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 53.8%. 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), but several authors have studied its presence in different PBMC subsets but no firm conclusions have yet been reached. HIV-1 can infect the macrophage-monocyte fraction because CD4 receptors are expressed in these cells and the virus has also been cultured from monocytes of infected patients. 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. 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. 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
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. The adherent cells were washed twice in PBS and incubated for 1 h at 60°C in a lysis buffer which amplifies a 104-bp fragment from the LTR gag region of 1 14 bp gene. The reaction was performed in 10 x 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 μM each; Taq polymerase 2.5 U; 25 μL of cell lysate containing 1 x 10⁶ T cells or monocytes, equivalent to 1 μg 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.
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 ACH₃ positive control cells.

Discussion

HIV-1 has been reported in many different localisations, but the main reservoirs are PBMC and lymphatic tissue. HIV-1 can produce a latent infection of CD4 lymphocytes but the virus is not present in CD8 cells; whether the virus is present or not in the monocyte fraction is not clear. 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. Following standard algorithms for HIV-1 PCR testing, 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. 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 monocytes and a lower percentage of infection in this cell subset, or even the absence of infection. 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. 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.

References