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Normal Telomere Lengths in Naive and Memory CD4$^+$ T Cells in HIV Type 1 Infection: A Mathematical Interpretation

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ABSTRACT

To study CD4$^+$ T cell productivity during HIV-1 infection, CD4$^+$ T cell telomere lengths were measured. Cross-sectional and longitudinal analysis of HIV-1-infected individuals with CD4$^+$ T cells counts >300 cells/mm^3 showed normal average telomeric restriction fragment (TRF) length and normal shortening rates of CD45RA$^+$ naive and CD45RO$^+$ memory CD4$^+$ T cells. These TRF data were interpreted in terms of CD4$^+$ T cell production by means of a mathematical model. This model resolves previous criticisms arguing that the normal TRF length of CD4$^+$ T cells in HIV-1 clinical latency is due to the killing of dividing CD4$^+$ T cells by the virus. Only an increased priming rate of naive CD4$^+$ T cells to become memory cells may elongate the average TRF length of memory CD4$^+$ T cells, and may therefore mask the shortening effect of increased turnover in the CD4$^+$ memory T cell compartment. The data are more compatible with the notion that during HIV-1 clinical latency the turnover of CD4$^+$ T cells is not markedly increased, however, and that HIV-related interference with renewal from progenitors plays a role in CD4$^+$ T cell depletion. In such a "limited renewal" scenario disease progression is no longer a consequence of markedly increased CD4$^+$ T cell production.

INTRODUCTION

A HALLMARK OF HIV-1 infection is the decline of CD4$^+$ T cells. The rate of CD4$^+$ T cell decline is slow and relatively constant in asymptomatic HIV-1-infected individuals, with a more rapid decline related to the emergence of syncytium-inducing (SI) variants.[1] The basis for this decline, however, is not known. It has been proposed that during HIV-1 clinical latency the production of CD4$^+$ T cells is increased by more than an order of magnitude,[2,3] and that this markedly increased production ultimately leads to proliferative exhaustion.[2] The typical recovery rates of 2×10^9 CD4$^+$ T cells/day during the first weeks of highly active antiretroviral therapy (HAART)[2] were based on measurements in peripheral blood. However, several authors suggested that this could be confounded by a redistribution of lymphocytes during HAART.[4–7] Direct measurements of CD4$^+$ T cell numbers in peripheral blood and lymphoid tissue during HAART indeed suggest that during the first weeks of treatment the percentage of CD4$^+$ T cells in the lymphoid tissue drops from 99% to the normal 98% in 3 weeks.[8] Such a 1% change is sufficient to explain the typical CD4$^+$ T cell recovery rates in the peripheral blood.[7] Moreover, the normal total body CD4$^+$ T cell production, as estimated by Ki67$^+$CD4$^+$ T cells in lymphoid tissue,[8,9] or by the peripheral enrichment of deuterated glucose-labeled CD4$^+$ T cells,[10] is about 10^9 CD4$^+$ T cells/day. The observed recovery of 2×10^9 CD4$^+$ T cells/day[2] would therefore maximally represent a twofold increase only, which seems insufficient to cause proliferative exhaustion.[10]

Evidence against markedly increased CD4$^+$ T cell production and proliferative exhaustion has come from telomere measurements.[11,12] Telomeres are TTAGGG repeats at the very end of chromosomes, which shorten with each cell division,[13,14] and can therefore be used as a tool to study CD4$^+$ T cell replication.[15] In HIV-infected individuals with CD4$^+$ T cell counts >100 cells/mm^3, normal to increased CD4$^+$ T cell telomere re-

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striction fragment (TRF) lengths in HIV-1 infection were reported.[11,12]

However, the use of telomere length as a marker of turnover in HIV infection has been debated.[10,16] Indeed, data on telomere length change must be interpreted with care. First, telomerase, a nucleoprotein enzyme that can add telomeric repeats onto chromosomal ends, is expressed at low levels in lymphocytes and can be upregulated after activation.[17–20] Normal telomerase activity and normal induction of expression have been reported in T lymphocytes from HIV-infected individuals.[11,12,21] There is therefore, as yet, no indication that increased telomerase activity is compensating for increased telomere loss in HIV^+ patients. Second, because HIV-1 preferentially infects dividing $CD4^+$ T cells it was argued that the populations surviving viral infection should on average have longer TRF lengths, which would mask the telomere shortening by the increased production.[16,22] Third, a preferential depletion of either $CD45RA^+$ naive, or of $CD45RO^+$ memory, $CD4^+$ T cells could have an impact on the measured population average TRF lengths. In $CD4^+$ T cells, the average TRF length of $CD45RA^+$ naive cells is about 1.4 kb longer than that of $CD45RO^+$ memory cells.[23] Although HIV-1 predominantly infects and replicates in the memory $CD45RO^+$ subset of $CD4^+$ T cells,[24] the naive $CD45RA^+$ subset is depleted during HIV-1 infection.[25,26]

We address these issues here by measuring TRF lengths of both $CD45RA^+$ naive and $CD45RO^+$ memory $CD4^+$ T cells in asymptomatic HIV-infected individuals with $CD4^+$ T cell counts >300 cells/mm^3. To investigate if the data on telomere length could still allow for high turnover rates in $CD4^+$ T cells, leading to exhaustion of renewal and therefore to $CD4^+$ T cell depletion, we developed mathematical models.

MATERIALS AND METHODS

Patient population

For cross-sectional analysis, fresh blood samples were obtained from 12 HIV-infected individuals who were in a clinically latent stage, with $CD4^+$ T cell counts varying from 300 to 800 cells/mm^3 (mean $\pm$ SD, 460 $\pm$ 170 cells/mm^3) and with duration of infection from 1 to 12 years (mean $\pm$ SD, 5.7 $\pm$ 3.6 years). Three patients had received treatment with nucleoside analogs. As healthy controls, fresh blood samples were obtained from laboratory workers. Longitudinal analysis of TRF length was performed on frozen blood samples from eight HIV-infected men. Patients 232, 1120, 6140, and 6049 received treatment with nucleoside analogs and patient 82 started triple therapy (Table 1). All HIV-infected men were participating in the Amsterdam cohort study on HIV infection in homosexual men.

Cell separation

Purified $CD4^+$ T cells were obtained from either freshly isolated (Ficoll-Hypaque gradient) or frozen peripheral blood mononuclear cells (PBMCs) by positive selection with magnetic microbeads (CD4 multisort kit, Mini-MACS; Miltenyi Biotec, Sunnyvale, CA) as described,[11] resulting in 97–99% purity. To separate $CD45RA^+$ and $CD45RO^+$ T cells, two subsequent positive selections were performed: first, purified $CD45RA^+$ T cells were obtained with magnetic microbeads (Mini-MACS) conjugated with CD45RA monoclonal antibodies (MAbs), and the remaining fraction was further purified with magnetic microbeads conjugated with CD45RO MAbs. Purity was checked by fluorescence-activated cell sorting (FACS) staining. Purity of $CD45RA^+$ cells was, on average, 94 and 90% and purity of $CD45RO^+$ cells was, on average, 82 and 76% for, respectively, control and HIV^+ samples.

Determination of telomeric restriction fragment length

DNA was isolated from 10^6 PBMCs by the Qiagen blood and body fluid protocol (Qiagen, Hilden, Germany). Genomic DNA (3 μg) was digested with 40 U of *Hin*fI and *Rsa*I (GIBCO-Life Technologies, Breda, The Netherlands). Southern blot analysis was performed as described previously.[11] The telomeric probe $(TTAGGG)_5$ was radiolabeled with [α-^{32}P]dCTP, using terminal transferase (Boehringer Mannheim, Almere, The Netherlands) and hybridized to the blots.[11] After washing, blots were exposed to Phosphor-Imager screens (Fuji, Kanagawa, Japan) for 4 hr or overnight. The integrated signal of the area

TABLE 1. DATA ON PATIENTS: CLINICAL AND LABORATORY CHARACTERISTICS AND TELOMERE RESTRICTION FRAGMENT LENGTH CHANGE OF NAIVE AND MEMORY $CD4^+$ T CELLS[a]

Patient	Follow-up (years after SC)	AIDS (years after SC)	$CD4^+$ cells (cells/μl)	$CD4^+$ cell decline (cells/μl per year)	TRF length change		Antiviral treatment (years after SC)	SI phenotype (years after SC)
					CD45RA (bp/year)	CD45RO (bp/year)		
H-6140*	0.5–3.4	—	530	3	0	ND	3.1	—
H-1024	1.8–6.8	—	390	4	0	80	—	—
H-6094*	3.5–5.7	—	450	32	136	−45	—	—
H-6134*	0.1–3.7	—	430	36	−83	−111	3.4	—
H-232	0.7–5.7	—	320	38	ND	0	1	—
H-82	2.0–9.3	—	340	48	−27	27	9	—
H-169	1.7–10.9	—	360	157	43	54	—	—
H-1120	0.1–3.8	4.7	870	165	−27	−189	1	3.8

[a]Follow-up is the time from first to last sample of TRF length analysis in years after seroconversion (SC) or, for patients about whom seroconversion time is unknown (*), years after entry. $CD4^+$ T cell counts of the last sample analysis are shown. $CD4^+$ T cell decline and TRF length change are the average changes per year during follow-up. Start of antiviral therapy and switch to SI phenotype are shown in years after seroconversion. SI, Syncytium-inducing variant; ND, not determined.

above the background and the positions of the molecular weight marker were analyzed by Phosphor-Imager software (Tina; Raytest, Straubenhardt, Germany) and mean telomere length was calculated.

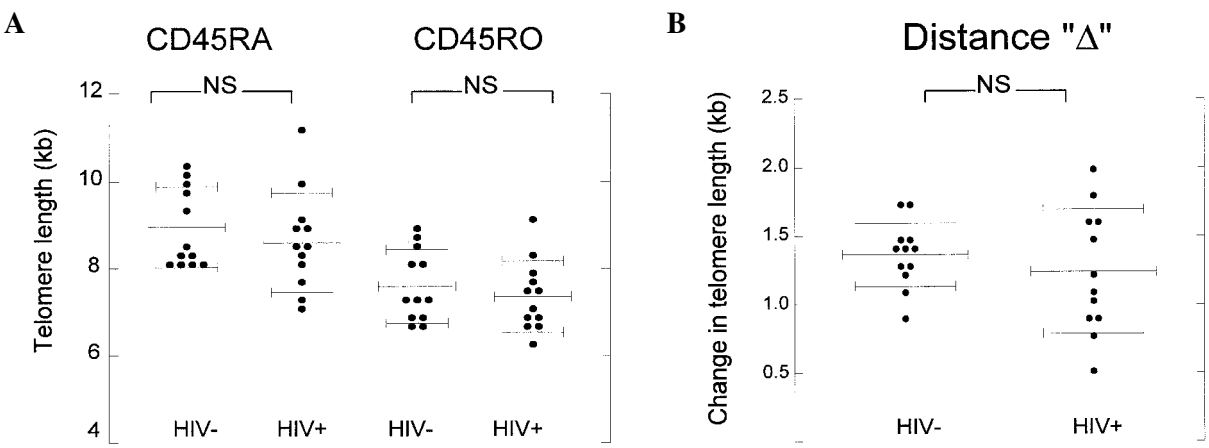
Correcting for CD45RA/RO impurity

We developed an algorithm to correct for the disproportionally high signals due to increased binding of the telomere probe $(TTAGGG)_5$ to longer telomeres,[27] and for the contamination of the naive and memory subsets with cells of the other subset (details available on request). Because the effect of the correction has a similar impact on both study groups, and has no substantial impact on any other results (data not shown), all results presented are the raw data.

RESULTS

Normal telomeric restriction fragment length in naive and memory CD4$^+$ T cells

TRF length was analyzed in purified CD45RA$^+$and CD45RO$^+$ CD4$^+$ T cells from healthy controls and HIV-infected individuals (see Fig. 1). All HIV-infected individuals were in a clinically latent stage at the time of analysis, with CD4$^+$ T cell counts >300 cells/mm^3 and with duration of infection from 1 to 12 years. Mean telomere length of CD4$^+$CD45RA$^+$ and of CD4$^+$CD45RO$^+$ T cells from HIV-infected individuals was not significantly different from that found in healthy controls (CD4$^+$CD45RA$^+$ T cells [mean ± SD], 8.6 ± 1.1 and 9.0 ± 0.9 kb; CD4$^+$CD45RO$^+$ T cells, 7.4 ± 0.8 and 7.5 ± 0.9 kb, respectively). In healthy controls, the mean difference (Δ) in telomere length between CD45RA$^+$ and CD45RO$^+$ CD4$^+$ T cells was 1.4 kb (Fig. 1B), which is in agreement with published data.[23] In HIV-infected individuals, the mean Δ was 1.2 kb. In Fig. 1C, the difference in TRF length of naive (lanes 1) and memory (lanes 2) T cells is illustrated for two HIV-negative controls (A and B) and four HIV-infected individuals (H1–4). Although the range of Δ values for the HIV$^+$ group was larger than in healthy controls, this difference was not significantly different (Fig. 1B). Furthermore, this larger variation in Δ in HIV-infected individuals was not related to CD4$^+$ T cell counts, to naive and memory CD4$^+$ T cell counts, to disease progression and treatment, or to the impurity of the naive and memory subsets (data not shown).



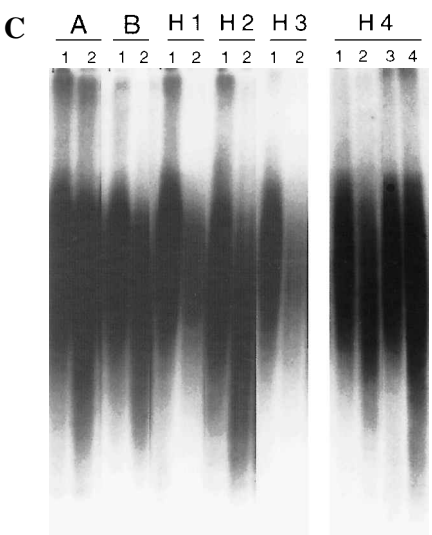


FIG. 1. Comparative analysis of purified CD4$^+$CD45RA$^+$ (naive) and CD4$^+$CD45RO$^+$ (memory) TRF length in 12 HIV controls and 12 HIV-positive individuals. TRF length analysis was performed by Southern blotting using the radiolabeled $(TTAGGG)_5$ probe, and analyzed by Phosphor-Imager for the mean TRF length. **(A)** Telomere lengths of CD4$^+$CD45RA$^+$ and CD4$^+$CD45RO$^+$ T cells from 12 HIV$^+$ and 12 HIV$^-$ subjects were compared. **(B)** The distance Δ between the TRF lengths of CD45RA$^+$ and CD45RO$^+$ cells from the HIV$^-$ and HIV$^+$ individuals in **(A)**. Statistical analysis was performed with the Mann–Whitney *U* test, and $p < 0.05$ was considered significant. NS, Not significant. The horizontal bars indicate means ± SD (standard deviation). **(C)** A representative Southern blot of telomeric DNA of CD4$^+$CD45RA$^+$ (lanes 1 and 3) and CD4$^+$CD45RO$^+$ (lanes 2 and 4) T cells from two healthy controls (A and B) and three HIV-infected individuals (H1 to H3). Two sequential samples (lanes 1 and 2, 2 months lanes 3 and 4, 3.7 years after entry) are shown for H4, which corresponds to patient 6134 depicted in Fig. 2.

No accelerated telomeric restriction fragment length loss in naive and memory CD4$^+$ T cells

The statistical analysis of cross-sectional data is hampered by the considerable variation in average TRF length of different individuals. To confirm that in asymptomatic HIV-1 infection naive and memory CD4$^+$ T cell TRF lengths shorten at a normal rate, we analyzed naive and memory CD4$^+$ T cell TRF length in sequential samples from eight HIV-infected individuals (Fig. 2). During follow-up, all eight HIV-infected individuals were in a clinically latent stage, with declining CD4$^+$ T cell counts remaining above 300 cells/mm^3 at the last time point (Table 1). The mean change in TRF length was 6 ± 64, and -26.3 ± 89 bp/year (mean $\pm$ SD), in naive and memory CD4$^+$ T cell subsets, respectively. This is normal compared with the 33 bp/year reported in a cross-sectional study of healthy individuals,[23] and in the normal range of the -100 to 100 bp/year we found previously in a longitudinal study of healthy HIV$^-$ controls.[11] Only patient 1120 exceeded the normal TRF length loss in the memory cells (i.e., a TRF length loss of 189 bp/year; see Table 1). Thus, the average TRF length and the rate of TRF length shortening in naive and memory CD4$^+$ T cells is not altered in asymptomatic HIV-infected individuals. These results extend previous findings,[11,12,28] with the demonstration that the average telomere length of both naive and memory CD4$^+$ T cells in HIV-infected individuals having CD4$^+$ T cell counts above 200 cells/mm^3 remains in the normal range.

MATHEMATICAL MODELS

Because HIV-1 preferentially infects activated CD4$^+$ T cells[29] that are about to shorten their telomeres by dividing, it was argued that HIV-1 infection will increase the mean TRF lengths of CD4$^+$ T cells, which could be masking the shortening effect of an increased production.[16,22] We here analyze mathematical models to show that, in a quasi-steady state scenario,[2] this TRF-elongating effect of HIV-1-associated killing does not mask TRF loss because most CD4$^+$ T cells that are killed must be replaced by further cell division, i.e., by further telomere shortening. Thus, for the quasi-steady state scenario where the killing of CD4$^+$ T cells is in almost perfect balance with novel CD4$^+$ production,[2] we formally prove that HIV-1-associated killing can only shorten the population average TRF length.

In our models we assume that dividing CD4$^+$ T cells are most vulnerable to lethal interactions with HIV-1. Thus, dividing cells have a probability $(1 - \beta)$ of surviving the division cycle, where β is an HIV-1-associated killing probability of dividing cells. This killing rate β may be more general than just infection. Because the death of one dividing cell implies the loss of two daughter cells, one obtains terms of the form $(1 - 2\beta)$ in the differential equations for the total cell numbers.

A one-compartment model

To illustrate basic principles, and to test the suggestion that HIV-1 associated killing might mask cell division, we first extend our previous one-compartment model[30] with HIV-1-associated killing (see the Appendix). Thus, a population of renewing CD4$^+$ T cells can be described by two differential equations, one for the total number of cells in the population T, and one for the average "telomere loss index" μ. Increases in μ are proportional to decreases in the average telomere length measured in experiments (see the Appendix), i.e., μ increases with the expected loss in TRF length per cell division.

For CD4$^+$ T cells that divide/renew at a rate α, and which die at a rate δ, we derive in the Appendix the following two simple differential equations:

$$dT/dt = T[\alpha(1 - 2\beta) - \delta] \quad (1)$$

$$d\mu/dt = 2\alpha(1 - \beta) \quad (2)$$

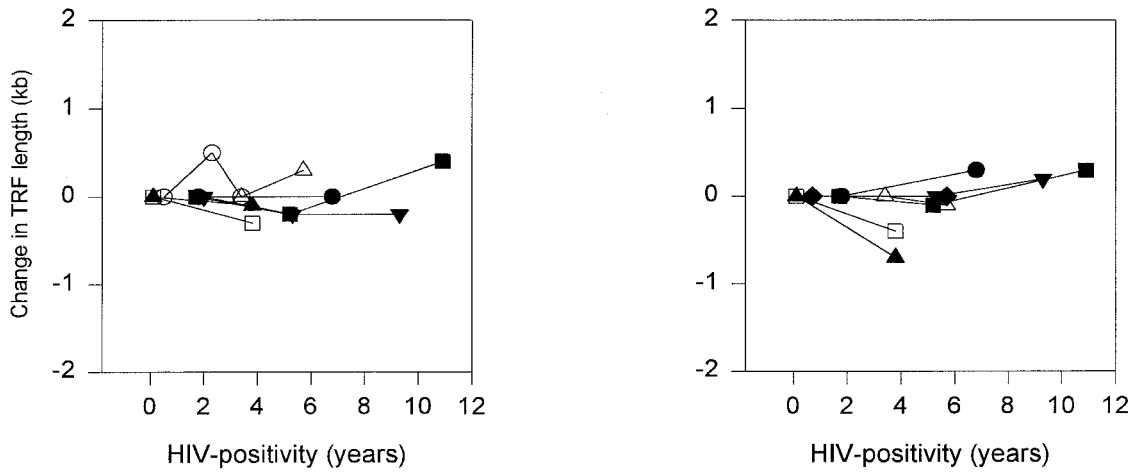


FIG. 2. Longitudinal analysis of TRF length of (left) CD4$^+$CD45RA$^+$ and (right) CD4$^+$CD45RO$^+$ T cells from eight HIV-infected men with CD4$^+$ T cell counts greater than 300 cells/mm^3. The first sample was considered the baseline TRF length (defined as 0), and sequential samples were defined as the change from baseline in kilobases. HIV positivity is expressed in years after seroconversion or entry. (●) 1024; (◆) 232; (■) 169; (▲) 1120; (▼) 82; (□) 6134; (△) 6094; (○) 6140.

for the changes in the total number T of $CD4^+$ T cells, and their average telomere loss index μ, respectively. An implication of this renewal model is that, under the quasi-steady state scenario,[2] the killing rate β of dividing T cells cannot be larger than 0.5. For $\beta > 0.5$ there is no longer a (quasi) steady state, because the renewal term $\alpha(1 - 2\beta)$ changes into a loss term, implying that T cells would be rapidly depleted.

The rate of change of the average division index [Eq. (2)] has a straightforward interpretation. For a constant killing probability β, we see that any increase in the $CD4^+$ T cell productivity α will increase the rate of telomere length shortening of the $CD4^+$ T cells [Eq. (2)]. However, when the $CD4^+$ T cell productivity is not increased, i.e., when α remains constant in HIV^+ patients, the telomere loss index will grow at a slower rate owing to the killing rate β. Thus, in the absence of any increase in the $CD4^+$ T cell division rate α, the $CD4^+$ T cell TRF lengths are indeed expected to remain longer in HIV^+ patients than in HIV^- controls.[16,22]

It was, however, argued that $CD4^+$ T cell levels are at quasi-steady state with HIV-1-associated killing by a markedly increased $CD4^+$ T cell production,[2,3] i.e., it was argued that $dT/dt \cong 0$. One can calculate what this increase in production (i.e., in α) implies for TRF length shortening by solving the killing probability $\beta = 0.5(1 - \delta/\alpha)$ from the $dT/dt = 0$ equation, and substituting this into Eq. (2) to find that $d\mu/dt = \alpha + \delta$. Thus if the $CD4^+$ T cell numbers remain in steady state by a markedly increased $CD4^+$ T cell production α, we do expect markedly decreased average telomere lengths of $CD4^+$ T cells during clinical latency. In retrospect this is an intuitive result because the continuous replacement of cells killed by HIV-1 can only decrease the average TRF lengths.

For example, in HIV^- controls $d\mu/dt = 2\alpha = 2\delta$ [see Eq. (2) and Ref. 30]. Thus, if the rate of $CD4^+$ T cell production were to be 10-fold increased, and if δ remains normal, one expects the rate of telomere length shortening during clinical latency to be increased by a factor $(10\alpha + \delta)/(2\alpha) = 5.5$. Similarly, if α were 100-fold increased, μ would run 50.5-fold faster.

Multicompartment models

Earlier work on the TRF shortening rates in models allowing for naive and memory T cell compartments demonstrated, however, that the TRF shortening rates of the memory compartment is expected to approach that of the naive compartment.[30] This conclusion is in good agreement with cross-sectional data from healthy volunteers, showing a consistent 1.4-kb difference between naive and memory $CD4^+$ T cell TRF length.[23] Because the compartment of naive $CD4^+$ T cells may be at least partly maintained by an earlier progenitor compartment (e.g., thymocytes),[31,32] similar results apply to the naive compartment, i.e., the telomere shortening rates of naive $CD4^+$ T cells are expected to approach that of their progenitors.[30] The effect of HIV-1 infection can be included in this multicompartment model.

Model structure, and analytical results

By extending our previous approach,[30] we have developed a novel three-compartment model (Fig. 3).[32a–c] In each compartment there may be cell division (at a rate α), HIV-1-associated killing of dividing cells (at a rate β), normal cell death (at a rate δ), and transfer to the subsequent compartment (at a rate γ). The maturation of thymocytes into immunocompetent naive $CD4^+$ T cells, and the priming of naive T cells into memory $CD4^+$ T cells, allows for a clonal expression C. Telomerase activity during clonal expansion[18] should limit TRF shortening during clonal expansion, but this compensation need not be complete. Hence, the mean telomere loss index μ after clonal expansion is taken to be increased by K units.[30] Both C and K may depend on HIV-1-associated killing β. For each cell subset, the model consists of an infinite set of equations describing the numbers of cells indexed by the number of TRF-shortening events they went through. As before,[30] and as already described, the full model can be distilled into just two equations per cell subset, for the population numbers and the mean TRF loss index per subset.

For the population sizes of the progenitor P, naive V, and memory M compartments we find

$$dP/dt = P[\alpha_P(1 - 2\beta_P) - \delta_P - \gamma_P]$$

$$dV/dt = V[\alpha_V(1 - 2\beta_V) - \delta_V - \gamma_V] + \gamma_P C_P P$$

$$dM/dt = M[\alpha_M(1 - 2\beta_M) - \delta_M] + \gamma_V C_V V \qquad (3)$$

Likewise, the equations for the mean of the TRF index per T cell subset are

$$d\mu_P/dt = 2\alpha_P(1 - \beta_P)$$

$$d\mu_V/dt = 2\alpha_V(1 - \beta_V) - \gamma_P C_P\ (P/V)\ (\mu_V - \mu_P - K_P)$$

$$d\mu_M/dt\ = 2\alpha_M(1 - \beta_M) - \gamma_V C_V\ (V/M)\ (\mu_M - \mu_V - K_V) \qquad (4)$$

Note that the most "upstream" compartment P follows the same equations as the one-compartment model already described—the only formal change is the extra loss by priming, with rate γ_P. The equations for the two downstream compartments (V,M) are almost identical in form.

Analogous to our earlier results,[30] one expects the TRF shortening rates $d\mu_V/dt$ and $d\mu_M/dt$ to approach that of the progenitors $d\mu_P/dt$. Eventually, all rates $d\mu/dt$ will depend on α_P and β_P only, while all downstream compartment parameters become irrelevant for the TRF shortening rates. This approach to equal TRF shortening rates implies an approach to constant distances between the average TRF lengths μ of all compartments. Importantly, these steady state distances Δ do depend on the parameters of the downstream compartments, and may thus provide information on the respective division rates. Defining $\Delta_V = \mu_V - \mu_P$ and $\Delta_M = \mu_M - \mu_V$ one obtains[30] for the steady state distance

$$\Delta_V = K_P + \alpha'_V - \alpha'_P/\gamma_P C_P\ V/P$$

$$\Delta_M = K_V + \alpha'_M - \alpha'_V/\gamma_V C_V\ M/V \qquad (5)$$

where α'_x is the one-compartment TRF shortening rate, i.e., $\alpha'_x \equiv 2\alpha_x(1 - \beta_x)$ for $x = P,V$, and M.

In the previous model[30] we assumed that the naive $CD4^+$ T cell compartment was maintained largely by renewal, i.e., by the α_V term, and that the contribution of the progenitor com-

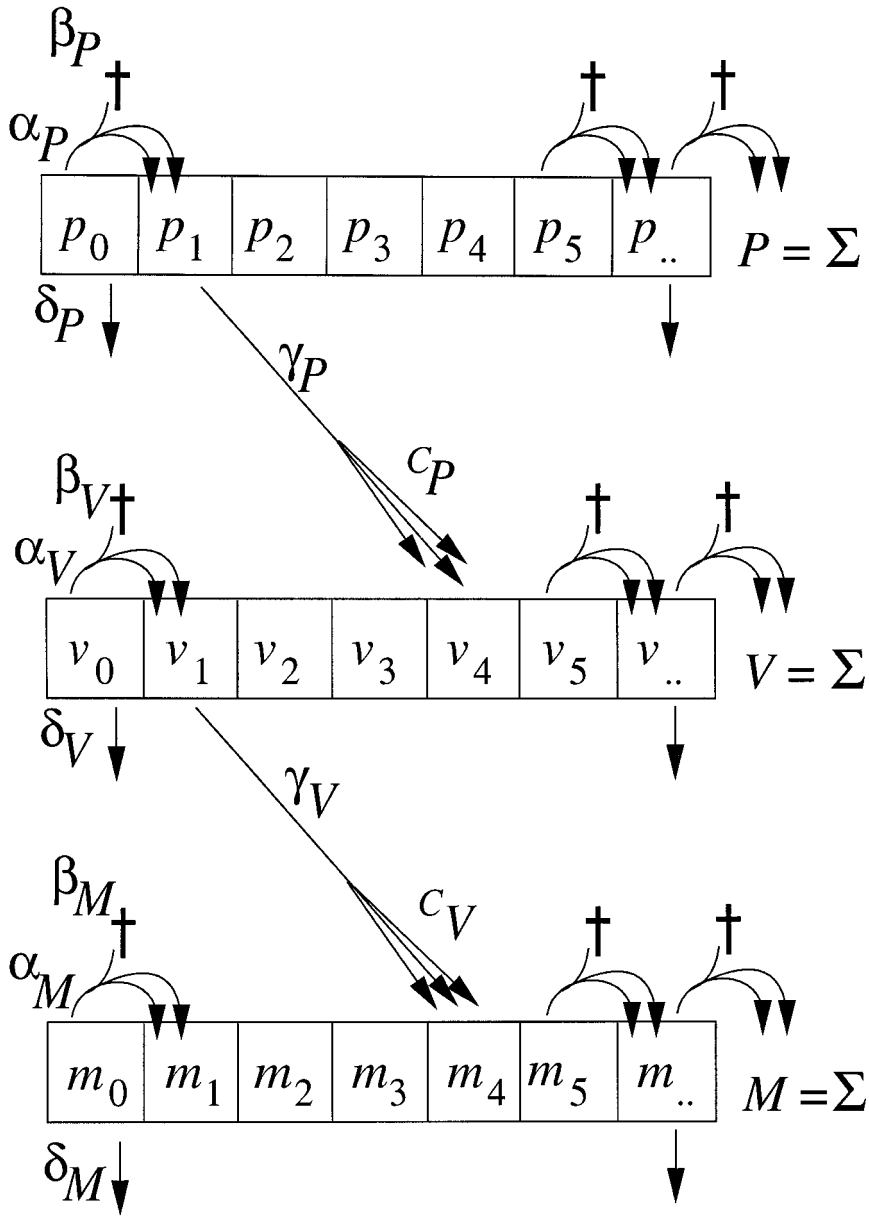


FIG. 3. A scheme of the mathematical model. Each box p_i, v_i, and m_i represents the number of *p*rogenitor, naive, or *m*emory $CD4^+$ T cells that have gone through precisely i cell divisions. The total number of progenitor, naive, or memory T cells, P, V, and M, respectively, is simply the sum of the corresponding array of boxes. The arrows α, δ represent the cell division and death rates, respectively. These may be arbitrary homeostatic functions of the total T cell density within the compartment. The parameters β represent the probabilities that a dividing T cell interacts with HIV and dies by infection, apoptosis, or otherwise. The parameters γ represent the maturation rate of progenitors (γ_P), and the priming rate (γ_V) of naive T cells. Thus, γ_V represents the probability that a naive T cell becomes primed by a foreign antigen to expand into a clone of memory T cells under the influence of telomerase activity. Similarly, γ_P is the probability that a progenitor matures into a small clone C_P of naive $CD4^+$ T cells. Because dividing $CD4^+$ T cells can be killed by the virus, the number of progeny generated per $CD4^+$ T cell is an unknown decreasing function $C[\beta]$ of the killing rate β. Owing to telomerase activity by antigen activation, the telomeres of the clonal progeny need not be much shortened. In our model we write that clonal expansion has a "cost" K in the telomore loss index, where K is probably small. Moreover, this shift should also be a function of the killing rate β because the same degree of clonal expansion requires more cell division when proliferating cells are killed by HIV. This function $K[\beta]$ is therefore probably small. Although in a rat model it has been suggested that in the absence of antigenic stimulation memory $CD4^+$ T cells revert to a naive phenotype,[32a] this appears to be insignificant for human $CD4^+$ T cells.[32b,c] Thus in our model memory $CD4^+$ cells never revert to the naive phenotype.

partment, i.e., $\gamma_P C_P P$, plays a negligible role. This simplifies the current three-compartment model into a two-compartment model for V and M, with V acting as the "upstream" compartment. This special case $\gamma_P C_P P = 0$ merely requires replacing all symbols P by V, and all V by M, while all M equations can then be dropped. Because for human adults it remains an open question whether naive CD4$^+$ T cells are maintained by renewal or by a progenitor compartment like the thymus, we will consider both possibilities.

Interpreting telomere data in terms of the model

Our earlier conclusions about the one-compartment model carry over to the upstream compartment (i.e., P in the three-compartment model and V in the two-compartment model). Thus, if cell division in the upstream compartment, say α_P, increases enough to maintain its population size despite HIV-associated killing, i.e., if $dP/dt = 0$, the telomere shortening rate $d\mu_P/dt = \alpha_P + \delta_P + \gamma_P$ should increase. Conversely, if there is no increase in α_P, then HIV infection will reduce the shortening rate by a factor $1 - \beta_P$. With progressive depletion ($dP/dt < 0$), the TRF shortening rate falls between these two extremes [see Eq. (4)].

We argued above, and previously,[30] that the downstream TRF shortening rates $d\mu_V/dt$ and $d\mu_M/dt$ will ultimately approach the rate $d\mu_P/dt$ of the upstream compartment. Thus, downstream TRF loss rates ultimately depend only on the homeostasis of the upstream compartment. Since downstream parameters do influence the steady distances Δ in the average TRF lengths between the compartments, we consider Eq. (5). First note that an increase in the naive and memory division rates α_V and α_M, with no other changes of parameters, should increase the steady state distances Δ_V and Δ_M. Initially, this would be observed as an increased downstream TRF shortening rate. This is in contradiction with our data on Δ_M (Fig. 1), and on the naive and memory TRF length shortening rates (Fig. 2). It is, however, possible that the killing of dividing cells "masks" part of the effect of increased division on TRF lengths [i.e., in Eq. (5) this result depends on $\alpha_x(1 - \beta_x)$]. Because the killing coefficients β are at most one-half (otherwise a rapid collapse of the population sizes occurs), the maximum masking effect can only be a factor of 2.

Equation (5), however, identifies a number of other parameters that may confound the effect of an increased division rate α. The constant K is likely to be unchanged because telomerase activity is normal in HIV-1 infection.[11,12,21] The preferential depletion of naive CD4$^+$ T cells,[26] should increase Δ_M, which is, however, not observed (Fig. 1). Therefore, other parameters seem to be compensating for this increased M/V ratio. Indeed, HIV-1 clinical latency is associated with a generalized hyperactivation,[6,33,34] which may correspond to an increased priming of naive CD4$^+$ T cells. Such an increase in the priming rate γ_V could mask the increasing effect of preferential naive cell depletion,[26] and/or an increased α_M on Δ_M [see Eq. (5)]. Thus, normal TRF lengths in the memory CD4$^+$ T cells could be due to fairly normal division rates and limited killing rates,[11] but could also be caused by an increased priming rate γ_V of the naive CD4$^+$ T cells that is masking an unknown increase in memory division rate.

It has already been described that the naive CD4$^+$ T cell compartment can be maintained by renewal (i.e., by the α_V term), by a progenitor compartment (i.e., by the $\gamma_P C_P P$ term), or by both. If the naive compartment is largely maintained by cell division in the progenitor compartment (i.e., if $\alpha_V << \alpha_P$), one surprisingly expects the average TRF length of the naive CD4$^+$ T cells to be larger than that of their progenitors. This perhaps counterintuitive result is in fact precisely what one would expect if naive cells hardly divide (and thus keep their original TRF length), while their population size is maintained by influx from a progenitor compartment with frequent cell division (which means telomere shortening). Importantly, this has implications for the effect of an HIV-induced reduction in the flux $\gamma_P C_P P$ from the progenitor to the naive compartment.[11,16] If naive CD4$^+$ T cells are largely maintained by progenitors, progenitors have the shortest TRF lengths. Thus, HIV-associated downregulation of the flux $\gamma_P C_P P$ from progenitor into the naive subset will reduce the entry of cells with relatively short TRF lengths into the naive population, leading to a reduced rate of TRF-shortening $d\mu_V/dt$. Conversely, if naive CD4$^+$ are largely maintained by self-renewal, such an interference with progenitors might have a shortening effect on the average naive TRF lengths.

In the latter scenario the naive cells act as the "upstream" compartment as already described, and the progenitors can be ignored. The fact that we find normal TRF lengths in naive CD4$^+$ T cells then suggests that during clinical latency there are only moderate changes in the naive CD4$^+$ T cell production and death rates. The former scenario, in which the naive CD4$^+$ T cell compartment is largely maintained by progenitors (e.g., thymocytes), is in good agreement with data, however. First, it is in perfect agreement with our data (Figs. 1 and 2) that the TRF length-shortening rates of naive CD4$^+$ T cells seem somewhat slower than normal. Second, it would explain the preferential depletion of naive CD4$^+$ T cells[26] while memory CD4$^+$ T cells become preferentially infected.[24] Third, increasing priming of naive CD4$^+$ T cells (i.e., increased γ_V) would add on to this preferential depletion, and may mask the somewhat increased division rate in the memory CD4$^+$ T cell compartment.

DISCUSSION

To summarize, the normal telomere lengths and shortening rates in CD45RA$^+$ naive and CD45RO$^+$ memory CD4$^+$ T cells in HIV$^+$ patients are fully consistent with our conjecture that during HIV-1 clinical latency the production of CD4$^+$ T cells is not markedly increased, and that HIV-associated interference with the CD4$^+$ T cell progenitors plays an important role in CD4$^+$ T cell depletion.[11,35] The average TRF lengths can, however, be confounded by changes in the flux between the progenitor, naive, and memory compartments. Because one expects similar changes in these fluxes in the CD8$^+$ T cell compartment, and CD8$^+$ T cells have decreased TRF lengths,[11,36] these confounding factors need not play a significant role however.

Measurements of CD4$^+$ T cell division by the Ki67 MAb, and by deuterated glucose enrichment, confirm the notion that CD4$^+$ T cell production is maximally severalfold increased.[8–10,37] In HIV$^+$ patients the total fractions of

$Ki67^+CD4^+$ T cells in lymphoid tissue are approximately normal,[9] to maximally severalfold increased.[8,38] Measurements of the fraction of $Ki67^+CD4^+$ T cells in peripheral blood tend to be somewhat higher than those in lymphoid tissue,[9,37] but also suggest a maximally severalfold increased proliferation rate. Bromodeoxyuridine (BrdU) labeling studies in SIV-infected macaques also suggested a severalfold increase in $CD4^+$ T cell turnover. The BrdU labeling studies, however, demonstrated that this not only applies to the $CD4^+$ target cells, but also to $CD8^+$ T cells, B cells, and natural killer (NK) cells.[34,39] These data therefore suggest that the increased production is due to a generalized hyperactivation of the immune system,[34] rather than to a homeostatic response to compensate specifically for $CD4^+$ T cell killing by viral infection.[2] Finally, the fact that the fraction of $Ki67^+CD4^+$ T cells in the blood and lymphoid tissue decreases during the first weeks of HAART[8,9] suggests that HIV-1-associated killing of dividing $CD4^+$ T cells has little impact on the pretreatment percentages of $Ki67^+CD4^+$ T cells.

According to the "quasi-steady state" and "proliferative exhaustion" scenario,[2] HIV-1 clinical latency involves a highly stressed homeostatic response of a markedly increased $CD4^+$ T cell production that almost fully compensates for the high rate of $CD4^+$ T cell destruction by the virus. This is called a *quasi*-steady state because compensation is *almost* complete. Because the percentage of productively infected $CD4^+$ T cells is low,[8,40] the HIV-1-associated killing rates need not be so high, however. In addition, in human adults the regenerative capacity of the $CD4^+$ T cell repertoire is limited.[31,32,41–43] Thus, even a severalfold increased production could be maximal, and still be insufficient to compensate fully for the additional HIV-1-associated cell death. This is the "limited renewal" scenario.[8,10,11,16,35,44] The two scenarios differ in the mechanism for disease progression, and in the relevance of therapies aiming at immune reconstitution. In the limited renewal scenario disease progression is due to insufficient production in the $CD4^+$ T cell compartment, whereas in the proliferative exhaustion scenario it is the highly stressed homeostatic response that causes the regenerative capacity to ultimately, and irreversibly, burn out.[2]

In HIV^+ patients, the innate limited renewal seems further impaired by interference of HIV with $CD4^+$ T cell progenitors.[10,11,16,35,44–47] The production of naive T cells in the thymus remains functional in adult $HIV\text{-}1^+$ patients. This was demonstrated by the increase in recent thymic emigrants (as identified by DNA excision circles produced during T cell receptor [TCR] gene rearrangement) during HAART,[46] and by the positive correlation between the amount of thymic tissue (as measured by chest computed tomography) and peripheral naive $CD4^+$ and $CD8^+$ T cell counts.[44] The thymus is, however, infected, infiltrated with macrophages, B cells, and $CD8^+$ effector cells, and lymphodepleted.[45,47,48] Indeed, both the peripheral $CD4^+$ and $CD8^+$ naive T cell counts,[26,44,49] and the number of recent thymic emigrants in the periphery,[47] are reduced during HIV-1 infection. Moreover, when PBMCs from HIV-infected individuals were cultured in a fetal thymic organ culture (FTOC) system, both $CD4^+$ and $CD8^+$ T cell regeneration were impaired, but the $CD4^+$ T cell regeneration was the most affected.[50] As already explained, such an interference with $CD4^+$ T cell progenitor production is fully compatible with our telomere data, and explains the preferential depletion of naive $CD4^+$ T cells while memory $CD4^+$ T cells are most readily infected.

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APPENDIX

For arbitrary density-dependent α and δ the model in Fig. 3 can be translated into an infinite system of differential equations for the subpopulations of "telomere loss index" i, where i measures the telomere loss in units of the expected loss per cell division (in the absence of telomerase). Thus, if b is the expected number of base pairs lost per cell division, a cell of index i has reduced its TRF length by bi base pairs. For these $CD4^+$ T cell subpopulations one obtains

$$dT_i/dt = 2\alpha(1-\beta)T_{i-1} - (\alpha+\delta)T_i$$

where α and δ denote the division, and the death rates, respectively. Summation yields the dynamics of the total number of $CD4^+$ T cells T, i.e., Eq. (1) in the text.

The "average telomere loss index" is naturally defined as a weighted sum of all subpopulations T_i, i.e., as $\mu = \sum_i^\infty = iT_i/T$, where T is the total number of cells. The rate of change of the average telomere loss index is

$$\frac{d\mu}{dt} = \frac{T\sum iT_i' - T'\sum iT_i}{T^2} = \frac{\sum iT_i' - T'\mu}{T}$$

where T_i' and T' are shorthand for dT_i/dt as given above, and dT/dt in Eq. (1).

The first sum term in the numerator is evaluated as

$$\begin{aligned}\sum_{i=1} iT_i' &= 2\alpha(1-\beta)\sum_{i=1} iT_{i-1} - (\alpha+\delta)\sum_{i=1} iT_i \\ &= 2\alpha(1-\beta)[T_0 + \sum_{i=1}(i+1)T_i] - (\alpha+\delta)T\mu \\ &= 2\alpha(1-\beta)T + [\alpha(1-2\beta) - \delta]T\mu\end{aligned}$$

Since the second term in the numerator equals $[\alpha(1-2\beta) - \delta]T\mu$ we obtain Eq. (2) in the text. Note that the measured av-

erage telomere length L is related to μ by $L = L_0 - b\mu$, where L_0 is the starting telomere length, and b is the number of base pairs lost per cell division in the absence of telomerase.

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