Human Immunodeficiency Virus Type 1 Modulates Telomerase Activity in Peripheral Blood Lymphocytes

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The effect of human immunodeficiency virus type 1 (HIV-1) on telomerase activity in peripheral blood lymphocytes (PBL) was examined. Telomerase is an enzyme that is involved in mechanisms that control cell life span and replicative potential. HIV-1 reduced telomerase activity in in vitro–infected PBL and impaired enzyme activation upon cell stimulation. Telomerase activity was significantly lower in PBL from 23 HIV-1–infected patients than in PBL from healthy donors and significantly increased during highly active antiretroviral therapy (HAART) in 10 patients who had both a virological and an immunological response and in 5 and 8 patients with a virological or an immunological response, respectively. Further analyses of fractionated cells revealed that telomerase activity increased mainly in $CD4^+$ lymphocytes. Overall, these findings demonstrate that HIV-1 infection down-modulates telomerase activity and suggest that both the HIV-1 decline and immunorestoration in response to HAART contribute to increased telomerase activity in $CD4^+$ lymphocytes.

Human immunodeficiency virus (HIV) type 1 infection is characterized by a progressive decrease in $CD4^+$ T cells and immune dysfunctions that ultimately leads to AIDS. The mechanism underlying the decline in $CD4^+$ cell counts (i.e., $CD4^+$ cell decline) is not well understood. Some studies that reported an increase in T cell proliferation [1–3] suggested that the primary cause of declining $CD4^+$ T cells is the progressive exhaustion of cell renewal capacity due to the persistent increase in T cell turnover [1]. However, in studies that addressed the kinetics of circulating T cells by analyzing telomere length [4, 5], Ki67 staining [6], and in vivo [^{2}H]glucose labeling [7], $CD4^+$ cell decline was attributed to a shortened half-life and impairments in the mechanisms of regeneration that consequently could not compensate adequately for the cell loss. An inefficient renewal of $CD4^+$ cells is probably due to several mechanisms, including the loss of T lymphocyte precursors in the thymus as a direct result of HIV-1 infection in these cells [8, 9] and HIV-1–dependent lesions in the hematopoietic progenitor cell (HPC) compartment; even uninfected HPCs from HIV-1–infected patients are severely compromised in their clonogenic and differentiation capacities [10–12].

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Telomerase, an RNA-dependent DNA polymerase that synthesizes telomeric repeats [13, 14] and thus prevents progressive telomeric shortening at each cell division [15, 16], is believed to play a determinant role in controlling cell life span and replicative potential [17, 18]. Telomerase activity in HPCs is associated with self-renewal potential. It is constitutively expressed at relatively high levels in lymphoid lineage-committed progenitor cells and at a low level in peripheral blood lymphocytes (PBL), in which it can be transiently up-regulated by cell activation [19, 20]. The finding of impaired telomerase activity in $CD34^+$ HPCs from HIV-1–infected patients may help explain why these cells show a compromised replicative capacity and clonal expansion [21]. Data are not clear on telomerase activity in PBL of HIV-1–infected patients. In some studies, telomerase activity was normal in T lymphocyte subsets [4, 5, 22]; however in $CD4^+$ cells, the preferential target of HIV-1 infection, lower telomerase activity levels have been described [22]. Moreover, after cell stimulation, telomerase expression is impaired in some HIV-1–infected patients but not in others [5, 22]. Most of these studies were cross-sectional, and results may be due to different patient characteristics (i.e., early and late disease stage). Low levels of telomerase activity were found in patients with advanced disease [23], and the few data available in sequentially studied patients suggested a decrease in telomerase activity as disease progressed [4, 22].

None of these studies addressed the relationship between telomerase activity and HIV-1 burden in the peripheral blood compartment; moreover, whether HIV-1 infection in itself modulates telomerase activity in PBL has not been investigated. By using in vitro–infected PBL from healthy donors, we studied whether HIV-1 might modulate telomerase activity. We also evaluated

telomerase activity in relation to virological and immunological responses in sequential samples collected from 23 HIV-1–infected patients before and during antiretroviral therapy.

Patients and Methods

In vitro HIV-1 infection. In vitro HIV-1 infection was done as reported elsewhere [24]. In brief, PBL were isolated from the blood of 3 healthy HIV-1–seronegative donors by using Ficoll-Paque (Pharmacia) gradient centrifugation. Cells from each donor were resuspended to a concentration of 2×10^6 cells/mL and were stimulated with phytohemagglutinin (PHA; 1 μg/mL) for 48 h. PBL then were washed, were resuspended to a concentration of 6×10^6 cells/mL in RPMI 1640 medium supplemented with 10% fetal calf serum and 10% T cell growth factor, and were plated in 12-well plates (Costar) at 0.5 mL/well. Cell cultures were exposed to a dose equivalent to 30 ng of p24 protein of R5 type prototype HIV-1_{BAL} for 2 h at 37°C, and then were washed twice and resuspended in 2 mL of complete medium. Control cell cultures derived from the same donors were exposed to 0.5 mL of medium rather than to virus inoculum. HIV-1–infected and control cell cultures were maintained for 13 days. On postinfection days 1, 2, 4–7, 9, and 13, cells were harvested for telomerase activity analysis. Culture supernatants were collected and analyzed for the presence of HIV-1 p24 antigen by enzyme-linked immunosorbent assay (HIV-1 p24 Core Profile ELISA; DuPont Medical Products). In parallel experiments, HIV-1–infected and control cell cultures were restimulated on postinfection day 4 with PHA (1 μg/mL) for 24 h; telomerase activity and p24 antigen were determined on days 5–7, 9, and 13.

Patients. We assessed 23 HIV-1–infected patients who began highly active antiretroviral therapy (HAART). The HAART regimen consisted of a triple-drug combination and included 2 reverse-transcriptase (RT) inhibitors (azidothymidine, dideoxyinosine, or dideoxycytidine) and 1 protease inhibitor (indinavir, ritonavir, or saquinavir), which were administered according to current guidelines [25]. At HAART entry, all patients had AIDS-defining conditions according to the 1993 Centers for Disease Control and Prevention classification system; 20 had previously experienced antiretroviral regimens with RT inhibitors. The patients' $CD4^+$ T cell counts and HIV-1 plasma RNA levels were followed over time. Virological responders were defined as persons in whom the HIV-1 RNA load decreased to undetectable levels (<200 copies/mL). Immunological responders were defined as persons in whom the $CD4^+$ T cell count increased >30%, compared with that at baseline, with an absolute value >100 cells/μL. Telomerase activity was determined in cryopreserved PBL samples collected at HAART entry and at the time of virological and immunological responses (i.e., the first time at which plasma HIV-1 RNA levels decreased to undetectable levels and the point when the $CD4^+$ cell count reached the maximum increase, respectively). The analysis was done on total PBL and on fractionated $CD4^+$ and $CD4^-$ cells separated from total PBL by using anti-CD4 immunomagnetic beads (Dynal), according to the manufacturer's instructions.

Quantification of HIV-1 RNA. We determined the plasma HIV-1 RNA copy number by using the Amplicor HIV-1 Monitor test (Roche Diagnostic System), according to the manufacturer's instructions.

Telomeric repeat amplification protocol (TRAP) assay. Cells were washed once in PBS, were centrifuged at 700 *g* for 10 min at 20°C, were resuspended in wash buffer (10 m*M* Hepes-KOH [pH 7.5], 1.5 m*M* $MgCl_2$, 10 m*M* KCl, and 1 m*M* dithiothreitol), and were centrifuged at 2000 *g* for 10 min. Cells then were resuspended (10^6 cells/20 μL) in ice-cold lysis buffer (0.5% CHAPS, 10 m*M* Tris HCl, pH 7.5, 1 m*M* $MgCl_2$, 1 m*M* EGTA, 0.1 m*M* phenylmethylsulfonyl fluoride, 5 m*M* β-mercaptoethanol, and 10% glycerol) and were kept on ice for 30 min. Cell lysates then were centrifuged at 16,000 *g* for 1 h at 4°C, and the supernatants were collected and stored at −80°C. The protein concentration in cell lysates was determined by the detergent-compatible protein assay kit (Bio-Rad); the same amount of extracted protein (5 μg, corresponding to ~10^5 cells) was analyzed for each sample.

Telomerase activity was determined by a TRAP-based quantitative assay, in which an internal telomerase assay standard (ITAS) was introduced, as reported elsewhere [26]. In brief, each cell extract was tested in a 50-μL reaction mixture containing 10 m*M* Tris HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 100 μM of a mix of deoxynucleotide triphosphate, 0.1 μg of forward TS primer (5′-AATCCG-TCGAGCAGAGTT-3′), 0.1 μg of reverse CX primer (5′-CCCTTA-CCCTTACCCTTACCCTTA-3′), 2.5 U of Taq Gold polymerase (Perkin Elmer), 2 μCi ^{32}P dCTP (3000 Ci/mmol), and 1 μg of T4 gene 32 protein. The reaction mixture was incubated for 20 min at 23°C for telomerase-mediated extension of TS primer and for 12 min at 94°C to activate taq gold polymerase. The telomerase reaction products were amplified by polymerase chain reaction (PCR) in a thermal cycler (model 2400; Perkin Elmer), and 34 amplification cycles were run at 45 s at 94°C, 30 s at 50°C, and 1.5 min at 72°C.

The ITAS was prepared according to the method of Wright et al. [27]. In brief, myogenin cDNA was amplified with RM-TS (5′-AATCCGTCGAGCAGAGTTGTGAATGAGGCCTTC-3′) and RM-CX (5′-CCTTACCCTTACCCTTACCCTTATAGGCGCTC-AATGTA-3′) oligonucleotides that contained an additional 15-bp sequence at their 3′ end, which overlapped with sequences encoding amino acids 97–132 of rat myogenin. The 150-bp product generated could be reamplified by using the same TS and CX primers used to amplify the telomerase ladder in the standard TRAP assay, without interfering with the visualization of the telomerase ladder. We included 5×10^3 attograms of ITAS in the PCR mix, which was coamplified with the telomerase reaction products in the standard TRAP assay. In total, 20 μL of the PCR reaction mixture was electrophoresed in 0.5× Trisborate EDTA on 10% polyacrylamide nondenaturing gel, followed by quantitative analysis on a InstantImager (Packard). For each sample, the cell extract pretreated with RNase (0.5 μg RNase-DNase–free/10 μL cell extract) and heat inactivation was used to prepare the negative control. The amount of telomerase activity in each sample was expressed as the ratio between the counts per minute (cpm) relative to the telomerase ladder (cpm TL) and the standard control (cpm ITAS). The cpm TL:cpm ITAS ratios provided a more accurate quantification of telomerase levels and a wider range of linear relationships than those obtained by comparing sample values against an external standard control [26].

Statistical analysis. We expressed telomerase values as mean ± SD. We compared data by using the nonparametric Wilcoxon signed-rank test for paired data and by using the Mann-Whitney *U* test for

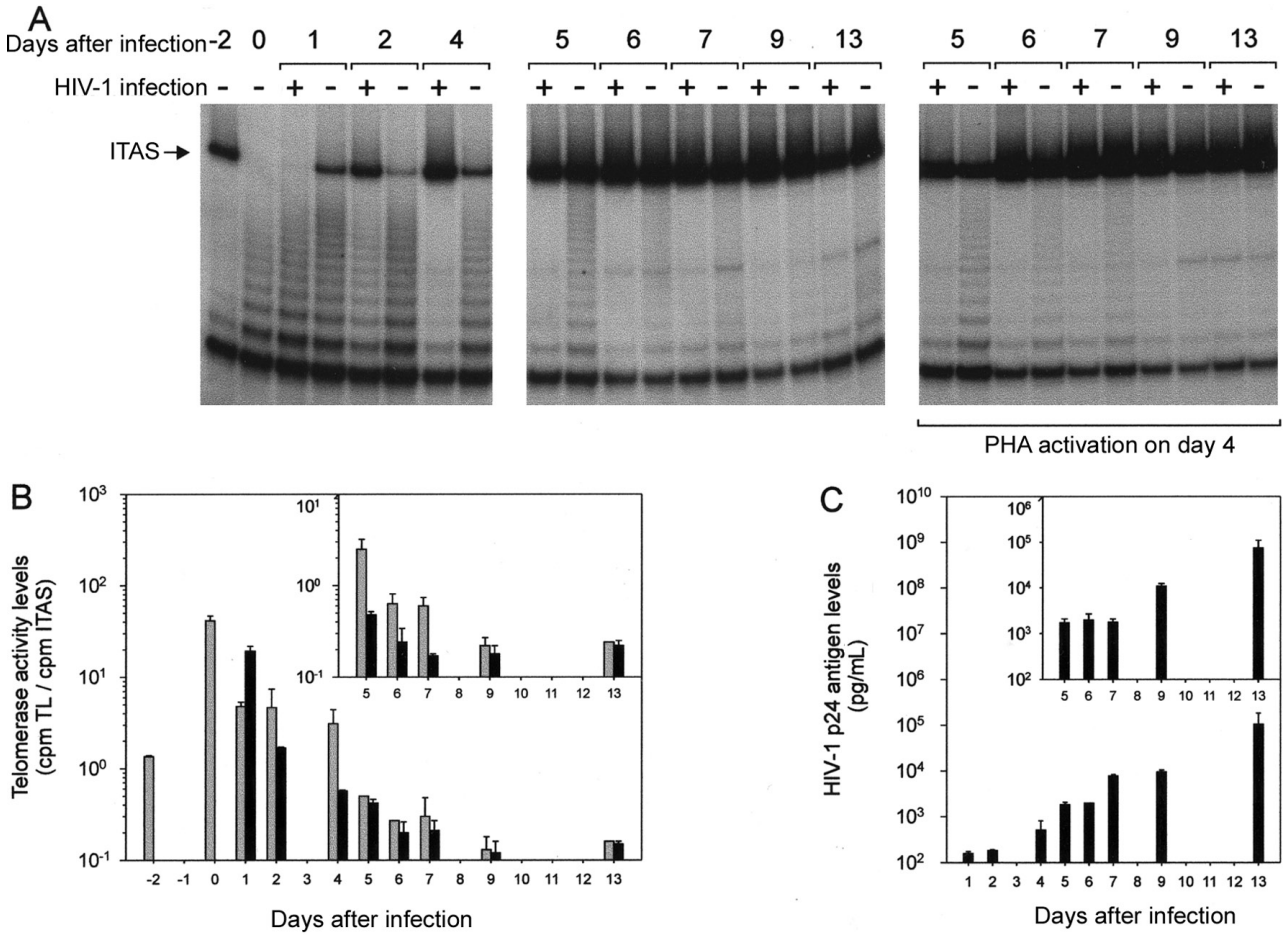

Figure 1. Quantitative analysis of telomerase activity in human immunodeficiency virus type 1 (HIV-1)–infected and HIV-1–uninfected phytohemagglutinin (PHA)–stimulated peripheral blood lymphocytes of healthy donors. *A,* Autoradiograph of 1 representative experiment. Cell extracts, corresponding to 10^5 HIV-1–infected and HIV-1–uninfected cells harvested at days indicated, were assayed in the presence of 5×10^3 attograms of internal telomerase assay standard (ITAS) in a telomeric repeat amplification protocol assay. Telomerase activity was calculated as the ratio between counts per minute (cpm) of telomerase ladder (TL) and cpm of ITAS. *B,* Telomerase activity of HIV-1–infected *(black bars)* and HIV-1–uninfected *(gray bars)* cells. *C,* p24 Antigen level in supernatant of HIV-1–infected cells. *B* and *C,* Data are mean ± SD from 3 independent experiments. *Inserts,* Telomerase activity and p24 levels in cell cultures restimulated with PHA on postinfection day 4.

unpaired data when appropriate. Analyses were done with SAS Institute statistical software (SAS/STAT, version 6.12).

Results

Telomerase activity in in vitro HIV-1–infected PBL. To evaluate whether HIV-1 infection modulates telomerase activity in PBL, PHA-stimulated PBL from healthy donors were infected with HIV-1_{BAL} isolate and were cultured in parallel with HIV-1–unexposed PHA-stimulated PBL derived from the same donors. Figure 1*A* shows a representative experiment; the results of 3 independent experiments are reported in figure 1*B*. Unstimulated PBL expressed very low levels of telomerase activity (day −2, 1.35 ± 0.04) which, in agreement with previous observations [19, 20], increased significantly after 48 h of PHA stimulation (day 0, 41.2 ± 5.42). This increase was followed by a decline over time in both HIV-1–infected and HIV-1–uninfected cells, but with different kinetics. On postinfection day 1, when cell culture supernatants were still negative for viral protein expression and virion release (figure 1*C*), telomerase levels were higher in HIV-1–exposed (19.3 ± 2.35) than in HIV-1–unexposed cell cultures (4.77 ± 0.55); thereafter, along with the establishment of a productive infection, as indicated by increasing values of HIV-1 p24 protein in culture supernatants (figure 1*C*), telomerase levels decreased more rapidly in HIV-1–infected than in HIV-1–uninfected cells. On day 2, telomerase levels decreased to 1.68 ± 0.04 in HIV-1–infected cells but remained fairly stable in HIV-1–uninfected cells (4.64 ± 2.74); on day 4, telomerase levels were sig-

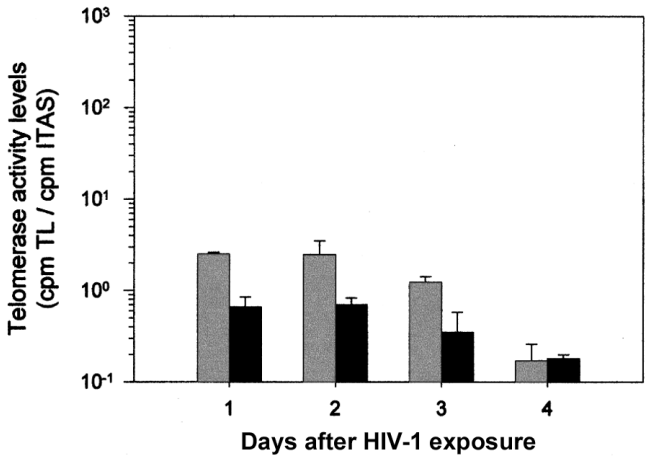

Figure 2. Quantitative analysis of telomerase activity in human immunodeficiency virus type 1 (HIV-1)–exposed and HIV-1–unexposed unstimulated peripheral blood lymphocytes from healthy donors. Telomerase activity of HIV-1–exposed *(black bars)* and HIV-1–unexposed *(gray bars)* cells are mean ± SD of values from 2 independent experiments. ITAS, internal telomerase assay standard; TL, telomerase ladder.

nificantly lower in HIV-1–infected (0.57 ± 0.01) than in HIV-1–uninfected cells (3.1 ± 1.3; $P = .04$, Mann-Whitney U test).

To further investigate whether HIV-1 infection impaired mitogen-induced activation of telomerase activity, HIV-1–infected and HIV-1–uninfected cells were restimulated on post-infection day 4 with PHA. Although telomerase activity was up-regulated in uninfected cells (2.5 ± 0.71 vs. 0.50 ± 0.01 without PHA stimulation), no substantial change was observed in HIV-1–infected cells (0.48 ± 0.04 vs. 0.42 ± 0.04 without PHA stimulation; figure 1*A* and figure 1*B, insert*).

Taken together, these data indicate that HIV-1 infection down-regulates telomerase activity and impairs enzyme activation after mitogenic stimuli, although the higher levels of telomerase activity in HIV-1–infected versus HIV-1–uninfected cells 1 day after HIV exposure might suggest that exposure to HIV-1 led to a transient activation of telomerase. To investigate whether cell antigenic stimulation by HIV-1 itself activated telomerase enzyme, unstimulated PBL from 2 different donors were exposed to HIV-1_{BAL}. In agreement with previous observations [28], the unstimulated cells were refractory to infection by HIV-1, and no p24 antigen could be detected in the supernatants of cells kept in culture for 14 days. Telomerase activity in HIV-1–unexposed cells remained stably low for the entire period; exposure to HIV-1 did not increase telomerase levels, which remained persistently lower than in control cells (figure 2).

Telomerase activity in PBL of HIV-1–infected patients. To investigate whether telomerase activity in PBL of HIV-1–infected patients could be modified in relationship to virological and immunological responses, 23 AIDS patients were studied before and during HAART. Mean telomerase activity at baseline was lower than that in 10 HIV-1–uninfected age-matched healthy donors (0.62 ± 0.2 vs. 1.25 ± 0.4; $P = .002$, Mann-Whitney U test).

Ten of 23 patients showed both virological and immunological responses, as defined in Materials and Methods (figure 3*A*). Before treatment, their median plasma HIV-1 burden was 130,000 RNA copies/mL (range, 16,000–1,210,000 RNA copies/mL), and they had a median $CD4^+$ cell count of 88 cells/μL (range, 5–400 cells/μL); 9 of 10 patients had a $CD4^+$ cell count <200 cells/μL. After HAART initiation, HIV-1 RNA levels decreased to undetectable levels over 1–7 months, accompanied by increases in $CD4^+$ cell counts. At the time of virological responses, patients had a median $CD4^+$ cell count of 189 cells/μL (range, 95–650 cells/μL); these cells further increased and reached a plateau in a median of 3 months (range, 1–15) after the virological response. At the time of immunological response, the median $CD4^+$ cell count was significantly higher (275 cells/μL; range, 190–650) than at the virological response and baseline ($P = .015$ and $P = .010$, respectively, Wilcoxon signed-rank test). Telomerase activity was evaluated at HAART entry and at times of virological and immunological responses. At virological response, telomerase activity levels were higher than at baseline (1.53 ± 0.68 vs. 0.46 ± 0.10; $P = .09$, Wilcoxon signed-rank test); they further increased and, at the time of immunological response, were significantly higher (2.11 ± 0.64) than at virological response or HAART entry ($P = .046$ and $P = .003$, respectively, Wilcoxon signed-rank test).

Five patients did not have a significant $CD4^+$ cell count increase during follow-up, although HIV-1 RNA levels decreased to undetectable levels (figure 3*B*). At HAART entry, their median HIV-1 RNA level was 12,800 copies/mL (range, 1130–167,000 copies/mL); within a median of 3 months (range, 1–5 months), it decreased to undetectable levels. Median $CD4^+$ T cell count at baseline was 80 cells/μL (range, 12–501 cells/μL) and did not increase at the time of virological response (median, 80 cells/μL; range, 40–208 cells/μL; $P = .87$, Wilcoxon signed-rank test) or during follow-up (≥5 additional months), although the plasma HIV-1 RNA level remained undetectable. Telomerase activity levels at the time of virological response tended to be significantly higher than at HAART entry (1.16 ± 0.19 vs. 0.65 ± 0.17; $P = .06$, Wilcoxon signed-rank test).

Despite the lack of a virological response, in 8 of 23 patients, $CD4^+$ cell counts significantly increased during HAART (figure 3*C*). At the time of the immunological response (achieved in a median of 12 months; range, 5–23), the median $CD4^+$ cell count was significantly higher than at baseline (248 [range, 160–540] vs. 63 cells/μL [range, 14–238]; $P = .007$, Wilcoxon signed-rank test). Telomerase activity increased in all but 1 immunological responder and was significantly higher at the time of the immunological response than at HAART entry (2.14 ± 0.61 vs. 0.72 ± 0.17; $P = .02$, Wilcoxon signed-rank test).

Our results demonstrate that telomerase activity levels in PBL of HIV-1–infected patients significantly increased during

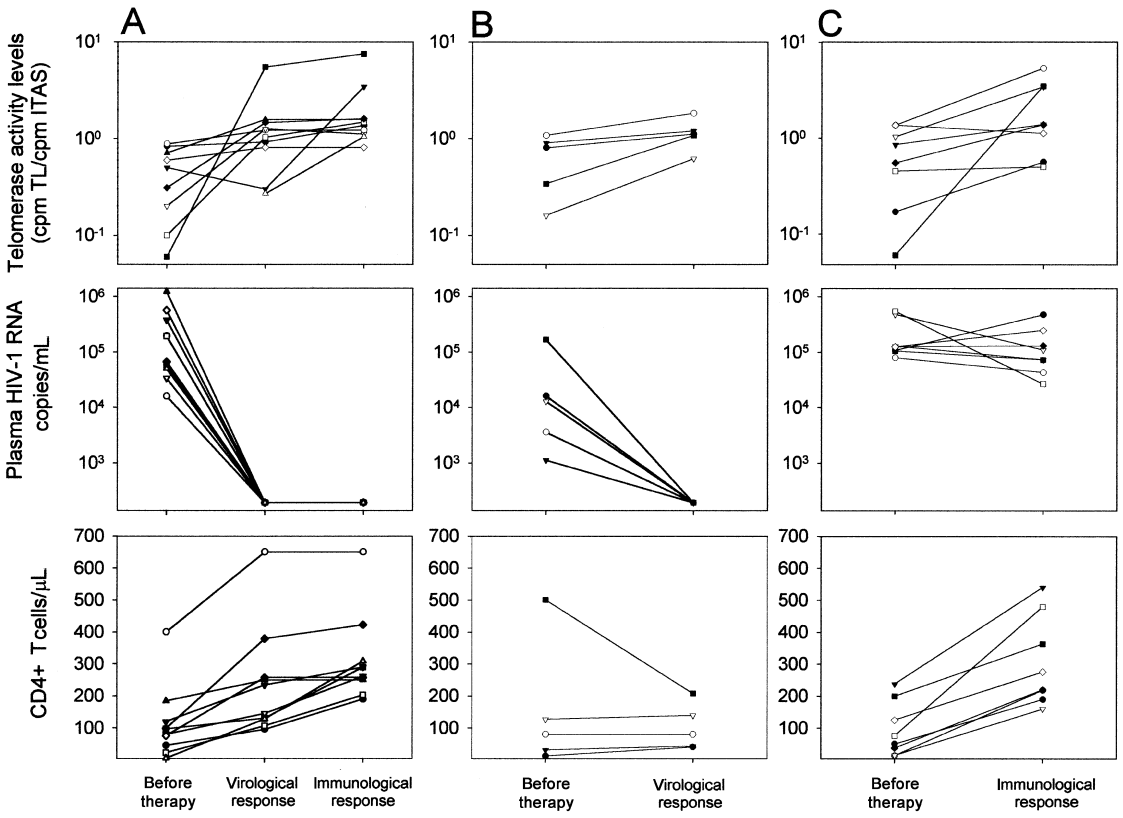

Figure 3. Telomerase activity levels and virological and immunological data. Patients were subgrouped (see Patients and Methods) as virological and immunological *(A),* virological *(B),* and immunological *(C)* responders. Telomerase activity levels were evaluated at entry into highly active antiretroviral therapy and at times of virological and immunological responses. Each symbol represents a different patient. HIV-1, human immunodeficiency virus type 1; ITAS, internal telomerase assay standard; TL, telomerase ladder.

HAART. This increase was also observed in patients who showed only a virological or immunological response, which suggests that both the HIV-1 RNA level decrease and the $CD4^+$ cell count increase may have contributed to the increase in telomerase activity levels. If so, then the HIV-1 RNA rebound should suffice to determine a decrease in telomerase activity. During follow-up, in 5 patients, HIV-1 RNA levels rebounded to pretherapy levels (median, 164,000 copies/μL; range, 38,400–574,000 copies/μL; figure 4). When HIV-1 rebounded, telomerase activity levels decreased significantly (0.46 ± 0.31) compared with values at the time of immunological (1.41 ± 0.15) or virological (1.01 ± 0.08) responses ($P = .04$ and $P = .05$, respectively, Wilcoxon signed-rank test; figure 4).

Telomerase activity in $CD4^+$ and $CD4^-$ cell fractions. The finding that telomerase activity increased as HIV-1 RNA levels declined or when $CD4^+$ cell counts increased in quantity strongly suggested that this phenomenon occurred in the $CD4^+$ cell fraction of PBL. To address this point, we analyzed telomerase levels in $CD4^+$ and $CD4^-$ cell fractions of PBL obtained at baseline and at times of virological and/or immunological responses in 8 patients who had sufficient PBL samples for study. In agreement with a previous report [22], telomerase activity levels were significantly higher in $CD4^-$ than in $CD4^+$ cells at baseline (1.60 ± 1.04 vs. 0.68 ± 0.54; $P = .01$, Mann-Whitney U test; figure 5). A parallel analysis of 5 healthy donors showed that telomerase levels were higher in $CD4^+$ (2.51 ± 0.85) than in $CD4^-$ cells (0.88 ± 0.27; data not shown), but the difference was not significant ($P = .1$, Mann-Whitney U test). Compared with those of healthy donors, telomerase values of HIV-1–infected patients were higher in $CD4^-$ cells (1.60 ± 1.04 vs. 0.88 ± 0.27; $P = .28$, Mann-Whitney U test) and were significantly lower in $CD4^+$ cells (0.68 ± 0.54 vs. 2.51 ± 0.85; $P = .01$, Mann-Whitney U test). During HAART, the decline in HIV-1 RNA level in virological responders was accompanied by a decrease in telomerase activity in $CD4^-$ cells (figure 5*A* and 5*B*); however, telomerase activity in this fraction remained at relatively high levels in 2 patients who showed only immunological responses (figure 5*C*), a finding that might be consistent with the proposal that HIV-1–driven antigenic stimulation can up-regulate telomerase activity in $CD8^+$ cells [4]. Telomerase activity increased in the $CD4^+$ cell fraction in virological and immunological responders (figure 5*A*) and in patients showing

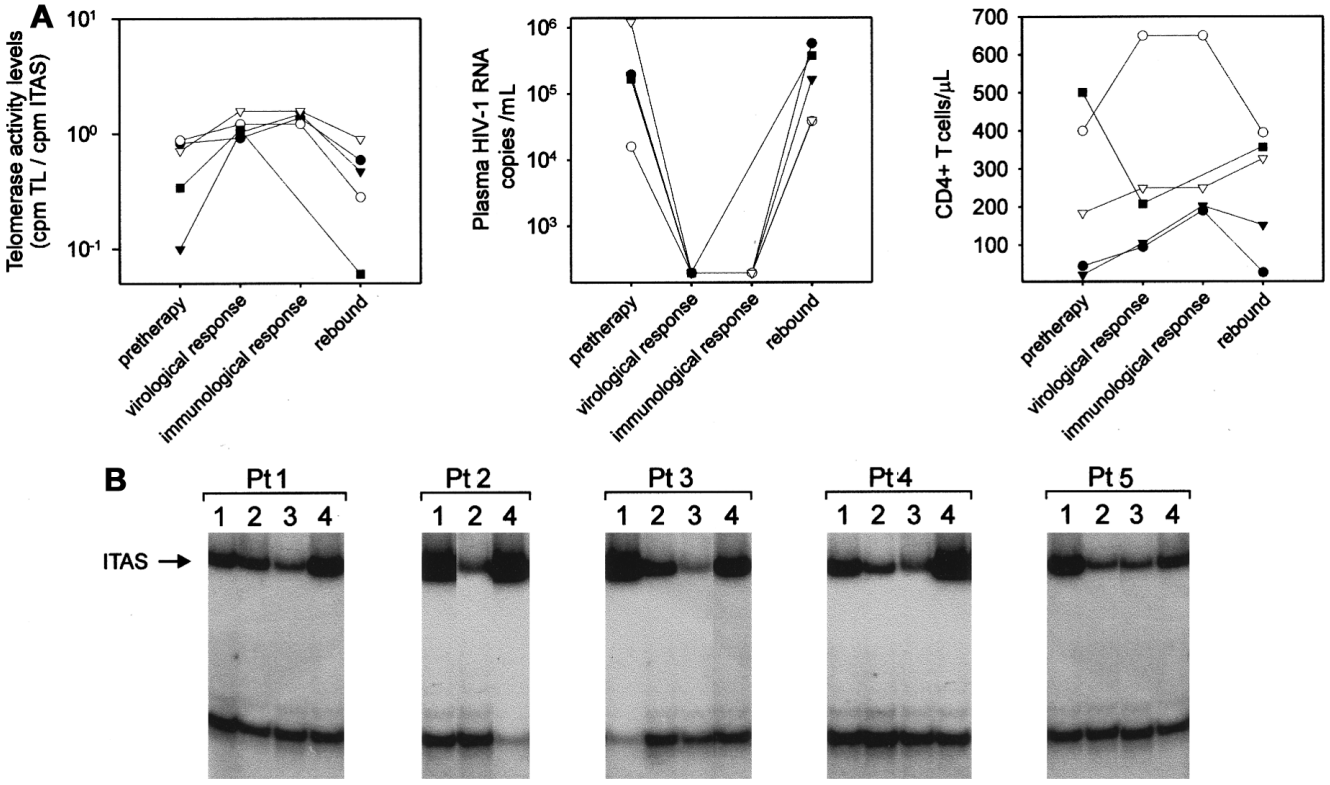

Figure 4. *A,* Telomerase activity levels and virological and immunological data of patients who had a rebound of plasma human immunodeficiency virus type 1 (HIV-1) RNA levels during follow-up. *B,* Autoradiographs of telomeric repeat amplification protocol assays; patients (Pt) 1, 3, 4, and 5 were virological and immunological responders; Pt 2 was a virological responder. Telomerase activity levels, plasma HIV-1 burden, and $CD4^+$ T cell counts were evaluated at entry into highly active antiretroviral therapy (*lane* 1), at times of virological (*lane* 2) and immunological (*lane* 3) responses, and at HIV-1 RNA rebound (*lane* 4). ITAS, internal telomerase assay standard; TL, telomerase ladder.

only a virological (figure 5*B*) or immunological response (figure 5*C*), thus supporting the hypothesis that the increase in telomerase activity observed in total PBL was mainly ascribable to the $CD4^+$ cells.

Discussion

$CD4^+$ lymphocytes from HIV-1–infected patients have a shorter half-life than $CD4^+$ lymphocytes from HIV-1–uninfected healthy donors [7, 29] and undergo apoptosis more rapidly [30]. Because telomerase activity compensates for telomere shortening at each cell division, thus influencing cell life span and replicative potential, it was advanced that an impairment in this enzyme might play a role in the $CD4^+$ T cell decline during HIV-1 infection. Although this occurs in HPCs [21], data regarding telomerase activity in PBL are discordant, mostly because the studies were cross-sectional and because the characteristics of the patients differed. Shortened telomere length and impaired telomerase activity were reported in patients with advanced disease [23, 31], whereas normal telomerase activity levels were found in patients in an early stage of disease [4, 31]. Furthermore, because telomerase expression may differ according to cell activation and stage of differentiation, the effects of HIV-1 infection in itself might be misinterpreted. We investigated the impact of HIV-1 infection on telomerase activity by evaluating enzyme levels in in vitro–infected PBL and in sequential PBL samples collected from patients whose $CD4^+$ cell counts and HIV-1 RNA levels were modulated by antiretroviral therapy.

The in vitro studies disclosed that HIV-1 down-regulated both the spontaneous and PHA-induced telomerase activity levels and impaired telomerase activation upon cell stimulation. The finding that PHA-stimulated PBL showed transiently higher values of telomerase activity after HIV-1 exposure was intriguing. The possibility that antigenic stimulation by HIV-1 without productive infection might have transiently activated telomerase was ruled out by the finding that exposure of unstimulated PBL to HIV-1 resulted in prompt down-modulation of telomerase. Since unstimulated PBL are refractory to HIV-1 replication [28], this may also suggest that a productive infection is not required to down-regulate enzyme activity. Of note, HPCs from HIV-1–infected patients had impaired telomerase levels, despite the lack of HIV-1 infection in these cells, and exposure to recombinant gp120 sufficed to lower telomerase activity in HPCs of healthy donors [21].

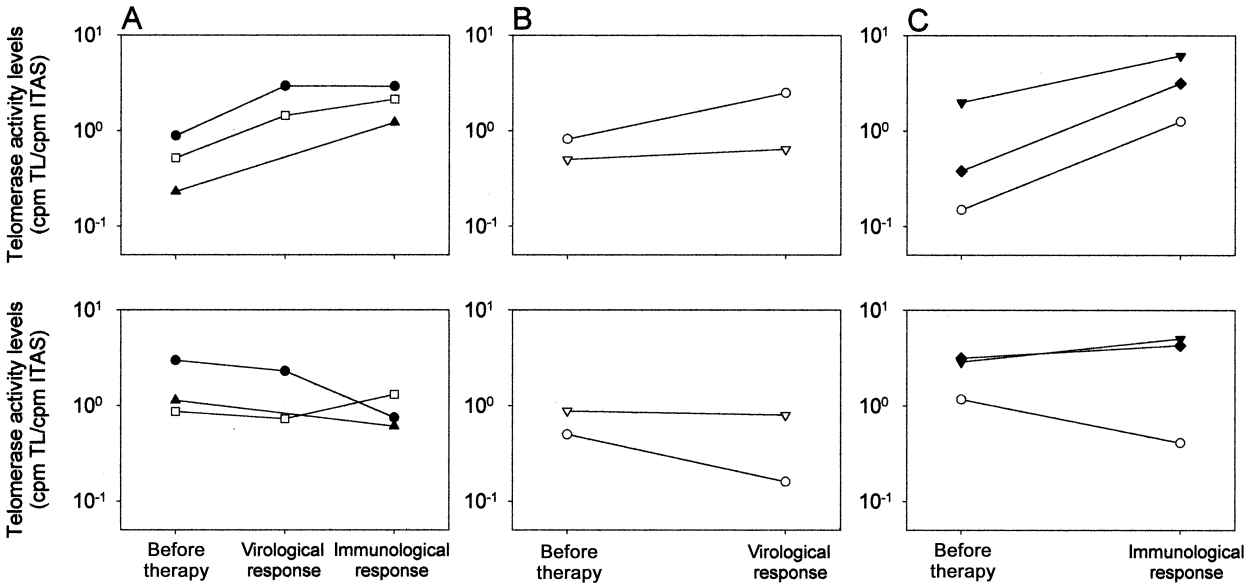

Figure 5. Telomerase activity levels in $CD4^+$ *(upper panel)* and $CD4^-$ *(lower panel)* cells by response group: 3 virological and immunological *(A)*, 2 virological *(B)*, and 3 immunological *(C)*. Each symbol represents a different patient. Analysis was done on fractionated samples collected at the beginning of highly active antiretroviral therapy and at time of virological and immunological responses. ITAS, internal telomerase assay standard; TL, telomerase ladder.

Longitudinal studies in patients provide evidence that even in vivo HIV-1 infection down-modulates telomerase activity, thus supporting the in vitro observations. At HAART entry, telomerase activity was lower in PBL of HIV-1–infected patients than in PBL of healthy donors and, in contrast to healthy donors, were lower in $CD4^+$ than in $CD4^-$ cells. The decrease in HIV-1 to undetectable levels during antiretroviral treatment was consistently associated with increased telomerase activity in PBL. This increase occurred mainly in $CD4^+$ lymphocytes. In persons who had both virological and immunological responses, the increase in telomerase levels was probably due to the composite effect of HIV-1 decrease and immune restoration. That HIV-1 in itself modulates telomerase was supported by the findings of an increase in patients who only showed a virological response during HAART and that the HIV-1 rebound to pretherapy levels was accompanied by decreased telomerase activity. Moreover, the finding of increasing levels of telomerase activity and increasing $CD4^+$ cell counts in virological and immunological responders and increased telomerase activity in patients with immunological restoration, despite virological failure, suggests that immune restoration also contributes to an increase in telomerase.

Spontaneous telomerase activity decreases with cell differentiation and is higher in thymocytes than in peripheral blood cells [19, 20]. It appears to be a general rule that both memory cell redistribution and output of newly produced naive cells contribute to peripheral immune reconstitution. Thus, it seems likely that the increased telomerase activity in our study was mainly due to the output of newly generated cells in the peripheral blood compartment. Preliminary findings indicate a higher and persistent output of thymic cells (evaluated by T cell receptor rearrangement excision circles analysis [32]) in viremic immunological responders than in virological and immunologic responders (unpublished data). This output of newly generated cells from the thymus might overcome the negative effect on telomerase exerted by HIV-1 persistence.

Telomerase is a cellular reverse transcriptase enzyme that uses its own RNA template. Its activity in ciliates [33] and in human cell lines [34] is repressed by reverse transcriptase inhibitors. Down-regulation of telomerase activity by a concurrent antiretroviral therapy with reverse transcriptase inhibitors was suggested in one study [22] but not supported in another [35]. Our finding that telomerase activity increased during HAART strongly indicates that the positive effect on telomerase stemming from either the RNA decrease or the $CD4^+$ increase overcame the possible negative effect of the transcriptase inhibitors included in the HAART; that the rebound of HIV-1 RNA levels was accompanied by a down-regulation of telomerase, with no modifications in the antiretroviral treatment, supports this concept.

The finding that HIV-1 down-regulates telomerase activity in $CD4^+$ lymphocytes suggests an additional mechanism by which HIV-1 could increase the apoptotic propensity of hematologic cells and lead to immune system dysfunction. In this regard, our finding of increased telomerase activity during HAART might fit with the recent observation of lower levels of $CD4^+$ T cell apoptosis in HIV-1–infected patients undergoing HAART [36]. Understanding the mechanisms responsible for

HIV-1–mediated inhibition of telomerase could provide new insight into immune system impairment during HIV-1 infection.

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