cells (PBMC) and proviral DNA from PBMC (PBMC-DNA) from the same HIV-1 infected individual. Interestingly, in one of the four patients studied, the viral population evolved in two separate lineages: viral population 1 (VP-1) and viral population 2 (VP-2). This has been observed previously for co-existing CCR5- (R5) and CXCR4-using HIV-1 variants. In our currently studied patient, R5 variants were present in both lineages, but CXCR4-using variants were only found in VP-2. VP-1 was constituted by the majority of the viral serum-RNA sequences from the first two time points studied and two PBMC-DNA sequences from the third time-point, lacked progeny at later stages of the infection, suggesting negative selection of those viral variants. VP-2, initially mainly made up of viral sequences obtained from PBMC, did lead to progeny at later time points, both in serum and PBMC (Figure 10.1A). This individual had a typical clinical course of infection (Figure 10.1B). To understand the mechanisms contributing to the negative selection of the majority of the viral population present in serum in the period between year 4 and 7 after seroconversion (SC), we compared molecular and phenotypic properties of the initially co-existing HIV-1 populations that did or did not successfully generate progeny virus that persisted in peripheral blood.

From longitudinally obtained blood samples (9 years of seropositive follow-up, 4 different time points; figure 10.1B), a total of 29 gp160 envelope (env) sequences were generated from serum-RNA, 37 env sequences from PBMC-DNA, and 19 env sequences from CV as described previously. Genbank numbers GU455456-GU455475 and HQ231027-HQ231090.

Differences between amino acid sequences of viral variants from VP-1 and VP-2 were...
Figure 10.1: Maximum-likelihood tree of gp160 env sequences from viral RNA in serum, PBMC proviral DNA and clonal HIV-1 variants, and clinical parameters

(A) ML tree was rooted using the root that maximized the correlation of root-to-tip divergence as a function of sampling time. Bootstrap support with values >70% are shown. The scale bar (horizontal line) indicates branch length corresponding to 0.01 substitutions per site. Sequences used to clone NL4-3/Env chimeras are circled and the names are given. The two viral populations are indicated. Figure is edited from Edo-Matas et al. 26. (B) The CD4⁺ T-cell counts are shown in black with the legend on the left y-axis, while viral RNA load data are indicated in gray with the legend on the right y-axis. Time of AIDS diagnosis is indicated by an arrow, sampling time points are indicated with a dot. Figure is edited from van Gils et al. 7.
Figure 10.2: The molecular *env* characteristics of VP-1 and VP-2

(A) Sequence logos depicting the amino acid conservation pattern across a multiple alignment of the first and second variable loops (V1V2) of all sequences of VP-1 and all sequences of VP-2. The height of the letter indicates the degree of conservation of the most common amino acid at that position. Weblogo (http://weblogo.berkeley.edu/) was used to create the sequence logos. * indicates a potential N-linked glycosylation site. (B) Longitudinal analysis of changes in number of PNGS and length of gp160 and the V1V2 region of viral variants from VP-1 and VP-2. Each dot represents one virus variant, grey dots represent X4 variants. The horizontal bars indicate average values per time point and *P*-values were calculated using a nonparametric t-test for independent samples. * indicates that *P*-value is not significant when R5 and X4 variants are analyzed separately. AA, amino acid.
found mainly, although not exclusively, in the first and second variable loops (V1V2) (Figure 10.2A) and the third constant region of env. Some of those mutations altered the number of potential N-linked glycosylation sites (PNGS) resulting in a significantly higher number of PNGS in viruses from VP-2 than in VP-1, in particular in the V1V2 region (Figure 10.2B). Additionally, the gp160 env of VP-1 viruses was significantly shorter than the gp160 env from VP-2 viruses. Within VP-2, CXCR4-using (X4) variants had longer gp160 envs than CCR5-using (R5) variants. However, even when the analysis was restricted to R5 variants, the gp160 env genes in VP-2 viruses were still longer, albeit only statistically significant at the 83 month time-point (Figure 10.2B). Increases in envelope length and number of PNGS have previously been described to decrease the neutralization sensitivity of HIV-1 \(^{8,9,23,24,29-31}\). Therefore we tested the sensitivity of several virus variants from both VP-1 and VP-2 to neutralization by four broadly neutralizing antibodies and autologous serum.

HIV-1 gp160 env sequences obtained from serum-RNA at 47 \((n=2)\), 68 \((n=2)\), and 83 \((n=1)\) months post-SC, and from CV at 47 \((n=2)\), 68 \((n=2)\), 83 \((n=2)\) and 107 \((n=1)\) months post-SC were cloned into the viral backbone NL4-3Δenv to create replication competent chimeric viruses (NL4-3/Env chimeras) through recombination, as described previously \(^7\). The NL4-3/Env chimeras were tested for their neutralization sensitivity by three-fold serial dilutions of broadly neutralizing antibodies (BrNAbs) b12, 2G12, 2F5 and 4E10, with a starting concentration of 25µg/ml in triplicate, and two-fold serial dilutions of autologous serum obtained at 68 and 83 months post-SC, with a starting dilution of 1:50 in triplicate \(^7\). The NL4-3/Env chimeras with the gp160 env from VP-1 viruses (VP-1-chimeras) showed similar sensitivity to neutralization by the four BrNAbs tested as compared to the NL4-3/

### Table 10.1: Neutralizing sensitivity of NL4-3/Env chimeras from VP-1 and VP-2

<table>
<thead>
<tr>
<th>Months since SC</th>
<th>NL4-3/Env chimeras</th>
<th>IC(_{50}) values µg/ml</th>
<th>4E10</th>
<th>2F5</th>
<th>2G12</th>
<th>b12</th>
<th>68 months</th>
<th>83 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>47_S1 VP-1</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td></td>
<td>390</td>
<td>354</td>
</tr>
<tr>
<td></td>
<td>47_S2 VP-1</td>
<td>2.05</td>
<td>2.18</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>55</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>47_C1 VP-2</td>
<td>0.89</td>
<td>0.15</td>
<td>&gt;25</td>
<td>3.79</td>
<td>&lt;50</td>
<td>288</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47_C2 VP-2</td>
<td>16.74</td>
<td>5.13</td>
<td>&gt;25</td>
<td>1.13</td>
<td>&lt;50</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>68_S1 VP-1</td>
<td>19.63</td>
<td>0.63</td>
<td>&gt;25</td>
<td>1.80</td>
<td></td>
<td>52</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>68_S2 VP-2</td>
<td>1.80</td>
<td>0.45</td>
<td>&gt;25</td>
<td>0.76</td>
<td>&lt;50</td>
<td>64</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>68_C1 VP-2</td>
<td>&gt;25</td>
<td>0.47</td>
<td>&gt;25</td>
<td>0.85</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>68_C2 VP-2</td>
<td>5.74</td>
<td>0.37</td>
<td>&gt;25</td>
<td>7.75</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>83_S1 VP-2</td>
<td>10.16</td>
<td>0.76</td>
<td>&gt;25</td>
<td>8.37</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83_S1 VP-1</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>&gt;25</td>
<td>2.23</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83_C2 VP-2</td>
<td>&gt;25</td>
<td>1.07</td>
<td>&gt;25</td>
<td>1.15</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td></td>
</tr>
<tr>
<td>107</td>
<td>107_C1 VP-2</td>
<td>&gt;25</td>
<td>1.85</td>
<td>&gt;25</td>
<td>4.56</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td></td>
</tr>
</tbody>
</table>

C = NL4-3/Env chimera with env from clonal HIV-1 variant
S = NL4-3/Env chimera with env from viral RNA from serum
IC\(_{50}\) = 50% inhibitory concentration

In bold, IC\(_{50}\) more than three-times the negative control
Env chimeras with the gp160 env from VP-2 (VP-2-chimeras) (Table 10.1). However, VP-1-chimeras showed a higher sensitivity to neutralization by autologous serum than VP-2-chimeras (Table 10.1). The VP-1- and VP-2-chimeras from the earliest time-point showed higher sensitivity to autologous neutralization than the VP-1- and VP-2-chimeras from later time points, respectively, suggesting escape of both virus populations from the autologous neutralizing antibody response. There was no difference in neutralization sensitivity to the BrNABS and autologous serum between the R5 and X4 variants. The difference in sensitivity to autologous but not to heterologous neutralization between VP-1 and VP-2 suggests that viruses of VP-1 are not more sensitive to antibody neutralization in general, but only to the neutralizing activity in autologous serum. This implies that the neutralizing antibody response of this patient may have played a role in the negative selection of those viral variants.

Serum from this individual was previously demonstrated to have cross-reactive neutralizing activity (i.e.: capable of neutralizing HIV-1 variants from different clades), which was already present 23 months post-SC. Cross-reactive neutralizing activity is considered to be directed against epitopes that are conserved amongst HIV-1 variants from different clades, and are therefore probably essential for the virus. Given that the higher sensitivity to autologous serum neutralization of the viral variants that did not persist in peripheral blood (VP-1) coincided with shorter env V1V2 regions with less PNGS, cross-reactive neutralizing activity seems to select for virus variants with longer variable loops that carry more glycans, which supports the occlusion of targeted epitopes in the conserved regions as a mechanism of viral escape. The epitope specificities for the cross-reactive neutralizing activity in this patient are currently being studied, which may help to elucidate whether viral escape also occurs by mutations of specific residues in the conserved epitopes themselves.

Figure 10.3: In vitro replication kinetics of NL4-3/Env chimeras from VP-1 and VP-2
Replication rates of 1 or 2 NL4-3/Env chimeras per time point per population are expressed as the p24 production during the logarithmic expansion after infection of PHA-stimulated PBMC. The replication capacity was tested with 100 50% tissue culture infective doses (TCID50) per virus. Similar relative differences between chimeric viruses were observed when an inoculum of 500 TCID50 was used (data not shown). S, NL4-3/Env chimera with env from viral RNA from serum. C, NL4-3/Env chimera with env from clonal HIV-1 variant.
Next we analyzed the replication kinetics of the VP-1- and VP-2-chimeras in a PBMC based replication assay, as described previously. Interestingly, VP-1-chimeras showed lower replication kinetics as compared to VP-2-chimeras. This could suggest that the combination of lower replication kinetics and higher sensitivity to autologous neutralizing activity may have been detrimental for the persistence of VP-1. The lower replication kinetics of VP-1 may have prevented those viruses from acquiring the mutations that would have allowed the escape from the broad neutralizing humoral immune response of this patient. Alternatively or in addition, mutations required for the escape from neutralizing humoral immunity in the background of VP-1 virus variants may have come at a larger fitness cost for the virus, resulting in a viral population with lower replicating capacity, which consequently was outcompeted by VP-2.

The half life of HIV-1 in plasma is about 1.3 hours indicating that the virions that were present in serum must have been produced shortly before we detected them. We failed to detect, however, the cells that produced some of those viruses in vivo as VP-1 viruses were not represented in CV and were only in low abundance in PBMC-DNA, suggesting that the cellular source for VP-1 was outside the peripheral blood compartment. The sensitivity of VP-1 viruses to autologous neutralizing antibodies may have contributed to their vulnerability in the cell-free state and may have interfered with their infection of PBMC, halting their survival in peripheral blood. The persistence of these viruses, even throughout the third time-point analyzed, suggests that they were indeed continuously produced, probably spreading through cell-to-cell transmission outside the peripheral blood compartment to avoid the cell-free state. However, we did not detect any VP-1 virus variants after 83 months post-SC. This patient developed AIDS with CD4+ T-cell counts below 200 cells/ml at 84 months post-SC (figure 10.1B). At this stage of disease, target cell availability may become limiting and viral properties such as replication capacity may exert an even greater impact on viral survival. The inability of VP-1 viral variants to compete with the more fit VP-2 viruses for limited target cells may explain their inability to persist in peripheral blood after progression to AIDS.

In summary, analysis of longitudinally obtained HIV-1 gp160 env sequences from a single individual with reported cross-reactive neutralizing activity in serum revealed that HIV-1 variants that were unable to persist in peripheral blood were more sensitive to autologous serum neutralization, had shorter envelope glycoproteins with fewer potential N-linked glycosylation sites and showed lower replication kinetics than successfully evolving HIV-1. This suggests a role for neutralizing antibody pressure on the negative selection of those viral variants. Our observations may not be unique, but merely a reflection of the common adaptation of HIV-1 in response to the host selective pressures. The detailed collection of patient materials may have provided us with the unique opportunity to study in detail the phenotypic characteristics of HIV-1 in interplay with its host humoral immune environment.
ACKNOWLEDGEMENTS

This work is financially supported by the Netherlands Organization for Scientific research (NWO), grant number 918.66.628, the European Community’s Six Framework Programme Europrise (FP6/2007-2012), grant number 037611, and the European Community’s Seventh Framework Programme NGIN (FP7/2007-2013), grant number 201433. The Amsterdam Cohort Studies on HIV infection and AIDS, a collaboration between the Amsterdam Health Service, the Academic Medical Center of the University of Amsterdam, Sanquin Blood Supply Foundation, the University Medical Center Utrecht, and the Jan van Goyen Clinic are part of the Netherlands HIV Monitoring Foundation and financially supported by the Center for Infectious Disease Control of the Netherlands National Institute for Public Health and the Environment. The funding organizations had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank José Alcami for his kind gift of the pNL4-3 vector.

REFERENCES


202


