Characterization of CXCR4-using HIV-1 during primary infection by ultra-deep pyrosequencing

Stéphanie Raymond1–3*, Adrien Saliou1, Florence Nicot1,3, Pierre Delobel1,2,4, Martine Dubois1,3, Romain Carcenac3, Karine Sauné1–3, Bruno Marchou4, Patrice Massip4 and Jacques Izopet1–3

1INSERM, UMR1043, Toulouse, France; 2Université Toulouse III Paul-Sabatier, Faculté de Médecine Toulouse-Purpan, Toulouse, France; 3CHU de Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse, France; 4CHU de Toulouse, Hôpital Purpan, Service des Maladies Infectieuses et Tropicales, Toulouse, France

*Corresponding author. CHU de Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse, France. Tel: +33-5-67-69-04-24; Fax: +33-5-67-69-04-25; E-mail: raymond.s@chu-toulouse.fr

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Objectives: R5 viruses have long been thought to account for almost all strains present in primary HIV-1 infections (PHIs), but recent studies using sensitive phenotypic assays have revealed that 3%–6.4% of subjects also harbour CXCR4-using viruses. Phenotypic assays provide only qualitative results: the presence or absence of CXCR4-using viruses. We have therefore used ultra-deep pyrosequencing (UDS) to determine the frequency of CXCR4-using viruses among HIV-1 quasispecies.

Methods: We first screened 200 patients for HIV-1 tropism using a sensitive phenotypic assay during PHI and identified 11 infected with an R5X4 dual/mixed (D/M) virus population. We then used UDS of the V3 env region with the geno2pheno algorithm (false positive rate = 5.75) to identify the HIV-1 quasispecies.

Results: CXCR4-using viruses were detected in all but 1 of the 11 patients by UDS, and accounted for 0.2%–100% of the virus populations. The frequency of CXCR4-using viruses was <20% in six subjects and 100% in four subjects. Virus populations containing 100% CXCR4-using variants during PHI persisted for at least 1–2 years after the acute phase. The CCR5 Δ32 heterozygous genotype was similarly prevalent in patients infected with D/M (27%) and R5 (15%) viruses.

Conclusions: UDS and the phenotype were concordant for determining HIV-1 coreceptor usage. UDS analysis indicated large differences in the percentage of CXCR4-using viruses in the HIV-1 quasispecies during PHI. Further studies should examine the impact of the proportion of CXCR4-using viruses on disease prognosis.

Keywords: HIV quasispecies, V3 genotype, chemokine receptor, HIV tropism, HIV envelope protein gp120

Introduction

HIV-1 enters CD4-expressing cells using one or both of the chemokine receptors CCR5 and CXCR4. Virus strains can be classified as R5, X4 or R5X4 variants depending on their use of one or both coreceptors.1 But the complexity of the virus quasispecies makes it difficult to determine virus coreceptor usage in a given HIV-infected individual. Population-based assessments of HIV coreceptor usage do not determine whether HIV isolates that use both CCR5 and CXCR4 coreceptors are mixtures of pure R5 and X4 monotypic variants or contain truly R5X4 dual-tropic virus clones. Neither do these approaches estimate the proportion of each type of variant in a mixture. Phenotypic and genotypic methods have been developed to assess HIV-1 coreceptor use.2 Bulk phenotyping can discriminate between pure R5 populations, pure X4 populations and R5X4 dual/mixed (D/M) populations.3–5 Bulk genotyping of the V3 env region can predict the presence of viruses that use CXCR4 for entry and is easier to perform in clinical practice.6,7 But the poor sensitivity of bulk genotyping for detecting minor variants among the quasispecies may lead to the virological failure of treatment with a CCR5 antagonist.8,9 Genotyping can be improved by ultra-deep pyrosequencing (UDS), which provides a new method for studying the clones within an HIV-1 population.8,10

R5 viruses predominate during the early stage of HIV-1 infection, probably by selective transmission or because R5 virus infections are more readily established.11 CXCR4-using viruses emerge later and are associated with the accelerated decline of CD4+ T lymphocytes and progression to AIDS.12–14 However, CXCR4-using viruses have been found even during primary infection, with a prevalence estimated at 3%–6.4% by bulk phenotyping and up to 17.2% by bulk
genotyping methods.\textsuperscript{15–19} The transmission of CXCR4-using viruses may be favoured by a defect in the expression of CCR5 at the cell surface. Individuals who are homozygous for a 32 bp deletion in CCR5 cannot be infected by R5 viruses but are susceptible to infection with CXCR4-using viruses.\textsuperscript{20} Moreover, a primary HIV-1 infection (PHI) with CXCR4-using viruses is associated with a more rapid disease progression than is a primary infection with pure R5 viruses.\textsuperscript{16} The transmitted D/M virus populations have not been well characterized and the proportion of CXCR4-using viruses may influence disease progression.

We have used UDS to determine the composition of the virus quasispecies during PHI in patients infected with D/M viruses identified using a sensitive phenotypic assay. We determined the evolution of the CXCR4-using viruses in the quasispecies in untreated patients. Lastly, we looked for a link between the transmission of CXCR4-using viruses and the Δ32 deletion in CCR5 gene of the host.

**Methods**

**Patient selection and clinical samples**

We selected 11 patients from a total of 200 patients screened for HIV-1 tropism during primary infection (Table 1) because of their D/M virus tropism determined using a sensitive bulk phenotypic assay. These patients were treated at the Department of Infectious Diseases of Toulouse University Hospital, France and all gave their informed consent for pathophysiological studies (ethics committee approval was not required). Recent HIV-1 seroconversion was defined as: (i) subjects with a negative anti-HIV-1 antibody test and positive plasma HIV-1 RNA; (ii) a positive HIV-1 antibody test and a negative or indeterminate immunoblot, later confirmed positive; or (iii) patients who were seronegative when tested once they had HIV-1 antibody test and positive plasma HIV-1 RNA; and (iv) patients who were infected with subtype B HIV-1 and two with non-B subtypes (CRF01-AE and CRF02-AG). The median age of the patients was 26 years and 64% were men. Their median CD4+ T cell count was 359 cells/mm\textsuperscript{3} (IQR, 287 – 618) and their median plasma HIV-1 RNA load was 5.8 log\textsubscript{10} copies/mL (IQR, 4.7 – 6.2). Plasma samples were collected from all 11 patients and stored at −80°C. The HIV transmission routes, virus subtypes, plasma HIV-1 RNA and CD4+ T cell counts are summarized in Table 1. The 189 blood samples with an R5 phenotype during PHI included 125 that were available to determine the CCR5 polymorphism in the Δ32 deletion.

**Phenotypic characterization of HIV-1 coreceptor usage**

HIV-1 tropism was determined using the Toulouse Tropism Test (TTT) recombinant phenotypic assay.\textsuperscript{21} Briefly, a fragment encompassing the gp120 and the ectodomain of gpl60 was amplified with RT–PCR using HIV-1 RNA isolated from plasma or with PCR using HIV-1 DNA taken from peripheral blood mononuclear cells (PBMCs). The PCR products then underwent nested PCR. The phenotype of HIV-1 coreceptor usage was determined using a recombinant virus entry assay with the pNL4-3-senf-Luc2 vector and the product of the nested PCR obtained from the challenged HIV-1-containing sample. The chimeric recombinant virus particles were used to infect U87 indicator cells bearing CD4 and either CCR5 or CXCR4. Virus entry was assessed by measuring the luciferase activity in lysed cells (as relative light units). The specificity of the positive signals was confirmed using the CXCR4 antagonist AMD-3100 obtained through the NIH AIDS Research and Reference Reagent Program\textsuperscript{22} and the CCR5 antagonist maraviroc (Pfizer). CXCR4-using viruses were detected when they accounted for 0.5% or more of the total population.

**UDS of V3 env**

UDS was performed using a 454 GS Junior. A fragment of 415 nucleotides, encompassing the V3 env region, was generated by nested RT–PCR carried out in duplicate as previously described.\textsuperscript{22} The nested PCR was performed with the Expand High Fidelity Plus PCR System (Roche Diagnostics) under the following conditions: 1 cycle of 94°C for 2 min, 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 3 min, followed by a final extension at 72°C for 7 min. The amplified PCR products were purified using Agencourt AMPure PCR purification beads (Beckman Coulter, Brea, CA) and quantified with the Quant-it PicoGreen dsDNA Assay Kit (Invitrogen) on a LightCycler 480 (Roche). Pooled PCR products were clonally amplified on capture beads in water-in-oil emulsion micoreactors. A total of 500000

**Table 1. Characteristics of 11 patients infected with R5X4 D/M viruses at the primary stage of HIV-1 infection**

<table>
<thead>
<tr>
<th>Patient</th>
<th>CCR5 polymorphism\textsuperscript{a}</th>
<th>Transmission route</th>
<th>Fiebig classification</th>
<th>HIV-1 subtype</th>
<th>Plasma HIV-1 RNA (log\textsubscript{10} copies/mL)</th>
<th>CD4+ T cell count (cells/mm\textsuperscript{3})</th>
<th>Antiretroviral treatment during PHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>wt/wt</td>
<td>sexual</td>
<td>V</td>
<td>CRF01</td>
<td>2.69</td>
<td>796</td>
<td>no</td>
</tr>
<tr>
<td>2</td>
<td>wt/wt</td>
<td>sexual</td>
<td>III</td>
<td>B</td>
<td>5.9</td>
<td>456</td>
<td>yes</td>
</tr>
<tr>
<td>3</td>
<td>wt/Δ32</td>
<td>IDU</td>
<td>V</td>
<td>B</td>
<td>5.58</td>
<td>940</td>
<td>no</td>
</tr>
<tr>
<td>4</td>
<td>wt/wt</td>
<td>sexual</td>
<td>III</td>
<td>B</td>
<td>3.83</td>
<td>412</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>wt/Δ32</td>
<td>IDU</td>
<td>V</td>
<td>B</td>
<td>5.78</td>
<td>420</td>
<td>no</td>
</tr>
<tr>
<td>6</td>
<td>wt/wt</td>
<td>sexual</td>
<td>V</td>
<td>B</td>
<td>4.34</td>
<td>776</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>wt/wt</td>
<td>sexual</td>
<td>III</td>
<td>B</td>
<td>3.5</td>
<td>359</td>
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</tr>
<tr>
<td>8</td>
<td>wt/wt</td>
<td>sexual</td>
<td>V</td>
<td>CRF02</td>
<td>5.89</td>
<td>333</td>
<td>yes</td>
</tr>
<tr>
<td>9</td>
<td>wt/wt</td>
<td>sexual</td>
<td>III</td>
<td>B</td>
<td>6.54</td>
<td>164</td>
<td>yes</td>
</tr>
<tr>
<td>10</td>
<td>wt/wt</td>
<td>sexual</td>
<td>IV</td>
<td>B</td>
<td>6.6</td>
<td>477</td>
<td>no</td>
</tr>
<tr>
<td>11</td>
<td>wt/Δ32</td>
<td>sexual</td>
<td>IV</td>
<td>B</td>
<td>4.10</td>
<td>759</td>
<td>no</td>
</tr>
</tbody>
</table>

\textsuperscript{a} wt, wild-type allele; Δ32, mutant allele. wt/wt is a homozygous genotype and wt/Δ32 is a heterozygous genotype.

IDU, intravenous drug use.
DNA-enriched beads were deposited in the wells of a full GS Junior Titanium PicoTiterPlate device and pyrosequenced in both forward and reverse directions. The 200 nucleotide cycles were performed in a 10 h sequencing run. Phylogenetic analyses excluded any possibility of sample contamination (data not shown).

**Genotypic prediction of HIV-1 coreceptor usage from UDS data**

The sequence reads of the V3 env regions were quantified with GS Amplicon Variant Analyzer (AV A) software Version 2.5p1 (Roche). The AV A software assigns each read to the proper amplicon and sample using multiplex identifiers. The sequence reads were aligned with the R5 strain HIV-1 BaL consensus sequence, and sequence alignments were manually edited to correct for insertions or deletions in homopolymeric regions that would result in a frame shift. The tropism of each virus clone was inferred from the V3 amino acid sequence by the geno2pheno tool (false positive rate = 5.75).22,23 The false positive rate can be adjusted for choosing how conservative the prediction of CXCR4 usage will be. With a false positive rate = 5.75, the specificity is expected to be around 95%. Geno2pheno is available at http://coreceptor.bioinf.mpi-sb.mpg.de/cgi-bin/coreceptor.pl (November 2012).

**Sensitivity of UDS for detecting minor V3 env variants**

The frequency of errors resulting from V3 amplification and deep sequencing was assessed by analysing the pyrosequencing data for a panel of 10 plasmid clones of env sequenced by the Sanger method. The mean error rate of pyrosequencing was 0.031% (99% CI, 0.005%–0.056%). The upper confidence limit of the error rate was used to calculate the sensitivity of pyrosequencing for a given number of reads. Poisson distribution was used to distinguish authentic variants from artefactual V3 sequences resulting from errors arising during PCR amplification and UDS. P values of <0.001 were considered to be statistically significant. The detection threshold of minor X4 variants varied according to the number of reads of V3 for each sample.

**Results**

**Characterization of HIV-1 quasispecies by UDS**

An average of 4737 analysable reads of V3 per sample were obtained by UDS. Nucleotide sequences were translated into amino acid sequences and HIV-1 tropism was predicted using the geno2pheno algorithm. The number of unique clones ranged from 1 to 11 per sample (Table 2). UDS detected CXCR4-using viruses in 10 samples in proportions ranging from 0.2% to 100% of the total virus quasispecies. Four samples contained only CXCR4-using clones. Sample 5 contained 18.4% CXCR4-using clones and five other samples contained minor CXCR4-using variants accounting for 0.2%–2.9% of the virus quasispecies. The last sample contained only one clone that used CCR5 for entry, although its phenotype was R5X4. This discordance between the results of genotyping by UDS and phenotyping by the TTT was confirmed in two independent experiments. This virus was subtype B and the subject was heterozygous for the CCR5 Δ32 deletion.

**Evolution of HIV-1 quasispecies during the early stage of infection**

After a median interval of 17 months we re-determined the HIV-1 tropism by UDS in five of the seven subjects who were not given

**Table 2. HIV-1 quasispecies during PHI and their evolution in untreated patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>no. of reads</th>
<th>no. of variants</th>
<th>percentage of CXCR4-using clones</th>
<th>timea (months)</th>
<th>no. of reads</th>
<th>no. of variants</th>
<th>percentage of CXCR4-using clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5330</td>
<td>2</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>4350</td>
<td>5</td>
<td>100</td>
<td>23</td>
<td>3445</td>
<td>25</td>
<td>98.52</td>
</tr>
<tr>
<td>3</td>
<td>4703</td>
<td>2</td>
<td>100</td>
<td>13</td>
<td>2391</td>
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<td>100</td>
</tr>
<tr>
<td>4</td>
<td>4618</td>
<td>5</td>
<td>100</td>
<td>—</td>
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<td>3381</td>
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<td>18.43</td>
<td>12</td>
<td>1296</td>
<td>10</td>
<td>0.4</td>
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<tr>
<td>6</td>
<td>6494</td>
<td>5</td>
<td>2.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>7</td>
<td>5938</td>
<td>5</td>
<td>1.2</td>
<td>22</td>
<td>5755</td>
<td>4</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>8</td>
<td>3647</td>
<td>10</td>
<td>1.04</td>
<td>17</td>
<td>2814</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>2172</td>
<td>4</td>
<td>0.8</td>
<td></td>
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<tr>
<td>10</td>
<td>6082</td>
<td>2</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>5395</td>
<td>1</td>
<td>&lt;0.07</td>
<td></td>
<td></td>
<td></td>
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</table>

aTime after diagnosis of PHI.
antiretroviral treatment during PHI (Table 2). Each of these second samples contained 4–25 unique clones, whereas samples from the same patients contained 1–5 variants at the time of primary infection. Two subjects infected with virus populations consisting of 100% CXCR4-using variants during PHI still harboured 98.52% and 100% CXCR4-using variants after 23 and 13 months of follow-up, respectively. One of the two subjects infected with virus populations consisting of CXCR4-using variants during PHI harboured only pure CCR5-using viruses after 22 months, while the other still harboured minor X4 variants (0.4% of the quasispecies) after 12 months. Patient 11 was infected with viruses that harboured no X4 genotypic determinants despite an R5X4 phenotype during PHI, but this genotypic–phenotype discordance disappeared in the follow-up; UDS detected 30% of viruses genotyped as CXCR4-using variants at month 17, in agreement with the phenotype.

Relationships between the percentage of CXCR4-using viruses and the patients’ characteristics

We determined whether the patients harbouring minor CXCR4-using variants (0.2%–18.4%, n=6) and a majority of R5 viruses differed from those harbouring exclusively CXCR4-using viruses (n=4) and no R5 viruses (Table 3). The HIV-1 transmission route, HIV-1 virus load and the CD4 T cell count at the time of PHI were similar in both groups. The four patients given antiretroviral treatment during PHI (Table 2). Each of these second samples contained 4–25 unique clones, whereas samples from the same patients contained 1–5 variants at the time of primary infection. Two subjects infected with virus populations consisting of 100% CXCR4-using variants during PHI still harboured 98.52% and 100% CXCR4-using variants after 23 and 13 months of follow-up, respectively. One of the two subjects infected with virus populations consisting of CXCR4-using variants during PHI harboured only pure CCR5-using viruses after 22 months, while the other still harboured minor X4 variants (0.4% of the quasispecies) after 12 months. Patient 11 was infected with viruses that harboured no X4 genotypic determinants despite an R5X4 phenotype during PHI, but this genotypic–phenotype discordance disappeared in the follow-up; UDS detected 30% of viruses genotyped as CXCR4-using variants at month 17, in agreement with the phenotype.

Impact of the Δ32 deletion in CCR5 on HIV-1 tropism during PHI

We determined the CCR5 polymorphism for the Δ32 deletion of the 11 D/M-infected patients (Table 1) and of 125 consecutive R5-infected patients screened during PHI. The D/M group was made up of eight patients with homozygous wild-type CCR5 and three that were heterozygous for Δ32. The 125 R5-infected patients included 19 who were heterozygous for Δ32. None of our patients was Δ32 homozygous. The prevalence of the Δ32 deletion was thus 27% in the D/M group and 15% in the R5 group (Fisher’s exact test, P=0.38).

Discussion

Primary infection is a crucial stage for understanding the pathogenesis of HIV-1 infection. Many studies have shown that CCR5-using viruses predominate during early infection, but that CXCR4-using viruses can also be transmitted. As CXCR4-using viruses have a negative impact on the clinical evolution of an HIV-1 infection and a primary infection with D/M viruses implies the presence of both CCR5-using and CXCR4-using viruses, it is important to know the percentage of CXCR4-using viruses in the quasispecies. In a previous study, we accurately detected CCR5-using and CXCR4-using viruses using UDS during PHI and showed a CXCR4-using virus frequency similar to that found using TTT. Thus we used UDS to analyse the components of D/M virus quasispecies during PHI and hence the composition of the HIV-1 population.

We used a phenotypic assay to screen 200 patients for HIV-1 tropism during PHI and selected 11 who were infected with D/M viruses. Their HIV quasispecies were then explored by UDS. The HIV-1 tropism determined using UDS and the phenotypic assay were concordant for 10 of the 11 patients, as previously shown in other populations. UDS revealed that four patients (36%) were infected with virus populations containing exclusively CXCR4-using variants, with no pure R5 variants. The genotypic algorithms cannot distinguish X4 from R5X4 clones because the determinants in their V3 env regions are similar. All are thus

![Figure 1](https://academic.oup.com/jac/article-abstract/68/12/2875/699656)

**Figure 1.** Proportion of V3 env variants among the quasispecies and prediction of HIV-1 coreceptor use during PHI. V3 sequences were generated by UDS and coreceptor usage was predicted using geno2pheno 5.75. Each pie chart represents the virus quasispecies in an individual subject. The sectors are proportional to the frequencies of the V3 amino acid variants whose tropism is indicated to be R5 (light grey) or X4 (dark grey). The number of reads obtained with UDS is indicated beneath each pie chart. The quasispecies harboured by subjects 1–10 contained CXCR4-using variants, whereas the quasispecies infecting subject 11 were pure CCR5-using viruses.
classified as CXCR4-using viruses by genotyping.25 The susceptibility to treatment with CCR5 antagonists of virus populations containing minor CXCR4-using variants during PHI (0.2%–18.4%) and a majority of R5 viruses could vary, but the clinically relevant cut-off of CXCR4-using variants above which a CCR5 antagonist-based treatment leads to virological failure is still unknown. It may depend on whether CXCR4-using variants are pure X4-tropic clones, which seems quite unusual, or R5X4 clones. Moreover, the replication of R5X4 clones could be partly inhibited by CCR5 antagonists, depending on their affinities for CCR5 and CXCR4 receptors.

The phenotypic assay detected very low percentages (0.2% or 1%) of CXCR4-using variants. We reanalysed the virus populations of two of the five patients with <5% CXCR4-using clones after the PHI. The patient who harboured 2.9% X4 variants during PHI still harboured minor CXCR4-using variants (0.4%) after 12 months, while the patient with 0.2% CXCR4-using variants harboured exclusively R5 variants after 22 months. Perhaps a very low frequency of CXCR4-using variants during PHI indicates that these clones have an overall disadvantage that prevents their further expansion in competition with more-fit R5 viruses. If so, determining the percentage of CXCR4-using variants by ultrasensitive sequencing methods at PHI would provide substantial data for predicting the evolution of virus tropism in subsequent years.

Patient 11 was infected with an R5X4 virus according to the phenotypic assay, but with an R5 genotype according to UDS. The discordance could be explained by analysis of the short V3 env region for the genotypic prediction, whatever the prediction algorithm used, while determinants outside V3 could influence HIV-1 coreceptor use.26–28 The sensitivity of UDS for detecting minor variants (0.07%–0.2% in our dataset) and the sensitivity of the phenotypic assay (0.5%) were similar; hence this cannot explain the failure to detect CXCR4-using variants.5 Patient 11 unexpectedly harboured 30% CXCR4-using variants 17 months after PHI without any selective pressure from antiretroviral drugs. The detection of X4 variants shortly after PHI, plus a high CD4+ T cell count (550 cells/mm³), tends to confirm that these variants had been transmitted rather than emerged.

The proportion of patients with D/M HIV-1 infection during PHI has previously been estimated to be between 3 and 17%.15–17 The selective advantage of CCR5-tropic viruses in establishing an HIV-1 infection may be reduced if the host has a polymorphism in CCR5 that lowers the amount of CCR5 at the cell surface. The most frequent polymorphism in CCR5 is a 32 bp deletion whose heterozygous prevalence is estimated to be about 16% in the French Caucasian population.29,30 A few cases of PHI with CXCR4-using viruses have been reported in subjects homozygous for the CCR5 Δ32 deletion. The consequences of heterozygous carriage of this genotype on the transmission rate of CXCR4-using variants are less clear. We determined the prevalence of the Δ32 deletion in the group of D/M-infected patients and in 125 consecutive R5-infected patients at the early stage of infection. The prevalence of the Δ32 deletion in the D/M-infected patients (27%) and in the R5-infected patients (15%) was not different. However, the small number of D/M-infected subjects during PHI is a limiting factor in these studies. The lack of relationship between HIV-1 tropism and the Δ32 deletion should be confirmed in larger cohorts of patients at the stage of PHI. Another limitation is the potential influence of the route of transmission. Two of our D/M-infected patients were infected via the parenteral route and were Δ32 heterozygous. Previous studies have shown a correlation between infection with CXCR4-using viruses and the parenteral transmission of HIV-1,15,18,31,32 suggesting that R5 viruses cross the mucosal barrier most readily, while other variants can enter via the parenteral route.11 The Δ32 deletion in CCR5 may be an additional factor favouring the transmission of CXCR4-using viruses via the parenteral route, but further studies are needed. By contrast, the abundance of CCR5-expressing target cells in genital and rectal mucosae, where the CXCR4 ligand SDF-1 is highly concentrated, favours the sexual transmission of R5 viruses.

We monitored five patients given no antiretroviral treatment for about 17 months after PHI. One of the two patients harbouring <5% CXCR4-using variants during PHI was still infected with CXCR4-using minor variants, whereas the two patients infected exclusively with CXCR4-using variants still had these variants as a dominant population after the acute phase of the infection, as shown previously in patients infected with CXCR4-tropic viruses harbouring mutations associated with antiretroviral resistance during PHI.33 The dominance of CXCR4-using variants from PHI could negatively influence the rate at which an HIV-1 infection evolves, as already reported.18,34

In conclusion, we have determined the composition of 11 virus quasispecies with D/M tropism during PHI and observed various proportions of CXCR4-using variants that may influence subsequent disease progression. We found that a virus population containing only CXCR4-using viruses can persist long after PHI with no link to CCR5 Δ32 deletion. Further studies are needed to determine the impact of the proportion of CXCR4-using variants on disease progression and susceptibility to CCR5 antagonist-based therapy. Knowledge of the viruses present very early in an infection may lead to a better understanding of HIV pathophysiology and the design of antiretroviral therapies.

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Transparency declarations
None to declare.

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