Effects of a Combination of Zidovudine, Didanosine, and Lamivudine on Primary Human Immunodeficiency Virus Type 1 Infection

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A combination of zidovudine, didanosine, and lamivudine was used to treat 10 patients with primary human immunodeficiency virus type 1 (HIV-1) infection 5–28 days after the onset of symptoms. When therapy began, the mean plasma HIV-1 RNA level was 5.31 ± 0.33 log_{10} copies/mL and the mean CD4 T cell count was 630 ± 112 × 10^6/L. The plasma HIV-1 RNA level decreased rapidly, and levels dropped below the cutoff in each case after 108 ± 32 days. Lymph nodes from 5 patients were biopsied before therapy and during follow-up. Infectious HIV-1 could not be cultivated from any lymph node mononuclear cells taken on day 90, and HIV-1 RNA was at very low levels in lymph nodes after 1 year. In some cases, waning of the antibody response to HIV-1 was shown by Western blot after several months of undetectable plasma RNA. These data demonstrate that triple-drug therapy has a potent antiviral effect during primary HIV-1 infection.

Patients and Methods

Population. Ten patients with PHI were identified between March 1995 and June 1996. The criteria used were clinical symptoms of PHI with serum p24 antigen positivity (Coulter, Hialeah, FL) and a negative or indeterminate Western blot (HIV blot 2.2; Diagnostic Biotechnology, Singapore), or a positive blot with a negative test during the preceding 3 months. After an initial blood evaluation and, in 4 cases, surgical lymph node biopsy, the patients were given oral zidovudine (200 mg three times daily) plus didanosine (dDI, 200 mg twice daily) plus lamivudine (3TC, 150 mg twice daily). Therapy began 5 days after the first symptoms of PHI in 1 patient, 10 days in 4, 15 days in 1, and 28 days in 4. Serum from the first days of the symptoms was analyzed retrospectively for these last 4 patients, and Western blot results were indeterminate.

CD4 T cell counts. The CD4 T cell count was measured by flow cytometry (EpicaProfile; Coulter) using commercially available monoclonal antibodies (Dako, Trappes, France).

Plasma HIV-1 RNA. Plasma HIV-1 RNA levels were measured at the first visit and at regular intervals thereafter, using a polymerase chain reaction technique (AmpliC fec Monitor HIV-1; Roche Diagnostic Systems, Neuilly sur Seine, France). The cutoff value of this test for plasma is 200 copies/mL. When levels below this cutoff were obtained with therapy, the method was made more sensitive by increasing the sample size to 500 μL and the number of amplification cycles to 40. This procedure gave an analytic sensitivity approaching 20 copies/mL [6] (unpublished data).

Cellular infectious viremia. Infectious viremia in peripheral blood mononuclear cells (PBMC) was measured with a coculture technique recommended by the French Agency for AIDS Research (consensus protocol), and the results were expressed as infectious units (IU)/10^6 cells.

HIV-1 burden in lymph nodes. Four patients had palpable superficial lymphadenopathies and agreed to undergo surgical biopsies before and 3 months after therapy. Lymph node mononuclear cells (LNMC) were teased out with a scalpel and isolated by ficoll hypaque density-gradient centrifugation. Cells were then cultured...
as for PBMC to measure the infectious titer or analyzed by semi-quantitative polymerase chain reaction (PCR) using the Amplicor Monitor kit and a robotic workstation (Beckman, Gagny, France) to measure proviral DNA titers [7]. Results were expressed as number of PCR units per 10^6 cells. A fifth patient agreed to have only needle biopsies of the lymph nodes. This technique provides less material, enough to measure HIV-1 RNA only [8]. Furthermore, 4 patients also agreed to be similarly biopsied after 1 year of therapy.

Assessment of biologic phenotype. The biologic phenotype for syncytium-inducing (SI) or non-SI (NSI) HIV-1 was tested on MT-2 cells as described previously [9].

Statistical analysis. The mean plasma RNA concentrations of groups were compared using Student’s t test after logarithmic transformation of the data. The CD4 or CD8 T cell counts were compared with a paired t test during follow-up. Modeling of HIV-1 RNA decrease used linear regression analysis as previously reported [10]. P < .05 was considered significant in each calculation.

Results

We studied 5 men and 5 women (mean age ± SE, 31 ± 3 years). HIV-1 infection had been acquired by homosexual activity in 4 cases, heterosexual transmission in 5, and intravenous drug use in 1. The mean CD4 T cell count was 630 ± 112 × 10^6/L when therapy began, and the mean plasma HIV RNA level was 5.31 ± 0.33 log_{10} copies/mL. At the first biologic evaluation before antiretroviral therapy (1–2 days before treatment began), the mean infectious HIV-1 titer in PBMC was 58 ± 11 IU/10^6 cells. In 5 patients, the first symptoms of PHI began 1 week before admission and therapy was initiated within the 2 days following confirmation of the diagnosis. For 1 patient, diagnosis was established 7 days after the onset of symptoms, but the patient was referred to the hospital only 1 week later. For 4 patients, the general practitioners waited to have 2 Western blot assays confirming HIV infection before sending the patients to the hospital; therapy was initiated ∼28 days after the first symptoms. Patients treated before 15 days after the onset of symptoms tended to have a higher virus load in plasma than others did, but the difference was not statistically significant (mean, 5.67 ± 0.56 vs. 4.94 ± 0.34 log_{10} copies/mL; P = .30). This could be because, although the HIV-1 RNA levels were >1.2 × 10^6 copies/mL in 4 of these 5 patients, the last patient (patient 1) had only 2593 copies/mL. This woman attended the sexually transmitted disease clinic because of sexual contact with an intravenous drug user with HIV-1 infection and presented with minor symptoms (sore throat) of PHI. The first plasma and/or PBMC virus isolate before therapy was NSI in 9 patients and SI in 1.

The plasma HIV-1 RNA levels of all patients decreased dramatically after initiation of therapy (figure 1A) and dropped below the cutoff of 200 copies/mL after a mean of 108 ± 32 days. Undetectable plasma levels were obtained after 54 ± 18 days in patients treated before day 15 of symptoms and after 162 ± 55 days in others (paired t test, P = .10). The level of plasma HIV-1 RNA of each patient treated before day 15 of symptoms dropped below 20 copies/mL after analysis of the samples with the more sensitive procedure. The plasma HIV-1 RNA levels of 2 patients treated after day 15 of symptoms dropped below 20 copies/mL, and 3 others remained stable at ∼100 copies/mL. Serum or plasma samples from 4 patients in the weeks preceding the diagnosis of PHI were available, and HIV-1 RNA was assayed retrospectively. After linear regression analysis, the rates of RNA decrease were similar both before and after therapy in these patients (figure 1B).

Infectious titers of LNMC were measured before therapy (mean, 112 ± 71 IU/10^6 cells) and 3 months after in 4 patients, and results were negative at 3 months for all of them (table 1). The provirus titer in LNMC of these 4 patients decreased by 0.56 log after 3 months of therapy (difference not significant). These 4 patients agreed to have a needle biopsy of superficial lymph nodes after 1 year of therapy. The level of HIV-1 RNA was 172,400 copies/10^6 cells in 1 patient; the other 3 patients were negative for HIV-1 RNA. In fact, very low levels could be detected in these 3 lymph nodes without any dilution of the sample (table 1), as is necessary when high titers are present [8]. HIV-1 RNA was also quantified in the patient with an SI phenotype by four sequential needle biopsies of lymph nodes during the 4 months after initiation of the triple-drug regimen. The amount of HIV-1 RNA in lymph nodes decreased exponentially in this patient, with a mean half-life of 15.75 days (95% confidence interval, 14.14–17.77).

Infectious virus could not be cultivated from the PBMC of any patient 1–4 months after therapy, and there was no cellular viremia during the follow-up in all cases. The amount of proviral DNA in PBMC was measured in 4 patients. Titers declined initially, then stabilized, except for 1 patient, who had a transient increase. The amount of proviral DNA was at the limit of detection after 1 year in 1 patient (figure 1C).

The number of CD4 T cells tended to increase with therapy, reaching +243 × 10^6/L after 4 months, and the number of CD8 T cells tended to decrease. Consequently, a significant increase (paired t test, P < .05) in the mean CD4:CD8 ratio was observed after 2 months of therapy (figure 1D).

Samples from patients who were treated before day 15 of symptoms were negative by Western blot (1 patient) or were weakly positive for gp160 and p25 only (4 patients) at the start of the combination regimen. None developed a serologic response to all proteins during follow-up. Three patients remained seronegative for gp41, p66, p39, and p17 at 6 months. One patient remained seronegative for gp41, p66, p39, and p31 at 10 months and then became negative for p17, p51, and p55. The Western blot of patient 1 showed more positive bands until the third month, and then gradually lost some seroreactivity. At 14 months, this patient was clearly positive for only gp160 (figure 2). The HIV-1 Western blot showed all bands within a few weeks after initiation of therapy in 2 patients treated after...

The combination of zidovudine, ddI, and 3TC was used, as it can block HIV-1 replication completely in vitro [11] and appeared to be the most potent triple-drug regimen when this protocol was initiated (before protease inhibitors became available in France). However, this combination is still under investigation in large-scale trials, and no data are available to compare this regimen with triple-drug combinations that include a protease inhibitor. The 10 patients with PHI described here all had major reductions in plasma HIV-1 RNA levels after the start of therapy, reaching at least $-5 \log_{10}$ in some patients whose virus load decreased to <20 copies/mL. As we do not have a control group, it is not possible to determine the relative contribution of therapy, immune responses, and loss of target cells in this decrease. However, in >60 untreated primary HIV-infected patients tested so far, viremia remained detectable at day 15 of symptoms and lacked only p66 positivity in the 3 others. One of these patients became seronegative for p17, p39, p55, and gp41 after 10 months of therapy. There were no changes during follow-up for the other patients.

The mean follow-up for these 10 patients was 10.7 ± 1.3 months. The antiretroviral therapy for all patients had been uninterrupted, and no patient developed toxicity, except for severe nausea and diarrhea in 1 during the first 2 months.

Discussion

The combination of zidovudine, ddI, and 3TC was used, as it can block HIV-1 replication completely in vitro [11] and appeared to be the most potent triple-drug regimen when this protocol was initiated (before protease inhibitors became available in France). However, this combination is still under investigation in large-scale trials, and no data are available to compare this regimen with triple-drug combinations that include a protease inhibitor. The 10 patients with PHI described here all had major reductions in plasma HIV-1 RNA levels after the start of therapy, reaching at least $-5 \log_{10}$ in some patients whose virus load decreased to <20 copies/mL. As we do not have a control group, it is not possible to determine the relative contribution of therapy, immune responses, and loss of target cells in this decrease. However, in >60 untreated primary HIV-infected patients tested so far, viremia remained detectable at...
Table 1. Virus load in lymph node (LN) samples from 4 patients presenting with primary HIV-1 infection and treated with a combination of zidovudine, didanosine, and lamivudine.

<table>
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<th>Patient no.</th>
<th>Infectious virus*</th>
<th>Proviral DNA†</th>
<th>Infectious virus*</th>
<th>Proviral DNA†</th>
<th>Total LN, Month 12</th>
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<td>25</td>
<td>1647</td>
<td>0</td>
<td>130</td>
<td>640</td>
</tr>
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</table>

* No. of infectious units/10⁶ cells.
† No. of polymerase chain reaction units/10⁶ cells.
‡ RNA copies/10⁵ cells.

levels of 10³–10⁷ HIV-1 RNA copies/mL [4]. In the trial of zidovudine versus placebo that involved patients treated after 25.1 days (mean) following the onset of symptoms of PHI [4], plasma HIV-1 RNA titers decreased by 1.44 log₁₀ in the zidovudine group and by 0.93 log₁₀ in the placebo group after 6 months, but the difference was not statistically significant. In another study of 24 patients with PHI, plasma HIV-1 titers decreased by ~2 log₁₀ without therapy 3–12 months after the acute syndrome [2]. The lymph node biopsies from 4 patients just before therapy showed high titers of infectious virus and proviral DNA in LNMC during the acute phase, confirming the early establishment of HIV-1 infection in lymphoid tissues. The decrease in plasma HIV-1 replication was generally associated with a decrease in lymph node virus load, as infectious virus was no longer cultivated from LNMC from any of the 4 patients analyzed at 3 months. Furthermore, HIV-1 replication was very low in 3 cases in lymph nodes analyzed by PCR after 1 year of therapy. The possibility of obtaining a decrease in the proviral DNA titer in LNMC, although not significant in the short period of time studied, and in PBMC, is encouraging. Because several of the cells that harbor provirus are believed to have a long half-life, decreasing proviral titers to undetectable levels will probably take several years.

The plasma HIV-1 RNA clearance of patients with chronic HIV-1 infection who receive a combination of zidovudine, ddI, and 3TC follows a two-compartment model, the second phase having the same kinetics as HIV-1 RNA decay in lymph nodes [8, 12, 13]. This decrease could be more rapid in patients with PHI if therapy is initiated early and perhaps viral dissemination* is still limited, although there were too few patients in our study for statistical significance to be reached.

The 4 patients from whom samples were obtained before diagnosis showed similar rates of virus decline before and after therapy. Early therapy during PHI possibly works mainly by decreasing viral dissemination in the body and preventing the establishment of a steady state in plasma [14].

The changes in the serologic markers of HIV-1 infection in patients with very early therapeutic intervention are striking. Patients receiving early intervention did not show seroreactivity by Western blot to all proteins, and some bands disappeared after several months of therapy. The exact significance of this waning antibody response, which was also recently reported by Perrin et al. [15] and Markowitz et al. [16], is not clear but could be related to the complete inhibition of HIV-1 replication. The declining antibody response was observed only in patients whose plasma HIV-1 RNA remained at <20 copies/mL.

The changes in the lymphocyte subsets of our patients showed an increase in the CD4 cell subset and a decrease in

Figure 2. HIV-1 Western blot patterns of 1 patient from 5 days after onset of symptoms of primary HIV-1 infection (blot 19) to 14 months of therapy (blot 34). Blot 35, positive control; blot 3, negative control.
the CD8 cell subset, leading to a CD4:CD8 ratio >1 in most cases within 3 months. Similar tendencies were observed during zidovudine monotherapy in this setting [4]. Because the drop in CD4 T cell numbers is primarily driven by HIV-1 replication [10, 12], the early immune reconstitution is probably due to the rapid decrease in cytopathic virus following therapy [17], as attested by the cultures of PBMC and LNMC from our patients becoming negative within a few months.

These preliminary results are encouraging because they show a large viral decrease in each compartment studied, leading to a waning of antibody response in some cases. Initiating early therapy during PHI could thus reduce or prevent the immune damage caused by HIV.

Acknowledgments

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References