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HIV Coreceptor Tropism in Paired Plasma, Peripheral Blood Mononuclear Cell, and Cerebrospinal Fluid Isolates from Antiretroviral-Naïve Subjects▽

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A survey of HIV coreceptor usage in cerebrospinal fluid (CSF) samples, peripheral blood mononuclear cells (PBMCs), and plasma samples from naïve seropositive patients was conducted. One hundred patients were enrolled in this study. Of the 100 patients, 36 had a primary or recent infection (P-RI), 31 had an early chronic infection (>350 CD4 cells) (ECI), and 33 had a late chronic infection (LCI). All 3 compartments were sampled in a subset of 33 participants, while the remaining 67 patients provided plasma samples and PBMCs only. Seventy-seven patients harbored the R5 virus in plasma samples and had a significantly higher median and percentage of $CD4^+$ T cells than patients with X4 virus (437 and 281 cells/μl, respectively; $P = 0.0086$; 20.6% and 18.6%, respectively). The X4 strain was detected more frequently in patients with LCI than in patients with P-RI or ECI (39.3%, 19.4%, and 9.6%, respectively; $P = 0.0063$). PBMC and plasma tropism was concordant in 90 patients, and 73 had the R5 strain. Among patients with discordant results, 4 had the R5 virus in their plasma and the X4 virus in PBMCs; 6 showed the opposite profile. Plasma, PBMC, and CSF tropism determinations were concordant in 26/33 patients (21 patients had R5, and 5 had X4). The tropism was discordant in 5/33 patients, with the X4 virus in plasma and R5 in CSF; the HIV tropism in PBMCs was X4 in 3 patients. The remaining 2/33 patients had the R5 virus in plasma and PBMCs and the X4 virus in CSF; one of these patients had a P-RI. The discordant tropism in CSF and blood may have implications for chemokine (C-C motif) receptor 5 (CCR5) antagonist use in patients with limited response to antiretroviral therapy (ART) or in responding patients evaluated for simplification of treatment.

The outcome of exposure to human immunodeficiency virus type 1 (HIV-1) varies greatly between individuals. One of the factors determining this variability in outcome is the cellular tropism or viral phenotype (8, 28), as the pathogenesis of HIV-1 is critically influenced by the cell types that the virus is capable of infecting. HIV-1 requires two cellular receptors for entry, CD4 and one of a family of chemokine receptors (coreceptor).

In vivo, the major coreceptors used by HIV-1 are chemokine (C-C motif) receptor 5 (CCR5) (3, 15, 16) and chemokine (C-X-C motif) receptor 4 (CXCR4) (20). Individual viruses are classified on the basis of their ability to use CCR5 (R5 variants), CXCR4 (X4 variants), or both (R5X4) (7). Viral populations that can use both receptors are designated dual/mixed (D/M), as they may contain any mixture of these three types (51).

Most HIV strains that infect macrophages utilize CCR5 (23, 39). CXCR4-utilizing strains mainly infect lymphocyte lines, although some X4 isolates have been shown to infect macrophages and microglia (15, 16). In general, R5 viruses predominate during early infection. X4 viruses are most often detected later in the course of infection and may be associated with more-rapid $CD4^+$ T cell loss, although it is unknown whether this virus subtype is the cause or result of a change in the course of T cell attrition (33, 37). Therefore, detection of the emergence of virus using CXCR4 has potential value for predicting pathogenesis, monitoring disease progression, and making treatment decisions. Moreover, the detection of R5 variants is required for the optimal use of the recently available CCR5 antagonist (Maraviroc) (35).

Coreceptor utilization is likely to be important in central nervous system (CNS) HIV infection and related neurological morbidity. Most HIV strains isolated from individuals with HIV encephalitis and AIDS dementia complex (ADC) are R5 viruses (2, 26, 30, 32), and this is consistent with the central role of macrophages in brain infection (10, 41, 47, 52) and with the pathogenic role of coreceptor binding in brain injury (4, 5, 6, 31, 50).

The good CNS penetration of Maraviroc identifies the drug as a compound to consider for use in patients with HIV infection and CNS involvement in the presence of an R5 isolate.

As there are only case reports or studies of patients with primary infection on the HIV tropism analyzed in paired plasma and peripheral blood mononuclear cell (PBMC) samples (12, 42) or in plasma and cerebrospinal fluid (CSF) samples (27, 45), we undertook this study to survey concurrent HIV coreceptor usage in CSF samples, PBMCs, and plasma

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TABLE 1. Characteristics of 90 patients with concordant HIV tropism in plasma samples and PBMCs

Characteristic	No. of patients or parameter value		
	All patients	Patients with R5 virus	Patients with X4 virus
No. of patients	90	73	17
No. of CD4 cells/μl [median (range)]	362 (1–1,100)	388 (20–1,100)	230 (1–705)
% CD4 cells [median (range)]	19.6 (0–41)	22 (2–40.2)	18 (0–41)
Plasma viremia, log no. of HIV RNA copies/ml [median (range)]	5.07 (2.96–7.0)	4.53 (2.96–7.0)	5.001 (2.97–6.19)
Recent infection (P-RI)	32	28	4
Early chronic infection (ECI)	30	27	3
Late chronic infection (LCI)	28	18	10

samples from a large sample of treatment-naïve HIV-seropositive patients with the ultimate aim of assessing the concordance of tropism of viral strains isolated from different sites.

MATERIALS AND METHODS

Subjects and protocols. One hundred antiretroviral-naïve patients were consecutively recruited in five Italian Centres for Infectious Diseases in 2008 and 2009. Paired plasma and PBMC samples were collected from all patients; CSF samples were obtained from 33 patients.

To be eligible for this study, the patient had to be >18 years old and had never been given an antiretroviral drug (antiretroviral naïve).

A primary or recent HIV infection (P-RI) was defined by the presence of either one of the following: (i) a negative or indeterminate HIV antibody enzyme-linked immunosorbent assay (ELISA) result and HIV RNA-positive result from plasma or (ii) an initially negative HIV antibody test result, followed by positive serology within 18 months.

All subjects underwent general medical and neurological assessments. Lumbar puncture was justified by the presence of symptoms or signs of possible involvement of the CNS at clinical examination (headache, neurocognitive disorders, depression, etc.). CSF samples were obtained with concurrent phlebotomy along with clinical assessments and analyzed for the following parameters: cell counts, cell differentials, total protein levels, and albumin levels. CSF samples for virological studies were centrifuged at 1,200 × *g* for 10 min to remove cells and stored at −80°C in individual aliquots.

Plasma samples and PBMCs were separated and stored for viral assays conducted in parallel with assays of CSF.

The protocol was approved by the local ethics committees, and informed consent was obtained from all participants.

General virological methods. HIV-1 RNA levels were measured in cell-free CSF and plasma by the Roche Cobas AmpliPrep-Cobas TaqMan HIV-1 assay, version 1 (F. Hoffmann-La Roche, Diagnostics Division, Basel, Switzerland). HIV-1 RNA concentrations were transformed to $\log_{10}$ values for all calculations.

Genotypic prediction of viral tropism. Genotypic analysis of viral tropism was performed as previously described (14). Briefly, V3 sequences were amplified using nested PCR with 1F1 and 1R1 as outer primers and 3F3 and 2R2 as inner primers. The generated V3 sequences were then interpreted using the bioinformatic tool Geno2pheno with false-positive rates of 10%. Geno2pheno is available at http://coreceptor.bioinf.mpi-sb.mpg.de (accessed June 2010). According to the recommendations on the geno2pheno website, all predictions were made using the maximum sensitivity value for recognizing X4; when choosing the significance levels, we selected a 10% false-positive rate (10% probability of classifying an R5 virus falsely as X4).

Statistical analysis. The following variables were reported and evaluated for each patient: age, blood CD4 lymphocyte count/μl, plasma viral load (number of HIV RNA copies/ml), viral load in CSF, presence of the X4 variant in plasma, presence of X4 in PBMCs, presence of X4 in CSF, recent HIV infection, early chronic HIV infection, and late HIV infection. Subsequently, the variable "X4_any" was derived and was set at 1 for any positive finding of X4 in plasma, PBMCs, or CSF or to 0 for the uniform absence of X4 and the presence of the R5 variant in the same compartment.

An exploratory, pairwise approach was carried out for all "original" variables. *P* values were obtained after a bivariate linear regression if at least one variable was continuous or after a Pearson's chi-square test if both variables were binary. This was used to detect any existing correlation among the aforementioned variables, with particular focus on X4/R5 viral phenotype in several body compartments.

In addition, the role of the X4/R5 phenotype was used to compare the corresponding values assumed by age, viral load, and CD4 lymphocyte count in the relevant populations using the Student *t* test. Conventional descriptive statistics were also applied when appropriate.

RESULTS

At enrollment in the study, 36 patients had a primary or recent infection (P-RI), while 64 had chronic infection. Of these 64 patients, 31 patients had a CD4 cell count of more than 350 cells/μl (early chronic infection [ECI]), and 33 had a late chronic infection (LCI) (<350 CD4 cells/μl).

The median age was 39 years (interquartile range [IQR], 32 to 44 years), with a CD4 cell count of 363 cells/μl (IQR, 188 to 580 cells/μl) and a CD4 cell percentage of 20.9% (IQR, 14.6 to 26.2%). The median viral load of plasma viremia was 5.07 $\log_{10}$ of the number of HIV RNA copies per ml (log HIV RNA/ml) (IQR, 4.24 to 5.30 log HIV RNA/ml).

The inferred HIV tropism study in plasma demonstrated that 77 patients harbored the R5 virus, while 23 patients had the X4 variant. Patients with the R5 strain had significantly higher median CD4 cell counts and higher CD4 cell percentages than did patients with the X4 variant (437 and 281 cells/μl, respectively; $P = 0.0086$; 20.6% and 18.6%, respectively). Patients with the X4 strain were significantly older than patients with the R5 strain (45.2 and 37.4 years, respectively; $P = 0.0008$).

Moreover, the X4 strain was detected more frequently in patients with LCI (13 of 33 patients [39.3%]) than in patients with P-RI (7 of 36 patients [19.4%]) or ECI (3 of 31 patients [9.6%]). Detection of X4 strain in plasma significantly correlated with LCI ($P = 0.0063$).

Viral tropism in PBMCs and plasma was concordant in 90 patients (Table 1): 73 had the R5 virus in both compartments, and 17 had the X4 virus in both compartments. In the 10 patients with discordant tropism in the two compartments, 4 patients (1 patient with P-RI, 1 with ECI, and 2 with LCI) had the R5 virus in plasma and the X4 virus in PBMCs (Table 2). The remaining 6 patients (3 P-RI and 3 LCI) with discordant tropism had the X4 virus in plasma and the R5 virus in PBMCs.

The inferred HIV tropism in paired samples of plasma, PBMCs, and CSF was successfully determined in 33 patients; of the 33 patients, 11 had P-RI, 10 had ECI, and 12 had LCI. The median age was 40 years (IQR, 34 to 44 years), with a CD4 cell count of 321 cells/μl (IQR, 84 to 413 cells/μl) and a CD4

TABLE 2. Characteristics of 10 patients with discordant HIV tropism in plasma samples and PBMCs[a]

Patient	Age (yr)	No. of CD4 cells/μl	% CD4	Plasma viremia (log no. of HIV RNA copies/ml)	Phase of infection	HIV tropism in:	
						Plasma samples	PBMCs
795	39	516		1.98	P-RI	R5	X4
646	41	469	29.1	1.65	ECI	R5	X4
514	36	110		4.65	LCI	R5	X4
877	54	30	2	4.37	LCI	R5	X4
420	33	380	24	4.93	P-RI	X4	R5
477	45	46	9	5.14	LCI	X4	R5
861	30	704	32	5.30	P-RI	X4	R5
842	41	390	31	3.44	P-RI	X4	R5
599	62	2		5.30	LCI	X4	R5
870	45	170		3.44	LCI	X4	R5

[a] PBMCs, peripheral blood mononuclear cells.

cell percentage of 17% (IQR, 9 to 22%). The median plasma viral load was 5.27 log HIV RNA/ml (IQR, 4.94 to 5.45 log HIV RNA/ml), while the median CSF viral load was 3.72 log HIV RNA/ml (IQR, 3.20 to 4.34 log HIV RNA/ml). Among the 33 CSF strains analyzed, 26 (78.8%) were R5 and 7 (21.2%) were X4. The frequency of X4-positive HIV strains in CSF was not significantly different in patients with P-RI, ECI, and LCI (3/11, 2/10, and 2/12, respectively; $P = 0.81$). Moreover, there was no significant difference in the CD4 cell count or percentage by comparing CSF samples from X4-positive and R5-positive HIV strain carriers ($P = 0.84$ and $P = 0.71$, respectively).

Plasma, PBMC, and CSF tropisms were concordant in 26 of 33 patients (78.8%): 21 patients with R5 strains and 5 patients with X4 strains in all compartments. In 7 patients with discordant tropism in the three compartments (Table 3), 5 patients had the X4 virus in plasma and the R5 virus in CSF; 3 of these patients had LCI, one had ECI, and one had P-RI. In these 5 patients, the HIV tropism in PBMCs was X4 in 3 patients and R5 in the remaining 2. The other 2 patients with discordant tropism had the R5 virus in plasma and PBMCs and the X4 virus in CSF; one of the 2 patients had P-RI.

DISCUSSION

This study demonstrated that the majority of antiretroviral-naïve patients in our series (77%) harbored the R5 strain.

HIV-1 coreceptor use is an important determinant of disease progression in the natural course of infection (28). Most coreceptor use data obtained from longitudinal samples collected during the natural course of disease have robustly defined the association between the emergence of virus using CXCR4 and accelerated disease progression (8, 9, 28, 36, 43).

In patients with recent infection, the X4 strain was detected in plasma samples from 19.4% of patients. Moreover, an additional patient with the R5 variant in plasma had the X4 strain in PBMCs. Recent studies have reported various frequencies of X4 dual/mixed (D/M) dualtropic strains (3.2 to 17.5%) in plasma samples from recently infected patients in the United States and Spain (13, 19). A large French epidemiological study demonstrated that a high proportion of patients (62 of 390 patients [15.9%]) harbored X4 or dualtropic viruses in PBMCs at the time of primary infection, suggesting the existence of a cellular X4 viral reservoir that could persist for a lengthy period of time (21). Further studies are needed to evaluate the impact on the outcome of HIV infection of detecting such strains at the time of primary infection.

Discordant tropism between plasma and PBMCs was detected in 10% of antiretroviral-naïve patients. Four patients had the R5 virus in plasma and the X4 virus in PBMCs; 3 of these patients had a chronic HIV infection. The remaining 6 patients had the X4 virus in plasma and the R5 virus in PBMCs; 3 of these patients had a recent HIV infection.

A previous study showed that PBMC and plasma tropisms were concordant in 10 viremic patients (48). Concordant results were identified in 98% of 133 patients with primary HIV-1 infection who were screened for HIV-1 coreceptor us-

TABLE 3. Characteristics of 7 patients with discordant HIV tropism in plasma samples, PBMCs, and CSF samples[a]

Patient	Age (yr)	No. of CD4 cells/μl	% CD4	Viral load (log no. of HIV-RNA copies/ml) in:		Phase of infection	HIV tropism in:		
				Plasma samples	CSF samples		Plasma samples	PBMCs	CSF samples
420	33	380	24	4.93	4.33	P-RI	X4	R5	R5
477	45	46	9	5.14	1.94	LCI	X4	R5	R5
502	63	22	2	5.001	3.44	LCI	X4	X4	R5
312	37	84	12	4.71	3.49	LCI	X4	X4	R5
410	40	413	22	3.67	3.71	ECI	X4	X4	R5
419	30	290	18	5.30	3.20	P-RI	R5	R5	X4
513	26	467	35	4.00	2.32	ECI	R5	R5	X4

[a] PBMCs, peripheral blood mononuclear cells; CSF, cerebrospinal fluid.

age in plasma samples and PBMCs using both genotypic and phenotypic methods (42). Therefore, it seems that the plasma virus population and the replication-competent virus population in PBMCs may actually be quite comparable. Indeed, virus variants using CXCR4 may emerge later in plasma than they do in PBMCs (12). The detection of the R5 variant in plasma and the X4 strain in PBMCs in the chronic phase of infection could represent the emergence of the most fit virus. A recent study suggested that CXCR4-using viruses were more frequent in PBMCs than in plasma samples from patients with advanced disease (49). 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 is similar at this stage.

Conversely, the presence of the X4 virus in plasma and the R5 virus in PBMCs can represent an early switch of viral tropism in the progression of infection. Moreover, if virus variants using CXCR4 emerge first, they do not immediately dominate the virus population in either compartment (29). Instead, their presence may fluctuate for a time at levels near the detection limit of current assays. It is conceivable that virus variants using CXCR4 are present at even earlier time points at levels below the current assay detection limits.

Plasma, PBMC, and CSF tropisms were concordant in 78.8% of patients. Three out of seven patients with the X4 variant in CSF samples had a recent infection. This confirms that virus variants using CXCR4 can primarily infect the CNS.

Seven patients had discordant tropism in the three compartments: 5 patients had the X4 virus in plasma and the R5 virus in CSF, and all but one of these patients had a chronic HIV infection. The other 2 patients with discordant tropism had the R5 virus in plasma and the X4 virus in CSF; one of the 2 patients had a recent HIV infection.

Although R5 viruses predominate in the CSF, HIV-1 populations able to utilize CXCR4 are also present. In a cross-sectional study of CSF and blood samples from 46 HIV-infected subjects, most CSF HIV populations utilized CCR5 as the principal coreceptor, and the majority of subjects had concordant tropism in the 2 compartments (45). However, approximately one-fifth of subjects displayed the R5/X4 phenotype in one or both fluids, and 1/10 of subjects had discordant tropism in plasma and CSF. A detailed analysis of tropism and phylogenetic relationships among clones picked from paired specimens of plasma and CSF provided evidence of extensive, subtle discordance between and variation within compartments.

HIV-1 infection of the CNS occurs shortly after peripheral infection, most likely through the trafficking of infected lymphocytes and monocytes across the blood-brain barrier (34, 38). Previous studies have shown that virus detected in the CSF originates from both local CNS tissue and the peripheral blood (via the choroid plexus and meninges) (17, 18, 22, 24), indicating that the CSF may act as a site of mixing of virus present in the brain and periphery. In addition, genetic compartmentalization has been reported between blood, plasma, and CSF viral variants (40, 44, 46).

In this study, discordance was observed in both directions, including 2 examples of the R5 strain in plasma and the X4 strain in CSF. Increased CSF compartmentalization of the HIV-1 *env* gene could reflect independent HIV-1 replication and evolution within the central nervous system (25).

The cross-sectional design, the lack of clonal analysis of paired plasma, PBMC, and CSF samples, and the possible transition state of the virus may have biased this study. Moreover, a genotypic assay was used to detect the HIV tropism. Discordances in the HIV tropism in different compartments are likely due to low-level minority species present as quasispecies that are not detected with bulk sequencing. New sequencing technology, such as massive parallel pyrosequencing (11), may allow us to investigate this further.

In a recent report on a limited number of naïve and experienced subjects (1), cellular quasispecies were found to be more heterogeneous than those observed in plasma. In most patients eligible for CCR5 antagonist treatment, X4 variants were detected in proviral DNA, ranging from 1.0% to 52.7%. However, other studies have demonstrated discordance between HIV tropism in plasma samples and PBMCs or plasma and CSF samples.

Viral genetic compartmentalization could play an important role in defining subjects at risk of progression to CNS involvement in the absence of therapeutic intervention. Moreover, the discordant tropism in CSF and plasma may have implications for CCR5 inhibitor therapy even in the presence of an X4 tropism detected in plasma samples from patients with a limited response to antiretroviral therapy (ART), considering the good penetration of this drug in the CNS compartment.

Finally, discordance between plasma and PBMCs could be relevant in the simplification of therapy with CCR5 antagonists in patients with an undetectable level of viremia.

In conclusion, larger studies are required to confirm the relevance of the discordance in HIV tropism in different compartments and its impact on the evolution of infection and treatment efficacy.

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