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Increased Neutralization Sensitivity of Recently Emerged CXCR4-Using Human Immunodeficiency Virus Type 1 Strains Compared to Coexisting CCR5-Using Variants from the Same Patient

Evelien M. Bunnik, Esther D. Quakkelklaar, Ad C. van Nuenen, Brigitte Boeser-Nunnink, and Hanneke Schuitemaker*

Department of Clinical Viro-Immunology, Sanquin Research and Landsteiner Laboratory at the Academic Medical Center, University of Amsterdam and Center for Infection and Immunity Amsterdam (CINIMA), Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands

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CXCR4-using (X4) human immunodeficiency virus type 1 (HIV-1) variants evolve from CCR5-using (R5) variants relatively late in the natural course of infection in 50% of HIV-1 subtype B-infected individuals and subsequently coexist with R5 HIV-1 variants. This relatively late appearance of X4 HIV-1 variants is poorly understood. Here we tested the neutralization sensitivity for soluble CD4 (sCD4) and the broadly neutralizing antibodies IgG1b12, 2F5, 4E10, and 2G12 of multiple coexisting clonal R5 and (R5)X4 (combined term for monotropic X4 and dualtropic R5X4 viruses) HIV-1 variants that were obtained at two time points after the first appearance of X4 variants in five participants of the Amsterdam Cohort Studies on HIV-1 infection and AIDS. Recently emerged (R5)X4 viruses were significantly more sensitive to neutralization by the CD4-binding-site-directed agents sCD4 and IgG1b12 than their coexisting R5 viruses. This difference was less pronounced at the later time point. Early (R5)X4 variants from two out of four patients were also highly sensitive to neutralization by autologous serum (50% inhibition at serum dilutions of >200). Late (R5)X4 viruses from these two patients were neutralized at lower serum dilutions, which suggested escape of X4 variants from humoral immunity. Autologous neutralization of coexisting R5 and (R5)X4 variants was not observed in the other patients. In conclusion, the increased neutralization sensitivity of HIV-1 variants during the transition from CCR5 usage to CXCR4 usage may imply an inhibitory role for humoral immunity in HIV-1 phenotype evolution in some patients, thus potentially contributing to the late emergence of X4 variants.

Entry of human immunodeficiency virus type 1 (HIV-1) into a host cell is mediated by binding of the viral envelope glycoprotein 120 (gp120) to CD4 and a coreceptor, of which CCR5 and CXCR4 are the most important on primary cells (7, 8, 9). Primary HIV-1 infections are generally established by R5 viruses, which remain present throughout the course of infection (27). In approximately 50% of therapy-naive individuals infected with subtype B HIV-1, X4 viruses evolve from R5 variants, preceding an increased CD4+ T-cell decline and accelerated progression to AIDS (4, 24, 27, 30).

The relatively late appearance of X4 HIV-1 variants is poorly understood. R5 and X4 subtype B HIV-1 variants can genetically be distinguished by the absence or presence of a positively charged amino acid on positions 11 and/or 25 in the third variable loop (V3) of the gp120 envelope (10). In vitro experiments revealed that these mutations in V3 (6), as well as other single or double mutations in V3 and other domains of gp120 (2, 12), are sufficient to change coreceptor usage. However, in spite of the high mutation rate of HIV-1, X4 viruses do not evolve rapidly in vivo and not in all infected patients. Moreover, the earliest detectable X4 variants in vivo show more than only one or two amino acid substitutions compared to coexisting R5 variants (16), suggestive of compensatory mutations. These observations point towards the existence of selective pressure against X4 HIV-1 evolution, the exact nature of which remains to be established.

During the conversion of CCR5 to CXCR4 usage in vitro and in vivo, HIV-1 has to traverse a phase with reduced replicative capacity and less-efficient coreceptor usage (16, 22, 34), indicating that HIV has to overcome a significant genetic hurdle to evolve from an R5 to an X4 phenotype. In addition, HIV-1 phenotype conversion may be suppressed by host immunity. In a previous study, we demonstrated that X4 variants emerged only after CD4 counts had dropped below 400 cells/µl blood (15). X4 variants may thus be considered opportunistic viruses that emerge as a result of immune failure and subsequently give rise to an accelerated loss of CD4+ T cells. Since the interaction between the viral envelope proteins and the host cell receptors may be prevented by neutralizing antibodies, humoral immunity could play a role in the evolution of HIV-1 coreceptor usage by differentially neutralizing R5 and X4 variants.

In our present study, we compared the neutralization sensitivities of clonal R5 and (R5)X4 (this term is used throughout the paper for both monotropic X4 and dualtropic R5X4) HIV-1 variants that coexisted in vivo and that were isolated from five individuals around the moment of the first appearance of X4 variants and at a later time point during symptomatic disease. We show that R5 and (R5)X4 variants have different susceptibilities to CD4-binding-site (CD4bs)-directed
agents. For two out of four patients, the (R5)X4 variants isolated early after the appearance of X4 were potently neutralized by autologous serum. We postulate that the difference in neutralization by host humoral immunity may influence virus phenotype evolution in vivo in these patients. However, the absence of a detectable anti-X4 response in others argues against a major role for the humoral response in this process and indicates that other selective pressures are also involved.

**MATERIALS AND METHODS**

**Patients and viruses.** Patients ACH0039, ACH0171, ACH1120, and ACH6052 were homosexual male participants of the Amsterdam Cohort Studies on HIV-1 and AIDS who all developed X4 variants during a progressive disease course. Patient ACH9012 (female) was infected after a deliberate injection of a few ml of blood drawn from an AIDS patient (36). All patients were infected with subtype B HIV-1. None of the participants ever received multidrug antiretroviral therapy. Biological virus clones from time points early and late after the first seroconversion were potently neutralized by sCD4 and 4E10 (Polymun Scientific, Vienna, Austria), autologous serum neutralization were evaluated for each time point and viral variant detected. For analysis of the neutralization by sCD4 and monoclonal antibodies, IC_{50} was determined by linear regression.

**Statistical analysis.** For analysis of the neutralization by sCD4 and monoclonal antibodies, IC_{50} was evaluated per virus variant using the Mann-Whitney U test. Viruses with IC_{50} of <0.2 or >12.5 were assigned a value of 0.1 or 12.5, respectively, for calculations and statistical analyses. IC_{50} of the autologous serum neutralization were evaluated for each time point and viral phenotype using the Wilcoxon signed ranks test. For this test, the IC_{50} for the two variants used per time point and phenotype were linked per serum sample to the IC_{50} of two other viruses isolates of another time point or phenotype. Viruses with IC_{50} of <40 or >1,280 were assigned a value of 20 or 1,280, respectively.

**RESULTS**

**Patients and viruses.** Longitudinally obtained coexisting R5 and dualtropic R5X4 and X4 ([R5]X4) viruses from five participants of the Amsterdam Cohort Studies were available from previous studies (14, 33, 34, 36). From four patients infected via homosexual contact (ACH0039, ACH0171, ACH1120, and ACH6052), biological virus clones were isolated shortly after the first detection of X4 variants and at a later time point 2 years before (ACH0171) or during symptomatic disease (Table 1). Patient ACH9012 (female) was par-
ently infected with a mixture of R5 and X4 viruses that were first isolated 3.5 months after transmission (Table 1). Since X4 variants had already developed in the donor, the exact time since X4 appearance was not known, and these viruses were considered late post-X4 emergent (35) (see Materials and Methods).

Sensitivities of coexisting R5 and (R5)X4 variants to neutralization by CD4-binding-site-directed agents. The sensitivities of clonal coexisting R5 and (R5)X4 viruses to neutralization by sCD4 and the CD4-binding-site-directed MAb IgG1b12 were analyzed. R5 viruses obtained early after X4 emergence from all patients were resistant to sCD4 neutralization (IC50s ranging between 12.5 μg/ml) (Fig. 1), while their coexisting (R5)X4 viruses were relatively susceptible (median IC50s ranging between 1.40 and 4.02 μg/ml) (Fig. 1), although this approached significance only in patient ACH6052. In line with this observation, (R5)X4 variants obtained early after X4 emergence showed an increased sensitivity to IgG1b12 compared to their coexisting R5 variants, although this was not statistically significant for viruses from patient ACH1120 (Fig. 1).

For three out of five patients (ACH0039, ACH0171, and ACH1120), (R5)X4 viruses obtained late after X4 emergence still showed significantly higher sensitivities to neutralization by sCD4 and/or IgG1b12 than their coexisting R5 variants. For viruses isolated from the other patients, differential neutralization sensitivities were lost due to an increased susceptibility of the R5 viruses to IgG1b12 (although this reached statistical significance only for viruses from patient ACH6052 and not for those from patient ACH0039) and/or an increased resistance to sCD4 of the (R5)X4 variants (statistically significant for viruses from ACH6052 but not for viruses from ACH0171). X4 viruses from patient ACH9012 showed higher neutralization sensi-

![FIG. 1. Sensitivities for sCD4-, IgG1b12-, 2F5-, 2G12-, and 4E10-mediated neutralization of coexisting R5 and (R5)X4 HIV-1 obtained early or late after X4 emergence. Neutralization was determined by a 7-day culture of clonal virus isolates on PBMC in the presence or absence of serial dilutions of neutralizing agent, followed by analysis of p24 production by ELISA. Distribution of IC50 for individual virus clones as determined by linear regression is shown. Bottom, middle, and top horizontal lines represent the 25th, 50th, and 75th percentiles, respectively. Per time point, IC50 of R5 and (R5)X4 viruses were evaluated using the Mann-Whitney U test. Data shown are from one representative experiment out of two to three performed.](http://jvi.asm.org/)
analysis of p24 production by ELISA. IC50s were determined by linear regression
PBMC in the presence or absence of serial dilutions of serum, followed by
We next ana-
variants (data not shown).

differences in the replication rate between the various virus
sensitivity for sCD4 and IgG1b12 observed here were the result of
tivities to IgG1b12 and equal resistance to sCD4 compared to those of the coexisting R5 variants.

Equal sensitivities of coexisting R5 and (R5)X4 variants to
zidovudine excluded that the differences in neutralization sensi-
tivity for sCD4 and IgG1b12 observed here were the result of differences in the replication rate between the various virus variants (data not shown).

Sensitivities of coexisting R5 and (R5)X4 HIV-1 variants to
neutralization by non-CD4bs-directed agents. We next ana-
yzed whether coexisting R5 and X4 variants also had differ-
etial susceptibilities to neutralization via epitopes outside the
CD4-binding site. To this end we determined the sensitivities of the coexisting R5 and X4 viruses to neutralization by MAb
2G12 (recognizing a carbohydrate epitope on gp120) and monoclonal antibodies 2F5 and 4E10 (both binding to the
membrane-proximal external region of gp41). For 2G12, in-
creased neutralization sensitivity compared to that of their
coexisting R5 variants was observed for early and late (R5)X4
viruses in patient ACH1120 and for the early (R5)X4 viruses in
patient ACH6052 (Fig. 1). In contrast, late (R5)X4 viruses
from patient ACH0171 were significantly more resistant to
2G12 neutralization than their coexisting R5 variants. For
4E10, early (R5)X4 viruses from patient ACH0171 and early
and late (R5)X4 viruses from patient ACH1120 were more
sensitive to neutralization than their coexisting R5 viruses (Fig.
1). No differences were observed for 2F5 (Fig. 1). Overall,
some differences in neutralization susceptibilities were ob-
berved between R5 and (R5)X4 viruses for non-CD4bs-di-
rected agents but not to the same extent as seen for sCD4 and
IgG1b12.

Sensitivities of coexisting R5 and (R5)X4 HIV-1 variants to
neutralization by autologous serum. The differential neutralization sensitivities of coexisting R5 and (R5)X4 variants might suggest that X4 variants can emerge only in the absence of
neutralizing humoral immunity. To examine whether the
humoral immune response indeed deteriorated around the time
point of the appearance of X4, we analyzed the neutralization sensitivities of coexisting R5 and (R5)X4 viruses to autologous
serum samples obtained before and after the first appearance
of X4 variants. Since ACH6052 and ACH9012 carried X4
variants at the time of entry into the cohort, only serum sam-
bles obtained after X4 emergence were available from these
patients. Unfortunately, due to limited amounts of serum, it
was not possible to analyze all virus variants. We therefore
randomly chose two virus variants per time point and pheno-
type for determination of neutralization by autologous serum.
Early (R5)X4 variants from patients ACH0171 and ACH6052
were highly sensitive to neutralization by sera from all time
points (IC50 titers, >200) (Tables 2 and 3), which was signifi-
cantly different from neutralization of the early R5 variants
(for ACH0171, P = 0.018; for ACH6052, P = 0.027), as well as the late X4 variants (for ACH0171, P = 0.018; for ACH6052,
P = 0.026). Virus variants from patients ACH0039, ACH1120,
and ACH9012, irrespective of phenotype or time point of iso-
lation, either were not neutralized or were neutralized at
relatively low serum dilutions (Table 4, Table 5 and Table 6).
No differences between virus variants from different time points or
in viral tropism were observed for these patients (P > 0.050).

**DISCUSSION**

We compared the neutralization sensitivities of coexisting
R5 and (R5)X4 viruses isolated early and late after X4 emer-
gence. In a previous study, we (11) and others (3, 17, 20, 32)

### TABLE 2. Autologous serum neutralization titers of early and late post-X4 virus clones of ACH0171

<table>
<thead>
<tr>
<th>Clone</th>
<th>Time since X4 (mo)</th>
<th>Phenotype</th>
<th>Neutralization titer (IC50) at mo since X4&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(16.2) (7.5) (10.5) (27.0)</td>
</tr>
<tr>
<td>171.31.ROF6</td>
<td>4.5</td>
<td>R5</td>
<td>125 79 69 87</td>
</tr>
<tr>
<td>171.33.ROA1</td>
<td>10.5</td>
<td>R5</td>
<td>87 84 81 114</td>
</tr>
<tr>
<td>171.41.ROE2</td>
<td>27.0</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>171.41.ROCS</td>
<td>27.0</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>171.31.ROB9</td>
<td>4.5</td>
<td>R5X4</td>
<td>&gt;1,280 &gt;1,280 &gt;1,280 &gt;1,280</td>
</tr>
<tr>
<td>171.33.ROA2</td>
<td>10.5</td>
<td>R5X4</td>
<td>151 768 343 NT</td>
</tr>
<tr>
<td>171.41.D12</td>
<td>27.0</td>
<td>R5X4</td>
<td>84 &lt;40 82 &lt;40</td>
</tr>
<tr>
<td>171.41.RAF5</td>
<td>27.0</td>
<td>R5X4</td>
<td>51 &lt;40 &lt;40 80</td>
</tr>
</tbody>
</table>

<sup>a</sup> Months since X4 variant was detected.

### TABLE 3. Autologous serum neutralization titers of early and late post-X4 virus clones of ACH6052

<table>
<thead>
<tr>
<th>Clone</th>
<th>Time since entry (mo)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phenotype</th>
<th>Neutralization titer (IC50) at mo since entry&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0) (6.8) (12.5)</td>
</tr>
<tr>
<td>6052.IG2</td>
<td>0</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>6052.E2</td>
<td>0</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>6052.C4</td>
<td>32.1</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>6052.C3</td>
<td>32.1</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>6052.H4</td>
<td>0</td>
<td>X4</td>
<td>&gt;1,280 339 &gt;1,280</td>
</tr>
<tr>
<td>6052.IG3</td>
<td>0</td>
<td>X4</td>
<td>&gt;1,280 396 288</td>
</tr>
<tr>
<td>6052.A10</td>
<td>32.1</td>
<td>X4</td>
<td>&lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>6052.B11</td>
<td>32.1</td>
<td>X4</td>
<td>&lt;40 &lt;40 &lt;40</td>
</tr>
</tbody>
</table>

<sup>a</sup> Months since entry of patient into HIV<sup>+</sup> cohort.

<sup>b</sup> Neutralization was determined by 7-day culture of clonal virus isolates on
PBMC in the presence or absence of serial dilutions of serum, followed by
analysis of p24 production by ELISA. IC<sub>50</sub> were determined by linear regression
after subtracting the inhibition obtained using human pooled HIV-1-negative serum.

### TABLE 4. Autologous serum neutralization titers of early and late post-X4 virus clones of ACH1120

<table>
<thead>
<tr>
<th>Clone</th>
<th>Time since X4 (mo)</th>
<th>Phenotype</th>
<th>Neutralization titer (IC50) at mo since X4&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(18.0) (8.7) (6.0) (17.3)</td>
</tr>
<tr>
<td>1120.53.1B6</td>
<td>6.0</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>1120.53.1E4</td>
<td>6.0</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>1120.57.3E7</td>
<td>17.3</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>1120.57.C4</td>
<td>17.3</td>
<td>R5</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>1120.254</td>
<td>6.0</td>
<td>X4</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>1120.255</td>
<td>6.0</td>
<td>X4</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>1120.267</td>
<td>19.1</td>
<td>R5X4</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
<tr>
<td>1120.268</td>
<td>19.1</td>
<td>R5X4</td>
<td>&lt;40 &lt;40 &lt;40 &lt;40</td>
</tr>
</tbody>
</table>

<sup>a</sup> Months since X4 variant was detected.

<sup>b</sup> Neutralization was determined by 7-day culture of clonal virus isolates on
PBMC in the presence or absence of serial dilutions of serum, followed by
analysis of p24 production by ELISA. IC<sub>50</sub> were determined by linear regression
after subtracting the inhibition obtained using human pooled HIV-1-negative serum.
reported no difference in neutralization sensitivities between R5, X4, or dualtropic R5X4 HIV-1 from different patients. However, even if differences among the various viruses had been observed, these most likely would have reflected interpatient variability of the neutralization sensitivities of unrelated HIV-1 variants rather than differences determined by coreceptor usage. In our present study, we therefore compared R5 and (R5)X4 virus clones that had been isolated from the same patient at the same time points.

Our data show that (R5)X4 viruses obtained early after X4 conversion are more sensitive to CD4-binding-site-directed agents than their coexisting R5 variants, whereas this differential sensitivity was less pronounced at a later time point. Since the differential sensitivity for non-CD4-binding-site-directed antibodies was not a general phenomenon among the five patients included in this study, mutations in the (co)receptor binding site associated with evolution from CCR5 to CXCR4 usage most likely change the neutralization phenotype in this region but do not change the general envelope conformation. In addition, coexisting dualtropic R5X4 and X4 viruses early after X4 emergence, although available from only one patient, had comparable neutralization sensitivities (data not shown). We therefore propose that the acquisition of CXCR4 usage, rather than the loss of CCR5 usage, coincides with changes in gp120 that increase neutralization sensitivity. In line with this is an observation by Lusso et al. (19), who showed that X4 viruses constitutively expose the epitope for monoclonal antibody D19, whereas R5 viruses are neutralized by this antibody only after pretreatment with sCD4, which would indeed imply a difference in conformation between R5 and X4 variants.

The neutralization-sensitive conformation of the viral envelope during the evolution from an R5 phenotype to an X4 phenotype would suggest that transition is possible only when an efficient neutralizing-antibody response is absent or lost due to immune deterioration associated with disease progression. However, for two out of four patients, the early (R5)X4 variants were efficiently neutralized by autologous serum, suggestive of the presence of neutralizing antibodies in vivo. Late (R5)X4 variants from these patients were not neutralized, indicating that (R5)X4 viruses had evolved towards increased neutralization resistance. This is most clearly demonstrated with patient ACH0171, from whom the earliest (R5)X4 virus, isolated 4.5 months after X4 appearance, was neutralized very efficiently (IC_{50} titers of >1,280). A virus isolated 10.5 months after X4 appearance was partially adapted to the autologous antibody response (IC_{50} titers between 151 and 768), whereas the late viruses, isolated 27.0 months after X4 appearance, were almost completely resistant to autologous serum neutralization. We observed differential autologous neutralization only between recently emerged (R5)X4 viruses and their coexisting R5 variants. The use of X4 and R5 viruses from later time points in infection may explain the lack of differential neutralization by serum between R5 and X4 viruses, as observed in a previous study by others (28).

Neutralizing antibodies present in serum obtained at a certain time point in infection generally neutralize viruses found during earlier time points (25, 37). Continuous viral escape makes the neutralization of HIV-1 by contemporaneous serum a rare event (25, 37). It is therefore not surprising that the R5 and (R5)X4 virus variants that were isolated late after X4 emergence were not neutralized by serum obtained at the earlier time points. However, we had not expected that R5 viruses isolated early after X4 emergence would resist neutralization by serum obtained later in infection. This may point to a decreasing neutralizing humoral immune response late in infection.

We were also surprised that the (R5)X4 viruses isolated shortly after the first appearance of X4 variants in patient ACH0171 were potently neutralized by serum samples obtained at earlier time points, even before the appearance of X4 viruses. Possibly, the conformational requirements of an early X4 envelope led to the exposure of certain neutralization epitopes that were initially exposed on R5 trimeric envelopes before the R5 viruses escaped from the antibodies directed against these epitopes. Alternately, early X4 viruses might expose epitopes that are vulnerable to antibodies directed against other envelope structures, such as monomeric gp120, and initially have an oligomeric envelope conformation that resembles the oligomeric gp120 of neutralization-sensitive T-cell-line-adapted HIV-1. Indeed, a large proportion of the antibody repertoire against HIV-1 envelope protein is directed against nonneutralizing epitopes on shed, misfolded, or otherwise nonfunctional forms of gp120 (21, 26), which could explain why such antibodies are already present before X4 viruses have emerged. Another possibility for the presence of
antibodies directed against epitopes present on early X4 variants is that the virus has evolved towards CXCR4 usage earlier during infection but was previously inhibited by a potent humoral immune response. As a result of a decrease in selection pressure, the X4 variants may have been selected from the PBMC archive.

For three out of five patients, we did not observe effective neutralization of the early X4 viruses by serum samples from any time point. It has previously been shown that some patients fail to develop a humoral immune response against HIV-1 (25), which may explain the lack of neutralization observed in our study. Indeed, serum of patient ACH1120, which did not show neutralization of autologous virus, also did not contain broadly neutralizing antibodies (data not shown). In contrast, serum from ACH0171 displayed potent autologous neutralization as well as broadly neutralizing activity (data not shown). Patient ACH0039 rapidly progressed to AIDS (in 3.2 years after seroconversion), and may not have developed an effective immune response against HIV-1. As described above, patient ACH9012 became infected with a mixture of R5 and X4 variants, which prevented study of the role of autologous neutralization sensitivity in R5-to-X4 evolution. Interestingly, the proportion of X4 variants in serum from this patient increased preseroconversion but sharply decreased postseroconversion (5), suggestive for selective suppression of X4 variants by humoral immunity, although serum from ACH9012 did not show autologous neutralization. However, HIV-1 variants and the only serum from this patient were both obtained 3.5 months after seroconversion, again underscoring that neutralization of virus by contemporaneous serum is not commonly observed.

The observation that the emergence of X4 viruses is not prevented by the presence of neutralizing antibodies indicates that these viruses most likely appear in body compartments with lower antibody pressure than that in plasma. On the other hand, the early X4 variants used in this study have been isolated from PBMC, indicating that neutralization-sensitive X4 viruses were able to replicate in an environment containing neutralizing antibodies. The fact that X4 viruses are not hampered by the presence of neutralizing antibodies might suggest that these variants spread via direct cell-to-cell transmission, which limits the possibility of antibody neutralization.

In conclusion, we have shown here that early X4 variants are more susceptible to antibody neutralization than their coexisting R5 variants and that some patients mount a potent humoral immune response against these CXCR4-using variants. Although the humoral immune response in these patients was not sufficient to prevent the appearance of X4 variants, strong humoral immunity in certain patients could thus contribute to the inability of the virus to evolve to X4 usage. In this light, the much lower X4 conversion rate of subtype C HIV-1 (23) could be causally related to the stronger autologous neutralizing antibody titer for subtype C HIV-infected individuals (18). However, the observation that an anti-X4 response is absent for the other patients included in this study indicates that evolution of the virus phenotype may also be influenced by factors other than autologous humoral immunity.

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REFERENCES


