VIROLOGY

Human immunodeficiency virus type-1 can be detected in monocytes by polymerase chain reaction

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Summary. Lymphocytes and monocytes from 25 patients infected with human immunodeficiency virus type-1 (HIV-1)—13 asymptomatic, seven with the AIDS-related complex (ARC) and five with the acquired immunodeficiency syndrome (AIDS)—were lysed and subjected to PCR with three primer pairs: SK38/SK39 (*gag*), SK68/SK69 (*env*) and SK29/SK30 (*LTR*). Amplified DNA was solution-hybridised with ³²P-labelled probes (SK19, SK70 and SK31, respectively) and detected by PAGE-autoradiography. HIV-1 DNA was detected as follows. Asymptomatic patients: monocytes—*gag* 61.5%, *env* 100%, *LTR* 0%; lymphocytes—*gag* 100%, *env* 92.3%, *LTR* 53.84%. ARC patients: monocytes—*gag* 71.4%, *env* 57.1%, *LTR* 0%; lymphocytes—*gag* 100%, *env* 71.4%, *LTR* 71.4%. AIDS patients: monocytes—*gag* 80.0%, *env* 100%, *LTR* 0%; lymphocytes—*gag* 100%, *env* 60%, *LTR* 60%. The presence of HIV-1 DNA was confirmed in the monocyte fraction. In this cell subset, the *env* gene-directed primers were the most effective for amplification, whereas the *LTR* gene-directed primers failed to amplify HIV-1 DNA. The different pattern of amplification found in monocytes may suggest that these cells could be infected by a genetic variant of the virus.

Introduction

Human immunodeficiency virus type-1 (HIV-1) has been detected in peripheral blood mononuclear cells (PBMC);¹ several authors have studied its presence in different PBMC subsets^{2–4} but no firm conclusions have yet been reached. HIV-1 can infect the macrophage-monocyte fraction because CD4 receptors are expressed in these cells⁵ and the virus has also been cultured from monocytes of infected patients.⁶ However, HIV-1 has never been isolated from CD8⁺ lymphocytes, perhaps because this subset does not express CD4 receptors.

HIV-1 can produce a latent infection of CD4 cells and its DNA is frequently integrated into the genome of these cells; conversely, HIV-1 RNA is rarely detected in CD4 lymphocytes which again indicates latent infection. Although HIV-1 can produce infection of the monocyte-macrophage fraction of PBMC, HIV-1 DNA has not been shown, so far, to be present in these cell subsets.⁷ The possibility that HIV-1 may infect a different cell subset needs to be

examined. The finding of a different pattern of detection of genes from HIV-1 in peripheral blood subsets other than CD4 lymphocytes, in an individual patient, may indicate that naturally- or treatment-selected variants of the virus are present.⁸ If this is so, new treatment strategies need to be established.

Therefore, the detection of HIV-1 DNA in the macrophage-monocyte fraction may be a key to the solution of some problems related to HIV-1 infection. The aim of this investigation was to study the presence of HIV-1 in monocytes by means of the polymerase chain reaction (PCR).

Patients and methods

Blood was obtained from 25 HIV seropositive patients: 13 asymptomatic carriers, seven with the AIDS-related complex (ARC) and five with the acquired immunodeficiency syndrome (AIDS).

Isolation of cell subsets

The mononuclear cell fraction was separated from the red blood cell fraction by Ficoll-Hypaque density

Table I. CD4 and CD8 count and *gag*, *env* and *LTR* amplification in lymphocytes and monocytes from asymptomatic carriers of HIV

Case no.	CD4/CD8 count	Lymphocytes			Monocytes			AZT/IFN
		<i>gag</i>	<i>env</i>	<i>LTR</i>	<i>gag</i>	<i>env</i>	<i>LTR</i>	
1	1123/1556	+	—	—	—	+	—	No
2	839/952	+	+	+	+	+	—	—/+
3	765/1097	+	+	+	+	+	—	—/+
4	874/1014	+	+	—	—	+	—	No
5	579/984	+	+	+	+	+	—	No
6	602/859	+	+	+	—	+	—	No
7	369/876	+	+	—	—	+	—	—/+
8	623/930	+	+	+	+	+	—	No
9	650/858	+	+	—	—	+	—	+/-
10	766/1107	+	+	—	+	+	—	No
11	697/2010	+	+	+	+	+	—	No
12	512/1870	+	+	—	+	+	—	No
13	402/1460	+	+	+	+	+	—	+/-
Total (%)		100	92.3	53.84	61.5	100	0	

CD4/CD8, T cell count; AZT/IFN, treatment with azidothymidine/ α -interferon.

gradient centrifugation. T lymphocytes were separated from other mononuclear cells by the rosette test and a second Ficoll-Hypaque density gradient centrifugation. To isolate the monocyte fraction, mononuclear cells were cultured for 24 h to eliminate dendritic and B cells, and then allowed to adhere for 1 h on to plastic dishes and washed twice with RPMI 1640 medium with fetal calf serum 10%. The adherent cells were incubated for 20 min at 37°C in 3 mM EDTA. Monocytes were detached by vigorous pipetting and analysed by FACscan flow cytometry to detect contamination with T lymphocytes; only monocyte fractions without contaminating T cells were processed further. After isolation, cells were washed twice in PBS and incubated for 1 h at 60°C in a lysis buffer containing 50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl₂, Tween 20, 0.5%, Nonidet P-40 0.5% and 0.06 proteinase K g/L. After disruption, proteinase K was inactivated by heating at 95°C for 10 min.

Nucleic acid amplification

HIV-DNA was amplified with three sets of primers from different regions of the HIV-1 genome: SK38/39 (1551–1578 and 1638–1665), which amplifies a conserved *gag* region of 114 bp; SK68/69 (7801–7829 and 7922–7942) which amplifies a conserved 141-bp region of the *env* gene; and SK29/30 (501–518 and 589–604) which amplifies a 104-bp fragment from the *LTR* gene.⁹ The reaction was performed in 10 × *Taq* buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3, 25 mM MgCl₂, gelatin 0.1%); the rest of the components were: primers, 50 pmol each; dNTPs, 50 μ M each; *Taq* polymerase 2.5 U; 25 μ l of cell lysate containing 1 × 10⁶ T cells or monocytes, equivalent to 1 μ g of genomic DNA. The reaction, consisting of 30 cycles (94°C for 1 min, 55°C for 2 min and 72°C for 3 min) was carried out in a thermal cycler (Perkin-Elmer). Solution hybridisation with ³²P-labelled probes was performed (SK19, SK70 and SK31 for SK38/39, SK68/69 and SK29/30 products, respectively); the

hybridisation product was detected by polyacrylamide gel electrophoresis (PAGE) and autoradiography.

To test the suitability of the lysates, samples were also amplified with a set of HLA primers (GH26/27), which flank a conserved region of HLA-DQ (α) locus and hybridised with a ³²P-labelled probe (GH64).¹⁰ A set of positive (ACH₂ cell line containing one copy of HIV provirus/cell) and negative (uninfected cells and a blank control—reaction tube without cells) controls were used.

Results

Detection of *gag*, *env* and *LTR* genes in cell subsets from asymptomatic patients

HIV-1 DNA was detected in lymphocytes from all 13 patients with primers directed against the *gag* gene, 12 (92.3%) patients when *env*-directed primers were used and seven (53.8%) patients with *LTR*-directed primers. When cells from the monocyte fraction were tested, amplified fragments were detected in eight (61.5%) patients with *gag*-directed primers and 13 (100%) patients with primers directed against the *env* gene; primers from the *LTR* gene failed to amplify any DNA fragments. These results are shown in table I.

Detection of *gag*, *env* and *LTR* genes in cell subsets from ARC patients

HIV-1 DNA was detected in lymphocytes from all seven patients with primers directed against the *gag* gene, five (71.4%) patients when *env*-directed primers were used and five (71.4%) patients with *LTR*-directed primers. When cells from the monocyte fraction were tested, amplified fragments were detected in five (71.4%) patients with *gag*-directed primers and four (57.1%) patients with primers directed against the *env* gene; primers from the *LTR* gene failed to produce amplification products. These results are shown in table II.

Table II. CD4 and CD8 count and *gag*, *env* and *LTR* amplification in lymphocytes and monocytes from ARC patients

Case no.	CD4/CD8 count	Lymphocytes			Monocytes			AZT/IFN
		<i>gag</i>	<i>env</i>	<i>LTR</i>	<i>gag</i>	<i>env</i>	<i>LTR</i>	
1	302/1123	+	+	+	+	-	-	+/+
2	240/864	+	+	-	+	+	-	+/-
3	415/1263	+	-	-	+	+	-	+/+
4	237/2223	+	+	+	-	-	-	+/-
5	309/1875	+	+	+	+	-	-	+/-
6	464/1319	+	-	+	+	+	-	+/+
7	345/2196	+	+	+	+	+	-	+/-
Total (%)		100	71.4	71.4	71.4	57.1	0	

CD4/CD8, T cell count AZT/IFN, treatment with azidothymidine/ α -interferon.**Table III.** CD4 and CD8 count and *gag*, *env* and *LTR* amplification in lymphocytes and monocytes from AIDS patients

Case no.	CD4/CD8 count	Lymphocytes			Monocytes			AZT/IFN
		<i>gag</i>	<i>env</i>	<i>LTR</i>	<i>gag</i>	<i>env</i>	<i>LTR</i>	
1	198/899	+	-	-	-	+	-	+/+
2	181/462	+	-	-	+	+	-	+/+
3	92/827	+	+	+	+	+	-	+/-
4	200/758	+	+	+	+	+	-	+/-
5	214/867	+	+	+	+	+	-	+/-
Total (%)		100	60	60	80	100	0	

CD4/CD8, T cell count; AZT/IFN, treatment with azidothymidine/ α -interferon.

Detection of *gag*, *env* and *LTR* genes in cell subsets from AIDS patients

HIV-1 DNA was detected in lymphocytes from all five patients with primers directed against the *gag* gene, from three (60%) patients when *env*-directed primers were used and three (60%) patients with *LTR*-directed primers. When cells from the monocyte fraction were tested, amplification products were detected in four (80%) patients with *gag*-directed primers and five (100%) patients with primers directed against the *env* gene; primers from the *LTR* gene failed to amplify any DNA fragments. These results are shown in table III.

The three primer pairs could detect as little as one copy of HIV-1 proviral DNA, as assessed by serial dilutions of ACH₂ positive control cells.

Discussion

HIV-1 has been reported in many different localisations,¹¹⁻¹⁶ but the main reservoirs are PBMC¹⁷ and lymphatic tissue.¹⁸ HIV-1 can produce a latent infection of CD4 lymphocytes but the virus is not present in CD8 cells;¹⁹ whether the virus is present or not in the monocyte fraction is not clear.^{2-4,7} PCR technology is a useful tool to study the presence of HIV-1 DNA in organs and tissues from infected patients. In this investigation, the presence of HIV-1 DNA in lymphocytes and monocytes from 25 HIV-seropositive patients was studied. Primers from the

gag, *env* and *LTR* genes were used to confirm HIV-1 infection and to determine the pattern of amplification in both cell subsets.

When PCR assays are used to confirm the presence of HIV-1 DNA in PBMC, positive results with at least two primer sets are necessary, because genomic variability is frequent.^{17,20-22} Following standard algorithms for HIV-1 PCR testing,²² positive results were obtained with lymphocytes from 12 (92.3%) asymptomatic carriers, five (71.4%) ARC patients and three (60%) AIDS patients. In the monocyte fraction, following the same algorithms, samples from eight (61.5%) asymptomatic carriers, four (57.1%) ARC patients and four (80.0%) AIDS patients were positive. Therefore, it can be stated that HIV-1 DNA can be detected in monocytes from patients at all stages of HIV-1 infection. These results are similar to those reported by McElrath *et al.*^{4,23} who proposed that HIV-1 can produce a latent infection in monocytes in infected patients. However, others have reported a rather transient infection with HIV-1 in monocytes² and a lower percentage of infection in this cell subset,³ or even the absence of infection.⁷ Although these authors show discordant results, some aspects of their reports need to be discussed. Firstly, for monocyte isolation we used a method that assures the absence of contaminating T lymphocytes, so the results for the monocyte fraction are representative. Secondly, those who propose that HIV-1 is not frequently present in monocytes used only primers from the *gag* gene

(SK38/SK39) for amplification, because this is the most sensitive primer set to study HIV-1 in PBMC. In the present study, the most sensitive primer set for studying the presence of HIV-1 in monocytes was the *env* gene-directed set (SK68/SK69). Thus, if this primer set is not used, a low percentage of detection may result. When the SK68/SK69 primer set is used, the rate of detection of HIV-1 DNA in monocytes from infected patient rises. To determine whether the infection is replicative or latent, HIV-1 RNA should also be detected.

The *env* gene-directed primers were the most sensitive for HIV-1 DNA amplification in monocytes. Usually, when HIV-1 DNA is assayed for PCR in PBMC, *gag* gene-directed primers are the most sensitive, followed by *env* gene primer pairs and, finally, the *LTR* gene-directed primer pairs which are the least sensitive.²² In this study, when these three primer pairs were used for PCR testing in lymphocytes, the same pattern was observed (asymptomatic carriers—*gag*, 100%, *env*, 92.3%, *LTR*, 53.84%; ARC patients—*gag*, 100%, *env*, 71.4%, *LTR*, 71.4%; AIDS patients—*gag*, 100%, *env*, 60%, *LTR*, 60%); however, when they were used for PCR testing of the monocyte fraction, a different pattern of amplification was

observed. In the monocyte fraction, *env* gene-directed primers were the most sensitive primer set (asymptomatic carriers, 100%; ARC patients, 57.1%; AIDS patients, 100%), followed by *gag* gene primer pairs (asymptomatic carriers, 61.5%; ARC patients, 71.4%; AIDS patients, 80%), and, surprisingly, no amplification was observed when *LTR* gene-directed primer pairs were used. This pattern of amplification, which is different from the usual PBMC pattern and from the lymphocyte pattern, might suggest that a variant of HIV-1 infects the monocyte fraction. The most highly conserved gene in the genome of HIV-1 is the *gag* gene. The finding of patients whose monocytes harbour a virus where the most effective amplification is achieved with *env*-directed primers but not with *gag*-directed primers adds support to our observation, as does the fact that *LTR* gene-directed primers always failed to amplify HIV-1 DNA from monocytes. However, further studies with quantitative PCR and virus isolation followed by DNA amplification and sequencing are necessary. In the present study, this different pattern was observed in both treated and untreated patients, but whether this variant is naturally- or treatment-selected also needs further investigation.

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