fractions were applied to linked Beckman SW2000-SW3000 molecular exclusion columns, equilibrated, and eluted with 0.5× phosphate-buffered saline/15% acetonitrile. (iv) Fractions eluting at apparent masses of 20 to 30 kD were loaded onto fast-flow reverse-phase Poros 20 R1 equilibrated at 4 ml/min in 15% acetonitrile/0.1% trifluoroacetic acid (TFA). After a 2-min wash, the bound proteins were eluted with a linear gradient from 15 to 60% acetonitrile in 2 to 24 min. (v) Fractions eluting from Poros R1 at 32 to 36% acetonitrile were refractionated on wide-pore Vydac C4 (250 mm by 4.6 mm, 5 μm particle size) at 1 ml/min, in a gradient of acetonitrile in 0.1% TFA (Fig. 1B). Amino acid sequence analysis was performed on blotted Coomassie stained bands, or reverse phase purified peptides from Endo-LysC digest, by automated Edman degradation on an Applied Biosystems 473A system. Methods for MALDI-MS were as described (*17*).

9. Degenerate oligonucleotide primers encoding all possible codons for TSDPKGWF (*18*) (sense, NH_2-terminal) and PYTVPNPY (*18*) (antisense, internal peptide sequence) were used for PCR on *Lymnaea* CNS cDNA. A 230–base pair (bp) product was cloned, sequenced, and used to screen a CNS cDNA library, resulting in isolation of a 472-bp cDNA clone. The nucleotide sequence of CRNF has been deposited in Genbank (accession number U72990). Mouse polyclonal antisera were raised against RSNLKYPKQILM (*18*) (residues 109 through 120 of the amino acid sequence). An expression construct was made by PCR amplification of the CRNF coding region, which was subcloned into pBAC (Clontech). Recombinant baculovirus were generated from the pBAC construct, using Clontech reagents according to manufacturer's instructions. Recombinant protein was produced in baculovirus-infected insect cells (*19*) and purified according to (*8*).
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18. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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20. Immobilization of NGF or CRNF to BIAcore CM5 sensor chips was done by amine coupling in 20 mM acetate buffer (pH 5.6 or 3.6). Binding of Sp75 to immobilized ligand was monitored in a BIAcore 2000 Biosensor (Pharmacia) at 20°C, with a flow of 5 ml/min, in Hepes-buffered saline. Kinetic analyses were done with BIAevaluation software, version 2.0 (Pharmacia).
21. Riboprobes for RPA and in situ hybridization were generated from linearized 380-bp subclones of CRNF cDNA in pCDNA3 (Stratagene). RPA was performed with equal amounts of total RNA, using RPAII reagents (Ambion). Equal loading was verified on ethidium bromide–stained gels and by parallel RPA with riboprobes for ubiquitously expressed *Lymnaea* mRNAs [fructose 1,6-biphosphate aldolase (Genbank accession number U73114) for the experiment of Fig. 3A and a CNS tyrosine kinase (A. G. M. Bulloch, unpublished data) for Fig. 3B].
22. Dedicated to the memory of Professor Håkan Persson (1952–1993), who was one of the most enthusiastic initiators of this project. We thank G. Hauser, R. van Elk, A.-S. Nilsson, E. van Kesteren, C. Popelier, and A. Ahlsen for technical support; L. Johanson and T. Laan for secretarial help; A. Vlamis and A. Holmgren for their generous assistance with the BIAcore; K. Dreisewerd and F. Hillenkamp for sharing expertise in MALDI-MS; and all members of the Molecular Neurobiology group at Vrije Universiteit Amsterdam for cheerful assistance in snail milking. Sp75 baculovirus and antisera were the generous gift of G. Weskamp and L. Reichardt. Supported by grants from the Swedish Medical Research Council (MRC), the European Neuroscience Program, the Canadian MRC, the Canadian Neuroscience Network, the Canadian National Science and Engineering Research Council, and a special equipment grant from the Netherlands Organization for Research. M.F. was supported by a long-term fellowship from the European Molecular Biology Organization and subsequently by the Swedish MRC. A.G.M.B., N.I.S., and W.C.W. were supported by the Alberta Heritage Foundation for Medical Research.

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T Cell Telomere Length in HIV-1 Infection: No Evidence for Increased CD4$^+$ T Cell Turnover

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Progression to acquired immunodeficiency syndrome (AIDS) has been related to exhaustion of the regenerative capacity of the immune system resulting from high T cell turnover. Analysis of telomeric terminal restriction fragment (TRF) length, a marker for cellular replicative history, showed that CD8$^+$ T cell TRF length decreased but CD4$^+$ T cell TRF length was stable during the course of human immunodeficiency virus type–1 (HIV-1) infection, which was not explained by differential telomerase activity. This observation provides evidence that turnover in the course of HIV-1 infection can be increased considerably in CD8$^+$ T cells, but not in CD4$^+$ T cells. These results are compatible with CD4$^+$ T cell decline in HIV-1 infection caused by interference with cell renewal.

In the course of HIV-1 infection, CD4$^+$ T cells are progressively lost, CD8$^+$ T cell numbers gradually increase, and immune function is progressively disturbed (*1*). Chronic immune activation is reflected by an activated phenotype of CD8$^+$ T cells in blood and lymph nodes (*2*), high concentrations of circulating HIV-specific cytotoxic T lymphocyte (CTL) effectors that are highly activated (*3*), and activation-induced programmed cell death that affects both CD8$^+$ and CD4$^+$ T cells (*4*). CD4$^+$ T cell numbers decline at an accelerated rate about 1.5 to 2 years before the onset of AIDS (*5*). It has been proposed that HIV-induced rapid CD4$^+$ T cell turnover eventually leads to exhaustion of the regenerative capacity of the immune system (*6*, *7*).

To study T cell turnover, we analyzed telomeric TRF length. Telomeres are the extreme ends of chromosomes that consist of TTAGGG repeats, ~10 kb long in humans (*8*). After each round of cell division telomeric sequence is lost (*9–12*) because of the inability of DNA polymerases to fully replicate the 5′ end of the chromosome (*13*). Cross-sectional studies have revealed a loss of 30 to 50 base pairs (bp) per year for human leucocytes in vivo (*9*, *10*, *14*), and telomere length has been used as a marker for replicative history and the proliferative potential of cells (*9–11*, *15*, *16*). To overcome the considerable variation in lymphocyte telomere length between donors of the same age (*17*), we analyzed TRF length on sequential peripheral blood mononuclear cell (PBMC) samples. For these analyses, the subtelomeric probe pTH2Δ (*18*) was chosen because it does not result in disproportionally high signals for longer telomeric

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repeats. Hybridization with the pTH2Δ or $(TTAGGG)_4$ probe gave similar results within one individual.

Over 5 to 9 years, the change in TRF length ranged from −0.6 kb to +0.5 kb in seven HIV-seronegative men (Fig. 1A), which indicates a maximum range of normal change in TRF length from −100 to +100 bp/year (mean ± SD 4.7 ± 71 bp/year) (*19*). TRF length was analyzed longitudinally in PBMC samples from eight age-matched asymptomatic HIV-infected homosexual men with $CD4^+$ T cell counts >300 cells per cubic millimeter of blood, and six age-matched HIV-infected homosexual men who progressed to AIDS within 5 years after seroconversion. In five asymptomatic HIV-infected individuals and two progressors, loss of TRF length in PBMCs was in the normal range (<100 bp/year). Accelerated loss of TRF length in PBMCs was found in three asymptomatics (200 to 264 bp/year) and four progressors (114 to 309 bp/year, Table 1 and Fig. 1). For the individuals with HIV infection, the mean loss of TRF length was significantly increased compared with the change in TRF length in healthy donors, with a mean ± SD loss of TRF length of 114 ± 100 bp/year in the group of eight asymptomatics and 175 ± 105 bp/year in the group of six progressors compared with 4.7 ± 71 bp/year in healthy controls ($P = 0.04$ and $P = 0.008$ respectively, Mann-Whitney U test).

TRF length in PBMCs is a composite of TRF length in different leucocyte populations. To compare TRF length between $CD4^+$ and $CD8^+$ T cells, we performed a cross-sectional study. In nine healthy controls, no significant difference was found in TRF length between $CD4^+$ and $CD8^+$ T cells [mean ± SD of 9.8 ± 1.3 kb and 9.9 ± 1.9 kb, respectively (Fig. 2)]. However, in significantly more persons in the group of asymptomatic HIV-infected men, TRF length in $CD8^+$ T cells was shorter than in $CD4^+$ T cells (Fig. 2) ($P = 0.03$, Wilcoxon matched-pairs signed-ranks test; mean ± SD $CD4^+$ TRF length was 10 ± 1.4 kb compared with a mean $CD8^+$ TRF length of 8.8 ± 1.3 kb).

Furthermore, longitudinal analysis of T cells from 11 of the 14 HIV-infected individuals included in Fig. 1 showed accelerated loss of TRF length almost exclusively in $CD8^+$ T cells, whereas the TRF length of $CD4^+$ T cells did not decline (Fig. 3). The mean loss of TRF length in $CD8^+$ T cells was 211 ± 187 bp/year compared with only 28 ± 114 bp/year in $CD4^+$ T cells in the group of HIV-infected persons. Within individuals the loss of $CD8^+$ T cell TRF length was significantly increased compared with loss in $CD4^+$ T cell TRF length ($P = 0.007$, Wilcoxon matched-pairs signed-ranks test). TRF length loss in $CD4^+$ T cells from one HIV-infected person was >100 bp/year. Results from the analysis on sequential samples from two asymptomatic HIV-infected men (ACH-1024 and ACH-232) and two progressors (ACH-1094 and ACH-54) are shown in detail in Fig. 3C. Thus, accelerated loss of PBMC TRF length in HIV infection could be accounted for by accelerated loss of $CD8^+$ T cell TRF length.

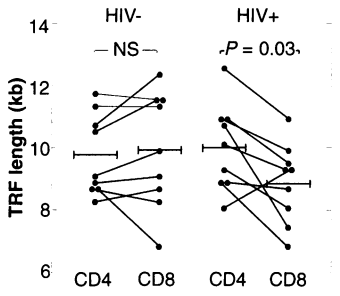
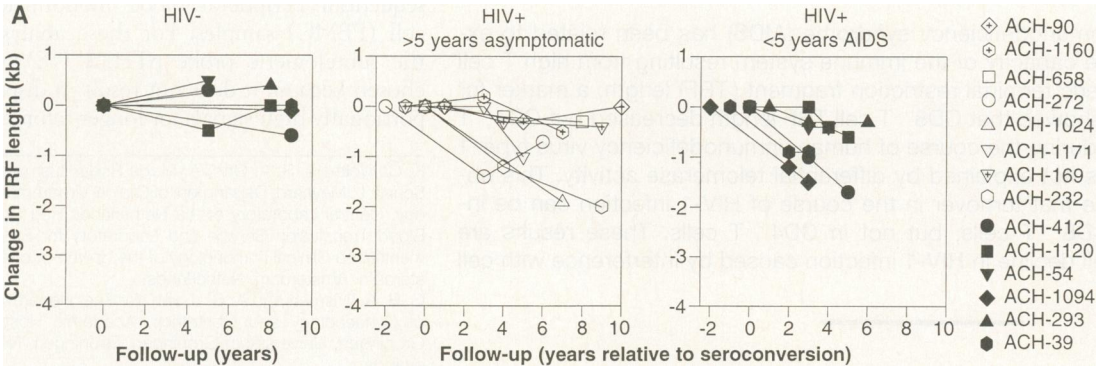


Fig. 2. Comparative analysis of $CD4^+$ and $CD8^+$ TRF length. Purified $CD4^+$ and $CD8^+$ T cells were obtained from nine healthy controls (HIV−) and nine asymptomatic HIV-infected individuals (HIV+) by magnetic separation over columns (MiniMACS, Miltenyi Biotec, Sunnyvale, California). PBMCs were incubated for 15 min with magnetic microbeads conjugated with monoclonal antibody to CD8 and passed over a MiniMacs separation column. $CD8^+$ T cells were obtained by flushing out the retained cells. The depleted fraction then was used to obtain $CD4^+$ T cells by labeling the cells with microbeads conjugated with monoclonal antibody to CD4 and repeating the protocol. With this technique, the purity of $CD4^+$ T cells was on average 80% with 10% contamination of $CD8^+$ T cells, and 90% purity of $CD8^+$ T cells with less than 1% $CD4^+$ T cells. TRF length was analyzed as described in Fig. 1. Statistical analysis was performed with the Wilcoxon matched-pairs signed-ranks test, and $P < 0.05$ was considered significant. NS, not significant. The horizontal bars indicate means.



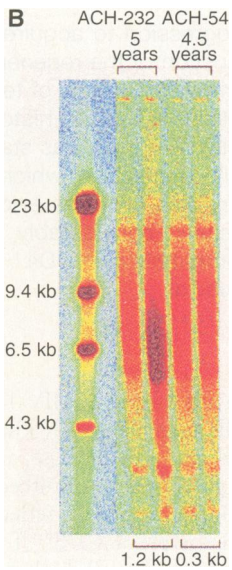


Fig. 1. Longitudinal analysis of TRF length in PBMCs. (**A**) Two or three sequentially frozen blood samples were taken from four healthy noninfected blood bank donors and three HIV-negative homosexual cohort participants as controls (HIV−) and 14 HIV-infected homosexual men (HIV+) (eight remained asymptomatic and six progressed to AIDS during follow-up). PBMCs were analyzed for TRF length. The first sample was considered the baseline TRF length (defined as 0), and sequential samples were defined as the change from baseline in kilobases. For HIV-infected individuals, follow-up is expressed in years relative to seroconversion. (**B**) A representative TRF blot analyzed by Phosphor-Imager (Fuji) of telomeric DNA from two sequential samples taken over 4.5 to 5 years from two HIV-infected individuals. Decrease in mean TRF length is indicated at the bottom. Molecular sizes are indicated in the left column. TRF length was analyzed by Southern (DNA) blot technique. Bgl II–digested total genomic DNA (8 μg) obtained from PBMCs was electrophoresed on 0.6% agarose gels (30 mA for 48 hours), blotted to Genescreen Plus (DuPont), cross-linked (Stratalinker), and hybridized to a radiolabeled subtelomeric probe pTH2Δ (*18*). Hybridization was at 65°C in 0.5 M Na_2HPO_4, 7% SDS (pH 7.2). Blots were washed with 3× standard saline citrate (SSC), 0.5% SDS gradually decreasing to 0.1× SSC, 0.5% SDS (15 min at 65°C). Blots were exposed to Phosphor-Imager screens (Fuji) for 4 hours or overnight, and analyzed by Phosphor-Imager software (TINA, from Raytest, Straubenhardt, Germany). The point of highest resolution was taken as the peak TRF length, and the mean TRF length was calculated by marking the area above the background. Usually a gaussian distribution was obtained, and the mean position was very close to the peak value. If an initial peak was followed by a broad shoulder, mean TRF length was compared.

The loss of TRF length in the total group of HIV-infected individuals could not be related to progression to AIDS or disease progression markers, including $CD4^+$ and $CD8^+$ T cell counts, HIV-1 phenotype (SI-NSI), viral RNA load, T cell reactivity (*20*), or to zidovudine (ZDV) therapy (Table 1 and Fig. 3C).

Stable TRF length is found in germline cells and immortal cells that express telomerase, a ribonucleoprotein DNA polymerase that carries its own RNA template and adds telomeric repeats to chromosomal ends to maintain telomeric length (*21–24*). It has been shown that telomerase activity is detectable at low levels in haematopoietic cells, decreases with age, and is inducible in PBMCs after in vitro stimulation (*25–27*). We tested whether stable TRF length of PBMCs and lymphocytes in HIV-1 infection correlated with increased telomerase activity. To detect telomerase activity in PBMCs and $CD4^+$ and $CD8^+$ T cells from seronegative controls and HIV-infected individuals of the same age, we used extracts equivalent to >1500 cells per assay. Telomerase activity could be detected in as little as 13 cell-equivalents per assay of a lung carcinoma cell line (GLC-4) (Fig. 4). Telomerase activity in PBMCs from patients ACH-293 and ACH-1120 was only detectable in 150,000 cell-equivalents per assay, comparable with telomerase activity in PBMCs from a healthy control. Telomerase activity in PBMCs from patient ACH-169 was still detected in the equivalent of 15,000 cells, a level 10 times that in cells from the control (Fig. 4A). Thus, levels of telomerase activity were low compared with the reference GLC-4 cell line and did not differ in PBMCs with stable TRF length (ACH-293) compared with PBMCs with decreasing TRF length (ACH-1120). In addition, stable TRF length in $CD4^+$ T cells from HIV-infected men could not be explained by elevated telomerase activity. Results from one patient, representative for seven patients, are shown in Fig. 4B. Telomerase activity in $CD4^+$ T cells was not increased compared with telomerase activity in $CD8^+$ T cells, or with telomerase activity in $CD4^+$ T cells from a healthy control. These results were confirmed by nonradioisotopic TRAP assay (*28*) where an internal amplification standard (ITAS) was included, showing that inhibition of Taq polymerase was not observed in samples of 15,000 cell-equivalents or less (*29*). This indicates that low telomerase activity measured by the TRAP assay in $CD4^+$ T cells from HIV-infected persons is not the result of Taq inhibitors in the samples.

Our analyses, in which TRF length is used as a measure of the replicative history of T cells, are consistent with chronic immune activation in HIV-1 infection. TRF length was shorter in the $CD8^+$ T cells compared with $CD4^+$ T cells of HIV-infected persons. Moreover, in a proportion of the persons that we studied longitudinally, a decrease of TRF length in the $CD8^+$ T cell population was seen over the course of HIV-1 infection. In agreement with this, Effros and co-workers have observed substantial decreased TRF length in the expanded population of activated effector $CD8^+CD28^-$ T cells, with a less pronounced TRF length decrease in the nonactivated $CD8^+CD28^+$ T cells (*30*). Increased turnover of $CD8^+$ T cells is not only due to HIV-1 specific cells, but may be caused by extensive bystander responses. Vigorous antiviral responses can induce a several thou-

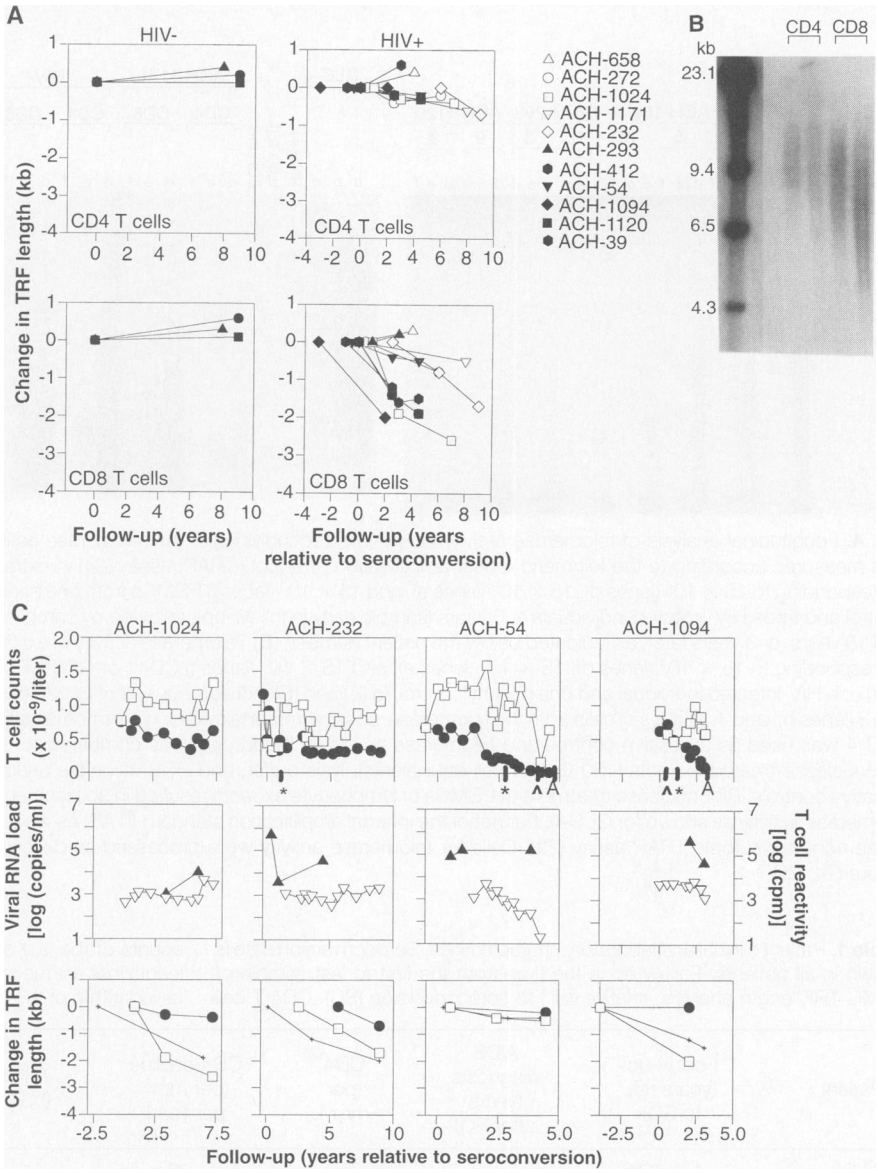


Fig. 3. Longitudinal analysis of TRF length in $CD4^+$ and $CD8^+$ T cells. (**A**) Two or three sequentially frozen blood samples were taken from a healthy noninfected blood bank donor and two HIV-negative homosexual cohort participants as controls (HIV−), and from 11 of the 14 HIV-infected individuals (HIV+) included in Fig. 1. $CD4^+$ and $CD8^+$ T cells were analyzed for TRF length as described in Figs. 1 and 2. Corresponding symbols for HIV-infected individuals depicted in Figs. 1 and 3 were used. (**B**) A representative Southern blot hybridized to the $(TTAGGG)_4$ probe. $CD4^+$ and $CD8^+$ T cell telomeric DNA from two sequential samples taken over 4 years from one HIV-infected individual progressing to AIDS. The last sample was taken right before the onset of AIDS with $CD4^+$ T cell counts of 150 per cubic millimeter of blood. (**C**) Longitudinal analysis of four HIV-infected individuals. $CD4^+$ (●) and $CD8^+$ (□) T cell counts, viral RNA load in plasma (▲) as determined by Q-NASBA (Organon), T cell reactivity to monoclonal antibody to CD3 (▽), and change in TRF length in PBMCs (+) and $CD4^+$ (●), and $CD8^+$ (□) T cells are shown. TRF length was expressed relative to TRF length in samples taken at early time points, which may however differ in analyses for the different cell subsets. In the top row, the asterisk indicates ZDV therapy; the inverted V, an SI variant; and A, AIDS diagnosis.

sand–fold expansion of virus-specific $CD8^+$ T cells, and overall $CD8^+$ T cell numbers may increase severalfold through nonspecific stimulation by cytokines (*31, 32*). The extent of this $CD8^+$ T cell response may vary between individuals and may be related to variable immune activation by HIV-1 and host genetics, regulating the magnitude of $CD8^+$ T cell activation.

Our data show that the $CD4^+$ T cell TRF lengths are stable. How can that be interpreted in the light of the now available information on $CD4^+$ T cell TRF length dynamics and the current ideas of high $CD4^+$ T cell turnover in HIV infection? In normal individuals, it is well established that T cells lose TRF length with age, despite some expression of telomerase during activation and division (*33*). Weng *et al.* (*34*) showed that with age, both naïve and memory $CD4^+$ T cells lose TRF length at the same rate. This finding shows that for the maintenance of these compartments by homeostasis, cell division in the naïve T cells or their precursors is required. This in turn suggests that, in a normal individual upon antigenic encounter, naïve cells are recruited to differentiate, and this is compensated for by proximal cell division either of the naïve cells, if the T cells are considered to be self-sufficient as has been suggested (*35*), or of a precursor feeding progeny into the naïve pool (*34, 36*).

Daily rapid turnover of large numbers of HIV-infected $CD4^+$ T cells would have a substantial effect and would cause a major flux of naïve and memory $CD4^+$ T cells. According to the data reported by Weng *et al.*, this distal consumption will be compensated for proximally and would thus result in accelerated TRF length loss in the overall $CD4^+$ T cell population. In HIV-1 infection, the half-life of productively infected cells that are mainly dividing memory cells (*37*) has now been estimated to be on the order of 1.6 days (*38*). If this loss of memory cells that have relatively short TRF length would be an order of magnitude greater than in healthy controls, which may be compensated for by expansion or influx of naïve cells with relatively longer TRF length, this would initially result in a stable or even slightly increased TRF length of the remaining peripheral T cells. Because progenitor cells also lose TRF length with age (*11*), with time the bulk of the T cells however would eventually show significant shortening. Our results show that TRF length of $CD4^+$ T cells in HIV-1 infection is stable, and that this is not because of increased telomerase activity. Although telomerase-independent mechanisms for telomere maintenance cannot be formally excluded, in primary human cells telomerase

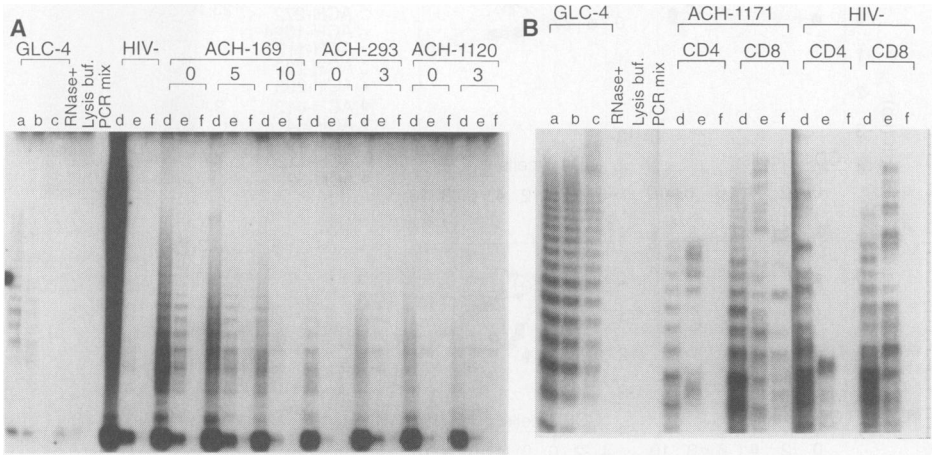


Fig. 4. Longitudinal analysis of telomerase activity in HIV-infected individuals. (**A**) Telomerase activity was measured according to the telomeric repeat amplification protocol (TRAP) assay (*24*) in extracts corresponding to 15×10^4 (lanes d), 15×10^3 (lanes e), and 15×10^2 (lanes f) PBMCs from one healthy control and three HIV-infected individuals at 0 years (sample early in follow-up), followed by sampling 5 and 10 years, or 3 years later, as indicated below the patient number. (**B**) Telomerase activity in extracts corresponding to 15×10^4 (lanes d), 15×10^3 (lanes e), and 15×10^2 (lanes f) $CD4^+$ or $CD8^+$ T cells from one HIV-infected individual and one healthy control. In (A) and (B) extracts equivalent to 130 (lanes a), 13 (lanes b), and 1.3 (lanes c) cells from the telomerase-positive immortalized lung carcinoma cell line GLC-4 was used as a positive control, and telomerase activity in blood cells was compared to this. Ribonuclease-treated extracts (130 GLC-4 cell-equivalents), lysis buffer, and PCR mix were used as negative controls. Ribonuclease treatment of PBMCs or lymphocyte extracts resulted in abolishment of telomerase activity as shown for GLC-4, but not of the internal amplification standard (ITAS) as we used in the nonradioisotopic TRAP assay (*29*). Cells for telomerase activity were processed as described before (*24, 25*).

Table 1. Patient clinical and laboratory characteristics. Seroconversion date is known in all patients. Follow-up is the time from the first to last sample of PBMC TRF length analysis, relative (rel.) to seroconversion (SC). CD4 T cell counts of the last sample analysis are shown. CD4 T cell decline and TRF length loss are the mean declines per year during follow-up. Viral load is shown as logarithm of the mean viral load of three timepoints during follow-up.

Patient	Follow-up (years rel. to SC)	AIDS diagnosis (years after SC)	CD4 (per mm^3)	CD4 decline (per mm^3 per year)	TRF loss (bp/year)	Viral load (copies of RNA/ml)	ZDV (years after SC)	SI-NSI† phenotype
ACH-90	1.7–10.6	–	750	8	0	3.0	No	NSI
ACH-1160*	−1.0– 6.9	–	580	38	64	3.0	No	NSI
ACH-658	0.1– 8.2	–	840	45	37	4.0	No	NSI
ACH-272*	−3.3 – 5.3	–	440	45	82	3.6	No	NSI
ACH-1024*	0.0– 6.5	–	420	51	264	3.7	No	NSI
ACH-1171	0.2 – 5.2	–	600	62	200	3.0	No	NSI
ACH-169	0.8 – 9.3	–	530	91	43	4.9	No	NSI
ACH-232	0.1– 8.9	–	300	69	225	5.2	1	NSI
ACH-293	0.7– 6.0	4.0	10	38	50	5.4	3	SI
ACH-412	0.5– 4.8	4.7	500	58	309	6.0	No	NSI
ACH-54	0.3– 4.3	4.0	10	128	89	5.1	2	SI
ACH-1094*	−4.0– 3.0	3.3	70	183	214	5.4	2	SI
ACH-1120	0.1– 5.3	4.7	30	279	114	5.7	1	SI
ACH-39*	−0.7– 2.6	3.0	260	436	273	5.5	No	SI

*TRF length analysis before and after seroconversion. †SI, syncytium-inducing variant; NSI, nonsyncytium-inducing variant.

activity most likely is the dominant mechanism (*39*). The telomere hypothesis on replication and aging holds up for $CD8^+$ T cells as we and Effros *et al.* (*30*) have shown. In addition, $CD4^+$ T cell clones obtained from an HIV-infected individual exhibit progressive TRF length loss of 26 to 75 bp per population doubling after culturing in vitro [mean ± SD of 43 ± 16 bp per mean population doubling (MPD), $n = 8$ clones], which is in the same range as TRF length loss of $CD4^+$ T cell clones ($n = 8$) obtained from this individual before seroconversion (26 to 89 bp/MPD, mean ± SD of 50 ± 20 bp/MPD, $P = 0.44$, Mann-Whitney U test). Altogether, these results indicate that it is unlikely that in HIV infection high T cell turnover does exist but is not reflected by loss of TRF length. Thus, our data do not support the idea of high rates of production and destruction of $CD4^+$ T cells as depicted in the "sink model" as proposed by Ho *et al.* (*7*). The supposedly short half-life of HIV-infected cells (*38*), and their presumed high level of turnover, does not seem to have an effect on turnover of the total $CD4^+$ T cell population. In conclusion, our data indicate that the bulk of the $CD4^+$ T cells in HIV-infected men is turning over at the same rate as in healthy persons.

Why then is the $CD4^+$ T cell count gradually declining? We suggest that HIV-1 infection is slowing down the flow of the tap, that is, the generation of new cells from an as yet undefined precursor source that could be in the naïve compartment as well. It would mean that even if distal cell death occurs at the same rate in HIV-1 infection, without an adequate proximal influx of $CD4^+$ T cells, the $CD4^+$ T cell count will decline. This could be brought about in two ways: (i) by interference with the generation of $CD4^+$ T cells in indirect ways through infection of stromal cells or microenvironmental damage or both; or (ii) by infection of dividing $CD4^+$ T cell precursors, which would thus abort the influx of new cells. However, there is as yet little evidence that directly supports these ideas. The stable $CD4^+$ T cell TRF length demonstrates that the increased turnover of $CD8^+$ T cells seen in a large fraction of the patients is supported by peripheral expansion and is not dependent on replenishment from a $CD4^+CD8^+$ precursor source.

The steep rise in $CD4^+$ T cell counts after treatment with potent inhibitors of HIV-1 replication has been interpreted to reflect pretreatment steady-state dynamics of $CD4^+$ T cell turnover, implying that in HIV-infected persons $\sim 2 \times 10^9$ $CD4^+$ T cells are destroyed and replenished per day (*6*, *7*). Our findings do not support the pretreatment steady-state interpretation. The initial increase in $CD4^+$ T cell counts after antiviral treatment may not be due to repopulation by newly generated cells but may be a redistribution of activated memory $CD4^+$ T cells, in agreement with kinetics of $CD4^+$ T cell repopulation after chemotherapy, which takes at least a year (*40–42*).

Our results are compatible with $CD4^+$ T cell depletion caused by interference with renewal. In this view, the major damage that the virus does to the immune system is not distal but proximal in the $CD4^+$ T cell life cycle and may not necessarily involve high rates of destruction. If this is the case, current ideas about repopulation and immune reconstitution after therapy need to be re-evaluated.

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