Monitoring Responses to Antiretroviral Treatment in Human Immunodeficiency Virus Type 1 (HIV-1)–Infected Patients by Serial Lymph Node Aspiration

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Fine-needle aspiration was used to collect lymph node cells (LNC) from 9 antiretroviral-naive patients entering a double-blind single- or combined-drug study of zidovudine, zalcitabine, and saquinavir. LNC were obtained twice before and 1 and 6 months after initiation of treatment. The effect of antiretroviral treatment on virus load ranged from no response to a dramatic decrease in plasma and LNC human immunodeficiency virus (HIV) RNA levels. The decrease in unspliced or spliced (or both) HIV RNAs in LNC was correlated with but consistently smaller than the decrease in plasma viremia. When present, the increase in blood CD4 T cells was, in general, moderate and transient. However, a striking rise in blood CD4 T cell count and in LNC CD4:CD8 ratio was observed in the 1 patient with the deepest sustained decrease in HIV RNA level in both plasma and lymph nodes.

Recent work has revealed that viral replication is about two orders of magnitude more intense in lymphoid tissue than in blood cells [1–4] and that the bulk of plasma virions is produced in compartment(s) other than blood, most probably the lymphoid tissue [5]. However, the precise contribution of each compartment to plasma viremia is not yet clearly defined. It appears, therefore, of prime importance to directly investigate how far antiretroviral drugs are effective in decreasing viral replication in one of the major lymphoid compartments, the lymph nodes (LNs). In addition, the determination of virus load in LNs might prove a valuable alternative quantitative parameter in patients in whom plasma viremia is no longer detectable, as the result of antiretroviral treatment.

Here, we have applied our recently described technique of fine-needle aspiration of LN cells (LNC) [1] to monitor the effect of antiretroviral treatment on human immunodeficiency virus (HIV) replication in LNs.

Methods

Patients. Nineteen antiretroviral-naive patients in the Swiss HIV Cohort Study and eligible for starting antiretroviral treatment were considered for this investigation. Seven could not be included because they lacked accessible enlarged LNs ($n = 6$) or because LN enlargement was due to Hodgkin's disease ($n = 1$). Fine-needle aspiration of LNC was attempted in the remaining 12 subjects and was successful in 10. One of those 10 chose to leave the study after the first two aspirations. Thus, 9 subjects could finally be investigated. Seven belonged to CDC clinical category A, 1 to category B (patient 8), and 1 to category C (patient 3). At baseline, the mean blood CD4 cell count was 229/μL (range, 70–372) and median plasma viremia was 46,196 copies/mL (range, 3032–479,300).

Patients 4, 5, 7, and 8 undertook zidovudine monotherapy (500 mg/day). The other 5 subjects (patients 1–3, 6, 9) were enrolled in a phase III, randomized, and still double-blinded study of saquinavir, a protease inhibitor [6], in combination with zidovudine or zidovudine plus zalcitabine, compared with zidovudine alone or zidovudine plus zalcitabine (zidovudine, 600 mg/day; zalcitabine, 2.25 mg/day; saquinavir, 1800 mg/day). This study is referred to as SV14604. Patients 1 and 2 received zidovudine alone for 28 days and were then moved to the SV14604 protocol. For patients 7 and 8, treatment was discontinued after 96 and 16 days, respectively. Patient 5 was lost to follow-up after the 1-month visit.

Fine-needle aspiration of LNC. Needle aspiration and LNC processing were done as described [1]. Blood was drawn (and EDTA-anticoagulated) at the same time and was processed to produce platelet-depleted plasma [1]. To establish baseline values, patients had two separate LN aspirations during the 28-day period that preceded treatment initiation at day 0. Two additional aspirations were done 1 and 6 months after initiation of treatment. The location of the LNs aspirated was inguinal ($n = 17$), cervical ($n = 9$), or axillary ($n = 13$). When two different LNs were aspirated at the same time ($n = 4$), cells were pooled. The cell yield ranged from 1×10^4 to 5.7×10^6.

Cell immunophenotyping. Two-color flow cytometry was done with a cytometer (Epics Profile II or Epics XL; Coulter, Miami), antibodies (Dako Diagnostics, Zug, Switzerland), and standard procedures.

Quantitation of HIV-1 unspliced RNA by competitive reverse transcription–polymerase chain reaction (RT-PCR). Total RNA was ex-

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All patients were enrolled in the Swiss HIV Cohort Study, and all gave informed consent for the present investigation.

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tracted from LNC and from plasma as described [1]. Contamination of LNC RNA by HIV DNA was often not detectable. When present, the signal measured in the absence of reverse transcriptase was always ≤14.5% of that measured in the presence of reverse transcriptase.

RNA was reverse-transcribed and cDNA amplified by PCR [1]. A 260-bp fragment of the *gag* gene was amplified using degenerated GAG06 and GAG04 primers [7]. For every sample, 8 RT-PCR reactions were done in the presence of various amounts (31,623 down to 10 copies, by half-$\log_{10}$ dilution steps) of an internal standard, generated by in vitro transcription of pQP1Δ80 [1].

Quantification was achieved by including primer GAG06 (0.51 pmol) labeled with ^{33}P (0.6 μCi) at the 5′ end in addition to unlabeled primer (10 pmol) in the PCR buffer (final volume, 100 μL). Amplicons were separated by PAGE and radioactivity was measured (InstantImager; Packard Instrument, Meriden, CT).

Semiquantitative RT-PCR for HIV-1 spliced RNAs. Spliced RNAs were quantitated as described [1], using primers designed to produce amplicons (100–300 bp long) representing all singly and multiply spliced HIV-1 RNAs except the Nef1 mRNA [1].

HIV RNA levels were measured in the linear range of the RT-PCR methods (20–6500 copies, $r = .9945$, for unspliced RNA; equivalent of 30–10,000 8E5/LAV cells [8], $r = .9964$, for spliced RNAs). HIV RNAs in the 4 plasma or LNC samples from a given patient were assayed in the same run to avoid the effect of interassay variation.

Results

In general, there was good agreement between the two baseline (pretreatment) virus load values in the 9 subjects studied. In LNC, they differed on the average by 0.3 and 0.5 $\log_{10}$ for unspliced and spliced RNAs, respectively, compared with 0.06 $\log_{10}$ for unspliced RNA in plasma. The mean difference between the two pretreatment CD4:CD8 T cell ratios was 0.3 in LNC and 0.07 in peripheral blood mononuclear cells.

Minimal changes relative to baseline values were observed in 3 patients (5, 7, and 8) after treatment, including the 2 who discontinued treatment. In patient 7 for example, there was only a small decrease in plasma viremia, little change in LN levels of viral RNAs or blood CD4 T cell number, and a decrease in the LN CD4:CD8 T cell ratio (figure 1).

Four patients (1, 3, 4, and 6) responded to treatment by moderate and essentially transient changes in their virologic and immunologic parameters. The data from patient 6 are depicted in figure 1.

Two patients (2 and 9) experienced a marked and long-lasting decrease in plasma viremia. In patient 2, the decrease in HIV RNA level was smaller in LNs than in plasma, the increase in blood CD4 T cell number was very limited, and the increase in the LN CD4:CD8 T cell ratio was marked (figure 1). Patient 9 contrasted with all other subjects by showing dramatic improvement of all virologic and immunologic parameters (figure 1).

In all patients, results were essentially unchanged when HIV RNA levels in LNC were expressed as copies per 10^5 CD4 T lymphocytes instead of copies per 10^5 total LNC.

Levels of spliced HIV RNAs in LNC were clearly correlated with those of unspliced HIV RNA (Spearman's $r = .50$, $P < .003$), and changes in the level of the two kinds of RNA species were, in general, similar.

As shown in figure 2, changes in viral RNA levels in LNC with therapy were strongly correlated with changes in plasma viremia. However, the slope of the regression lines was <1 (0.75 and 0.42 for unspliced and spliced RNAs, respectively), indicating that the decrease of LNC RNAs was smaller than the decrease of plasma RNA.

Discussion

The present study demonstrates that LN needle aspiration allows assessment of the effect of antiretroviral treatment on virus load and T cell subsets in the lymphoid tissue. Indeed, our sampling procedure appeared to give reproducible results, as judged by the comparison of the two baseline (pretreatment) values. The mean difference between the two baseline levels of virus load was only slightly higher than that reported using LN mononuclear cells isolated from surgical biopsies of LNs in untreated patients [9]. The observation of concerted virus load decreases in LNs and plasma in response to treatment also supports the validity of our LN virus load assessment by fine-needle sampling.

One major finding of this study is that the decrease in viral RNA levels in LNs with treatment was, in general, blunted compared with the decrease in plasma viremia, even though the two changes were correlated. This observation does not contradict the proposal that most virions present in plasma are produced in LNs [2, 3, 5]. Indeed, the regression lines shown in figure 2 may describe the complex relationship between the rate of viral replication in lymph nodes and the resulting plasma viremia. Alternatively, our results could reveal the existence of another compartment, such as the spleen, where a fraction of plasma virions would be produced and which would respond more dramatically than LNs to antiretroviral treatment.

We found that the levels of unspliced and spliced RNAs in LNC were correlated with each other. Unspliced HIV RNA in LNC represents the mRNA for the *gag* and *gag-pol* precursors and therefore reflects the level of replication in CD4 T cells. However, it may also correspond to genomic RNA present in extracellular virions bound to follicular dendritic cells (FDC) [2, 3, 10, 11]. In contrast, spliced HIV RNAs represent exclusively viral replication in infected cells. Thus, the correlation we observed between both kinds of RNA may indicate either that changes in the number of FDC-trapped virions paralleled changes in viral replication or that LNC obtained by our procedure did not contain significant numbers of FDC.

The results presented here are not inconsistent with those reported by Cohen et al. for patients starting zidovudine [9] or adding didanosine to zidovudine [12] treatment. Indeed, these investigators found that zidovudine alone had little effect on HIV RNA levels in LN mononuclear cells (LNMC) while it reduced plasma viremia in 2 of 6 patients [9]. They reported that, upon addition of didano-

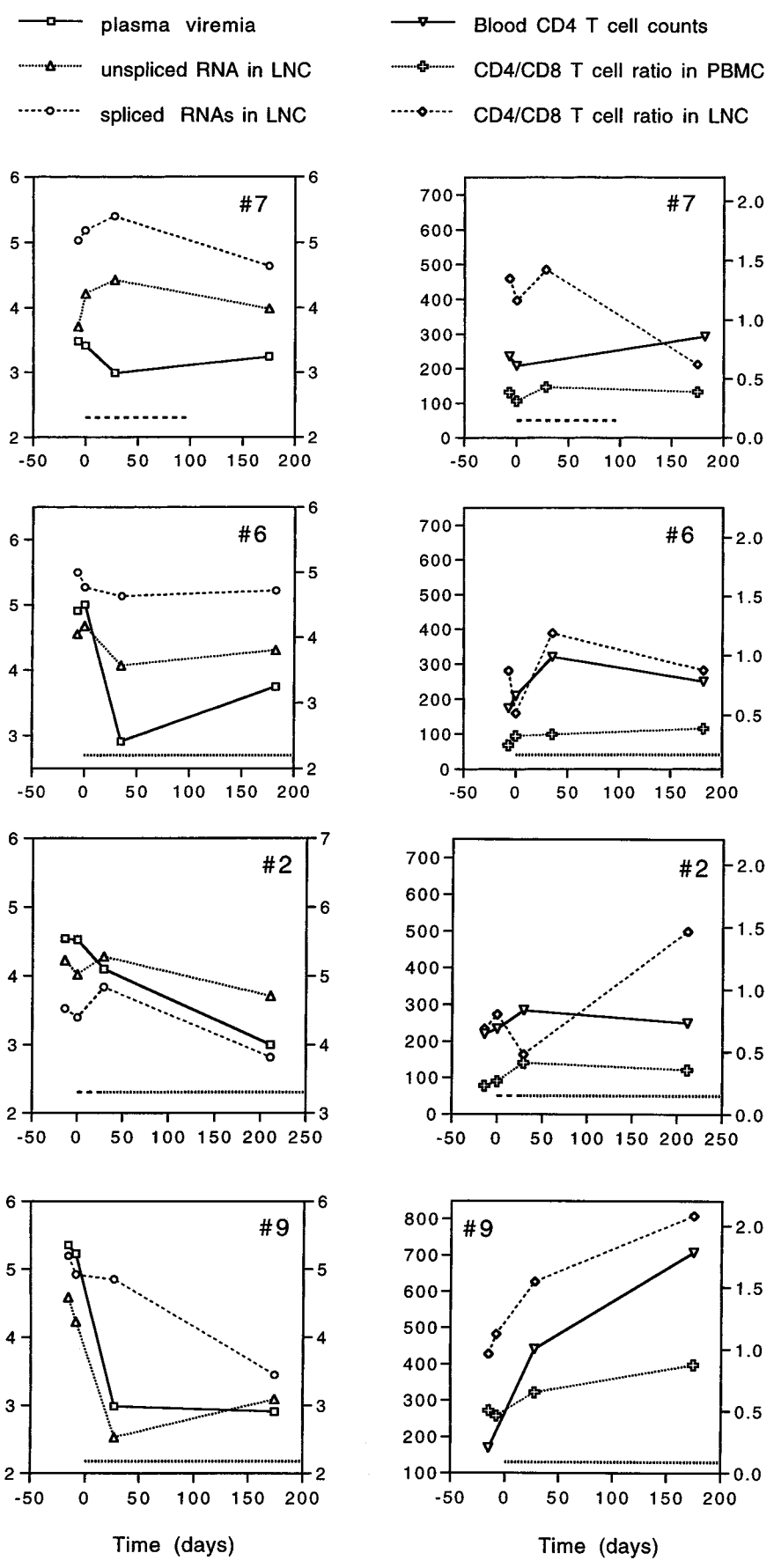

Figure 1. Changes in virologic (left panels) and immunologic (right panels) parameters with treatment in patients 7, 6, 2, and 9. In left panels, plasma viremia is expressed as log HIV RNA copies/mL and unspliced HIV RNA in lymph node cells (LNC) as log copies/10^5 cells (left y axes) and spliced HIV RNAs in LNC as log counts/10^4 cells (right y axes). In right panels, blood CD4 T cell counts are expressed as cells/μL (left y axes), and CD4:CD8 T cell ratios are shown (right y axes). Horizontal dashed line, zidovudine treatment; horizontal dotted line, SV 14604 protocol; PBMC, peripheral blood mononuclear cells.

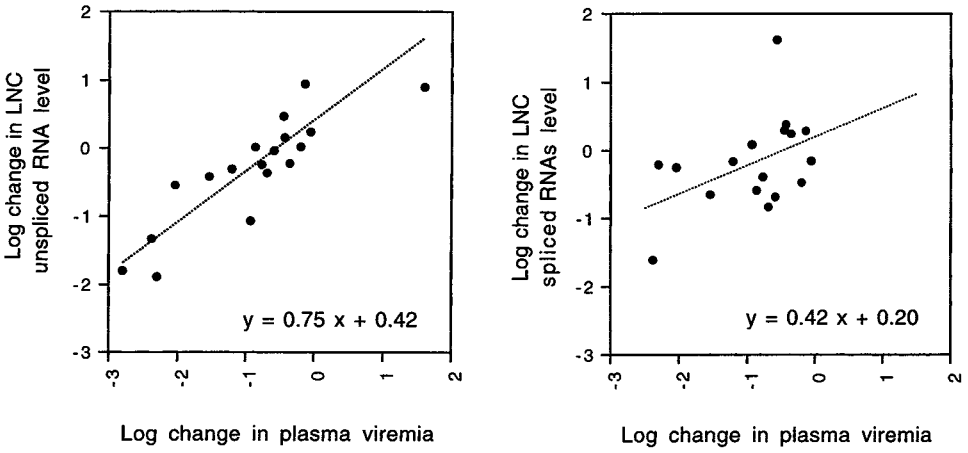

Figure 2. Correlation between changes in plasma viremia and changes in level of HIV unspliced RNA (left, $r = .877$, $P < .0001$) or spliced RNAs (right, $r = .493$, $P = .045$) in lymph node cells (LNC) after log transformation.

sine to zidovudine treatment, HIV RNA levels in LNMC and plasma decreased correlatively in only 3 of 6 patients [12].

Our second important finding is that a major increase in CD4 T cell number in blood occurred only in the patient in whom treatment induced a sharp and sustained decrease of HIV RNA levels in both LNC and plasma. The increase in CD4 T cell counts in blood with therapy has been attributed by some to redistribution of the cells from lymphoid tissue to peripheral blood rather than to a true increase in number by proliferation or differentiation [13–15]. In the present study, we found that 2 of the 3 patients with the highest increases in blood CD4 T cell number had a concomitant increase in the CD4:CD8 T cell ratio in LNC. This argues against the hypothesis of mere redistribution between the two compartments.

In summary, our results indicate that monitoring the effect of antiretroviral treatments in a relatively noninvasive manner in lymphoid tissue is possible in ~50% of patients. We are currently investigating whether ultrasonic guidance could increase the rate of success of LNC aspiration. This technique could provide a quantitative assessment of residual virus load, even after plasma viremia has become undetectable following the use of highly efficacious antiretrovirals.

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