CXCR4-using viruses in plasma and peripheral blood mononuclear cells during primary HIV-1 infection and impact on disease progression

Stéphanie Raymond[a,b,c], Pierre Delobel[a,b,d], Maud Mavigner[a], Michelle Cazabat[a,c], Stéphanie Encinas[a,c], Corinne Souyris[a,c], Patrick Bruel[a,c], Karine Sandres-Sauné[a,b,c], Bruno Marchou[d], Patrice Massip[d] and Jacques Izopet[a,b,c]

Objective: Cysteine-cysteine receptor 5 (CCR5)-using viruses classically predominate during HIV-1 primary infection but the frequency of cysteine-X-cysteine receptor 4 (CXCR4)-using viruses varies between studies and could be different between plasma and peripheral blood mononuclear cells (PBMCs). Thus, we determined HIV-1 tropism in both these compartments during primary infection and evaluated the impact of CXCR4-using viruses on disease progression.

Design: One hundred and thirty-three patients with primary HIV-1 infection were screened for HIV-1 coreceptor usage in plasma and PBMCs using both genotypic and phenotypic methods. The impact of CXCR4-using viruses' transmission on subsequent disease progression was assessed in a case–control study.

Methods: HIV-1 coreceptor usage was determined using a recombinant virus phenotypic entry assay and V3-based genotypic algorithms. We also monitored $CD4^+$ T-cell count, clinical events and therapeutic intervention.

Results: There was 6.4% of CXCR4-using HIV-1 in plasma during primary infection as measured by a phenotypic assay and combined criteria from the 11/25 and net charge genotypic rules. Geno2pheno$_{10}$ overestimated the prevalence of CXCR4-using viruses (12%). HIV-1 tropism in plasma and PBMCs was 98% concordant. The HIV-1 RNA load and $CD4^+$ T-cell count during primary infection were not related to virus tropism. Primary infection with CXCR4-using viruses was associated with an accelerated rate of disease progression, estimated by a faster decline of $CD4^+$ T-cell count under 350 cells/μl and by a reduced delay in initiating a first antiretroviral treatment.

Conclusions: Plasma or PBMC samples can be used for determining HIV-1 tropism during primary infection. CXCR4-using viruses are rare during primary infection but increase the risk of disease progression.

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Keywords: coreceptor, disease progression, entry phenotype, HIV-1 tropism, primary infection, V3 genotype

Introduction

Human immunodeficiency virus type 1 (HIV-1) enters CD4-expressing cells using one or both of the chemokine receptors cysteine-cysteine receptor 5 (CCR5) and cysteine-X-cysteine receptor 4 (CXCR4). CCR5-using viruses are classified as R5 variants, CXCR4-using viruses are classified as X4 variants, and viruses using both

[a]INSERM, U563 Toulouse, [b]Université Toulouse III Paul-Sabatier, Centre de Physiopathologie de Toulouse Purpan, [c]CHU de Toulouse, Hôpital Purpan, Laboratoire de Virologie, and [d]CHU de Toulouse, Hôpital Purpan, Service des Maladies Infectieuses et Tropicales, Toulouse, France.

Correspondence to Jacques Izopet, CHU de Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse F-31300, France.
Tel: +33 5 67 69 04 24; fax: +33 5 67 69 04 25; e-mail: izopet.j@chu-toulouse.fr
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coreceptors are classified as R5X4 dualtropic variants [1]. A virus population may use both coreceptors because it contains R5X4 dualtropic clones and/or a mixture of pure R5 and X4 virus clones (dual/mixed R5X4 phenotype). R5 viruses usually predominate early in a HIV-1 infection, whereas R5X4 or X4 viruses emerge in half the patients at late stages [2,3]. The X4 variants present late in the infection are associated with the accelerated decline of $CD4^+$ T lymphocytes and progression to AIDS [2,4,5]. HIV-1 tropism in recent seroconverters seems to be restricted to R5 variants whatever the transmission route and the tropism of the variants in the source [6]. The mechanisms underlying the selective advantage of CCR5-tropic viruses in establishing an HIV-1 infection are poorly understood. It may be that target cells and tissues important for establishing the initial infection preferentially express CCR5 and/or there is an immune response preferentially directed against CXCR4-using viruses. The classic view that CCR5-tropic viruses massively predominate during primary HIV-1 infection has been challenged by recent finding that X4/R5X4 viruses are not necessarily rare during HIV-1 primary infection. Frequencies of 3–17.2% have been reported depending on the assay used to measure HIV-1 tropism [7–10].

HIV-1 coreceptor usage can be assessed by phenotypic assays, mainly using recombinant virus assays, or by culturing the HIV-1 isolate on MT-2 cells [11–13]. Genotypic assays that analyze the V3 region of *env* have also been developed for predicting HIV-1 tropism [14–16]. Phenotypic methods indicated that the plasma of patients with recent seroconversion contained about 3% of CXCR4-using viruses [9,10]. By contrast, genotypic methods found 13.4–17.2% of CXCR4-using viruses in the plasma of primary infected patients [7,17]. Another recent study using genotypic methods found 15.9% of CXCR4-using viruses in the peripheral blood mononuclear cells (PBMCs) of recently infected patients [18]. Cases of rapid disease progression after the transmission of CXCR4-using variants have been reported [5,19,20]. But the impact of the tropism of transmitted virus on the subsequent clinical evolution has rarely been studied in cohorts [10]. Moreover, characterization of HIV-1 tropism in primary infection is now of therapeutic interest because CCR5 antagonists have been developed.

The aim of this study was to determine the HIV-1 coreceptor usage in the plasma and PBMCs of patients with a primary HIV-1 infection using both phenotypic and genotypic approaches. We also monitored the biological and clinical outcomes of these patients to evaluate the impact of HIV-1 tropism on disease progression.

Methods

Study population and samples

We studied 133 consecutive patients with a primary HIV-1 infection treated at Toulouse University Hospital, France, between 1995 and 2008. They all gave their informed consent for virological studies. Plasma samples were collected from each patient and stored at −80°C. Samples of peripheral whole blood were also collected after 2000, and the Ficoll-separated PBMCs were stored at −80°C. Recent HIV-1 seroconversion was defined as patients with a negative anti-HIV-1 antibody test and positive plasma HIV-1 RNA; a positive HIV-1 antibody test and a negative or indeterminate immunoblot, confirmed positive later; HIV-1 seropositivity that was seronegative when tested within the previous 6 months. Plasma HIV-1 RNA was measured by Amplicor HIV-1 Monitor until June 2004 and by COBAS Ampliprep/COBAS Taqman HIV test (Roche) thereafter. Genotypic resistance analyses were performed by the consensus method of the AC11 ANRS Resistance Group (http://www.hivfrenchresistance.org). $CD4^+$ T cells were counted by flow cytometry.

Case–control study

We studied the impact of R5X4/X4 virus transmission on subsequent disease progression. Patients who were given antiretroviral treatment during the first 6 months following primary infection were excluded from this analysis. Each patient infected with an R5X4/X4 virus was matched with five control patients infected with an R5 virus for age (± 5 years) and HIV-1 RNA load at the time of primary infection (± 0.5 log copies/ml) because these two factors are associated with disease progression. Patients' follow-up was censored when antiretroviral therapy was started.

Phenotypic characterization of HIV-1 coreceptor usage

Phenotypic characterization of HIV-1 tropism was performed using the Toulouse Tropism Test assay [21]. Briefly, a fragment encompassing the gp120 and the ectodomain of gp41 was amplified by RT-PCR using HIV-1 RNA isolated from the plasma or by PCR from HIV-1 DNA taken from PBMCs. The PCR products then underwent nested PCR. Two amplifications were performed in parallel from each sample and pooled to prevent sampling bias of the assessed virus population.

The phenotype of HIV-1 coreceptor usage was determined using a recombinant virus entry assay with the pNL43-Δ*env*-Luc2 vector. 293T cells were cotransfected with NheI-linearized pNL43-Δ*env*-Luc2 vector DNA and the product of the nested PCR obtained from the challenged HIV-1-containing sample. The chimeric recombinant virus particles released into the supernatant were used to infect U87 indicator cells bearing CD4 and either CCR5 or CXCR4. Virus entry was assessed by measuring the luciferase activity in lyzed cells [as relative light units (RLUs)]. Minor X4 variants were detectable when present at 0.5% or higher. The reproducibility of the assay was assessed by 35 repeated experiments with the reference strains HXB2 and BaL; the coefficient of

variation was 3.4–6.2% in both U87 indicator cell lines.

Genotypic prediction of HIV-1 coreceptor usage

The V3 region was directly sequenced from bulk *env* PCR products in both directions by the dideoxy chain termination method (BigDye Terminator v.3.1, Applied Biosystems) on an ABI 3130 DNA sequencer. Results were analyzed with Sequencher (Genecodes), blinded to the phenotype. Minor species were detected when the automated sequencer electrophoregram showed a second base peak. Multiple alignments were performed with CLUSTALW 1.83 (Conway Institute UCD Dublin), and sequence alignments were manually edited using BioEdit software. Phylogenetic analyses of the PCR product sequences excluded any possibility of sample contamination (see supplementary figure, http://links.lww.com/QAD/A72).

We used a combination of criteria from the 11/25 and net charge rules to predict HIV-1 tropism from the V3 genotype [16]. One of the following criteria is required for predicting CXCR4 coreceptor usage: 11R/K and/or 25K; 25R and a net charge of at least +5; a net charge of at least +6. The V3 net charge was calculated by subtracting the number of negatively charged amino acids (D and E) from the number of positively charged ones (K and R). All possible permutations were assessed when amino acid mixtures were found at some codons of V3. The combination resulting in the highest net charge was used to predict the tropism. We also compared the performances of these combined criteria for detecting CXCR4-using viruses with that of the bioinformatic tool Geno2pheno with false-positive rates of 10 and 5.75% [22]. Geno2pheno is available at http://coreceptor.bioinf.mpi-sb.mpg.de/cgi-bin/coreceptor.pl (January 2010).

Statistical methods

We used STATA 8.0 (Stata Corporation, Grand Forks, North Dakota, USA) software for statistical analysis. Continuous variables were tested with the Mann–Whitney test. Categorical variables were tested by the chi-squared test or the Fisher's test. A statistically significant difference was defined as a P value of less than 0.05. Kaplan–Meier curves were used to provide a graphic representation of the time to disease progression according to virus tropism. Events were defined as a $CD4^+$ T-cell count dropping below 350 cells/μl, or the initiation of antiretroviral therapy. The log rank test was used to compare groups.

Nucleotide sequence accession numbers

The sequences reported here were given Genbank accession numbers HM239515 to HM239639 for plasma RNA sequences and HM246186 to 246241 for cellular DNA sequences.

Results

Patient characteristics

We identified and prospectively monitored 133 patients who were at the stage of primary infection during the 13-year study. The median follow-up time was 31 months [interquartile range (IQR) 12–68]. The clinical, virological, and immunological characteristics of the patients are shown in Table 1. Their median age was 35 years and 83.5% were men. Most (85%) were infected with HIV-1 subtype B, but 15% had non-B subtypes (CRF01, CRF02, A, C, D, F1 and G). The median HIV-1 virus load was 5.5 log copies/ml; (IQR 4.5–6.5). The median CD4 cell count was 479 cells/μl (IQR 325–653) and the percentage of CD4 cells was 24% (IQR 16–32). Ten per cent of the patients harbored HIV-1 viruses resistant to at least one class among the nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors or protease inhibitors of HIV-1.

Entry phenotype of HIV-1 at the time of primary infection

Phenotypic characterization of coreceptor usage from plasma HIV-1 RNA

The *env* gene was successfully amplified from plasma of 126 of the 133 patients. The phenotype was then determined for 125 of these 126 patients (94% of samples) and recombination failed for one sample. Amplification failed for three non-B subtype viruses and four subtype B viruses, probably because of mismatches in the priming site due to HIV-1 polymorphisms. We found 117 virus populations with an R5 phenotype and eight with a dual/mixed R5X4 phenotype. No virus had a pure X4 phenotype. There was thus 6.4% of CXCR4-using HIV-1 at the time of primary infection. We performed a phylogenetic analysis to exclude the possibility that the R5X4 variants belonged to a single transmission cluster; only two patients had related R5X4 viruses. The patients harboring subtype B viruses (7/109 subtype B, 6.4%) and those harboring non-B viruses (1/16 non-B subtypes, 6.2%) had similar percentages of CXCR4-using HIV-1.

Table 1. Baseline characteristics of 133 patients with primary infection.

Age (years), median (IQR)	35 (28.5–43)
Men, no. (%)	111 (83.5)
Transmission route, no. (%)	
Heterosexual	34 (25.6)
Homosexual	73 (54.9)
Intravenous drug use	4 (3)
Unknown	22 (16.5)
HIV-1 subtype B, no. (%)	113 (85)
HIV-1 resistance mutation to at least one drug, no. (%)	13 (10.2)
HIV-1 RNA load (log copies/ml)	
Median (IQR)	5.5 (4.5–6.5)
CD4 cell count (cells/μl)	
Median (IQR)	479 (325–653)
CD4 cell count (%)	
Median (IQR)	24 (16–32)

Table 2. RLU signals of CXCR4-using viruses from plasma and PBMCs.

Sample	Plasma HIV-1 RNA			Cellular HIV-1 DNA		
	Log RLU[a] signal			Log RLU[a] signal		
	$CCR5^+$ cells	$CXCR4^+$ cells	Tropism	$CCR5^+$ cells	$CXCR4^+$ cells	Tropism
BaL control	7.47	1.6	R5	6.94	1.35	R5
HXB2 control	2.05	7.49	X4	1.58	6.35	X4
Patient 15	6.58	6.85	R5X4	N/A[b]	N/A[b]	
Patient 23	4.13	6.02	R5X4	2.86	4.67	X4
Patient 55	7.41	6.62	R5X4	N/A[b]	N/A[b]	
Patient 87	6.84	7.13	R5X4	N/A[b]	N/A[b]	
Patient 93	7.06	6.6	R5X4	4.39	4.76	R5X4
Patient 95	7.51	4.12	R5X4	N/A[b]	N/A[b]	
Patient 106	7.62	4.94	R5X4	N/A[b]	N/A[b]	
Patient 111	5.85	5.39	R5X4	4.44	5.05	R5X4

N/A, not available; PBMCs, peripheral blood mononuclear cells; RLU, relative light unit.
[a]RLU, relative light units of luciferase.
[b]N/A, not available.

The only CRF01 strain was R5X4 and there was no R5X4 viruses among nine CRF02, one subtype A, one subtype C, one subtype D, one subtype F, one subtype G and one undetermined subtype. Five patients harbored dual/mixed R5X4 virus populations able to use both coreceptors with similar entry efficiencies (Table 2). In contrast, two patients (patients 95 and 106) harbored a dual/mixed R5X4 virus population that infected $CXCR4^+$ cells less efficiently than $CCR5^+$ cells; whereas one patient (patient 23) harbored a dual/mixed R5X4 virus population that infected $CXCR4^+$ cells more efficiently than $CCR5^+$ cells.

Phenotypic characterization of coreceptor usage from cellular HIV-1 DNA

Peripheral blood mononuclear cells were available for 74 patients, recruited after 2000, and gp140 was successfully amplified for 56 of them. The failure rate of amplification may be explained by mismatches in the priming site due to HIV-1 polymorphism and by the virus DNA load in cell samples being lower than the virus RNA load in the plasma. The phenotype was determined for 54 of the 56 amplified samples with 51 virus populations using exclusively CCR5, two dual/mixed R5X4 virus populations (patients 93 and 111) and one virus population using exclusively CXCR4 (patient 23). Thus the prevalence of CXCR4-using HIV-1 in PBMCs was 5.6%.

The 51 R5 and the two dual/mixed R5X4 virus populations identified in the PBMCs were concordant with the results for the plasma. Patient 23 harbored a virus population phenotyped as purely X4 in his PBMCs but dual/mixed R5X4 in his plasma. HIV-1 tropism in the plasma and PBMCs from patients at the stage of primary infection was thus 98% concordant.

Genotypic prediction of HIV-1 tropism during primary infection

Genotypic prediction of tropism from plasma HIV-1 RNA

We obtained the V3 genotype of plasma HIV-1 by direct sequencing from bulk *env* PCR products from 125 of the 126 patients for whom the *env* gene had successfully been amplified. The combined criteria from the 11/25 and net charge rules predicted 117 CCR5 and eight CXCR4-using virus populations (predicted prevalence of CXCR4-using viruses in plasma of 6.4%), whereas $Geno2pheno_{10}$ predicted 110 CCR5 and 15 CXCR4-using virus populations (predicted prevalence of CXCR4-using viruses in plasma of 12%). The capacity of the genotype to predict the observed phenotype was determined for 124 plasma samples that had been characterized by both assays (Table 3). The combined criteria from the 11/25 and net charge rules accurately predicted the entry phenotype of 113/116 CCR5-using viruses and 5/8 CXCR4-using viruses (concordance with observed phenotype 95.2%). $Geno2pheno_{10}$ accurately predicted 107 CCR5-using viruses and six CXCR4-using viruses (concordance 91.1%). Geno2pheno with an optimized cut-off at 5.75% accurately predicted 114/116 CCR5-using viruses and 5/8 CXCR4-using viruses (concordance 96%).

Genotypic prediction of tropism from cellular HIV-1 DNA

The V3 genotype was also characterized for the 56 patients whose HIV-1 DNA had been amplified successfully. The combined rule classified 53 CCR5 and three CXCR4-using virus populations (predicted prevalence of CXCR4-using viruses in PBMCs of 5.4%), whereas $Geno2pheno_{10}$ predicted 51 CCR5 and five CXCR4-using viruses (predicted prevalence of CXCR4-using viruses in PBMCs of 8.9%). The observed

Table 3. Comparison of the genotypic prediction of HIV-1 tropism and the observed entry phenotype in plasma and cellular viruses during primary infection.

Genotype	Phenotype			Concordance[a]
		R5	R5X4/X4	
Plasma HIV-1 RNA				
Combined 11/25 and net charge rule	R5	113	3	95.2%
	X4	3	5	
Geno2pheno$_{10}$	R5	107	2	91.1%
	X4	9	6	
Geno2pheno$_{5.75}$	R5	114	3	96%
	X4	2	5	
Cellular HIV-1 DNA				
Combined 11/25 and net charge rule	R5	51	0	100%
	X4	0	3	
Geno2pheno$_{10}$	R5	49	0	96.3%
	X4	2	3	
Geno2pheno$_{5.75}$	R5	51	0	100%
	X4	0	3	

[a]Concordance between each genotypic algorithm and the phenotype was calculated as follows: number of samples with a concordant R5 genotype and phenotype plus number of samples with a concordant R5X4/X4 genotype and phenotype, the whole divided by the total number of tested samples.

phenotype for 54 samples and the genotype determined with the combined rule or with Geno2pheno$_{5.75}$ were in complete agreement (100%), whereas the genotype determined with Geno2pheno$_{10}$ was almost as good (96.3%) (Table 3).

The V3 genotype predicted from HIV-1 DNA in the PBMCs correlated well with the tropism predicted from HIV-1 RNA in the plasma. Fifty-five viruses were concordant in the plasma and PBMCs using the combined rule or Geno2pheno to predict HIV-1 tropism. There was only one discordance between a plasma sequence predicted as CXCR4-using and the sequence of virus DNA predicted as CCR5-using. This difference is explained by a double population S/R at position 11 of V3 on the plasma virus, thus predicting a CXCR4-using virus, whereas there was only an S residue on the viral DNA at this position.

Relationships between HIV-1 tropism and characteristics of the patients at the time of primary infection

The characteristics of patients according to their plasma HIV-1 tropism are shown in Table 4. R5X4-infected patients were younger (median age 29 years), with fewer men (50%), than the R5-infected patients (37 years) and mostly men (88%). The HIV-1 transmission route was similar in both groups. But more of the R5X4 group were intravenous drug users (IDUs) (2/8 patients) than the R5 group (2 of 97 patients with known risk factors) ($P = 0.028$) (Table 4). In agreement with the higher frequency of IDUs among the patients harboring R5X4

Table 4. Relationships between HIV-1 tropism in plasma and patient characteristics at the time of primary infection.

Characteristics at baseline	Entry phenotype		*P* value
	R5X4 ($n = 8$)	R5 ($n = 117$)	
Age (years), median (IQR)	29 (19–37)	37 (30–43)	0.035
Men, no. (%)	4 (50)	103 (88)	0.015
Transmission route, no.			
Heterosexual	3	26	0.39
Homosexual	3	69	0.28
Intravenous drug use	2	2	0.028
Unknown	0	20	
T CD4$^+$ cell count (cells/μl), median (IQR)	482 (399–768)	481 (317–652)	0.36
Positive HCV RNA, no. (%)	4 (50)	3 (2.6)	<0.0001
HIV-1 RNA load (log copies/ml), median (IQR)	5.9 (4.0–5.8)	5.6 (4.6–6.6)	0.27
Patients with HIV non-B subtype, no. (%)	1 (12.5)	13 (11.5)	1
HIV resistant to antiretroviral drug, no.	0	13	0.6
Year of seroconversion			
1995–1999	3	14	
2000–2004	2	42	0.128
2005–2008	3	61	

viruses, more of these patients were infected with hepatitis C virus (HCV) than in the R5 group. The HIV-1 viral load, $CD4^+$ T-cell count and the frequency of drug-resistant viruses at the time of the primary infection were similar in the two groups. We also analyzed a subgroup of patients diagnosed at an early stage of infection, when the HIV-1 antibody test was negative or the immunoblot was indeterminate. The four R5X4-infected patients and the 65 R5-infected patients had similar HIV-1 virus loads and $CD4^+$ T-cell counts. The prevalence of CXCR4-using viruses during primary infection did not vary throughout the study period.

Impact of HIV-1 tropism during primary infection on the course of the subsequent infection

We studied the patients who were not given any antiretroviral treatment at the stage of primary infection to compare the natural evolution of the $CD4^+$ T-cell count and the delay before the first antiretroviral treatment in the patients infected with R5 and R5X4 viruses. Five R5X4-infected patients were analyzed and each of them was matched with five R5-infected control patients for age and baseline HIV-1 RNA load. We assessed HIV-1 coreceptor usage one year after the first characterization in four patients initially infected with dual/mixed R5X4 viruses. All four patients were persistently infected with R5X4 viruses (data not shown). Age and HIV-1 RNA load were similar in both groups, with medians of 30 years (IQR 22–39) in the R5X4 group and of 31 years (IQR 25–36) in the R5 group ($P = 0.8$), and medians of 5.0 log copies/ml (IQR 4.0–6.1) in the R5X4 group and of 4.9 log copies/ml (IQR 4.3–5.5) in the R5 group ($P = 0.9$). The CD4 cell counts at baseline were also similar, with medians of 477 cells/μl (IQR 386–850) in the R5X4 group and of 599 cells/μl (IQR 432–725) in the R5 group ($P = 0.88$). Kaplan–Meier estimate of the proportion of patients who progressed to $CD4^+$ T-cell count under 350 cells/μl, stratified according to HIV-1 tropism, revealed that infection with R5X4 viruses was associated with an accelerated rate of disease progression (log rank test $P < 0.001$) (Fig. 1a). Infection with R5X4 viruses was also associated with a reduced delay in initiating a first antiretroviral treatment (log rank test $P = 0.01$) (Fig. 1b).

Discussion

The determination of HIV-1 coreceptor usage is now of therapeutic interest as CCR5 antagonists have recently been developed. Conflicting results have recently been reported regarding the frequency of CXCR4-using viruses during HIV-1 primary infection. Previous studies have suggested that CCR5-using viruses overwhelmingly predominate during primary HIV-1 infection [2,3]. But recent studies using genotypic methods have found a prevalence of about 15% CXCR4-using viruses at this stage [7,17,18], particularly in PBMCs. We therefore investigated HIV-1 tropism during primary infection using an ultrasensitive phenotypic assay to examine the virus in both the plasma and PBMCs.

Phenotypic characterization of HIV-1 in the plasma indicated that dual-mixed R5X4 virus populations accounted for 6.4% of the total in patients with recent seroconversion, with the prevalence being similar in subtype B and non-B HIV-1 subtypes. These results are close to the 3% of CXCR4-using viruses in the plasma of recent seroconverters previously estimated with phenotypic methods [9,10]. More of the patients infected with dual-mixed R5X4 viruses were intravenous drug users than were those infected with R5 viruses. Phylogenetic analysis excluded a cluster of transmission with a unique strain of R5X4 viruses among the intravenous drug users.

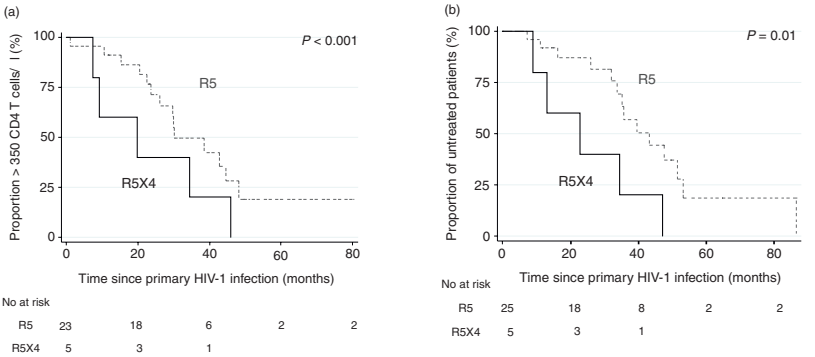

Fig. 1. Progression-free survival among untreated patients. (a) Progression was defined in Kaplan–Meier curve as a CD4 T-cell count of less than 350 cells/μl (log rank test). Patients harboring R5X4 viruses (black solid line, $n = 5$) were compared to controls harboring R5 viruses (gray dashed line, $n = 25$). The table below the graph indicates the number of patients at risk in each group at the different times of the study. (b) Progression was defined in Kaplan–Meier curve as initiating an antiretroviral treatment (log rank test). Patients harboring R5X4 viruses (black solid line, $n = 5$) were compared to controls harboring R5 viruses (gray dashed line, $n = 25$). The table below the graph indicates the number of patients at risk in each group at the different times of the study.

The increased frequency of R5X4 virus among the patients infected via the parenteral route could be linked to the $CXCR4^+$ $CD4^+$ T cells being more abundant than $CCR5^+$ $CD4^+$ T cells in the blood [23]. By contrast, CCR5-expressing target cells are abundant in genital and rectal mucosae. Nevertheless, counterselection of CXCR4-using viruses following their transmission via the parenteral route had been reported [24,25], suggesting that the global advantage of R5 viruses in establishing primary HIV-1 infection is not only related to the mucosal transmission step. The transmitted R5X4 viruses were not counterselected in our study and remained predominant after the acute phase of the infection in all four patients whose longitudinal samples were analyzed. Others have reported similar findings [7,26] but larger studies are required to explore viral tropism in this transmission group. In addition, the patients infected with R5X4 viruses were younger and more often female than those harboring R5 viruses. This was not related to any specific characteristics of the intravenous drug user population, as women and young people were not over-represented in this particular group. We have determined HIV-1 tropism using phenotypic methods in both plasma and PBMC samples taken from recently infected patients. HIV-1 tropism in the two compartments was 98% concordant. A recent study suggested that CXCR4-using viruses were more frequent in PBMCs than in the plasma of patients with advanced disease [27]. However, the virus population during the first few months of HIV-1 infection is more homogenous than later in the disease evolution. This may explain why the HIV-1 tropism in the cellular and plasma compartments are similar at this stage. Thus, data for the plasma compartment during primary infection do not underestimate the prevalence of CXCR4-using viruses.

Several studies have shown that genotypic methods can be used to accurately determine HIV-1 tropism in chronically infected patients [14,16,17,28]. But little is known about how they perform during primary infection. We sequenced the V3 region of *env* from plasma HIV-1 RNA and used the combination of the 11/25 and net charge rules to predict coreceptor usage [16]. The concordance between the observed phenotype and the combined rule was better than 95% using both plasma and PBMC samples. The $Geno2pheno_{10}$ bioinformatic algorithm also performed well with a concordance better than 91% using samples from both compartments.

Genotypic methods predicted the prevalence of CXCR4-using viruses in the plasma to be 6.4% with the combined rule, and 12% with $Geno2pheno_{10}$. The unexpected high prevalence of CXCR4-using viruses during primary infection found by others [7,17,18] may be due to the use of genotypic algorithms that overestimated the number of CXCR4-using viruses. We found that the $Geno2pheno_{10}$ positive predictive value was only 40% for plasma samples (six samples well predicted X4 divided by a total of 15 samples predicted X4 by $Geno2pheno_{10}$). Specificity was also significantly reduced by combining several genotypic algorithms in order to increase the sensitivity of detecting CXCR4-using viruses [7,8,18], and prevalence of CXCR4-using viruses was thus overestimated. Geno2pheno algorithm is now available with an optimized cut-off at 5.75% (false-positive rate). Using this new algorithm estimated better the prevalence of CXCR4-using viruses in this set of samples (5.6%).

CXCR4-using viruses are considered to be more pathogenic than R5 viruses, including at the time of primary infection in a few studies [7,19]. Previous studies have not agreed on the correlation between the $CD4^+$ T-cell count and HIV-1 tropism at the stage of primary infection [8,18]. HIV-1 coreceptor usage could influence the clinical evolution of these patients, as observed in other groups of patients [29,30]. We find that the $CD4^+$ T-cell counts in patients infected with R5 viruses and in those infected with R5X4 viruses are similar at the time of primary infection. We compared the clinical evolution of patients infected with CCR5 or CXCR4-using viruses in a case-control study. The patients infected with CXCR4-using viruses at the time of primary infection had an increased risk of disease progression, estimated by a $CD4^+$ T lymphocyte count under 350 cells/μl or by initiation of a first antiretroviral treatment.

In conclusion, the prevalence of CXCR4-using viruses at the time of primary infection was only of 6.4% in the plasma using an ultrasensitive phenotypic method. HIV-1 tropism in paired plasma and PBMC samples was essentially the same (concordance 98%) at the time of primary infection. The capacity of genotypic algorithms to predict CXCR4 usage by viruses identified during HIV-1 primary infection was similar to that previously described for viruses identified at a later stage of the disease. However, the use of low-specificity genotypic algorithms, or their combination to increase their sensitivity can lead to overestimation of the prevalence of CXCR4-using viruses. Finally, larger studies are required to confirm the negative impact of the transmitted CXCR4-using viruses on the subsequent evolution of HIV-1 disease.

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References

1. Berger EA, Doms RW, Fenyo EM, Korber BT, Littman DR, Moore JP, *et al*. **A new classification for HIV-1.** *Nature* 1998; **391**:240.
2. Schuitemaker H, Koot M, Kootstra NA, Dercksen MW, de Goede RE, van Steenwijk RP, *et al*. **Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population.** *J Virol* 1992; **66**:1354–1360.
3. Berger EA, Murphy PM, Farber JM. **Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease.** *Annu Rev Immunol* 1999; **17**:657–700.
4. Markowitz M, Mohri H, Mehandru S, Shet A, Berry L, Kalyanaraman R, *et al*. **Infection with multidrug resistant, dual-tropic HIV-1 and rapid progression to AIDS: a case report.** *Lancet* 2005; **365**:1031–1038.
5. Yu XF, Wang Z, Vlahov D, Markham RB, Farzadegan H, Margolick JB. **Infection with dual-tropic human immunodeficiency virus type 1 variants associated with rapid total T cell decline and disease progression in injection drug users.** *J Infect Dis* 1998; **178**:388–396.
6. van't Wout AB, Kootstra NA, Mulder-Kampinga GA, Albrecht-van Lent N, Scherpbier HJ, Veenstra J, *et al*. **Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission.** *J Clin Invest* 1994; **94**:2060–2067.
7. de Mendoza C, Rodriguez C, Garcia F, Eiros JM, Ruiz L, Caballero E, *et al*. **Prevalence of X4 tropic viruses in patients recently infected with HIV-1 and lack of association with transmission of drug resistance.** *J Antimicrob Chemother* 2007; **59**:698–704.
8. de Mendoza C, Van Baelen K, Poveda E, Rondelez E, Zahonero N, Stuyver L, *et al*. **Performance of a population-based HIV-1 tropism phenotypic assay and correlation with V3 genotypic prediction tools in recent HIV-1 seroconverters.** *J Acquir Immune Defic Syndr* 2008; **48**:241–244.
9. Eshleman SH, Husnik M, Hudelson S, Donnell D, Huang Y, Huang W, *et al*. **Antiretroviral drug resistance, HIV-1 tropism, and HIV-1 subtype among men who have sex with men with recent HIV-1 infection.** *AIDS* 2007; **21**:1165–1174.
10. Shepherd JC, Jacobson LP, Qiao W, Jamieson BD, Phair JP, Piazza P, *et al*. **Emergence and persistence of CXCR4-tropic HIV-1 in a population of men from the multicenter AIDS cohort study.** *J Infect Dis* 2008; **198**:1104–1112.
11. Coakley E, Reeves JD, Huang W, Mangas-Ruiz M, Maurer I, Harskamp AM, *et al*. **Comparison of human immunodeficiency virus type 1 tropism profiles in clinical samples by the Trofile and MT-2 assays.** *Antimicrob Agents Chemother* 2009; **53**:4686–4693.
12. Skrabal K, Low AJ, Dong W, Sing T, Cheung PK, Mammano F, Harrigan PR. **Determining human immunodeficiency virus coreceptor use in a clinical setting: degree of correlation between two phenotypic assays and a bioinformatic model.** *J Clin Microbiol* 2007; **45**:279–284.
13. Whitcomb JM, Huang W, Fransen S, Limoli K, Toma J, Wrin T, *et al*. **Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism.** *Antimicrob Agents Chemother* 2007; **51**:566–575.
14. Delobel P, Nugeyre MT, Cazabat M, Pasquier C, Marchou B, Massip P, *et al*. **Population-based sequencing of V3 env for predicting the coreceptor usage of HIV-1 quasispecies.** *J Clin Microbiol* 2007; **45**:1572–1580.
15. Jensen MA, Li FS, van't Wout AB, Nickle DC, Shriner D, He HX, *et al*. **Improved coreceptor usage prediction and genotypic monitoring of R5-to-X4 transition by motif analysis of human immunodeficiency virus type 1 env V3 loop sequences.** *J Virol* 2003; **77**:13376–13388.
16. Raymond S, Delobel P, Mavigner M, Cazabat M, Souyris C, Sandres-Saune K, *et al*. **Correlation between genotypic predictions based on V3 sequences and phenotypic determination of HIV-1 tropism.** *AIDS* 2008; **22**:F11–16.
17. Poveda E, Briz V, de Mendoza C, Benito JM, Corral A, Zahonero N, *et al*. **Prevalence of X4 tropic HIV-1 variants in patients with differences in disease stage and exposure to antiretroviral therapy.** *J Med Virol* 2007; **79**:1040–1046.
18. Frange P, Galimand J, Goujard C, Deveau C, Ghosn J, Rouzioux C, *et al*. **High frequency of X4/DM-tropic viruses in PBMC samples from patients with primary HIV-1 subtype-B infection in 1996–2007: the French ANRS CO06 PRIMO Cohort Study.** *J Antimicrob Chemother* 2009; **64**:135–141.
19. Dalmau J, Puertas MC, Azuara M, Marino A, Frahm N, Mothe B, *et al*. **Contribution of immunological and virological factors to extremely severe primary HIV type 1 infection.** *Clin Infect Dis* 2009; **48**:229–238.
20. Hayman A, Moss T, Arnold C, Naylor-Adamson L, Balfe P. **Disease progression in heterosexual patients infected with closely related subtype B strains of HIV type 1 with differing coreceptor usage properties.** *AIDS Res Hum Retroviruses* 2004; **20**:365–371.
21. Raymond S, Delobel P, Mavigner M, Cazabat M, Souyris C, Encinas S, *et al*. **Development and performance of a new recombinant virus phenotypic entry assay to determine HIV-1 coreceptor usage.** *J Clin Virol* 2010; **47**:126–130.
22. McGovern R, Dong W, Zhong X, Knapp D, Thielen A, Chapman D, *et al*. **Population-based sequencing of the V3-loop is comparable to the enhanced sensitivity trofile assay in predicting virologic response to maraviroc of treatment-naïve patients in the MERIT trial.** In *17th Conference on Retroviruses and Opportunistic Infections*. San Francisco, CA; 2010.
23. Moore JP, Kitchen SG, Pugach P, Zack JA. **The CCR5 and CXCR4 coreceptors: central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection.** *AIDS Res Hum Retroviruses* 2004; **20**:111–126.
24. Cornelissen M, Mulder-Kampinga G, Veenstra J, Zorgdrager F, Kuiken C, Hartman S, *et al*. **Syncytium-inducing (SI) phenotype suppression at seroconversion after intramuscular inoculation of a nonsyncytium-inducing/SI phenotypically mixed human immunodeficiency virus population.** *J Virol* 1995; **69**:1810–1818.
25. Pratt RD, Shapiro JF, McKinney N, Kwok S, Spector SA. **Virologic characterization of primary human immunodeficiency virus type 1 infection in a healthcare worker following needlestick injury.** *J Infect Dis* 1995; **172**:851–854.
26. Brumme ZL, Goodrich J, Mayer HB, Brumme CJ, Henrick BM, Wynhoven B, *et al*. **Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals.** *J Infect Dis* 2005; **192**:466–474.
27. Verhofstede C, Vandekerckhove L, Eygen VV, Demecheleer E, Vandenbroucke I, Winters B, *et al*. **CXCR4-using HIV type 1 variants are more commonly found in peripheral blood mononuclear cell DNA than in plasma RNA.** *J Acquir Immune Defic Syndr* 2009; **50**:126–136.
28. Poveda E, Briz V, Roulet V, Del Mar Gonzalez M, Faudon JL, Skrabal K, Soriano V. **Correlation between a phenotypic assay and three bioinformatic tools for determining HIV co-receptor use.** *AIDS* 2007; **21**:1487–1490.
29. Waters L, Mandalia S, Randell P, Wildfire A, Gazzard B, Moyle G. **The impact of HIV tropism on decreases in CD4 cell count, clinical progression, and subsequent response to a first antiretroviral therapy regimen.** *Clin Infect Dis* 2008; **46**:1617–1623.
30. Weiser B, Philpott S, Klimkait T, Burger H, Kitchen C, Burgisser P, *et al*. **HIV-1 coreceptor usage and CXCR4-specific viral load predict clinical disease progression during combination antiretroviral therapy.** *AIDS* 2008; **22**:469–479.