

HIV-2 viral tropism influences CD4+ T cell count regardless of viral load

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Background: HIV-2 infection is characterized by low plasma viraemia and slower progression to AIDS in comparison with HIV-1 infection. However, antiretroviral therapy in patients with HIV-2 is less effective and often fails to provide optimal CD4 recovery.

Methods: We examined viral tropism in persons with HIV-2 infection enrolled in the HIV-2 Spanish cohort. Viral tropism was estimated based on V3 sequences obtained from plasma RNA and/or proviral DNA.

Results: From a total of 279 individuals with HIV-2 infection recorded in the Spanish national register, 58 V3 sequences belonging to 42 individuals were evaluated. X4 viruses were recognized in 14 patients (33%). Patients with X4 viruses had lower median CD4+ cell counts than patients with R5 viruses [130 (17–210) versus 359 (180–470) cells/mm³; $P=0.007$]. This was true even considering only the subset of 19 patients on antiretroviral therapy [94 (16–147) versus 184 (43–368) cells/mm³; $P=0.041$]. In multivariate analysis, significant differences in CD4+ cell counts between patients with X4 and R5 viruses remained after adjusting for age, gender, antiretroviral therapy and viral load.

Conclusions: The presence of X4-tropic viruses in HIV-2 infection is associated with low CD4+ cell counts, regardless of antiretroviral treatment. Along with CD4+ cell counts, viral tropism testing may assist decisions about when to initiate antiretroviral therapy in HIV-2-infected individuals.

Keywords: CCR5 antagonists, proviral load, maraviroc

Introduction

HIV-2 infection is endemic in certain areas of West Africa, where 5%–10% prevalence rates have been reported in some populations, but the virus has limited spread worldwide.¹ Compared with HIV-1, HIV-2 infection results in lower viral loads, slower CD4 declines and slower progression to AIDS.² However, HIV-2-infected individuals are at risk for developing opportunistic conditions once severe immunodeficiency develops. Given that antiretroviral drugs have mainly been optimized for the treatment of HIV-1 infection, therapies for HIV-2 are currently limited. HIV-2 is naturally resistant to non-nucleoside reverse transcriptase inhibitors³ and fusion inhibitors,⁴ and less susceptible to most protease inhibitors, such as fosamprenavir, atazanavir and tipranavir.⁵ Fortunately, clinical and *in vitro* studies show that most nucleos(t)ide reverse transcriptase inhibitors and integrase inhibitors exhibit good antiviral activity against HIV-2.^{6,7} In this scenario,

information on the susceptibility of HIV-2 to CCR5 coreceptor antagonists is important, since these agents may provide a desirable alternative option within the HIV-2 armamentarium.

Primary CCR5-tropic isolates of HIV-2 are inhibited *in vitro* by maraviroc.⁸ Because CCR5 antagonists only block infections produced by R5 viruses, it is mandatory to assess viral tropism before testing any susceptibility of HIV-2 to maraviroc. A recent report has identified four major genetic determinants of tropism in the V3 loop of the env gp105 of HIV-2.⁹ Furthermore, prediction of HIV-2 tropism using viral sequences obtained from proviral DNA collected from peripheral blood mononuclear cells (PBMCs) has shown to be accurate and therefore a suitable tool in HIV-2 patients with low or undetectable viral load.¹⁰

Besides its therapeutic interest, viral tropism has been shown to influence disease progression in HIV-1 infection; in fact the presence of X4 viruses has been associated with low CD4+ cell counts,¹¹ even when patients are receiving antiretroviral

therapy.¹² A similar effect might occur in HIV-2 infection. The aim of our study was to examine viral tropism in patients belonging to the HIV-2 Spanish cohort and determine its influence on disease progression.

Patients and methods

The Spanish HIV-2 national register is a publicly funded database that collects information from all individuals diagnosed with HIV-2 infection in Spain since 1989. A centralized repository of stored clinical samples, including PBMCs and plasma, functions in parallel and was used for the current study. The study was approved by the hospital ethics committee.

Plasma HIV-2 RNA was quantified using a non-commercial real-time PCR assay at Hospital Carlos III. The region amplified was the long terminal repeat region, for which the primers and probe have been described elsewhere.¹³ Both HIV-2 groups A and B are reliably detected with this assay. RNA was extracted from 1 mL of plasma (QIAmp viral RNA Mini Kit, Westburg, Germany); then it was eluted in a volume of 50 µL. Every sample had an internal positive control (TaqMan Exogenous Internal Positive Control Reagent, Applied Biosystems, Foster City, CA, USA), which was added to the buffer before nucleic acid extraction. Thermal cycling conditions consisted of an initial step of 2 min at 50°C and an activation step at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. For each run, a standard curve was generated using a stock of HIV-2 strain NIHZ, which was counted by electron microscopy and used as the standard (Advanced Biotechnology Inc., Columbia, MD, USA). Prior to lysis, the stock solution contained 7.2×10^{10} virus particles/mL. The HIV-2 NIHZ stock solution was diluted to obtain 5 000 000, 500 000, 50 000, 5 000, 500 and 50 copies/µL to generate the standard curve. The HIV-2 copy number in each clinical sample was estimated by interpolation from the regression curve. All samples were run in duplicate. The results were expressed as HIV-2 RNA copies/mL. Each hospital periodically sent plasma samples to Hospital Carlos III for viral load determination, along with a completed case report form recording clinical and laboratory information, including CD4+ cell counts at the time of sample extraction. Blood samples from more than half the individuals were collected at the time of HIV-2 diagnosis and before they began antiretroviral therapy, the rest being drawn from patients already on antiretroviral therapy.

Viral tropism in HIV-2 was estimated based on V3 sequences obtained from plasma RNA and/or proviral DNA from PBMCs collected from HIV-2 individuals. The V3 region was amplified using a nested PCR method reported elsewhere.¹⁴ X4 or X4/R5 tropism was assigned in the presence of any mutation at residue L18, mutation V19K/R, V3 global net charge >6 and/or insertions at position 24.⁹

Statistical analysis

Results are given as percentages and median values with IQRs. Comparisons were made using the χ^2 test, with Fisher's correction when appropriate or the Mann-Whitney *U*-test for the comparison of

quantitative variables. Univariate and multivariate analyses were performed by logistic regression including gender, age, viral load, CD4+ cell count and antiretroviral therapy as variables. Differences were considered as significant only when *P* values were <0.05. All analyses were performed using SPSS software (version 15.0).

Results

From a total of 279 patients with HIV-2 recorded in the Spanish national register, 58 V3 sequences belonging to 42 distinct individuals were evaluated. Forty of these samples were drawn from plasma RNA and 18 from proviral DNA. Lack of plasma and/or PBMCs and low plasma viraemia or proviral DNA precluded obtaining results in the remaining subjects owing to unsuccessful amplification of the genetic material.

The median age of the study population at HIV-2 diagnosis was 44 years; 28 (67%) were male, 38 were infected with group A virus, 27 (64%) were immigrants from sub-Saharan Africa, 8 (19%) were native Spaniards, 4 (9.5%) were from Portugal and 1 was born in Costa Rica (the country of origin was not known for 2 patients). At the time of testing, the median plasma HIV-2 RNA was 3.28 (2.16–4.10) log copies/mL and the median CD4+ cell count was 227 (111–444) cells/mm³. A total of 19 (45%) patients were on antiretroviral therapy at the time of viral tropism assessment. There were no significant differences in terms of age, gender, country of origin and HIV-2 viral subtype comparing the overall cohort (279 patients) and the patients selected for this analysis. Regarding viral load and CD4+ cell counts, the selected patients tended to have lower CD4+ cell counts (227 versus 315) and higher viral load values (3.28 versus 2.74) than the global population, although this did not reach statistical significance.

Overall, 28 (67%) individuals were estimated to be infected with R5 viruses whereas 14 (33%) harboured X4 HIV-2 strains. Any mutation at residue L18 was found in 12 patients, mutation V19K/R was present in 8 individuals, V3 global net charge >6 was found in 10 patients and/or insertions at position 24 were present in 6 patients. Viral tropism could be characterized in both RNA and DNA drawn from blood collected at the same time in 16 patients. Lack of frozen PBMCs precluded obtaining results in the remaining subjects. The overall concordance between tropism prediction testing using plasma RNA and proviral DNA was 15/16 (94%). One subject exhibited X4 tropism on DNA testing but R5 on RNA testing. The virus of this patient was considered to be X4 tropic in further analysis.

As shown in Table 1, patients with X4 viruses had a lower median CD4+ cell count than patients with R5 viruses (130 versus 359 cells/mm³; *P*=0.007). They also exhibited greater median plasma HIV-2 RNA (3.8 versus 3.1 log copies/mL; *P*=0.1).

Table 1. Main characteristics of the HIV-2 study population according to viral tropism

	Total	R5	X4	<i>P</i> value
Patients, <i>n</i> (%)	42	28 (67)	14 (33)	—
Male gender, <i>n</i> (%)	28 (67)	17 (61)	11 (79)	0.73
Age (years), median (IQR)	44 (39–50)	43 (38–48)	45 (40–50)	0.73
HIV-2 RNA (log copies/mL), median (IQR)	3.3 (2.2–4.1)	3.1 (2.1–3.9)	3.8 (2.9–4.2)	0.1
CD4 count (cells/mm ³), median (IQR)	227 (111–444)	359 (180–470)	130 (17–210)	0.007
On antiretroviral therapy, <i>n</i> (%)	19 (45)	11 (39)	8 (57)	0.5

Table 2. Factors associated with X4 tropism in HIV-2-infected patients

	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Older age	1	0.95–1.06	0.94	1.01	0.95–1.08	0.70
Male gender	1.45	0.36–5.9	0.6	1.53	0.26–9.01	0.64
HIV-2 RNA (log copies/mL)	1.79	0.94–3.4	0.07	1.5	0.73–3.09	0.26
CD4 count (cells/mm ³)	0.99	0.98–1	0.034	0.99	0.99–1	0.044
Under HAART	0.6	0.17–2.15	0.43	0.57	0.11–2.84	0.50

HAART, highly active antiretroviral therapy.

Differences in CD4 counts and viral load were also recognized when comparing X4 with R5 viruses in the subset of 19 individuals on antiretroviral therapy. The median time on antiretroviral therapy in this subset of patients was 42 (15–78) months. X4 viruses were found in eight of these patients. The median CD4+ cell count in these patients compared with patients with R5 viruses was 94 (16–147) versus 184 (43–368) cells/mm³ ($P=0.041$), respectively, and the median plasma HIV-2 RNA was 3.84 (3.80–4.34) versus 3.1 (2.08–4.19) log copies/mL ($P=0.07$), respectively.

In multivariate analysis, the association between HIV-2 tropism and CD4+ cell counts remained significant ($P=0.044$) after adjusting for age, gender, viral load and being on antiretroviral therapy (Table 2).

Discussion

This study has characterized viral tropism in a relatively large population of patients with HIV-2 living in Spain. Based on a previously validated genotypic tool,⁹ X4 viruses were present overall in one-third of the population, a rate somewhat comparable to that reported in HIV-1 populations with similar CD4+ cell counts.¹⁵ As expected, X4 viruses tended to be less prevalent in newly diagnosed HIV-2 patients, naive for any antiretroviral drug, as was shown in a recent French study that reported 10% rates.¹⁶

Hypothetically, up to two-thirds of patients with HIV-2 in our series could benefit from CCR5 antagonists as part of their antiretroviral therapy. This information is relevant given that antiretroviral drug options are quite limited for patients with HIV-2. Furthermore, we found a good correlation between HIV-2 tropism testing using plasma RNA and proviral DNA, in agreement with that demonstrated in HIV-1 infection¹⁷ and one small HIV-2 series.¹⁰ This observation is particularly relevant for clinical practice, given that a substantial proportion of individuals with HIV-2 harbour low or even undetectable plasma viraemia.¹⁸

The presence of X4 viruses in HIV-1 infection is consistently associated with low CD4+ cell counts, with some controversy regarding the causal relationship.¹⁹ In our study, conducted in individuals with HIV-2, we found a similar correlation between X4 tropism and low CD4+ cell counts. This association remained even when considering exclusively patients on antiretroviral therapy and when adjustments were made for gender, age and viral load. Thus, despite the cross-sectional nature of our study design, we are confident that our results underscore that X4 viruses are associated with low CD4+ cell counts in patients with HIV-2 infection.

Compared with patients with HIV-1, CD4 recovery after the initiation of antiretroviral therapy in patients with HIV-2 is generally poorer.²⁰ This fact might be further accentuated in HIV-2-infected individuals with X4 viruses. Our findings support that along with CD4+ cell counts and viral load, testing for viral tropism in HIV-2 patients could assist the decision when to initiate antiretroviral therapy. Individuals with X4 viruses might warrant earlier initiation of treatment based on their increased risk for faster disease progression and poor CD4 recovery with antiretroviral therapy.

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Transparency declarations

None to declare.

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