T Cell Renewal Rates, Telomerase, and Telomere Length Shortening[1]

Rob J. De Boer[2] and André J. Noest[1]

Measurements on the average telomere lengths of normal human naive and memory T cells suggested that 1) naive and memory human T cells have similar division rates, and 2) that the difference between naive and memory cells reflects the degree of clonal expansion during normal immune reactions. Here we develop mathematic models describing how the population average of telomere length depends on the cell division rates of naive and memory T cells during clonal expansion and normal renewal. The results show that 1) telomeres shorten with twice the cell division rate, 2) that the conventional approach of estimating telomere length shortening per mean population doubling gives rise to estimates that are 39% larger than the "true" loss per cell division, 3) that naive and memory T cells are expected to shorten their telomeres at rates set by the division rate of the naive T cells only, i.e., irrespective of the division rate of memory T cells, 4) that the measured difference in the average telomere length between naive and memory T cells may largely reflect the difference in renewal rates between these subpopulations rather than the clonal expansion, and 5) that full telomerase compensation during clonal expansion is consistent with all data on the shortening of telomere length in, and between, naive and memory T cells. Thus we reconcile the apparent contradictions between the demonstrated difference in division rates between human naive and memory T cells and their similar rates of telomere shortening, and the demonstrated telomere shortening in the presence of telomerase activity. ***The Journal of Immunology*****, 1998, 160: 5832–5837.**

The maintenance of the T cell repertoire is an important unresolved issue in immunology. Experiments using mice suggest that at least part of the memory T cells are relatively short lived and are maintained by active proliferation, i.e., by renewal (1, 2). The nature of the ligands stimulating memory cells to divide is unknown, and may vary from persisting pathogens (3), environmental (e.g., food) Ags (2), to self-Ags (4). Memory T cells can indeed be very cross-reactive (5, 6). Naive T cells, on the other hand, seem to live much longer (2, 7), and are maintained by production of novel naive cells in the thymus and by cell division (2); the former are the dominant source during early life (2).

Cell division of memory cells is also the dominant maintenance mechanism for the T cell repertoire in human adults (8). Division rates of human naive $CD45RA^+$ and memory $CD45RO^+$ T cells have been estimated by the rates at which patients treated with radiotherapy lose lymphocytes with chromosome damage (9, 10). It was thus estimated that memory $CD45RO^+$ T cells divide once every 22 wk (10). The $CD45RA^+$ naive T cells were estimated to divide every 3.5 yr (10). Having an order of magnitude of 10^{11} naive T cells in either the $CD4^+$ or the $CD8^+$ compartment, dividing once every 1000 days amounts to a production of order of magnitude of 10^8 naive T cells/day in each subclass of T cells. Similarly, an average division rate of memory T cells of once every 100 days amounts to a production of about 10^9 memory T cells/day. Note that these numbers are probably upper estimates because these patients are recovering from radiotherapy and have not yet attained normal steady state peripheral T cell counts.

Cancer patients treated with chemotherapy typically fail to reconstitute a normal T cell repertoire, and the reconstitution rates correlate with the enlargement of the thymus and the appearance of $CD45RA^+$ $CD4^+$ naive T cells (11). For example, the three oldest patients in this study (11) are 24 yr old, and have daily recovery rates of 0.54, 0.26, and 0.63 $CD4^+$ T cells/μl blood. For adults having approximately 5 liters of blood, and having about 2% of the $CD4^+$ T cells in the circulation (12), these figures indicate an average total body production of about 10^8 $CD4^+$ T cells per day during this recovery phase. This is similar to the naive T cell production estimated above by chromosome damage (9). Similarly, multiple sclerosis patients treated with anti-CD4 mAb have markedly depleted peripheral $CD4^+$ T cell counts (13). Upon withdrawal of the treatment the $CD4^+$ T cell counts again recover very slowly, with similar recovery rates of $CD45RO^+$ memory and $CD45RA^+$ naive T cells (13).

Materials and Methods

Recent studies provide a novel and independent perspective on T cell renewal by documenting the rates of telomere length shortening of $CD45RA^+$ naive and $CD45RO^+$ memory $CD4^+$ T cells (14), and of $CD28^+$ and $CD28^-$ $CD8^+$ T cells (15). Telomeres are unique structures at the ends of chromosomes that are involved in cellular proliferative capacity (16, 17). Each cycle of cell division results in a loss of 50 to 100 terminal nucleotides from the telomere end of each chromosome (14, 17–21). Thus, measuring the rates of telomere shortening may reveal insights into the dynamics of T cell renewal (14, 15). Both studies reveal a 1400-base pair difference in the average telomere lengths of $CD45RO^+$ naive and $CD45RA^+$ memory $CD4^+$ T cells and of $CD28^+$ and $CD28^-$ $CD8^+$ T cells. Furthermore, this difference is independent of the average telomere length and the age of the donor (14). Given a loss of 50 to 100 base pairs per cell division, this difference was interpreted to reflect a clonal expansion of 14 to 28 population doublings (14, 15). The average telomere lengths of both naive and memory $CD4^+$ T cells decrease at a rate of 33 bp/yr (14). For the naive T cells, such a division rate is in agreement with the data cited above (10). The apparently equal average division rate of

Theoretical Biology, Utrecht University, Utrecht, The Netherlands

Received for publication September 8, 1997. Accepted for publication February 18, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] A.J.N. was financially supported by the Life Sciences Foundation (SLW) of the Netherlands Organization for Scientific Research (NWO). R.J.D.B. is supported by a Scientific Collaboration Grant from NATO (GRC960019).

[2] Address correspondence and reprint requests to Rob J. De Boer, Theoretical Biology U.U., Padualaan 8, 3584 CH Utrecht, The Netherlands. E-mail address: rdb@alive.bio.uu.nl

0022-1767/98/$02.00

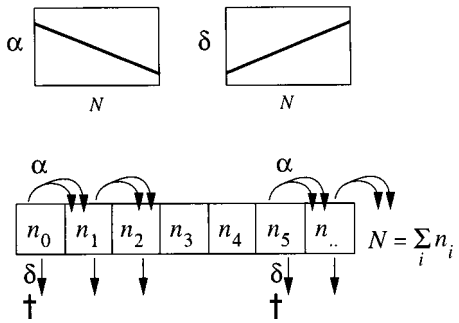

FIGURE 1. A scheme of the one-compartment model. Each box n_i represents the number of T cells that have gone through precisely i cell divisions. N is the total number of T cells. The arrows α and δ represent division and death rates, respectively. Both may be arbitrary homeostatic functions of the total T cell density, i.e., α is some decreasing, and δ some increasing function of the total number of T cells $N = \Sigma_i n_i$. Two linear examples are depicted in the figure.

memory $CD4^+$ T cells, however, is almost an order of magnitude lower than the one estimated above (10).

More recently, it became clear that activated T cells can express telomerase activity (22–27), and avoid telomere shortening during the first 10 to 12 divisions in culture (23, 27). Thus, the $CD4^+$ T cell telomere shortening of 33-bp $year^{-1}$ (14) and the 1400-bp difference between the T cell subpopulations (14, 15) paradoxically coexist with apparent telomerase activity. This implies that there should be cell division that is not fully compensated by telomerase activity. Developing a mathematical model in which naive and memory T cells are maintained both by renewal and clonal expansion, we are able to resolve this paradox by showing that one can obtain full consistency with the data even if telomerase activity fully compensates during clonal expansion. If memory T cells do divide more frequently than naive T cells during renewal (10), we obtain similar telomere shortening rates of both subpopulations as a steady state solution of our model. This accounts for the fixed difference of average telomere length between T cell populations, irrespective of division rate of the memory T cells, and irrespective of telomerase activity during clonal expansion.

Results

To model the dynamics of telomere shortening in peripheral populations of human T cells, we start by defining a "telomere loss index", i, which 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 shortened its telomere length by bi base pairs. We formulate our model by calculating the numbers n_i of T cells with a telomere loss index i. The numbers n_i change as cells die, divide, express telomerase, or switch phenotype (see Fig. 1). Below, we define an "average telomerase loss index," μ, which is proportionally related to the population-mean telomere loss. To illustrate basic principles, we first develop a simple "one-compartment" model in which the naive and memory T cell subsets are combined into one compartment. Subsequently, a more elaborate model is developed in which naive and memory cells are undergoing cell division and death at independent rates, and in which naive cells may be primed to become memory cells.

One-compartment model

Let n_i be the number of T cells of telomere loss index, i, in which $i = 0$ denotes cells that have an initial maximal telomere length. Cells may die, with rate δ, or divide, with rate α. Division of a cell of index i clearly removes it from the cell count n_i, while its two daughter cells are added to cell count n_{i+1}. The basic dynamics of our model can thus be expressed by the following system of differential equations

$$\frac{dn_i}{dt} = 2\alpha n_{i-1} - (\alpha + \delta)n_i, \text{ for } i = 0,1, \ldots,\infty, \quad (1)$$

in which, formally, $n_{-1} \equiv 0$. The cellular division rates α and/or death rates δ are expected to be regulated homeostatically. For deriving our results, it is not required to specify these functions, however.

The dynamics of the total number of T cells, N, is found simply by summing the n_i equations

$$\frac{dN}{dt} = \sum_i \frac{dn_i}{dt} = N(\alpha - \delta), \quad (2)$$

which recovers a standard model disregarding the division-class structure. Our very simple model so far is similar to another model that was previously derived (28). Our major simplification is to ignore the so-called Hayflick limit (29, 30), which is the minimal telomere length at which cells can still divide successfully. Our approach can be extended to include Hayflick limit effects, but at a cost in simplicity and ease of interpretation.

One advantage of this simplification is that one can derive a very simple differential equation describing the growth of the "average telomere loss index," μ. This index, μ, should be proportional to the average loss of telomere length (i.e., $L = L_0 - b\mu$, where L is the measured average telomere length, L_0 is its starting value, and b is the number of base pairs lost per cell division. The average division index, μ, is simply the mean value of the telomere loss index, i.e.,

$$\mu = \frac{1}{N}\sum_{i=0}^{\infty} in_i . \quad (3)$$

Differentiating Equation 3 with respect to time, and using Equation 1 and Equation 2, one obtains the satisfyingly simple result (see Appendix A)

$$\frac{d\mu}{dt} = 2\alpha , \quad (4)$$

which says that the average telomere loss index increases at a rate that is twice the average cellular division rate. This is because two daughter cells with shortened telomeres replace one parent cell. Likewise, the average telomere length decreases at a rate $dL/dt = -2b\alpha$. According to this model, the telomere-shortening rate is independent of the average telomere length. In tumor cells, additional factors have recently been described, such as the telomeric repeat-binding factor TRF1 (31), which are involved in repairing telomere shortening dependent on the telomere length. We have not incorporated such factors in our model because it seems unlikely that similar factors play a significant role in human T cells, which are well known to suffer from telomere shortening, and, ultimately, senescence, by cell division (14, 15, 22–27).

Telomere loss per cell division

Experimental measurements of the average rate of telomere loss are based upon a longitudinal in vitro expansion of cell lines (17–21). Implicitly assuming synchronous cell divisions, and ignoring cell death, one typically computes the number of bases lost per mean population doubling (MPD)[3] as

$$b_{MPD} = \frac{\Delta_L}{\log_2[N_t/N_0]}, \quad (5)$$

[3] Abbreviation used in this paper: MPD, mean population doubling.

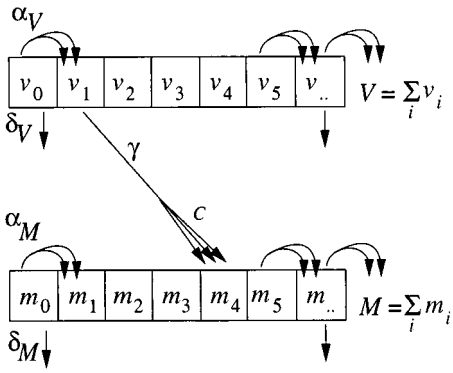

FIGURE 2. A scheme of the two-compartment model. The boxes v_i and m_i represent the number of naive T cells, and memory T cells, respectively, that have gone through precisely i cell divisions. The arrows α_V, α_M, δ_V, and δ_M represent their (homeostatically controlled) division and death rates, respectively. The arrow γC represents the priming and proliferation involved in normal immune reactions to foreign Ags.

where Δ_L is the total telomere loss over the experiment, N_0 is the number of cells the culture started with, and N_t is the final population size. These experiments have yielded $50 \leq b_{MPD} \leq 100$.

Because cell division is not expected to be synchronous, this b_{MPD} is not equal to the number of base pairs lost per cell division (i.e., to our parameter b). We here calculate the difference between the two. Solving Equation 2, one obtains that $(\alpha - \delta)t = \ln [N_t/N_0]$. Employing the solution of Equation 4, i.e., $\mu = 2\alpha t$, and our definition that $b = \Delta_L/\mu$, and also ignoring cell death, i.e., assuming $\delta \ll \alpha$, we obtain the expression

$$b = \frac{\Delta_L}{2\ln[N_t/N_0]} \tag{6}$$

for the average number of base pairs lost per cell division. Thus, in comparison with Equation 5, our Equation 6 requires taking the natural logarithm and correcting by the factor 2 obtained in Equation 4. The ratio of this "true" estimate of the telomere loss to the "MPD"-based estimate is

$$\frac{b}{b_{MPD}} = \frac{\log_2[N_t/N_0]}{2\ln[N_t/N_0]} = \frac{1}{2\ln[2]} \simeq 0.72\,. \tag{7}$$

Thus, the true average rate of telomere loss per cell division is 72% of the previous estimates of telomere loss per MPD. The existing data (17–21) then yield an estimate of $35 \leq b \leq 70$. For definiteness we use $b = 50$ bp/cell division from now on. The rate at which a population of dividing T cells loses its telomere base pairs (i.e., $2b\alpha$) can now be equated to the experimental value of 33 base pairs/yr (14). This yields a division rate of $\alpha = 1/3$ per year. Fortuitously, this is identical to the previous estimate (14).

Finally, as total T cell numbers tend to remain at steady state, we may put $dN/dt = 0$ in Equation 2 and obtain $\alpha = \delta$, as expected. Hence, the steady state average cellular life span $(1/\delta)$ equals $1/\alpha = 3$ yr.

Two-compartment model

In Figure 2 we generalize the simple one-compartment model to a model describing two coupled compartments, for "naive" and "memory" T cells, respectively. We assume separate homeostatic control of division and/or death rates for the naive and the memory cell types, since one would otherwise expect one of the two types to outcompete the other (32, 33). Additionally, mouse experiments do suggest that the naive and memory subsets are regulated independently (34). The human telomere data (14) demonstrate that naive $CD4^+$ T cells (or at least their precursors) are, indeed, slowly dividing cells. Experiments with mice also argue for slow division rates of $CD45RA^+$ naive T cells (2, 35). Although in a rat model it has recently been suggested that, in the absence of antigenic stimulation, memory $CD4^+$ T cells revert to a naive phenotype (36), it remains unclear whether one should allow for a similar reversal in the human situation. In the present model, we do not allow for such a reversion of the memory marker (see alternative model under *Discussion*). Thus, we devise a two-compartment model in which naive T cells cannot only divide or die, but can also be primed by external Ags to expand into a clone of memory T cells.

Denoting the naive cell numbers by v_i and the memory cell numbers by m_i, we write

$$\frac{dv_i}{dt} = 2\alpha_V v_{i-1} - (\alpha_V + \gamma + \delta_V)v_i\,, \tag{8}$$

where $v_{-1} \equiv 0$ as before. Extending our previous notation, the α_V and δ_V denote the division and death rates, which will probably be homeostatically controlled by the total count of naive cells $V \equiv \Sigma_i v_i$. The new parameter, γ, is the probabilistic rate at which naive T cells are externally primed to become a proliferating clone of activated T cells. Clearly, all results of the one-compartment model carry over to the naive cells of this two-compartment model. We need only repeat the analysis of the one-compartment model to obtain for the total number of naive cells

$$\frac{dV}{dt} = V(\alpha_v - \delta_v - \gamma), \tag{9}$$

and for the average telomere loss index $\mu_V \equiv \Sigma_i i v_i/V$

$$\frac{d\mu_V}{dt} = 2\alpha_V\,. \tag{10}$$

As before, the naive telomere lengths decrease with twice the naive T cell division rate. The only difference is that at a steady state of Equation 9 we obtain $\alpha_V = \delta_V + \gamma$, which is simply the "net turnover rate" of naive cells. The "priming" rate, γ, thus increases the naive cell telomere-shortening rate, but this is estimated to be a negligible contribution (see below).

Turning to the memory cell compartment, we have to consider clonal expansion. The 1400-base pair difference in average telomere lengths between human memory and naive T cells was interpreted to reflect a clonal expansion of 14 to 28 cell divisions (14, 15). In our model, we consider naive T cells that may be primed with external Ags at a probabilistic rate γ per day, to ultimately yield a clone of C memory T cells. Due to the telomerase activity evoked by the activation with Ag and/or the costimulatory factors (22–27), the degree of clonal expansion is unlikely to be reflected in a proportional degree of telomere loss (23, 27). Thus, we write that right after clonal expansion a memory T cell has shifted its telomere loss index by an amount K. Note that perfect compensation by telomerase activity during clonal expansion would correspond to $K = 0$.

In Appendix B, we derive that the total number of memory cells M satisfies

$$\frac{dM}{dt} = M(\alpha_M - \delta_M) + \gamma CV \tag{11}$$

and that the differential equation for the average telomere loss index is

$$\frac{d\mu_M}{dt} = 2\alpha_M - \gamma C\frac{V}{M}(\mu_M - \mu_V - K), \tag{12}$$

which says that telomere-shortening rate of memory cells is not just reflecting their division rate α_M, but depends on the clonal expansion, the naive:memory ratio, and their difference in average telomere lengths. Thus, memory telomere lengths are much less directly related to the division or turnover rates than the very simple proportionality Equation 10 found for the naive cells.

The difference Δ in naive and memory telomere lengths

For memory T cells, it is apparent from Equation 12 that the shortening rate slows down when μ_M exceeds $\mu_V + K$, which is the mean index of cells that have just been produced by clonal expansion of a primed (former naive) cell. To analyze this effect, it is helpful to write a differential equation for the distance between the naive and memory telomere loss index, which we define as $\Delta \equiv \mu_M - \mu_V$. Subtracting Equation 10 from Equation 12 gives

$$\frac{d\Delta}{dt} = 2(\alpha_M - \alpha_V) - \gamma C\frac{V}{M}(\Delta - K)\,. \qquad (13)$$

Inspecting this result, one notices a "time constant" $\tau = M/(\gamma CV)$, and a "source" term $\sigma = 2(\alpha_M - \alpha_V) + K/\tau$. Since we only consider data from adults (14), in whom memory and naive T cell counts tend to remain in steady state, σ and τ can be treated as finite constants allowing us to simplify Equation 13 into

$$\frac{d\Delta}{dt} = \sigma - \Delta/\tau\,, \qquad (14)$$

which will ultimately approach the steady state

$$\Delta = \sigma\tau = K + 2\tau(\alpha_M - \alpha_V) = K + \frac{2(\alpha_M - \alpha_V)}{\gamma C}\,\frac{M}{V} \qquad (15)$$

on a time scale, τ. Comparing these results to experimental data, we can draw several interesting conclusions.

Importantly, our results imply that the steady state telomere-shortening rate of memory cells should approach that of the naive cells, after a time period of a few times τ. The empirical results on $CD4^+$ T cells (14) demonstrate precisely this similarity of shortening rates. Hence, in steady state, the rate of telomere shortening in memory T cells should approach $2\alpha_V$, i.e., that of the naive T cells, and should not have any relation to the parameters of the memory T cells. There is, indeed, good agreement between both telomere-shortening rates and the average division rates $\alpha_V \approx 0.0008\ \text{day}^{-1}$ of human naive T cells (9, 10, 14). This resolves what appeared to be a contradiction between measured memory cell telomere-shortening rates and data suggesting that memory T cells are dividing considerably more frequently than naive T cells (1, 2, 9, 10, 35, 37), seem to be cross-reactive (5), and have less stringent maintenance requirements (38).

Intuitively, this result can be understood in terms of the influx of primed naive cells with long telomeres into the memory compartment. If memory T cells are dividing more frequently than naive T cells their average telomere length will initially shorten more rapidly than that of the naive T cells. The average memory telomere length cannot "run freely," however, because it is bound to the average naive telomere length by the influx of primed naive cells. This bond ultimately leads to a steady distance Δ between the two average telomere lengths. It is also natural therefore that this distance Δ depends on the difference in naive and memory T cell division rates, on the clonal expansion, and on the ratio of total numbers of naive and memory T cells.

The effect of telomerase activity during clonal expansion on the naive-memory distance Δ can be understood completely in terms of the parameter K. One extreme case is "perfect telomerase compensation" corresponding to $K = 0$. By Equation 15 one sees that $K = 0$ does allow for $\Delta > 0$, i.e., a fixed telomere length difference Δ between naive and memory T cells remains a natural result even if there is no telomere shortening at all during clonal expansion. This does require that memory cells divide more frequently than naive T cells, i.e., $\alpha_M > \alpha_V$, which is indeed supported by data (1, 2, 9, 10, 35, 37, 38). This resolves the apparent contradiction between transient telomerase activity during clonal expansion (23, 27) and the measured telomere shortening: the latter could, in principle, all be due to telomere loss during renewal. Obviously, the parameter K need not be zero, allowing the 1400-base pair difference (14, 15) to reflect both clonal expansion and differences in renewal rates.

Parameter estimation

The time constant, τ, and the priming rate of naive T cells, γ, can both be estimated from Equation 11. Arguing from the viewpoint that in adults memory T cells are maintained largely by renewal, and only marginally by the priming of naive T cells (1, 2, 6, 8, 35, 37), we obtain from Equation 11 that $\gamma CV \ll \alpha_M M$. Because in most adults the ratio of memory over naive T cells is approximately one (39, 40), i.e., $M/V \simeq 1$, this simplifies into $\gamma C \ll \alpha_M$. By the same reasoning the time constant simplifies to $\tau \simeq 1/(\gamma C)$. Employing the estimated memory division frequency of once every 22 wk, i.e., $\alpha_M \approx 0.006$ (10), we therefore obtain that $\tau > 22$ wk. Approaching the steady state telomere distance Δ in Equation 15 may therefore take several years. Since the experimental data were all from human adults (14, 15) such a time scale could indeed be consistent with our steady state approximation. Assuming that T cell precursor frequencies generally increase about a 1000-fold following a typical immune reaction (41), i.e., assuming $C \approx 1000$, we obtain for the priming rate that $\gamma \ll 6 \times 10^{-6}$ per day. In other words, on average, a naive T cell would run a chance of less than $10^{-6}\ \text{day}^{-1}$ of being primed by a foreign Ag to clonally expand.

Discussion

Summarizing, we find that naive T cells reduce their average telomere lengths at a rate reflecting twice their cell division rate, that the conventional approach of estimating telomere length shortening per MPD gives rise to estimates that are 39% larger than the "true" loss per cell division, that—irrespective of their division rates—memory T cells are expected to have the same rate of losing their telomere ends as naive T cells, and that the average difference in telomere lengths between naive and memory T cells need not reflect the clonal expansion telomerase activity of Ag-primed naive T cells. As a consequence, the recent telomere data (14) are most informative about the division rates of naive T cells, and should not be used for estimating the division rates of memory T cells. In combination, the current T cell data on telomere lengths (14, 15) and telomerase activity (22–27) suggest that memory T cells are indeed dividing more frequently than naive T cells (1, 2, 9, 10, 35, 37, 38).

The naive and memory T cell repertoires in our model are maintained by proliferative self-renewal, and we have shown that this is consistent with the available data. Alternatively, one could argue that both naive and memory T cells are long lived, and are only stimulated to divide by clonal expansion upon stimulation with specific Ag. The latter view is, in fact, supported by the slow recovery rate of memory T cells in adults, and the relationship of this recovery rate with the rate of naive T cell recovery (11, 13). Furthermore, it has recently been suggested that the so-called "memory" isoforms of the CD45R T cell markers reflect an activation stage rather then a true memory stage (36). Thus, in the absence of Ag, memory cells would revert to a quiescent state with

a naive CD45R phenotype (9, 36). These revertant T cells could, in principle, account for the observed shortening of the average telomere length of naive $CD4^+$ T cells (14) in the absence of any cell division in the naive T cell compartment. The shortening of the average memory T cell telomere length could, indeed, be due to incomplete telomerase compensation during clonal expansion. Although it remains unclear whether this alternative scenario is realistic, we are currently developing alternative mathematic models to investigate its consistency.

In our model, we have not considered a possible source of naive T cells from thymic or extrathymic lymphoid compartment. It is, indeed, believed that such a source is small in human adults (8, 11). Our approach of a two-compartment model can, however, be reiterated for the progenitor T cells providing novel naive T cells. If the compartment of mature immunocompetent T cells would have a source term from a self-renewing stem progenitor compartment (20), we could reinterpret our two-compartment model for progenitor T cell and mature T cell renewal. Calling progenitor T cells v_i and mature T cells m_i, γC would represent the differentiation and proliferation of a progenitor cell into an immunocompetent mature T cell. Thus, the rate of mature T cell telomere length shortening should reflect twice the division rate of the progenitor cells, and should be quite independent of the mature T cell division rate.

Other groups (R. Antia and S. Frost, personal communication) are analyzing the same telomere data (14) with mathematic models incorporating a Hayflick limit (28). These authors interpret the data as showing a skew in the distribution of memory T cell telomere lengths in older humans, which would be due to the death of memory T cells when their telomeres become too short. Thus, memory T cells can be dividing more frequently than the rate estimated from the average shortening rate of their telomeres. Although the recent data on T cell telomerase activity cast some doubt on the relevance of the Hayflick limit for T cells, this remains a valid alternative interpretation of the data. By choosing to ignore the Hayflick limit, we have been able to demonstrate that the average rate at which memory T cells lose their telomere ends is expected to be independent of their division rate anyway. Our model can in principle be extended to include the effect of a Hayflick limit. The major effect of this is that the difference between the memory and naive telomere lengths need not be in perfect equilibrium, and may become smaller in elderly individuals. Thus, qualitatively, this hardly affects our results.

Appendix A. The One-Compartment Model

First, we derive Equation 4, which describes the dynamics of the "population average telomere loss index" $\mu \equiv \sum_{i=0}^{\infty} in_i/N = \sum_{i=1}^{\infty} in_i/N$. The rate of change of μ is

$$\frac{\mathrm{d}\mu}{\mathrm{d}t} = \frac{N\sum in_i' - N'\sum in_i}{N^2} = \frac{\sum in_i' - N'\mu}{N}, \tag{16}$$

where n_i' and N' are the derivatives given by Equation 1 and Equation 2, respectively. The first sum term in the numerator is evaluated as

$$\sum_{i=1} in_i' = 2\alpha \sum_{i=1} in_{i-1} - (\alpha + \delta)\sum_{i=1} in_i \tag{17}$$

$$= 2\alpha n_0 + 2\alpha \sum_{i=1} (i+1)n_i - (\alpha + \delta)N\mu$$

$$= 2\alpha N + (\alpha - \delta)N\mu\,.$$

Since the second term in the numerator of Equation 16 equals $(\alpha - \delta)N\mu$ we obtain Equation 4 in the text, which describes the μ dynamics.

For some purposes it may not be sufficient to know only the time-dependent average telomere loss index $\mu(t)$; the full solution of $n_i(t)$ may be required. The $\mathrm{d}n_i/\mathrm{d}t$ equations can, in fact, be solved explicitly, e.g., by Laplace transform methods (42). For the simplest initial condition (i.e., $n_0(0) = 1$, $n_i(0) = 0$; $i > 0$), and constant α and δ, the normalized age distribution is found as

$$p_i(t) \equiv \frac{n_i(t)}{N(t)} = \frac{(2\alpha t)^i}{i!}\mathrm{e}^{-2\alpha t}, \tag{18}$$

which is evidently the Poisson distribution with parameter $2\alpha t$. Indeed, this parameter equals the mean index $\mu(t)$, as found by integrating Equation 4 with constant α. One may also generalize to time variant α, δ, and find that $p_i(t)$ remains a Poisson distribution with parameter $\mu(t)$. Extension to general initial conditions requires an additional convolution only, because of linearity and invariance under i shifts.

Appendix B. The Two-Compartment Model

To write down the m_i dynamics we use the fact that naive populations v_i are Poisson distributed around the average μ_V. Thus, the clonal expansion of a clone of naive T cells should give rise to a clone of memory T cells with a telomere loss index that is Poisson distributed around the average $\mu_V + K$, where K is the average telomere loss during clonal expansion (allowing for a compensation by telomerase activity). We idealize slightly by assuming that each clonal expansion event happens much faster than the m_i dynamics, but that there is a steady stream of such events, each producing a small addition to the total count of memory cells. This enables us to write the m_i dynamics as

$$\frac{\mathrm{d}m_i}{\mathrm{d}t} = 2\alpha_M m_{i-1} - (\alpha_M + \delta_M)m_i + \gamma C v_{i-K}\,. \tag{19}$$

The M dynamics, Equation 11 in the main text, follows by summing over i. To derive the μ_M dynamics, we first write

$$\frac{\mathrm{d}\mu_M}{\mathrm{d}t} = \frac{\sum im_i' - M'\mu_M}{M}, \tag{20}$$

where m_i' and M' are the derivatives given by Equation 19 and Equation 11, respectively. The first term in the numerator evaluates as

$$\sum_i i\frac{\mathrm{d}m_i}{\mathrm{d}t} = 2\alpha_M \sum_{i=1} im_{i-1} - (\alpha_M + \delta_M)\sum_{i=1} im_i + \gamma C \sum_{i=1} iv_{i-K} \tag{21}$$

$$= 2\alpha_M m_0 + 2\alpha_M \sum_{i=1} (i+1)m_i$$

$$- (\alpha_M + \delta_M)\mathrm{M}\mu_M + \gamma CV(\mu_V + K)$$

$$= 2\alpha_M M + (\alpha_M - \delta_M)M\mu_M + \gamma CV(\mu_V + K).$$

Since the second term in the numerator is equal to $(\alpha_M - \delta_M)M + \gamma CV$, we obtain Equation 12 in the main text.

Acknowledgment

We thank Katja Wolthers and Frank Miedema from the CLB in Amsterdam for extensive discussions and valuable suggestions. In a forthcoming collaborative paper,[4] we generalize the current model to study the effects of an HIV infection on $CD4^+$ T cell telomere lengths (21).

References

1. Freitas, A. A., and B. B. Rocha. 1993. Lymphocyte lifespans: homeostasis, selection and competition. *Immunol. Today 14:25.*
2. Tough, D. F., and J. Sprent. 1994. Turnover of naive- and memory-phenotype T cells. *J. Exp. Med. 179:1127.*
3. Gray, D., and P. Matzinger. 1991. T cell memory is short-lived in the absence of antigen. *J. Exp. Med. 174:969.*
4. Vos, Q., L. A. Jones, and A. M. Kruisbeek. 1992. Mice deprived of exogenous antigenic stimulation develop a normal repertoire of functional T cells. *J. Immunol. 149:1204.*
5. Selin, L. K., S. R. Nahill, and R. M. Welsh. 1994. Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. *J. Exp. Med. 179:1933.*
6. Matzinger, P. 1994. Immunology: memories are made of this? *Nature 369:605.*
7. Kelly, K. A., and R. Scollay. 1990. Analysis of recent thymic emigrants with subset- and maturity-related markers. *Int. Immunol. 2:419.*
8. Mackall, C. L., F. T. Hakim, and R. E. Gress. 1997. T-cell regeneration: all repertoires are not created equal. *Immunol. Today 18:245.*
9. Michie, C. A., A., McLean, C. Alcock, and P. C. Beverley. 1992. Lifespan of human lymphocyte subsets defined by CD45 isoforms. *Nature 360:264.*
10. McLean, A. R., and C. A. Michie. 1995. In vivo estimates of division and death rates of human T lymphocytes. *Proc. Natl. Acad. Sci. USA 92:3707.*
11. Mackall, C. L., T. A. Fleisher, M. R. Brown, M. P. Andrich, C. C. Chen, I. M. Feuerstein, M. E. Horowitz, I. T. Magrath, A. T. Shad, S. M. Steinberg, L. H. Wexler and R. E. Gress. 1995. Age, thymopoiesis, and $CD4^+$ T-lymphocyte regeneration after intensive chemotherapy. *N. Engl. J. Med. 332:143.*
12. Westermann, J., and R. Pabst. 1990. Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? *Immunol. Today 11:406.*
13. Rep, M. H., B. W. Van Oosten, M. T. Roos, H. J. Ader, C. H. Polman, and R. A. Van Lier. 1997. Treatment with depleting CD4 monoclonal antibody results in a preferential loss of circulating naive T cells but does not affect IFN-γ secreting TH1 cells in humans. *J. Clin. Invest. 99:2225.*
14. Weng, N. P., B. L. Levine, C. H. June, and R. J. Hodes. 1995. Human naive and memory T lymphocytes differ in telomeric length and replicative potential. *Proc. Natl. Acad. Sci. USA 92:11091.*
15. Monteiro, J., F. Batliwalla, H. Ostrer, and P. K. Gregersen. 1996. Shortened telomeres in clonally expanded CD28-$CD8^+$ T cells imply a replicative history that is distinct from their $CD28^+CD8^+$ counterparts. *J. Immunol. 156:3587.*
16. Blackburn, E. H. 1991. Structure and function of telomeres. *Nature 350:569.*
17. Harley, C. B., A. B. Futcher, and C. W. Greider. 1990. Telomeres shorten during ageing of human fibroblasts. *Nature 345:458.*
18. Allsopp, R. C., H. Vaziri, C. Patterson, S. Goldstein, E. V. Younglai, A. B. Futcher, C. W. Greider, and C. B. Harley. 1992. Telomere length predicts replicative capacity of human fibroblasts. *Proc. Natl. Acad. Sci. USA 89:10114.*
19. Vaziri, H., F. Schachter, I. Uchida, L. Wei, X. Zhu, R. Effros, D. Cohen, and C. B. Harley. 1993. Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. *Am. J. Hum. Genet. 52:661.*
20. Vaziri, H., W. Dragowska, R. C. Allsopp, T. E. Thomas, C. B. Harley, and P. M. Lansdorp. 1994. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc. Natl. Acad. Sci. USA 91:9857.*
21. Wolthers, K. C., G. Bea, A. Wisman, S. A. Otto, A. M. De Roda Husman, N. Schaft, F. De Wolf, J. Goudsmit, R. A. Coutinho, A. G. Van der Zee, L. Meyaard, and F. Miedema. 1996. T cell telomere length in HIV-1 infection: no evidence for increased $CD4^+$ T cell turnover. *Science 274:1543.*
22. Hiyama, K., Y. Hirai, S. Kyoizumi, M. Akiyama, E. Hiyama, M. A. Piatyszek, J. W. Shay, S. Ishioka, and M. Yamakido. 1995. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J. Immunol. 155:3711.*
23. Bodnar, A. G., N. W. Kim, R. B. Effros, and C. P. Chiu. 1996. Mechanism of telomerase induction during T cell activation. *Exp. Cell Res. 228:58.*
24. Weng, N. P., B. L. Levine, C. H. June, and R. J. Hodes. 1996. Regulated expression of telomerase activity in human T lymphocyte development and activation. *J. Exp. Med. 183:2471.*
25. Buchkovich, K. J., and C. W. Greider. 1996. Telomerase regulation during entry into the cell cycle in normal human T cells. *Mol. Biol. Cell 7:1443.*
26. Weng, N., B. L. Levine, C. H. June, and R. J. Hodes. 1997. Regulation of telomerase RNA template expression in human T lymphocyte development and activation. *J. Immunol. 158:3215.*
27. Weng, N., L. D. Palmer, B. L. Levine, H. C. Lane, C. H. June, and R. J. Hodes. Tales of tails: regulation of telomere length and telomerase activity during lymphocyte development, activation and aging. *Immunol. Rev. In press.*
28. Pilyugin, S., J. Mittler, and R. Antia. 1997. Modeling T-cell proliferation: an investigation of the consequences of the Hayflick limit. *J. Theor. Biol. 186:117.*
29. Hayflick, L. 1989. Antecedents of cell aging research. *Exp. Gerontol. 24:355.*
30. Linskens, M. H., C. B. Harley, M. D. West, J. Campisi, and L. Hayflick. 1995. Replicative senescence and cell death. *Science 267:17.*
31. Van Steensel, B. and T. De Lange. 1997. Control of telomere length by the human telomeric protein TRF1. *Nature 385:740.*
32. De Boer, R. J., and A. S. Perelson. 1994. T cell repertoires and competitive exclusion. *J. Theor. Biol. 169:375.*
33. De Boer, R. J., and A. S. Perelson. 1997. Competitive control of the self-renewing T cell repertoire. *Int. Immunol. 9:779.*
34. Tanchot, C., and B. Rocha. 1995. The peripheral T cell repertoire: independent homeostatic regulation of virgin and activated $CD8^+$ T cell pools. *Eur. J. Immunol. 25:2127.*
35. Sprent, J., and D. F. Tough. 1994. Lymphocyte life-span and memory. *Science 265:1395.*
36. Bunce, C., and E. B. Bell. 1997. CD45RC isoforms define two types of CD4 memory T cells, one of which depends on persisting antigen. *J. Exp. Med. 185:767.*
37. Beverley, P. C., C. A. Michie, and J. L. Young. 1993. Memory and the lifespan of human T lymphocytes. *Leukemia 7:S50.*
38. Tanchot, C., F. A. Lemonnier, B. Perarnau, A. A. Freitas, and B. Rocha. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science 276:2057.*
39. Cossarizza, A., C. Ortolani, R. Paganelli, D. Barbieri, D. Monti, P. Sansoni, U. Fagiolo, G. Castellani, F. Bersani, M. Londei, and C. Franceschi. 1996. CD45 isoforms expression on $CD4^+$ and $CD8^+$ T cells throughout life, from newborns to centenarians: implications for T cell memory. *Mech. Ageing Dev. 86:173.*
40. De Paoli, P., S. Battistin, and G. F. Santini. 1988. Age-related changes in human lymphocyte subsets: progressive reduction of the CD4 CD45R (suppressor inducer) population. *Clin. Immunol. Immunopathol. 48:290.*
41. Ahmed, R., and D. Gray. 1996. Immunological memory and protective immunity: understanding their relation. *Science 272:54.*
42. Carslaw, H., and J. C. Jaeger. 1947. *Operational Methods in Applied Mathematics*. Oxford University Press, Oxford, U.K.

[4] K. C. Wolthers, A. J. Noest, S. A. Otto, F. Miedema, and R. J. De Boer. Mathematical modelling of $CD4^+$ T cell subset TRF length in HIV-1 infection allows for only a marginally increased T cell turnover. *Submitted for publication.*