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The number of HIV DNA-infected mononuclear cells is reduced under HAART plus recombinant IL-2

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Abstract

It is common opinion that, in addition to potent antiretroviral regimens which effectively reduce plasma viremia, new strategies should be developed to ensure the reduction of cell-associated HIV DNA load together with HIV RNA plasma levels. The present study explored whether the number of provirus-infected cells can be reduced by combined antiviral and immunomodulatory regimens. Thus, 14 naive patients (with CD4 $>400/\mu l$ and plasma HIV RNA copies $>5000/ml$) were randomly assigned to receive highly active antiretroviral therapy (HAART) alone or HAART plus rIL-2. Plasma viremia (measured by a commercial RT-PCR assay) and the number of provirus-infected cells (measured by an endpoint cell dilution PCR assay) were monitored at the enrollment and after 12 weeks of treatment. The results indicate that while HAART and HAART plus rIL-2 are both able to significantly reduce plasma viremia after 12 weeks of treatment, a significant reduction of the number of provirus-infected cells can be achieved only by treatment with HAART plus rIL-2.

Keywords: AIDS; HIV DNA-infected cells; Viral load; HAART; HAART plus rIL-2

It has been established that the course of HIV infection depends on the dynamics of viral replication, generally measured as the number of copies of HIV RNA per ml of plasma (Ho et al., 1995; Mellors et al., 1996). In fact, a correlation between plasma viral load and progression rate of disease has been demonstrated (Mellors et al., 1996; Craib et al., 1997). Moreover, previous clin-

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ical studies have shown that disease progression stops, or reverses, when viral replication is effectively inhibited (Mellors et al., 1996; Craib et al., 1997). However, despite a sustained fall of plasma viremia below detection, the load of proviral DNA may remain stable for a relatively long period of time (Finzi et al., 1997; Wong et al., 1997). Furthermore, after discontinuation of therapy, viral replication resumes promptly and often exceeds the pre-treatment plasma viral load (De Jong et al., 1997; Montaner et al., 1998). It has been proposed that the source of this viral reemergence are the long-lived provirus-infected cells, by definition not susceptible to current antiretroviral agents. The size of the replication-competent provirus-containing cellular reservoir may be sufficient, once activated, of reinitiate productive infection, even though many of these cells (from 50 to 99%) may contain a defective genome (Finzi and Siliciano, 1998; Levy, 1998). It has also been recently shown that even during antiviral therapy residual viral replication continuously occurs (Furtado et al., 1999; Zhang et al., 1999), possibly due to activation of integrated HIV provirus. The existence of a reservoir of long-lived provirus-infected cells, not substantially declining while on antiviral therapy (Pantaleo, 1997; Tamalet et al., 1997; Finzi et al., 1999; Zhang et al., 1999), may suggest new therapeutic strategies aimed at reducing not only viral replication but also the cell compartment harboring silent HIV DNA, mostly represented by quiescent T cells (Chun et al., 1998; Roederer, 1998).

Effective treatment of provirus-infected cells might be achieved by combining highly active antiretroviral therapy (HAART) with IL-2 treatment to activate them in a state that: (a) promotes HIV replication leading to their death by cytopathology or apoptosis, and (b) results in viral progeny that would be unable to propagate due to the HAART. Thus the combined treatment may decrease the load of provirus-infected cells without causing the enhancement of plasma viremia usually seen following mitogenic activation by IL-2 (Davey et al., 1997). We have addressed this possibility and show here that HAART combined with IL-2 is capable of reducing not only HIV RNA viremia but also cell-associated HIV DNA.

Briefly, 14 antiviral drug-naive patients with CD4+ lymphocyte counts >400/ul and plasma viral load >5000 HIV RNA copies per ml, were recruited at the Spallanzani hospital and randomly assigned to receive HAART (Indinavir 2400 mg/day, Stavudine 60–80 mg/day, and Epivir 300 mg/day) alone (arm 1) or HAART plus recombinant interleukin 2 (rIL-2, Aldesleukin, Chiron, 10^6 IU subcutaneously, 5 days per week at alternative weeks, arm 2). At 0 and 12 weeks, plasma viral load and the proportion of provirus-infected cells were evaluated as described below. Clinical, immunological (i.e. CD4 cell count, CD4/CD8 ratio, etc.), and virological (i.e. plasma viral load) characteristics of the two groups of patients were comparable at enrollment. All patients agreed to participate by signing an informed consent form and the study, conducted according to GCP standards, was approved by the Institutional Ethical Committee.

HIV RNA was extracted from plasma and quantified by a RT-PCR procedure (HIV-1 Amplicor Monitor Roche) performed according to the Manufacturer's instructions. Plasma levels of HIV-1 RNA were expressed as HIV RNA copies/ml with a threshold level of 400 copies/ml. Using frozen mononuclear cells obtained from peripheral blood (PBMC) of patients we measured by PCR the proportion of cells harboring HIV proviral DNA following a procedure already established (Dianzani et al., 1996). Briefly, cell suspensions to be amplified were diluted 10-fold starting from 10^6 to 10^0 cells. In each dilution 10^6 cells were reconstituted by adding the proper number of PBMC from a healthy donor. Each dilution was then pelleted, lysed, and subjected to PCR to detect *gag* gene by using the HIV-1 Amplicor kit (Roche). The results are expressed as values from five replicates calculated according to the Reed and Muench method. In each assay serial dilutions (from 10^4 to 10^0 cells) of 8E5 cells containing one copy of HIV DNA/cell (Folks et al., 1986), were processed as above and added as control. The limit of sensitivity of this assay is 0.47 Log cell/10^6 cells and the interassay and intra-assay variability is <0.3 Log. In control experiments using a known number of infected cells supplemented with a known number of unin-

fected cells, the assay permits to reveal differences in number of infected cells higher than 0.47 Log (i.e. the limit of sensitivity of the assay) even with baseline values < 2 Log infected cells/10^6 cells.

Fig. 1 shows the effects of therapy on plasma viremia (Panel A) and on number of provirus-infected cells (Panel B). It can be seen that in both arms plasma viremia fell below detection limit. In contrast, although the proportion of provirus-infected cells remained stable in patients receiving HAART, it fell significantly in patients treated with HAART plus IL-2 ($P < 0.01$). No significant variations were observed in the total number of PBMC during HAART or HAART + IL-2 treatment and both treatments were well tolerated with no significant differences between the two arms. No patient drop out occurred. CD4 cell number increased in both HAART-treated and HAART + IL-2-treated patients after 12 weeks of therapy (baseline values: arm $1 = 453 \pm 66.5$ cell count/μl, arm $2 = 497 \pm 56.1$ cell count/μl; 12 weeks values: arm $1 = 567 \pm 140$ cell count/μl, arm $2 = 826 \pm 226.1$ cell count/μl). No significant differences were observed between the increase of CD4 cell count in the two groups of patients (mean of fold increase: arm $1 = 1.19 \pm 0.33$; arm $2 = 1.7 \pm 0.6$, $P = 0.085$), probably due to the low dose of IL-2 used in the study and/or to the low number of patients examined.

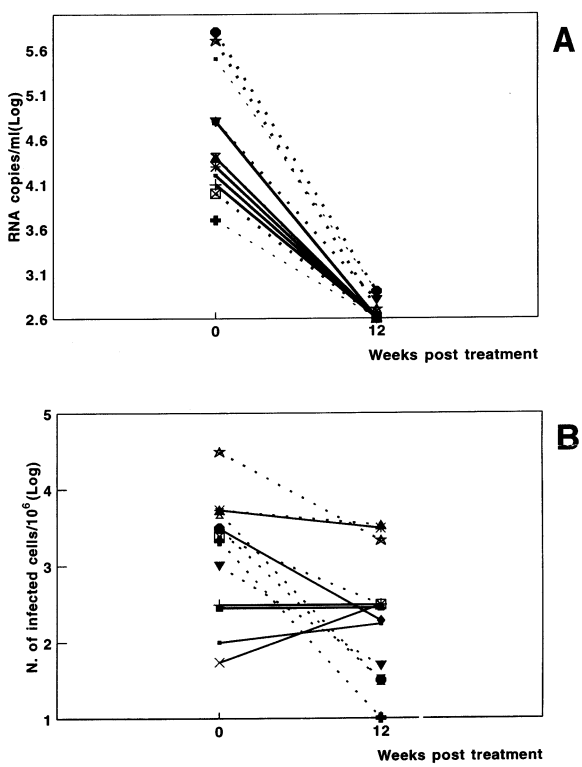

Fig. 1. Effect of HAART or HAART plus rIL-2 on plasma viral load (panel A) and on number of provirus-infected cells/10^6 PBMC (panel B). Continuous lines refer to patients enrolled in arm 1; dotted lines refer to patients enrolled in arm 2. The results of the number of provirus-infected cells are expressed as mean values from five replicates according to the Reed and Muench calculation. The changes of plasma viremia and proportion of provirus-infected cells in the two arms were evaluated by the Student's *t*-test.

The rate of decay of cell-associated HIV DNA in patients under HAART is not known in detail. It is likely that cell-associated HIV DNA decreases with a slower kinetic decay when compared with that of HIV RNA and, indeed, a significant reduction of cell-associated HIV DNA was recently reported only after 60 weeks of suppressive therapy, in plasma HIV RNA/negative, HIV DNA/positive patients (Izopet et al., 1998). Our data indicate that the addition of IL-2 to conventional HAART significantly and promptly reduces the number of cells harboring HIV DNA rather than simply decreasing the amount of circulating HIV RNA. Our observations are consistent with the recent data of Chun et al. (1999) and by Simonelli et al. (1999) concerning the ability of IL-2 plus antiretroviral treatment to significantly reduce the number of HIV DNA copies. In the latter study the doses of the IL-2 administered were much higher than those used in the present study and the number of cells harboring HIV DNA was not examined. Thus, all three studies indicate that the DNA viral load may be efficaciously affected by IL-2 treatment plus conventional HAART.

Unaddressed by our experiments is the nature of the proviral DNA affected or the cell lineage harbouring it. However, the lack of decrease of HIV DNA load under HAART therapy alone tends to rule out the possibility that most of proviral DNA is contained in productively infected T cells. It can then be inferred that the reduction of this parameter might have important

consequences in view of eventual viral eradication and/or deferred disease progression. Our data suggest that this reduction is achievable by adding even low doses of rIL-2 to HAART. Undetermined is whether rIL-2 acted as an activator of latently infected cells and/or as a potentiator of the immune-mediated killing of HIV infected cells. It remains also to be established how sustained this reduction will be and whether the response can be improved by higher rIL-2 dosages and/or prolonged treatment. Larger and more comprehensive studies are in progress.

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