Impact of antiretroviral therapy on viral tropism in HIV-infected patients followed longitudinally for over 5 years

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Background: Viral tropism plays a major role in HIV pathogenesis and may influence the activity of entry inhibitors. The impact of antiretroviral therapy use on the dynamics of viral tropism over time is still poorly understood.

Patients and methods: HIV co-receptor usage was determined longitudinally for over 5 years in 237 plasma specimens collected from 73 distinct HIV-1-infected drug-naive individuals, 42 of whom initiated antiretroviral therapy thereafter and 31 who remained untreated. Viral tropism was estimated genotypically using the phenotype predictor software webPSSM, considering as X4 virus populations those with pure X4 and dual/mixed X4/R5 variants.

Results: At baseline, the prevalence of X4 viruses was 3.2% and 14.6% in patients who remained untreated and in those who initiated antiretroviral therapy, respectively (P = 0.112). Mean plasma HIV-RNA was lower in the former compared with the latter (3.8 ± 0.9 versus 4.5 ± 0.9 log; P < 0.004), while conversely the mean CD4 count was greater in untreated than in those who had begun therapy (536 ± 191 versus 278 ± 192 cells/mm³; P < 0.001). During follow-up, switch in co-receptor use occurred overall in 26% of the study population, with no significant differences between the groups. Emergence of X4 viruses was significantly associated with lower CD4 counts regardless of antiretroviral treatment exposure.

Conclusions: The use of antiretroviral therapy does not seem to influence the selection of X4 viruses, which mainly occur in patients with low CD4 counts.

Keywords: HAART, entry inhibitors, pathogenesis

Introduction

Along with CD4 molecules, CCR5 and CXCR4 are the major co-receptors used by HIV-1 to enter into human cells.1,2 Based on co-receptor usage, HIV-1 variants are classified as CCR5-tropic (R5), CXCR4-tropic (X4) or dual tropic (R5/X4).3 R5 viruses predominate at early stages of disease and are responsible for the establishment of infection in vivo,4–6 whereas X4 viruses tend to appear at later stages and may be associated with a rapid decline in CD4+ T cell counts, accelerated disease progression and reduced survival in untreated individuals.7–9 Although HIV-1-infected individuals can progress to AIDS in the absence of X4 variants, disease progression has generally been associated with the recognition of X4 or R5/X4 viruses.10–11

Co-receptors CCR5 and CXCR4 are both expressed on cell and tissue targets of HIV-1, consistent with roles in disease transmission and progression.11 CCR5 is highly expressed in activated memory CD4+ T cells (CD4+RO+CCR5+), which are the major source of viral production in vivo. The gut-associated lymphoid tissue, which is rich in memory CD4+CCR5+ T cells, plays a critical role as an early site for HIV-1 replication and massive depletion of CD4+ T cells.12 In contrast, CXCR4 molecules are expressed preferentially in naive T cells, such as immature thymocytes.13–15 This different co-receptor expression could explain in part the higher depletion of CD4+ T cells and faster progression to AIDS seen in patients infected with X4 variants. Not surprisingly, increased plasma levels of interleukin-7 (IL-7) in patients with X4 viruses may just reflect the up-regulation of this cytokine when CD4+ T cells are rapidly lost, since this cytokine plays a pivotal role in T cell proliferation and survival.16

The dynamics of viral tropism during the course of HIV-1 infection in persons exposed to antiretroviral therapy are still

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unclear. Several studies have claimed a higher prevalence of X4 variants in patients exposed to antiretroviral drugs than in drug-naive individuals. It remains unclear how antiretroviral drugs might favour the selection of X4 viruses. Alternatively the variants may just act as surrogate markers of more advanced disease or faster progression, and this is why these patients required antiretroviral therapy. In order to assess the impact of antiretroviral therapy on viral tropism evolution, we examined longitudinally a relatively large group of HIV-1-infected drug-naive individuals who attended our institution, some of whom initiated highly active antiretroviral therapy and were followed for over 5 years.

Patients and methods

Study population

A retrospective search of the database of HIV-1-infected persons who attended Hospital Carlos III, a reference HIV/AIDS centre located in Madrid, was carried out to identify individuals followed for over 5 years who could be included in one of the following two categories: (i) chronically drug-naive HIV-1-infected individuals; and (ii) drug-naive individuals who initiated antiretroviral therapy and were on regular follow-up since then, and who had experienced at least one episode of virological failure. Baseline for drug-naive individuals was considered as the first time they were seen at our institution, while it was the time of initiation of therapy in the second group. Demographics, and clinical and laboratory parameters were recorded longitudinally.

Laboratory parameters

Frozen plasma samples were available for all individuals. Plasma HIV-RNA was measured using a commercial bDNA assay (Versant v3.0; Bayer, Barcelona, Spain), which has a lower limit of detection of 50 copies/mL. CD4+ T cells were counted by flow cytometry (Coulter, Madrid, Spain) using specific fluorescein-labelled antibodies.

Determination of HIV-1 co-receptor usage

HIV-1 co-receptor usage was determined based on V3 amino acid sequences obtained after genetic sequencing of the HIV-1 env region. The amplicon was obtained from plasma HIV-RNA by RT-nested PCR using E80 (5'-CCA ATT CCC ATA CAT TAT TGTG-3') and E105 (5'-GCT TTT CCT ACT TCC TGC CAC-3') as outer primers, and E125 (5'-CAA TTT CTG GGT CCC CTC CTG AGG-3') and ES7 (5'-CTG TTA AAT GGC AGT CTA GC-3') as inner primers. PCR amplicons were sequenced using the ABI PRISM dRhodamine Terminator Cycle Sequencing Kit (Celera Diagnostics, Foster City, CA, USA).

The webPSSM software (http://ubik.microbiol.washington.edu/computing/pssm) was used to predict viral co-receptor usage. This bioinformatic tool has shown good concordance with phenotypic testing of HIV co-receptor usage. For practical purposes in this study, V3 amino acid sequences were classified as R5 or X4, dual/mixed X4/R5 virus populations being considered as X4.

Determination of CCR5-Δ32 genotype

Human DNA extraction was carried out from frozen peripheral blood mononuclear cells using a commercial assay, the QiA

RNA Blood Mini Kit (Qiagen). A single round of PCR was then performed, using the following primers, which flank the CCR5-Δ32 region: CCR5-U (5'-CCT GGC TGT CGT TGC TG-3') as the forward primer and CCR5-D (5'-CCA GCA GCG GCA GGA CCA GC-3') as the reverse primer. PCR products were visualized by electrophoresis on a 2.5% agarose gel, in which they split into three categories: wild-type (wt)/CCR5-Δ32 (242 and 210 bp fragments), CCR5-Δ32/CCR5-Δ32 (210 bp fragment) and wt/wt (242 bp fragment).

Measurement of IL-7 plasma levels

Quantification of IL-7 in plasma was performed using an ultrasensitive commercial ELISA (Quantikine<sup>®</sup> HS Immunoassay; R&D Systems, Minneapolis, MN, USA), following the manufacturer’s instructions.

Statistical analyses

Baseline characteristics of the study population were recorded as absolute numbers and percentages and means ± SD for qualitative and quantitative variables, respectively. The association between qualitative variables was tested using Pearson’s χ² test or Fisher’s exact test, as appropriate. The Student’s t-test or analysis of variance (ANOVA) was used to compare the means of quantitative variables of two or more groups, respectively. The association between quantitative variables was tested using Pearson’s correlation coefficient. Switch-free survival times were estimated by the Kaplan–Meier method and the different subgroups were compared using the log-rank test. Statistical significance was assumed for P values below 0.05. All statistical analyses were performed using SPSS v15.0 (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the study population

Overall, 73 HIV-1-infected subjects were identified as fulfilling the study criteria and had available frozen plasma samples collected at least at three different time points separated by at least 12 months each. Forty-two subjects initiated antiretroviral therapy right thereafter and 31 were chronically HIV-1-infected subjects who remained untreated. Table 1 shows the main baseline characteristics of the study population. Overall, 83.6% (n = 61) were men and most had acquired HIV-1 infection through sexual contacts and less frequently following intravenous drug use. On average, patients who initiated antiretroviral therapy had been diagnosed with HIV-1 earlier than those who remained untreated (1993 versus 1997; P = 0.002). In the group of patients who initiated antiretroviral therapy, half had begun with mono or dual nucleoside analogue regimens before 1996.

At baseline, mean plasma HIV-RNA was lower in subjects who remained untreated compared with those who begun antiretroviral therapy (380.9 ± 174.9 versus 290.4 ± 177.7 cells/mm<sup>3</sup>; P = 0.034).
HIV-1 co-receptor usage

Amplicons to infer HIV co-receptor use could be obtained from 237 (93.7%) out of 253 plasma samples collected from the 73 individuals examined in this study. Three to six specimens were tested for each patient. One specimen was always the one taken at baseline. Overall, 148 (62.4%) samples belonged to patients who initiated antiretroviral therapy and 89 (37.6%) to chronically drug-naive subjects. At baseline, the proportion of X4 viruses was slightly higher in patients who initiated antiretroviral therapy compared with those who remained untreated (14.6% versus 3.2%; P = 0.112, respectively).

Predictors of HIV-1 co-receptor usage at baseline

In the univariate analysis, no association was found between HIV-1 co-receptor use and gender, transmission route, year of infection or age in the group of patients who initiated antiretroviral therapy. The CCR5-Δ32 polymorphism was not associated with HIV-1 tropism. No differences in baseline viral load or CD4 counts were observed in the subset of patients who had begun antiretroviral therapy according to co-receptor usage. Only one out of 31 subjects who remained untreated had X4 viruses.

Baseline IL-7 plasma levels could be measured in only 52 patients; 15 who remained untreated and 37 who begun antiretroviral therapy. Lack of enough plasma volume prevented results from being obtained for the rest. Although higher IL-7 plasma levels generally correlated with higher plasma viremia, a significant positive correlation between viral load and IL-7 plasma levels could only be found in the subset of patients who remained untreated (r = 0.6, P = 0.018). No association between IL-7 plasma levels at baseline and HIV co-receptor usage was found in untreated or in antiretroviral-experienced patients.

Switch in HIV-1 co-receptor usage

Overall, changes in co-receptor usage were recognized during follow-up in 26% (n = 19) of the study population. A similar frequency of switch in co-receptor usage was noted when comparing patients who remained untreated and antiretroviral-experienced patients [22.6% (n = 7) versus 28.6% (n = 12), respectively; P = 0.564].

Changes in viral tropism from R5 to X4 occurred in 78.9% (n = 15) of patients, regardless of antiretroviral exposure. In the drug-naive group (n = 7), switch in viral tropism from R5 to X4 was observed in 85.7% (n = 6). No significant differences in CD4 counts at baseline and at the time of switch were found (439 ± 50.9 versus 307.8 ± 164.1 cells/mm³; P = 0.273). Of note, most patients, 71.4% (n = 5), showed the switch in co-receptor usage in the first sample of follow-up.

In antiretroviral-experienced patients (n = 12), changes from R5 to X4 were observed in 75% (n = 9). No differences were found when comparing CD4 counts at baseline and at the time of shift (88.5 ± 91.7 versus 172.8 ± 109.6 cells/mm³; P = 0.174). A double switch in tropism from R5 to X4 and then back to R5 again, or vice versa, was seen in half of antiretroviral-experienced patients (n = 6). The higher rate of switch in viral tropism was observed at the time of first virological failure, 58.3% (n = 7), regardless of whether the treatment regimen contained protease inhibitors or non-nucleoside reverse transcriptase inhibitors along with nucleoside analogues. The prevalence of switches in viral tropism in second virological failures was 45% (n = 4), and only one patient (8.4%) presented a switch in viral tropism during a third failure.

Predictors of HIV-1 co-receptor usage during follow-up

The mean time of follow-up in patients who were on treatment was 84.2 months, whereas it was 76.7 months for individuals who remained untreated. Overall, the prevalence of switch in viral tropism after 12 months of follow-up was 4.1%. During the following 72 months, changes in viral tropism occurred at similar rates in patients on and off therapy (26.2% versus 16.1%), the proportion of shifts from R5 to X4 being 19% and 13%, respectively, during this period.

### Table 1. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Chronically drug-naive individuals</th>
<th>Patients who initiated antiretroviral therapy</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>73</td>
<td>31</td>
<td>42</td>
<td>0.541</td>
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<tr>
<td>Male gender, no. (%)</td>
<td>61 (83.6)</td>
<td>27 (87.1)</td>
<td>34 (81)</td>
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<td>Transmission route, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>intravenous drug use</td>
<td>27 (39.7)</td>
<td>11 (42.3)</td>
<td>16 (38.1)</td>
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<tr>
<td>homosexual men</td>
<td>24 (35.3)</td>
<td>8 (30.8)</td>
<td>16 (38.1)</td>
<td></td>
</tr>
<tr>
<td>heterosexuals</td>
<td>17 (25)</td>
<td>7 (26.9)</td>
<td>10 (23.8)</td>
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<tr>
<td>Mean age, years (SD)</td>
<td>42.5 (6.9)</td>
<td>41 (8.5)</td>
<td>44 (5.3)</td>
<td>0.079</td>
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<tr>
<td>Mean plasma HIV-RNA, log copies/mL (SD)</td>
<td>4.2 (0.9)</td>
<td>3.8 (0.9)</td>
<td>4.5 (0.9)</td>
<td>0.004</td>
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<tr>
<td>Mean CD4+ count, cells/mm³ (SD)</td>
<td>385.7 (229)</td>
<td>536 (191)</td>
<td>278 (192)</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean nadir CD4+ count, cells/mm³ (SD)</td>
<td>328.9 (180.9)</td>
<td>380.9 (174.9)</td>
<td>290.4 (177.7)</td>
<td>0.034</td>
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<tr>
<td>Mean year of infection (SD)</td>
<td>1994.7 (6.2)</td>
<td>1997 (6)</td>
<td>1993 (5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean IL-7 plasma levels, IU/mL (SD)</td>
<td>4.9 (4.3)</td>
<td>4 (4.5)</td>
<td>5.4 (4.2)</td>
<td>0.309</td>
</tr>
<tr>
<td>HIV-1 X4 variants, no. (%)</td>
<td>7 (9.6)</td>
<td>1 (3.2)</td>
<td>6 (14.6)</td>
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</tr>
<tr>
<td></td>
<td>Baseline follow-up</td>
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<tr>
<td></td>
<td>chronically drug-naive (n = 31)</td>
<td>patients who initiated HAART (n = 42)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>R5 (n = 30)</td>
<td>X4 (n = 1)</td>
<td>R5 (n = 36)</td>
<td>X4 (n = 6)</td>
</tr>
<tr>
<td>Mean plasma HIV-RNA, log copies/mL (SD)</td>
<td>5.3 (0.9)  — 4.6 (0.9)</td>
<td>4.2 (1.3)</td>
<td>3.5 (0.8)</td>
<td>3.9 (0.9)</td>
</tr>
<tr>
<td>Mean CD4 count, cells/mm$^3$ (SD)</td>
<td>525.5 (190.1) — 251.7 (172.6)</td>
<td>398.8 (247.8)</td>
<td>540.7 (179.7)</td>
<td>537.7 (198.6)</td>
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<tr>
<td>Mean nadir CD4 count, cells/mm$^3$ (SD)</td>
<td>377.8 (177) — 281.9 (180.5)</td>
<td>341.7 (164.9)</td>
<td>325.3 (177.2)</td>
<td>397.2 (174.6)</td>
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<td>Mean IL-7 levels, IU/mL (SD)</td>
<td>3.1 (3.2) — 5.5 (4.4)</td>
<td>4.5 (3.6)</td>
<td>4.8 (6.4)</td>
<td>3.6 (3.7)</td>
</tr>
<tr>
<td>Mean year of infection (SD)</td>
<td>1997.3 (6.4) — 1992.6 (5.5)</td>
<td>1991.8 (1.3)</td>
<td>1994.9 (5.6)</td>
<td>1998.2 (6.5)</td>
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<td>Male, n (%)</td>
<td>26 (86.7)  — 1 (100)</td>
<td>&gt; 0.9</td>
<td>29 (80.6)</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>40.6 (8.6) — 43.3 (5.1)</td>
<td>46.2 (6.5)</td>
<td>44.1 (8)</td>
<td>39.7 (8.5)</td>
</tr>
<tr>
<td>CCR5-D32 genotype, n (%)</td>
<td>wt/wt (97.5) — 1 (100)</td>
<td>&gt; 0.9</td>
<td>27 (93.1)</td>
<td>5 (83.3)</td>
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<tr>
<td>Transmission route, n (%)</td>
<td>parenteral (40) — 1 (100)</td>
<td>0.42</td>
<td>15 (41.7)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td></td>
<td>sexual (60)</td>
<td>—</td>
<td>21 (83.3)</td>
<td>5 (83.3)</td>
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<tr>
<td></td>
<td>switch R5 to X4 (n = 9)</td>
<td>no switch (n = 33)</td>
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<td>Mean plasma HIV-RNA, log copies/mL (SD)</td>
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<td>—</td>
<td>21 (83.3)</td>
<td>5 (83.3)</td>
</tr>
</tbody>
</table>

SD, standard deviation; HAART, highly active antiretroviral therapy.
Viral tropism in HIV-infected patients

No association was found between switch in co-receptor usage and gender, age, transmission route or year of infection. The CCR5-Δ32 polymorphism was not associated with changes in HIV-1 tropism. Mean CD4 counts could not be associated with a switch in co-receptor usage in the whole population. In drug-naive patients, changes in co-receptor use were not associated with CD4 counts at baseline (540.7 versus 537.7 cells/mm³; P = 0.968) or nadir CD4 counts (325.3 versus 397.2 cells/mm³; P = 0.347). In contrast, in antiretroviral-experienced patients, changes in co-receptor use were associated with both lower baseline CD4 counts (148.4 versus 329.9 cells/mm³; P = 0.016) and nadir CD4 counts (165.5 versus 340.4 cells/mm³; P = 0.003) (Table 2).

No significant correlation was found between viral load and IL-7 plasma levels in both antiretroviral-experienced patients and individuals who remained untreated on follow-up. Finally, IL-7 plasma levels were not associated with HIV co-receptor usage in any group of patients.

Discussion

The main purpose of this study was to assess the impact of antiretroviral therapy on viral tropism in HIV-1-infected patients. For this purpose, a longitudinal follow-up of HIV-1-infected subjects, some of whom initiated antiretroviral therapy and others who remained untreated, was carried out for over 5 years. The higher frequency of X4 viruses in antiretroviral-experienced patients in this study, both at baseline and during the follow-up, was clearly associated with the lower CD4 count of this population at any time point. This is in agreement with prior cross-sectional studies, which have found a higher prevalence of X4 viruses in antiretroviral-experienced patients compared with chronically drug-naive subjects and recent seroconverters.20–23,27,28

Our results also show that changes in HIV-1 co-receptor usage are relatively uncommon over time in both chronically drug-naive and antiretroviral-experienced patients, the incidence of switches in viral tropism being similar in both groups of patients (22.6% versus 28.6%, respectively). These results are in agreement with other preliminary studies conducted in patients with virological failure under antiretroviral treatment.29,30 However, longer follow-ups were examined in our study and comparisons with untreated individuals were made. In this way, our findings suggest that the emergence of X4 variants is independent of exposure to antiretroviral treatment, and that low CD4 counts at baseline and nadir CD4 counts are the main determinants of selection of X4 viruses rather than its consequence.

Antiretroviral-experienced patients who showed a switch in viral tropism from R5 to X4 had significantly lower CD4 counts at baseline as well as lower nadir CD4 counts than treated patients who remained with the same viral tropism over time. This observation is in agreement with the concept that selection of X4 variants is associated with profound immune deterioration.7–10 Our results also confirm the hypothesis that X4 variants emerge as a consequence of progressive immune damage instead of being responsible for a more rapid CD4+ T cell depletion, at least in the subset of patients under antiretroviral therapy. In contrast, in chronically drug-naive individuals, CD4 counts at baseline and nadir CD4 counts did not differ in patients who experienced a shift in viral tropism from R5 to X4 on follow-up and those who did not. This observation, however, should be viewed with caution since this subset of patients had relatively high CD4 counts at all time points. Therefore, in the absence of significant immune deterioration, factors other than CD4 counts could favour the selection of X4 viruses. In this regard, it should be noted that heterozygosis for the CCR5-Δ32 allele did not explain changes in viral tropism in our study. Assessment of IL-7 plasma levels did not shed any light on viral tropism switch in the drug-naive patient population.

Among other limitations of our study, it should be noted that viral tropism was estimated using a genotypic predictive tool. Although several recent studies have demonstrated a relatively high degree of concordance between genotype and phenotypic assays to determine viral tropism,11,31,32 especially using webPSSM,25 it is clear that phenotypic assays should at this time be considered as the gold standard.

In summary, our results show that a switch in viral tropism occurs only in a quarter of HIV-1-infected patients with detectable viraemia and that antiretroviral exposure does not seem to influence it. Shifts from R5 to X4 viruses are the most frequent and are mainly associated with low baseline and nadir CD4 counts. Our findings do not support that X4 viruses predict a more rapid depletion of CD4+ T cells at least within 5 years of follow-up.

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Transparency declarations

None to declare.

References


