Human Immunodeficiency Virus Type 1 Kinetics in Lymph Nodes Compared with Plasma

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As lymphoid organs are the major reservoir of human immunodeficiency virus type 1 (HIV-1), the rates at which HIV-1 RNA decreases from the plasma and from a series of lymph node biopsies from 4 patients treated with a combination of zidovudine, didanosine, and lamivudine were measured. The concentrations of HIV-1 RNA in the plasma and in lymph nodes declined exponentially, with mean half-lives of 1.88 ± 0.86 days for plasma and 6.01 ± 3.44 days for lymph nodes. These data show that most of the HIV-1 in lymphoid organs is due to the infection of new cells and demonstrate that a triple-drug combination is able to target this compartment.

Patients and Methods

Study population. Four patients with HIV-1 infection CDC stage A or B, who had been given no prior antiretroviral therapy and who had CD4 T cell counts <500 × 10⁶/L, were treated with oral zidovudine (200 mg thrice daily) plus didanosine (ddI, 200 mg twice daily) plus lamivudine (3TC, 150 mg twice daily). The therapy began in the evening of day 0, after the first blood and lymph node biopsies were taken.

HIV-1 viremia. Plasma HIV-1 RNA titers were measured before therapy, every 2 days until day 21, and each week thereafter by a polymerase chain reaction (PCR) technique (Amplicor Monitor; Roche Diagnostic Systems, Neuilly sur Seine, France) using an internal standard as control [5]. The cutoff value of this test in plasma is 200 copies/mL.

HIV-1 RNA in lymph nodes. All 4 patients had large superficial lymphadenopathies. Lymphoid tissue biopsies (~20 mg) were obtained with a large needle and ultrasonographic monitoring from patients under local anesthetic. The tissue was totally lysed by incubation with RNA-B (Bioprobe Systems, Montreuil sous Bois, France) for 2 h at 4°C, and the resulting liquid phase was diluted 1/100 with diethyl procarbonate water. This procedure should extract all the HIV-1 RNA in the sample, including the HIV-1 RNA in lymph node cells and in the virions on the surface of the dendritic cells. The sample was diluted 1/100 to ensure that the Amplicor kit gave linear amplification. The sample was then incubated with chloroform for 5 min on ice and centrifuged for 15 min at 12,000 g. The aqueous phase was collected and used for PCR as for the plasma samples.

Preliminary experiments on 3 patients showed that this procedure has a mean variability of 20% between experiments for the same sample and that repeated biopsies for a patient with stable disease have a mean variability of 25% (unpublished data). The amount of HIV-1 RNA found is expressed in terms of the number of lymph node cells, calculated after TE-SDS-Proteinase K (10 mM TRIS, 1 mM EDTA [pH 7.5], 1% SDS, 500 μg/mL Proteinase K) digestion of lymphoid tissue, extraction of genomic DNA with phenol-chloroform, and ethanol precipitation. DNA was measured by the optical density at 260 nm. The number of cells was then inferred from the total amount of genomic DNA measured using a standard curve established with known numbers of peripheral blood mononuclear cells.

Results

The mean plasma HIV-1 RNA at baseline was 179,184 copies/mL (range, 12,255–614,800). These titers declined exponentially after initiation of therapy (figure 1). The titers stabilized at ~500 copies/mL after day 16 in patient 1 and remained there until the last evaluation on day 114. HIV-1 RNA in patient 2 was low at baseline (12,255 copies/mL) and dropped to <200 copies/mL after day 11. An initial rapid decline was followed by a more gradual decrease to values below the cutoff of the test after 40 and 150 days of therapy in the other 2 patients.

The number of HIV-1 RNA copies in the lymph nodes of patient 1 on days 0, 4, and 7 were similar (day 0: 24,442,897;
Figure 1. Decrease in HIV-1 RNA titers in plasma (○) and lymph nodes (□) of 4 patients treated with zidovudine, didanosine, and lamivudine in combination. Treatment started in evening, day 0. Plasma HIV-1 RNA titers declined rapidly and stabilized at ~500 copies/mL in patient 1 (A). Similar drop in plasma titers was seen in patient 2 (B), but values were below test cutoff after day 11. Plasma HIV-1 RNA dropped below test cutoff after 40 days in patient 3 (C) and after 150 days in patient 4 (D). Decreases followed 2-compartment model. This triple-drug combination reached HIV-1 in lymph nodes and produced exponential decline in titers.

day 4: 29,106,646; day 7: 20,892,458 copies/10^5 cells). The equivalent HIV-1 RNA concentrations in plasma were 71,155, 1780, and 2300 copies/mL, respectively, on the same days. HIV-1 RNA titers on day 9 were 12,756,902 copies/10^5 cells in lymph nodes and 1450 copies/mL in plasma from this patient. Similarly, the HIV-1 RNA numbers in the lymph nodes from patient 2 were about the same on days 0 and 2 and began to decrease by day 5. The 4 patients studied showed an average decrease of 0.786 log_{10} in HIV-1 RNA titers in lymph nodes after 2 weeks of treatment. The HIV-1 RNA titers in lymph nodes of patient 3 dropped from 29,788,644 copies/10^5 cells on day 0 to 486,075 copies/10^5 cells on day 40.

The rates of viral clearance were analyzed by linear regression. Plasma changes were calculated from day 0 to the inflection point of the curve at which it was biphasic or to the last positive RNA titer. The mean ± SD half-life of viral RNA for the 4 patients studied was 1.88 ± 0.86 days in plasma and 6.01 ± 3.44 days in lymph nodes.

Discussion

HIV-1 concentrates in lymphoid organs during the first stages of the disease, mainly as immune complexes on the surfaces of the dendritic cell network [3]. In vitro studies indicate that these viruses may remain highly infectious for CD4 T cells circulating through these organs [6]. To our knowledge, there has been only one published study on the impact of antiretroviral therapy on the HIV-1 load in lymph nodes [7].
A combination of zidovudine and ddI was found to have a modest effect on lymph node HIV-1 RNA after 8 weeks [7]. We have also found no significant decrease in infectious HIV-1 content, HIV-1 RNA, or HIV-1 DNA in the lymph nodes of 8 patients after 24 weeks with a similar regimen, although no antiretroviral resistance developed [8]. These studies used a therapeutic combination that gives only a 1.4 log_{10} decrease in plasma HIV RNA, which is probably not enough to influence therapeutic combination that gives only a 1.4 log_{10} decrease in plasma HIV RNA, which is probably not enough to influence therapeutic combination that gives only a 1.4 log_{10} decrease in plasma HIV RNA, which is probably not enough to influence the HIV-1 burden in lymph nodes. Also, there were only 2 plasma HIV RNA, which is probably not enough to influence the HIV-1 burden in lymph nodes. Also, there were only 2 lymph node biopsies in each case. These biopsies were taken surgically, several months after the start of treatment, so a more rapid decline could have been missed.

Plasma HIV-1 RNA drops exponentially in response to potent HIV-1 inhibitors, such as nevirapine, or molecules targeting the viral protease, with a mean half-life of ~2 days [1, 2]. However, there is a rebound of viremia within a few weeks because HIV-1 rapidly develops resistance to these drugs [1]. Consequently, earlier studies on HIV-1 kinetics have focused on the first 2 weeks following the introduction of these drugs [1, 2, 9] and have described only the first phase of the drop in plasma HIV-1 RNA. Preliminary results of two recent clinical trials have shown that plasma RNA titers can be kept below the detection limit for several months with triple-drug combinations [10, 11]. In vitro screening of triple-drug combinations with potent inhibitors, such as zidovudine, ddI, zalcitabine, 3TC, nevirapine, saquinavir, and indinavir showed that the combination of zidovudine/ddI/3TC is one of the most effective combinations where HIV is present in different types of cells, including long-lived virus-producing macrophages [9].

This sustained antiretroviral effect was used to investigate the change in HIV-1 RNA in lymph nodes. Frequent biopsies were taken in 3 cases (patients 1, 2, and 4) during the first week after therapy to detect any rapid drop that might parallel the change in levels in plasma. However, there was a gap of several days between the responses of these two compartments, and the exponential declines in lymph node HIV-1 RNA occurred later. This delay is probably due to the time required for the drug to be distributed throughout the tissue. Lymph node biopsies were taken at intervals of ~10 days in patient 3, until day 40 after therapy. The HIV-1 RNA titers decreased exponentially 61.28-fold (1.78 log_{10}) during this time.

Virus is cleared from lymph nodes much more slowly than from plasma, although the rates are quite similar to those for the second plasma phase. This difference may be due in part to incorrect distribution of the drugs in tissues, as has been postulated from animal models [13], but it is more likely to reflect the presence of long-lived infected cells in lymph nodes and the longer half-life of virions on dendritic cells compared with free plasma virions. PCR for spliced RNA could give a better approximation of the half-life of the RNA in lymph node cells. The kit we used has the great advantage of standardization but amplifies only the genomic RNA and some spliced RNA from the gag region. It would also be interesting to monitor by DNA PCR the frequency of HIV-infected cells in lymph nodes. We plan to investigate these aspects in future studies with surgical biopsies, because samples obtained by the current protocol are too small for all of these tests.

Nevertheless, our preliminary results show that virus turnover was high in lymph nodes, as it is in plasma. Since the three drugs we used block the infection of new cells but not the production of virus by already infected cells, the high levels of HIV-1 RNA in lymph nodes appear to be due in part to the constant infection of new cells. The presence of a long-lived population of chronically infected cells in tissues could explain the slower decline in virus load in lymph nodes compared with plasma. However, if viral replication can be completely blocked with appropriate drug combinations, these cells will gradually die and be replaced by uninfected cells. As the pool of latently infected cells in HIV-1-infected patients appears to be very limited [14], it may be possible to obtain an eventual "burn-out" of the lymphoid compartment [9].

Acknowledgments

We thank G. C. Sayada of Roche Diagnostic Systems for helpful technical assistance.

References

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Direct Genetic Detection of Dobrava Virus in Greek and Albanian Patients with Hemorrhagic Fever with Renal Syndrome

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Blood samples were collected from an Albanian and a Greek patient with hemorrhagic fever with renal syndrome and tested by reverse transcriptase–polymerase chain reaction. The genetic detection assay amplified hantavirus-specific DNA fragments from RNA extracted from the blood of the patients; nucleotide sequence analysis revealed that the causative agent of the disease was Dobrava virus. These findings suggest that Dobrava virus (which was originally isolated from the lungs of an Apodemus flavicollis mouse in Slovenia) is endemic throughout the Balkan States and causes overt human disease.

Hemorrhagic fever with renal syndrome (HFRS) is an acute febrile nephropathy caused by closely related zoonotic viruses of the genus Hantavirus, family Bunyaviridae [1]. Three distinct hantaviruses have been clearly associated with the disease in humans: Hantaan virus, the cause of the most severe form of HFRS in Korea and China; Puumala virus, the cause of nephropathia epidemica, a milder form of HFRS commonly described in Scandinavia and Russia (west of the Ural Mountains); and Seoul virus, the cause of a moderately severe form of HFRS that occurs in China and Korea [2]. These viruses are all associated with specific primary rodent hosts. The prototype Hantaan virus is associated with Apodemus agrarius; Puumala and Seoul viruses are associated with Clethrionomys glareolus and domestic rats, respectively. Another distinct hantavirus, Dobrava, has been detected in Apodemus flavicollis in Slovenia [3], and the identical, or near identical, Belgrade virus [4] has been suggested to be associated with HFRS cases in the former Yugoslavia [5]. It is difficult to serologically differentiate between Hantaan and Dobrava virus infections except by neutralization assays. Several other hantaviruses, including Sin Nombre, Bayou, Black Creek Canal, Prospect Hill, and Thailand viruses, are found elsewhere in the world and are associated with other specific rodent species [6].

Cases of HFRS (mostly shown or suspected to be caused by Puumula virus) have recently been reported in several European locations outside Scandinavia and Russia, including France [7], Germany [8], and Belgium [2]. On the Balkan Peninsula, where A. agrarius, A. flavicollis, and C. glareolus can all be found, clinical cases of HFRS have been reported from Albania [9], Bulgaria [10], Romania [11], and the former Yugoslavia [3, 5, 6].

Received 21 December 1995; revised 20 March 1996.
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The Journal of Infectious Diseases 1996;174:407–10
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0022-1899/96/7402-0026$01.00