Residual Low-Level Viral Replication Could Explain Discrepancies between Viral Load and CD4+ Cell Response in Human Immunodeficiency Virus–Infected Patients Receiving Antiretroviral Therapy

Felipe Garcia,1 Carmen Vidal,2 Montserrat Plana,3 Anna Cruceta,1 M. Theresa Gallart, 3 Tomas Pumarola,2 Jose M. Miro,1,2 and Jose M. Gatell1

We report the evolution of chronic infection with human immunodeficiency virus type 1 (HIV-1) in a patient treated with stavudine plus didanosine, whose CD4+ lymphocyte count progressively decreased, despite a sustained plasma viral load <20 copies/mL. After 12 months of therapy, treatment was switched to zidovudine plus lamivudine plus nelfinavir. CD4+ T cell count decreased from 559 × 10^3/L at month 0 to 259 × 10^3/L at month 12. Plasma viral load decreased from 21,665 HIV-1 RNA copies/mL at baseline (month 0) to <20 copies/mL after 1 month of therapy with stavudine plus didanosine, and remained below 20 copies/mL until month 12, but always >5 copies/mL. Viral load in tonsilar tissue at month 12 was 125,000 copies/mg of tissue. After the change to triple-drug therapy, the plasma viral load decreased to 5 copies/mL, the CD4+ T cell count increased to 705 × 10^3/L, and the viral load in tonsilar tissue decreased to <40 copies/mg of tissue at month 24. A low level of HIV-1 replication could explain the lack of immunologic response in patients with apparent virological response.

With the introduction of highly active antiretroviral therapy (essentially triple-drug therapy with 2 nucleoside reverse transcriptase inhibitors plus a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor), it is possible to considerably suppress HIV-1 replication in a high percentage of compliant patients, leading to a quick and permanent drop in plasma viral load to <20 copies/mL and to an unprecedented and sustained increase in CD4+ T cell counts [1]. Discrepancies between immunologic and virological response have been reported in both directions, namely, a persistent CD4+ response despite an incomplete virological response [2, 3] or else a progressive decrease in CD4+ cell count despite an apparently complete virological response [4].

We report a case with a progressive drop in CD4+ lymphocyte count despite a sustained plasma viral load of <20 copies/mL. Persistent viral replication was demonstrated in both plasma and lymphatic tissue, and the situation was reverted when treatment with a more potent antiretroviral regimen containing a protease inhibitor was initiated.

Methods

Patient. We describe a patient with chronic asymptomatic HIV-1 infection (detected 48 months before the start of therapy) who was receiving stavudine plus didanosine and whose CD4+ lymphocyte count progressively decreased, despite a sustained plasma viral load <20 copies/mL. At month 12 stavudine and didanosine were withdrawn, and treatment with a new antiretroviral regimen with zidovudine, lamivudine, and nelfinavir was initiated.

Monitoring. Medical controls were performed at months 1, 2, 3, and 6 and every 3 months thereafter until month 24. These included clinical assessment and routine laboratory monitoring. Plasma HIV RNA levels were determined twice at baseline and at every time point by use of the Amplicor HIV-1 Monitor Ultra Sensitive Specimen Preparation Protocol Ultra Direct Assay (Roche Molecular Systems, Somerville, NJ; lower limit of quantification of 20 copies/mL). Those samples below the quantification limits of this test at 6, 9, 12, 18, 21, and 24 months were restested by means of a more sensitive method with a lower limit of quantification of 5 HIV-1 RNA copies/mL, as reported by Schockmel et al. [5]. Both procedures were repeated twice in order to confirm the results. Absolute numbers and percentages of CD4+ and CD8+ cells were also measured by flow cytometry at each time point.

Written consent of the patient was obtained for the invasive procedures and for closer control.

Substudies. Tonsillar biopsy was performed with a triangular adenoid punch-biopsy forceps that could obtain up to 80 mg of tissue. A sample was obtained from each tonsil. The weights of the samples were determined before they were processed. Each sample was split into 2 parts; one half was paraffin-embedded and then examined by a pathologist to confirm the presence of lymphoid tissue. The other half was immediately frozen and stored in liquid nitrogen.

Viral load in tonsilar biopsy specimens was determined by use
of the NucliSens HIV-1 RNA QT Assay (Organon Teknika, Turnhout, Belgium). The amount of RNA was expressed as copies per milligram of tissue. The procedure was repeated twice to confirm the results. CSF HIV RNA determinations were made by use of the Amplicor direct assay mentioned above (lower limit of quantification of 20 copies/mL).

Subpopulations of CD3⁺, CD4⁺, and CD8⁺ T cells were determined by 3-color flow cytometry. Lymphocyte-proliferation assays were performed as described elsewhere [6]. Analyses of genotypic resistance and viral phenotype and genotypic analysis of chemokine receptors were performed by means of standard methods [7–11].

Results

Plasma viral load decreased from 21,665 HIV-1 RNA copies/mL at baseline (month 0) to <20 copies/mL following 1 month of therapy with stavudine plus didanosine and remained <20 copies/mL until month 12. By use of a more sensitive method with a lower limit of quantification of 5 copies/mL, the plasma viral load was 13, 15, and 6 copies/mL at months 6, 9, and 12, respectively. The CD4⁺ T cell count decreased from 559 × 10⁶/L at month 0 to 259 × 10⁶/L at month 12. The percentage of CD4⁺ T cells dropped from 33% to 24% during the same period of time.

At month 12 a new therapeutic regimen was initiated: zidovudine plus lamivudine plus nelﬁnavir. Twelve months later (month 24), the plasma viral load remained below 20 copies/mL (in fact, an ultrasensitive method showed it was below 5 copies/mL at months 18, 21, and 24). The CD4⁺ T cell count increased up to 705 × 10⁶/L at month 24 (at this time point the percentage was 42%) (figure 1). The viral load in lymphatic tissue (as determined by tonsilar biopsy) was <40 copies/mg of tissue, and that in the CSF was <20 copies/mL. No mutations were detected in the protease and reverse transcriptase genes at baseline, and HIV-1 RNA could not be isolated at months 12 and 24. The virus was a nonsyncytium-inducing strain, and mutations at CCR5, CCR2, and SDF-1 genes were not observed (data not shown).

On the other hand, markers of immune activation (the CD8⁺CD38⁺ T cell subset) increased from 67% at month 0 to 78% at month 12 and then decreased to 42% at month 24. Moreover, CD8⁺CD28⁺ T cells decreased from 42% at month 0 to 34% at month 12 and then increased to 49% at month 24. From month 12 to month 24, the stimulation index to mitogens (phytohemagglutinin-M 1%, or PHA) increased from 28 to 61, and the stimulation index to speciﬁc antigens (cytomegalovirus) increased from 0.72 to 3.55.

Discussion

The pathophysiological basis for the discrepancy between a sustained decrease in plasma viral load to below the quantification level and a progressive decrease in the CD4⁺ T cell count is not clear. Potential explanations could involve genetic, virological, or immunologic factors or direct toxicity of antiretroviral therapy. A syncytium-inducing HIV-1 phenotype has been shown to be associated with a worse prognosis than a nonsyncytium-inducing viral phenotype [8]. Moreover, some immunologic responses could help control HIV-1 infection [12, 13], and the poor quality of this response could explain the discordance.

None of these hypotheses, however, could explain the discordance between the progressive fall in CD4⁺ T cell counts and the apparent virological response in our patient. The virus in our patient was not of a syncytium-inducing phenotype. Although toxic effects of the initial medication on lymphocytes cannot be excluded, such toxicity is unlikely since there was no decrease in the absolute number of lymphocytes; on the other hand, the percentage of CD4⁺ T lymphocytes decreased.

It could be hypothesized that potential differences exist in viral infectivity between patients. Since viral load only measures virus particles, this patient might have had a very high ratio of infectious particles to total viral particles. In any case, this
hypothesis is difficult to demonstrate since assays for replication competence are still not standardized. Therefore, the most likely explanation is the persistence of residual replication despite apparent response (plasma viral load <20 copies/mL). In fact, at months 6, 9, and 12, the viral load was 13, 15, and 6 copies/mL, respectively, and the lymphoid-tissue viral load was 125,000 copies/mg at month 12. Moreover, 1 year after the switch from double-drug to triple-drug therapy, an increase in CD4 + T cells was observed, from 259 to 705 × 10^6/L, and the plasma and lymphoid-tissue viral load decreased to below the detectable level (to <5 copies/mL and <40 copies/mg, respectively).

With respect to our case, it is noteworthy not only that a decrease occurred in the number of CD4+ T cells but also that after 1 year of antiretroviral therapy and persistence of the viral load at <20 copies/mL, the immune system deteriorated in comparison with the pretreatment level. Despite low replication in plasma, activation markers (percentage of CD6+ CD38+ cells) increased, whereas the percentage of CD28− CD8+ cells (which are involved in antigen presentation and therefore are required for correct T cell function) [14] decreased during this period. Conversely, 1 year after the change to a more effective regimen (from treatment with stavudine and didanosine to that with zidovudine, lamivudine, and nelfinavir), the percentage of CD8− CD38+ cells decreased while the percentage of CD28− CD8+ cells increased, as has been described with regard to asymptomatic patients receiving triple-drug therapy [15].

In addition, proliferation responses to mitogens (PHA) and specific antigens (cytomegalovirus) also increased during this period, reaching levels similar to those in asymptomatic patients after therapy [6, 15]. Therefore, a change to a more potent regimen seems to lead to recovery of immune system function.

A drawback of the study was that the data on immune activation were determined only at specific points (at months 0, 12, and 24), as were those on proliferation responses to mitogens and antigens (at months 12 and 24). Given the substantial variability in these assays, these data should be carefully considered. However, the changes in the different immunologic markers (proliferation responses to mitogens and antigens, as well as percentages of CD8− CD38+ and CD8+ CD28− T cells) were concordant, supporting the validity of the results.

In summary, a residual low level of HIV-1 replication could explain the discrepancy between an apparent complete virological response, as measured by current standards (durable response to <20 copies/mL), and a progressive decrease in CD4+ T cell count. Determinations of plasma viral load by more sensitive methods (with lower limits of quantification <5 copies/mL) or by lymphoid-tissue biopsies may be necessary to detect this residual replication. It is likely that changing or intensifying the therapy with a more potent regimen could prevent a decrease in the CD4+ T cell count and improve the function of the immune system.

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References