Mathematical models of human $CD4^+$ T-cell population kinetics

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ABSTRACT

We review how mathematical models help the interpretation of data measuring $CD4^+$ T-cell kinetics by two recently-developed techniques. Mathematical models are developed for the average content of T-cell receptor excision circles (TRECs) and the average telomeric restriction fragment (TRF) in T-cells in the peripheral blood. Changes in the TRECs were supposed to indicate changes in thymic production. The rate at which naive and memory $CD4^+$ T-cells erode their telomeres was supposed to reflect their respective division rates. Analysing the mathematical models, we show that rapid changes in the TRECs per naive T-cell are most likely due to changes in the division rates, and that the rates of telomere erosion fail to reflect naive and memory division rates. The model is applied to explain data showing that rheumatoid arthritis (RA) patients have abnormal TRECs and telomeres.

INTRODUCTION

Surprisingly little is known about the population dynamics, i.e. the production rates, the division rates, and the distribution of life-spans, of human lymphocytic populations. As a consequence, fundamental questions like the maintenance of memory, the maintenance of a diverse naive repertoire, and the role of homeostatic mechanisms, remain largely unresolved. Additionally, we fail to understand how human diseases (e.g. HIV-1 infection and rheumatoid arthritis (RA)) affect normal lymphocyte kinetics, with sometimes fatal consequences. Having so little insight into the normal lymphocyte population dynamics hampers our understanding of immune reconstitution after therapeutic interventions such as chemotherapy, irradiation and/or bone marrow transplantation. This review summarises current work on the characterisation of $CD4^+$ T-cell dynamics in healthy human adults, and discusses how diseases may interfere with the $CD4^+$ T-cell kinetics.

The dynamics of lymphocyte populations have largely been studied in mice.[1-3] The consensus seems to be that mouse naive T-cells are relatively long-lived and are mostly produced by bone marrow progenitors maturing and expanding in the thymus.[4,5] Memory T-cells can be long-lived or short-lived,[3] are generated during immune reactions and by 'homeostatic proliferation'[6] and are partly maintained by renewal, i.e. by low-level proliferation.[4,7] Total body counts of naive and memory $CD8^+$ T-lymphocytes seem to be regulated independently.[8] There is strong evidence in favour of a homeostatic regulation of total body lymphocyte counts by competition between lymphocytes for survival and/or renewal signals.[3,6,9] Apparently, there is no typical 'preprogrammed' lymphocyte life-span,[1,10,11] and not even a typical exponential half-life, because death and division rates are influenced by the total lymphocyte density. It remains unclear, however, how these rodent data translate to the human system.

The kinetics of human T-cells have recently been studied with two novel techniques. This first is measuring telomere lengths as a measure for the replicative history of naive and memory T-cells.[12,13] The second measures thymic production by excision circles generated when T-cell receptors recombine.[14-18] In this review we will show that these techniques provide data that can hardly be interpreted without analysis with appropriate mathematical models. Before embarking on the mathematics let us first consider some basic numbers.

TOTAL LYMPHOCYTE COUNTS AND PRODUCTION

Estimates for the total numbers of $CD4^+$ T-lymphocytes in a human body are calculated either by extrapolating from peripheral blood counts, or from small samples of lymphoid tissue. It is conventionally assumed that in a healthy

human adult 2% of the lymphocytes reside in the blood.[19] Considering that human adults have 5 litres of blood, with a typical $CD4^+$ T-cell count of a 1000 cells/μl, one obtains a total estimate of $1000 \times 50 \times 5 \times 10^6 = 2.5 \times 10^{11}$ $CD4^+$ T-cells.[20] Studies from the group of Haase[21] document the numbers of $CD4^+$ T-cells in peripheral blood and lymphoid tissue (i.e. mostly tonsils). The average of five healthy subjects yields a total of 2.24×10^{11} $CD4^+$ T-cells in the lymphoid tissue, and an average of 4.85×10^9 $CD4^+$ T-cells in the peripheral blood.[21] Thus, 2.1% of the $CD4^+$ T-cells were residing in the blood. From similar measurements in lymph nodes and peripheral blood we calculated that an average of 1.6% of the $CD4^+$ are residing in the blood.[22] Both are reassuringly close to the conventional estimate of 2%. Summarising, a fair estimate for the total count of $CD4^+$ T-cells in a human adult is 2.5×10^{11} cells. Since there is an approximately 1:1 ratio of the naive $CD45RA^+$ and memory/effector $CD45RO^+$ subpopulations,[23-25] we estimate that a typical healthy human adult harbours about 10^{11} naive and 10^{11} $CD4^+$ memory cells.

Total production rates have been estimated from the recovery of the $CD4^+$ T-cell counts in the peripheral blood following T-cell depletion by thoracic duct drainage,[26] flow centrifugation,[27,28] irradiation, chemotherapy, and monoclonal antibodies (mAb).[29] Typically, recovery rates are slow, i.e. about 10^9 cells/day,[29] and are largely due to proliferation of memory cells.[30,31] In human adults the recovery of naive $CD4^+$ T-cells following depletion is much slower than that of memory cells, and slower than the naive recovery in children, due to a decreasing *de novo* production of naive $CD4^+$ T-cells in the thymus.[30-34] The amount of productive tissue in the thymus decreases by 4-5% per year.[35] In a group of HIV-1$^+$ patients treated with HAART, the recovery rate of the naive $CD4^+$ $CD45RA^+$ subset indeed decreased about 5% per year.[36]

We previously estimated the $CD4^+$ naive and memory T-cell recovery rates from studies in which the $CD4^+$ counts of RA patients[37] and multiple sclerosis (MS) patients[38] were depleted by treatment with CD4mAb. In contrast to most previous studies, we found hardly any difference between the average recovery rates of naive $CD45RA^+$ and memory/effector $CD45RO^+$ $CD4^+$ T counts in these two patient groups.[29] Both naive and memory recovery rates were very low and amounted to 2.5×10^7 cells/day in MS patients and 1.5×10^8 in cells/day in RA patients.[29] The reasons for this exceptionally low memory production, and for the almost tenfold difference between the two studies, remain unclear. Mathematical models[9] suggest that the poor recovery of memory cells could be due to the oligoclonality of the T-cell repertoire in RA patients.[39]

The fraction of cells in division can be assessed with the Ki67 mAb. Measurements in the peripheral blood of healthy human adults' $CD4^+$ T-cells reveal that about 0.4-0.8% of the $CD45RA^+$ naive cells are $Ki67^+$,[40-42] and that 2.7-5.5% of the $CD45RO^+$ memory/effector cells are $Ki67^+$.[40,41] Memory cells therefore seem to divide at 3.5 to sevenfold higher frequencies than naive $CD4^+$ T-cells (which is in surprisingly good agreement with a classic early study of naive and memory T-cell division rates.[43]). Part of this difference may be due to the subset of memory/effector cells taking part in ongoing immune responses to antigen. Splitting $CD45RO^+$ $CD4^+$ T memory cells into $CD27^+$ and $CD27^-$ subpopulations,[44] we indeed found that $CD45RO^+$ $CD4^+$ T memory cells are heterogenous. The $CD27^+$ memory subpopulation had a twofold higher fraction of $Ki67^+$ cells.[45]

Assuming that cell division takes about one day, and extrapolating from the blood to the total body, these fractions amount to a normal production of 4×10^8 to 8×10^8 naive $CD4^+$ T-cells/day and 3×10^9 $CD45RO^+$ $CD4^+$ T-cells/day. These numbers are higher than those estimated above from the $CD4^+$ T-cell recovery rates following depletion. Ki67 measurements in the lymphoid tissue (LT) suggest that in healthy adults the fraction of $CD4^+$ T-cells in division is similar in the blood and in the LT.[21,22] In germinal centres and in the T-cell zones of the LT somewhat higher percentages of $Ki67^+$ $CD4^+$ T-cells have been reported.[45] Since these are the areas where immune responses take place these may be the cells undergoing clonal expansion.

Summarising, current estimates for the normal total production rates of $CD4^+$ naive and memory T-cells vary orders of magnitude. There is a consensus that memory/effector cells divide (at least fivefold) more frequently than naive $CD4^+$ T-cells. Additionally, it remains unclear whether this difference is due to memory/effector proliferation during clonal expansion, or whether naive and memory $CD4^+$ T-cells have different renewal kinetics. We first develop a mathematical model to pinpoint the different processes involved in $CD4^+$ T-cell kinetics, and to show that these processes may be density-dependent, i.e. homeostatic. This mathematical model will later be employed to analyse the more complicated techniques measuring TRECs and TRF lengths.

GENERAL MODEL

The peripheral population levels of naive N and memory/effector M $CD4^+$ T-cells can be described by the following general model

$$\frac{dN}{dt} = \sigma_N(t) + \rho_N(N)N - \delta_N(N)N - \alpha_N N \qquad (1)$$

$$\frac{dM}{dt} = \sigma_M(N) + \rho_M(M)M - \delta_M(M)M \qquad (2)$$

where

$$\sigma_M(N) \equiv C_N \alpha_N N_{t-\tau N} \qquad (3)$$

In Eq.(1) $\sigma_N(t)$ is the source of naive $CD4^+$ T-cells from the thymus, which is a decreasing function of the age (time). The two $\delta()$ terms represent death rates, and the two $\rho()$ terms represent renewal rates, which are density-dependent functions to allow for homeostasis. The $\alpha_N N$ term represents the activation/priming of naive T-cells by antigen. This is a stochastic process which is here accounted for by assuming that α_N is a smaller parameter. Activated T-cells expand by proliferation into a clone of memory/ effector cells, which is represented as a source term in Eq.(2). The C_N parameter in Eq. (3) represents the clonal expansion. Because this takes a few days one should allow for a time delay τ_N (because we here typically consider a time-scale of years we let $\tau_N \to 0$). Examples of the functions used in the model are

$$\sigma_N(t) = \sigma_N e^{-\epsilon t},\ \rho_X(X) = \frac{\rho_X}{1 + X/h_X},\ \text{and}\ \delta_X(X) = \delta_X + \epsilon_X X \qquad (4)$$

which represents an exponential decrease in thymic production,[35] an inverse Michaelis-Menten function for the density-dependent renewal, and a linear increase for the density-dependent death respectively.

The total production rates reviewed above allow for 'order of magnitude' estimates for the parameter values of this model for a human adult. For a normal human adult, a rough estimate for the daily production of naive $CD4^+$ T-cells is 10^8 cells/day,[29,36] i.e. at an age of t=30 years, and a steady-state total $CD4^+$ T lymphocyte count of $\bar{N}=10^{11}$ cells $\sigma_N(t)+\rho_N(\bar{N})\bar{N} \simeq 10^8$ cells/day. Most of this production is probably due to the thymus.[5,29,31,34,46,47] Note that this can only be true if for $\bar{N}=10^{11}$ the renewal $\rho_N(\bar{N})<0.001$/day; otherwise, most of the naive T-cell production is due to renewal. The Ki67 data reviewed above[40-42] suggest that in a normal human adult about 0.5% of the naive $CD4^+$ T-cells is in division, i.e. $\rho_N(\bar{N})=0.005$/day. Given that we can only estimate orders of magnitude, this would be in agreement with the 10^8 naive $CD4^+$ cells per day. There is a disagreement, however, with our assumption that normally most of the naive $CD4^+$ T-cells are produced in the thymus. The Ki67 data suggest peripheral division rates[40-42] can easily account for a daily production that is at least as large as that of the thymus.

Whatever the mechanism for the production of naive $CD4^+$ T-cells, a daily production of 10^8 cells, and a steady-state total of $\bar{N}=10^{11}$ cells, means that the death plus priming rate should obey $\delta_N(\bar{N})+\alpha_N \simeq 10^{-3}$/day. Hence naive $CD4^+$ T-cells live on average a thousand days before they die or become primed by antigen. Because priming should be a rare event, most naive $CD4^+$ seem to be lost by death, i.e. $\alpha_N \ll \delta_N(\bar{N})$ at the normal steady state. By the Ki67 measurements the daily production of memory $CD4^+$ T-cells seems at least fivefold higher than that of the naive cells. Moreover, except for the atypical RA and MS patients discussed above, the counts of memory cells recover much more rapidly from $CD4^+$ T-cell depletion than the counts of the naive $CD4^+$ T-cells. It is generally believed that memory $CD4^+$ T-cells are produced by clonal expansion ($\sigma_M(N)$) and by renewal ($\rho_M(M)$). Having the same steady state of $\bar{M}=10^{11}$ memory $CD4^+$ T-cells in a healthy human adult, and an estimated production of 10^9 cells/day, the death rate of memory $CD4^+$ T-cells would amount to $\delta(\bar{M}) \simeq 0.01$/day.

T-CELL RECEPTOR EXCISION CIRCLES

A recently-developed technique has provided evidence that in human adults there is ongoing production of naive $CD4^+$ T-cells in the thymus. Recent thymic emigrants (RTE) can be characterised by circles of DNA that are generated during the T-cell receptor (TCR) rearrangement in the thymus.[14-17,34] In healthy individuals aged from 0 to 80 years the average content of TRECs per $CD4^+$ T-cell decreases almost a hundredfold.[16,18] This observation could be biased by a shift in the proportions of naive and memory $CD4^+$ T-cells over this age range,[25] because memory cells have virtually no TRECs. The study of Poulin et al.,[17] showing a tenfold decline of the TRECs per $CD4^+$ $CD45RA^+$ $CD62L^-$ naive T-cells over 60 years, therefore provides the most conclusive evidence. Adult thymectomy leads to a significant (about tenfold) reduction of the TREC content per peripheral $CD4^+$ cell.[16] Reconstitution of naive $CD4^+$ T-cells after bone marrow transplantation is correlated with an increase in the TRECs, and both the naive $CD4^+$ T-cell recovery and the increase in the TRECs decrease with the age of the patient.[34] HIV-1 patients and RA patients tend to have a lower TREC content per $CD4^+$ T-cell.[16,48,49] Because TRECs seemed to be a good indicator of thymic function, this has been interpreted as evidence for impaired thymic function in these diseases.[16,49] We recently developed a model to demonstrate that this is more likely due to a dilution of the TRECs by increased division rates of naive $CD4^+$ T-cells.[42]

The total TREC content T of the naive $CD4^+$ T population increases by *de novo* production of T-cells in the thymus at rate $\sigma_N(t)$, and decreases when naive $CD4^+$ T-cells die (at rate $\delta_N(N)$), or become primed by antigen (at rate α_N), and by intracellular degradation (at rate δ_I):

$$\frac{dT}{dt} = c\sigma_N(t) - (\delta_N(N) + \alpha_N + \delta_I)T \qquad (5)$$

where c scales the TREC content of a RTE. Note that the total amount of TRECs in the population is not affected by naive T-cell division: only the average TREC content per naive T-cell decreases by division. We define $A \equiv T/N$

and find that the derivative of the quotient of Eq.(5) and Eq.(1) obeys

$$\frac{dA}{dt} = \frac{\sigma_N(t)}{N}\{c - A) - [\delta_I + \rho_N(N)]A \qquad (6)$$

which at steady state will approach

$$\bar{A} = \frac{c}{1 + [\delta_I + \rho_N(\bar{N})]\bar{N}/\sigma_N(t)} \qquad (7)$$

Figure 1 depicts a computer simulation showing that this model accounts for a very realistic decline of the TRECs per naive T-cell with age. Eq.(7) shows that increasing the division rate $\rho_N()$ and decreasing the thymic production $\sigma_N(t)$ both decrease the average TREC content per naive T-cell. Elsewhere[42] we show by numerical simulation that the effect of increasing the division rate on the TRECs is much more rapid than the effect of impairing thymic production. The effect of reducing thymic production is slow because naive $CD4^+$ T-cells are long-lived. The observed low TREC content per $CD4^+$ T-cell during human disease[16,49] is therefore more likely due to immune activation inducing naive $CD4^+$ T-cell division (see also *figure 2*).[42]

Additionally, this model provides evidence for naive T-cell homeostasis. In the absence of homeostasis $\bar{N} \propto \sigma_N(t)$, which would cancel the dependence on thymic production from Eq.(7). The mere fact that the average TREC content per naive $CD4^+$ T-cell decreases with age, i.e. with $\sigma_N(t)$, therefore demonstrates that there is homeostasis of naive $CD4^+$ T-cells. Finally, note that TRECs can only decrease when $\delta_N+\alpha_N > 0$, i.e. there has to be intracellular loss of the TRECs and/or division of naive T-cells;[42]; otherwise

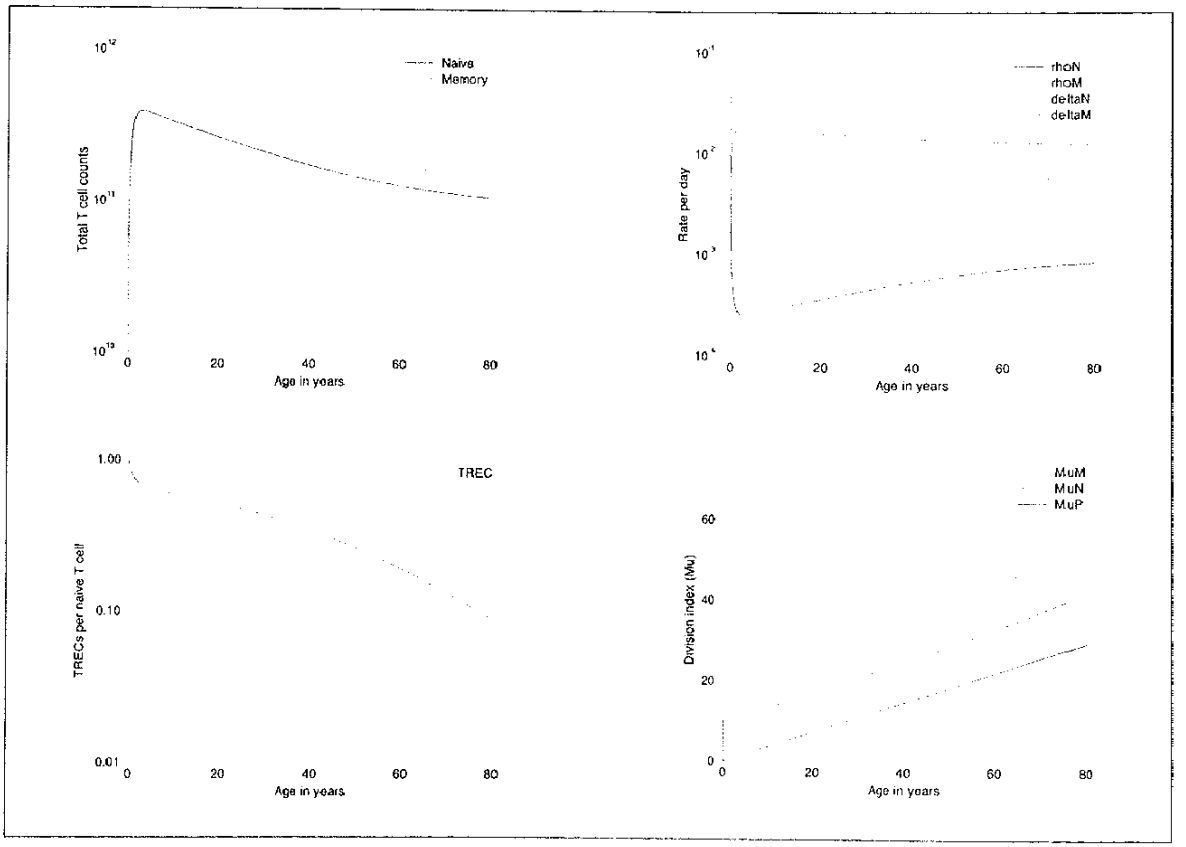

Figure 1
Normal aging of the immune system

As a function of age, we depict the total counts of naive and memory $CD4^+$ T-cells N and M, the renewal and death rates $\rho_N(N)$, $\rho_M(M)$, $\delta_N(N)$ and $\delta_M(M)$, the average TREC content of naive T-cells A, and the division indices μ_P, μ_N, and μ_M as a measure for the telomere erosion. We depict a scenario where the naive T population is largely maintained by thymic production (note in the figure that $\rho_N(N)<\delta_N(N)$), and where the memory population is largely maintained by priming and expansion of naive T-cells (note that $\rho_M(M)<\delta_N(M)$). Parameters: $\alpha_N = 10^{-6}$/day, $C_N = 10^4$ cells, $\delta_M = 0.005$/day, $\delta_N = 0.005$/day, $\delta_I = 0.001$/day, $\epsilon_M = \epsilon_N = 10^{-11}$/cell, $h_M = h_N = 10^{10}$ cells, $\kappa = 0.001$/day, $K_P = K_N = 10$ divisions, $\sigma_N(t) = 10^9 e^{-0.05t/365}$, i.e. a decline of 5% per year, $\rho_M = 0.1$/day, and $\rho_N = 0.01$/day. At age zero we start with an 'empty' immune system $N = M = \mu_P = \mu_N = \mu_M = 0$.

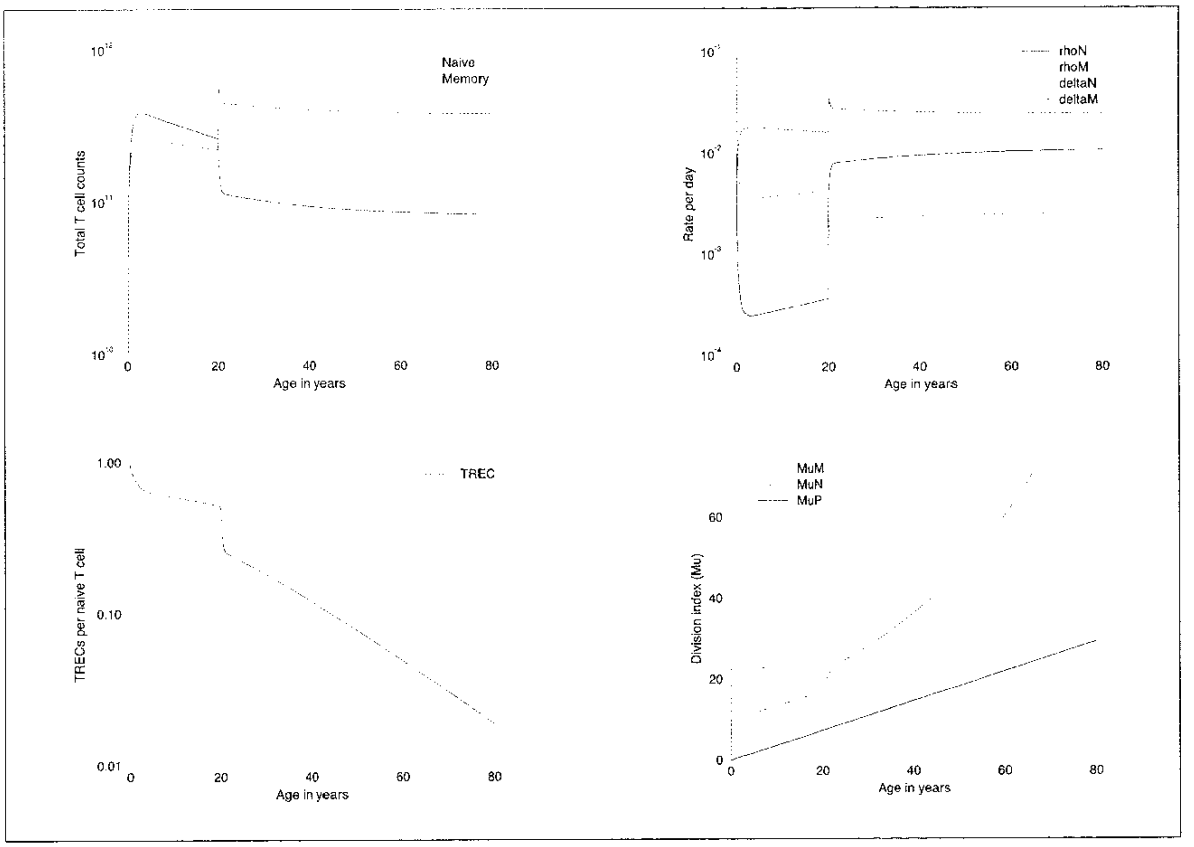

Figure 2
The effect of diseases leading to chronic activation of naive T-cells

Parameters as in *figure* 1, but at age 20 we increase ρ_N 10-fold and α_N 10^4-fold to simulate chronic activation of naive T-cells. To prevent a large increase in the memory population we compensate for increasing the priming rate by decreasing the clonal expansion 1000-fold.

$\bar{A}=c$. This also demonstrates that the observed decrease in the TRECs is no evidence for naive T-cell division. The average TREC content of naive T-cells also declines with age, i.e. with the thymic production $\sigma_N(t)$, when $\rho_N=0$ and there is density-dependent death $\delta_N(N)$ and intracellular degradation of the TRECs, i.e. $\delta_i > 0$ (Dutilh, in preparation).

TELOMERIC RESTRICTION FRAGMENTS (TRF)

The difference in the division rates between naive and memory $CD4^+$ T-cells has been studied by measuring telomere lengths. Telomeres are TTAGGG repeats at the very end of chromosomes, which shorten about a hundred bases per cell division.[50-52] In human $CD4^+$ T-cells, the average TRF length of $CD45RA^+$ naive T-cells is about 1400 bases longer than that of $CD45RO^+$ memory T-cells.[12] Although there is a large variation amongst individuals in the average TRF length of their $CD4^+$ $CD45RA^+$ naive T-cells, this distance of 1400 bases between the naive and memory cells is surprisingly constant.[12,13] A cross-sectional analysis of the average telomere lengths of $CD45RA^+$ naive and $CD45RO^+$ $CD4^+$ T-cells from individuals varying between 24 and 72 years of age demonstrated that in each compartment the loss rate was about 33 bases per year. It was therefore estimated that the frequency of cell division in both naive and memory $CD4^+$ T-cells is about once every three years.[12] This is surprising because most data reviewed above show that memory/effector cells divide at least five times more frequently than naive cells do.

We recently developed a mathematical model for telomere erosion and demonstrated that these telomere data cannot allow for the conclusion that naive and memory $CD4^+$ T-cells divide at a similar frequency.[53,54] The CD4 T-cell population is composed of a 'maturation' cascade of a progenitor population, naive T-cells, and memory $CD4^+$ T-cells. Because cells move through these compartments, the telomere loss rates of the different compartments are strongly linked. Provided there is a sufficient flux of cells

between the compartments, one expects the telomere loss rate in a compartment to approach that of the previous compartment, irrespective of the actual division rate within that compartment.[53] The empirical fact that the loss rate in the memory $CD4^+$ T-cell compartment equals that of the naive $CD4^+$ T-cells[12,13] is in full agreement with this.

To keep track of telomere lengths we have to rewrite Eq. (1) by substituting a true source of progenitor cells for the $\sigma_N(t)$ term. Although progenitor cells express telomerase, they do erode their average TRF length.[55] T-cell progenitors are produced by renewal in the bone marrow, migrate to the thymus where they undergo a cascade of cell divisions, and migrate into the periphery as immunocompetent naive T-cells. In our model this resembles the clonal expansion of naive cells contributing to the memory population. We therefore substitute in Eq. (1)

$$\sigma_N(t)=Cp(t)\alpha p(t)P_{t-\tau p} \qquad (8)$$

Here $Cp(t)$ represents the progenitor expansion in the thymus taking about τp days (by again considering a time-scale of years we will let $\tau p \rightarrow 0$) The αpP term represents the seeding of the thymus with progenitor cells, which may decrease by thymic involution.

We write a TRF model in terms of the average number μ of divisions the cells have completed.[53,54] The average TRF length decreases proportionally to μ. For the division index of progenitor cells, naive and memory $CD4^+$ T-cells we previously[54] derived

$$\frac{d\mu_P}{dt} = \kappa \qquad (9)$$

$$\frac{d\mu_N}{dt} = 2\rho_N(N) \cdot (\sigma_N(t)/N)(\mu_N - \mu_P - K_P) \qquad (10)$$

$$\frac{d\mu_M}{dt} = 2\rho_M(M) \cdot (\sigma_M(N)/M)(\mu_M - \mu_N - K_N) \qquad (11)$$

where κ is the daily telomere erosion of progenitors. Because bone marrow progenitors express telomerase,[55] κ is not equal to the progenitor renewal rate. K_P is the (unknown) telomere erosion due to the expansion of progenitor cells in the thymus and K_N is the average telomere erosion during clonal expansion. Because proliferating T-cells express telomerase,[56] K_N is less than the number of divisions required for the clonal expansion.

This model is conveniently analysed by considering the quasi-steady-state differences Δ between the average TRF length between naive T-cells and progenitors (Δ_{NP}), and memory T-cells and naive T-cells (Δ_{MN}) respectively. By Eqs. (10-11), the equations for the differences, $d\Delta_{NP}/dt=d\mu_N/dt-d\mu_P/dt$ and $d\Delta_{MN}/dt=d\mu_M/dt-d\mu_N/dt$, are expected to approach a quasi-steady state at time-scales $N/\sigma_N(t)$ and $M/\sigma_M(N)$ respectively. Since the quasi-steady states $\bar{N}$ and $\bar{M}$ obey $\bar{N}=\sigma_N(t)/[\delta_N(\bar{N})+\alpha_N-\rho_N(\bar{N})]$ and $\bar{M}=\sigma_M(\bar{N})/[\delta_M(\bar{M})-\rho_M(\bar{M})]$ respectively, one obtains quasi-steady state differences derived

$$\Delta_{NP} = K_P + \frac{2\rho_N(N) - \kappa}{\delta_N(\bar{N}) + \alpha_N - \rho_N(\bar{N})} \qquad (12)$$

$$\Delta_{MN} = K_N + \frac{2\rho_M(M) - \kappa}{\delta_M(\bar{M}) - \rho_M(\bar{M})} \qquad (13)$$

which are approached at time scales $1/[\delta_N(N)+\alpha_N-\rho_N(N)]$ and $1/[\delta_M(M)-\rho_M(M)]$ respectively.

Thus, whenever $\rho_N(N) \ll \delta_N(N)+\alpha_N$, i.e. whenever the naive T-cell population is largely maintained by the thymic production $\sigma_N(t)$, one expects Δ_{NP} to approach a bounded quasi-steady-state distance on a reasonably short time-scale. When Δ_{NP} has approached this quasi-steady state, the rate of telomere erosion of naive T-cells reflects that of the progenitor cells κ (see *figure 1*). If, on the other hand, naive T-cells are largely maintained by renewal, i.e. if $\rho_N(N) \approx \delta_N(N)+\alpha_N$ and $\sigma_N(t)/N \rightarrow 0$, the steady-state difference becomes very large, and will only be approached over an exceedingly slow time-scale. By Eq. (10) the average naive TRF length is then expected to shorten at a rate proportional to $2\rho_N()$. Because $\sigma_N(t)$ is declining by 5% per year, the maintenance of naive cells may slowly move from a thymic production scenario to a renewal scenario. Careful inspection of Fig. 1 indeed reveals that the telomere erosion of the T-cells is accelerating somewhat in old age. Note that the average TRF length of memory cells (i.e. μ_M) remains parallel to this acceleration.

Indeed, one expects Δ_{MN} to approach a bounded quasi-steady-state distance over a reasonably short time-scale whenever $\rho_M(\bar{M}) \ll \delta_M(\bar{M})$, i.e. whenever the memory T-cell population is largely maintained by the priming of naive T-cells and the subsequent clonal expansion $\sigma_M(N)$. Thus, for sufficiently small renewal rates of memory T-cells, one expects the TRF length difference Δ_{MN} to approach a fixed steady-state distance. This is confirmed by the simulation in *figure 1*, and is in excellent agreement with the data demonstrating the parallel decrease in TRF lengths of naive and memory $CD4^+$ T-cells with age.[12,13] When this steady state has been approached, the average TRF length of memory cells decreases at the same rate at which naive T-cells erode their telomeres. If, on the other hand, $\rho_M(M) \approx \delta_M(M)$ and $\sigma_M(N)/M \rightarrow 0$, one expects the memory telomeres to erode at a rate proportional to the memory division rate (see Eq. (11)).

Summarising, the telomere erosion of naive T-cells is only expected to reflect the naive T-cell division rate $\rho N()$ when the thymic production $\sigma_N()$ has a sufficiently small contribution to the maintenance of the naive T population.

Because this seems unrealistic for normal human adults,[5,29,31,34,46,47] we expect the observed telomere erosion of naive T-cells to reflect the rate of telomere erosion of progenitor cells κ. This is confirmed by the observation that granulocytes and naive T-cells have a parallel decline in TRF length with age.[13] The fact that the telomere erosion of naive and memory T-cells is experimentally observed to run at a similar rate[12,13] suggests that the memory population is largely maintained by priming of naive T-cells $\sigma_N()$, and not by the renewal $\rho_M()$.

COMPUTER SIMULATION

To confirm our analysis, and to illustrate the normal effects of aging, *figure 1* depicts a numerical simulation of the model. The total population sizes of naive and memory T-cells rapidly approach quasi-steady states of about 10^{11} cells. Although thymic production decreases from 10^9 cells/day in the newborn to 1.8×10^7 cells/day at age 80, the total population size of the naive T-cells varies only severalfold. This is due to a homeostatic increase in the renewal rate and decrease in the death rate (see *figure 1*). The TRECs per naive T-cell decrease very realistically by the combination of intracellular decay and density-dependent death and renewal (see Eq. (7)). Due to the decreasing thymic production, the naive T-cell division rate $\rho_N()$ increases later in life by homeostasis, which accelerates the loss of TRECs with age. To compensate for the decline of $\sigma_M(N)$ with age, which is due to the (small) decline of the naive T population with age, the memory renewal rate $\rho_M()$ also increases later in life. Note that renewal rates always remain smaller than the death rates: throughout life both naive and memory populations are largely maintained by the sources σ from progenitors and primed naive T-cells respectively. As predicted by Eq. (12) and Eq. (13), the naive and memory telomeres therefore run parallel to the TRF shortening of progenitors. If one were to measure dividing cells in this model, to simulate Ki67 measurements, the bulk of the $Ki67^+$ memory cells would be involved in clonal expansion (i.e. be represented in the $\sigma_M(N)$ term).

We have also studied the other scenarios where the naive and/or memory populations are largely maintained by renewal (i.e. scenarios where $\rho_X(X) \simeq \delta_X(X)$ and $\sigma_X() \to 0$). A hundredfold reduction of the thymic production, for instance, gives very similar population dynamics to those depicted in *figure 1*. The TRECs per naive T-cell decline by three orders of magnitude, however, and the naive division index μ_N no longer runs parallel to that of the progenitors, but at the rate $2\rho_N()$/day. The memory division index μ_N still runs parallel to that of the naive T-cells (not shown). Similarly, a hundredfold decrease in the naive priming rate α_N yields a picture very similar to *figure 1*, but with a memory telomere erosion that runs faster, i.e. at a rate proportional to $2\rho_M()$/day, than that of the naive and progenitor cells (not shown).

RHEUMATOID ARTHRITIS

Two recent papers describe the abnormal $CD4^+$ T-cell kinetics in RA patients.[39,49] Both naive and memory $CD4^+$ T-cell repertoires are oligoclonal, suggesting that some clones expand at the expense of others.[39] The TREC content of $CD4^+$ T-cells is lower than normal, and the TRF lengths of both naive and memory $CD4^+$ T-cells are shorter than normal.[49] By their TRECs and telomeres the RA patients seemed 20-30 years older than age-matched controls. The total naive and memory population sizes seem normal however.[49] These abnormalities appear very early in the disease, with no progression over the course of the disease.[49] The data was therefore interpreted as premature thymic involution, which by a subsequent homeostatic response increasing the renewal rate is causing premature erosion of TRECs and telomeres.[49]

We have shown elsewhere[42] that premature thymic involution fails to explain a rapid loss of the TRECs because naive T-cells are long-lived. Since the loss rate of TRECs with age was similar in RA patients and controls,[49] and because the difference Δ in TRF length between RA patients and controls was not increasing with disease duration,[49] it seems likely that the RA patients rapidly approach a new steady state. For instance, a new steady state could be brought about by chronic activation increasing naive T-cell division and priming rates, due to the inflammatory nature of RA. By Eq. (7) increasing $\rho_N()$ would rapidly[42] decrease the TRECs to a new quasi steady state, and by Eq. (12) increasing $\rho_N()$ would rapidly shorten the TRF lengths of naive T-cells. Since activation driven division of naive T-cells would also explain the oligoclonality of the repertoire,[39] there is no need to invoke a premature decline of thymic production to explain the data.

This is studied in *figure 2*, where we increase the naive T-cell activation of a normal individual at age 20 by increasing the renewal $\rho_N()$ tenfold and the priming rate $\alpha_N()$ 10^4-fold. The latter sets $\alpha_N > \delta_N(N)$ such that naive cells disappear by priming rather than by death. Since the increased priming of naive cells would increase the memory population, and because we aim to represent a chronic activation, we compensate for increasing $\alpha_N()$ by decreasing the clonal expansion C_N a thousandfold. *Figure 2* shows that such a chronic activation rapidly decreases the TRECs to a new steady state, after which they continue to decline at a rate similar to the normal rate depicted in *figure 1*. The naive and memory telomeres

shorten very rapidly during an initial transient. Later the naive and memory telomere erosion approach the same increased loss rate $2\rho_N(N)$, which is only somewhat faster than that of the progenitors (note in *figure 2* that $\rho_N(N) \simeq \delta_N(N) + \alpha_N \simeq 0.01$/day. Summarising, a chronic activation of naive T-cells seems sufficient to account for the observations of Koetz et al.[49]

DISCUSSION

We have reviewed several cases where the correct interpretation of experimental results required analysis by mathematical modelling. Results that at first seemed intuitive were proven to be inconclusive when analysed with an appropriate mathematical model. Current mathematical models also remain inconclusive, however, because most of their parameters remain very rough guesses. Modelling efforts can therefore be improved by appropriate experiments allowing a better characterisation of human lymphocytic dynamics, and interpretation of experiments can be improved significantly by mathematical modelling. Both call for an increase in the collaborative work between experimentalists and theoreticians in immunology.

A major unresolved issue in the area of $CD4^+$ T-cell dynamics remains the division of naive cells.
Some argue that naive T-cells will only divide when stimulated by their cognate antigens.[3] Others argue for 'homeostatic proliferation'[6] in naive T-cell compartments. We published data that healthy Ethiopians have lower than normal naive $CD4^+$ T-cell counts in the blood, and found that the lower the count the higher the fraction of $Ki67^+$ naive $CD4^+$ T-cells in division.[42] HIV-1-infected patients also have low naive T-cell counts and a similar relation between the count and the naive T-cell division rate.[40-42]

Homeostatic proliferation is not truly indicative for naive T-cell homeostasis. Most publications demonstrate that the T-cells lose their naive CD45RA markers and hence contribute to the $CD45RO^+$ memory population.[6] This is supported by observations showing that the recovery of the $CD4^+$ T-cell repertoire diversity following irradiation is largely due to the slow recovery of $CD45RA^+$ naive T-cells, and is not represented in $CD45RO^+$ memory T-cells.[30,31] Additionally, the total number of naive $CD4^+$ T-cells in the blood correlates positively with the size of the thymus as assessed by computed tomography (CT) scans.[47] Finally, naive recovery in human adults is slow, and correlates with thymic function and age and with an increase in the TRECs.[34]

The fact that naive T-cell division rates in HIV-1 infected patients and in healthy Ethiopians correlate negatively with the naive T-cell counts,[40-42] seems to suggest a major role for homeostatic renewal within the naive compartments. Several lines of evidence argue against this however. First, low $CD4^+$ T-cell counts are strongly associated with high HIV-1 loads, and with other infections activating the immune system. Thus the correlation between the naive CD4 T-cell count and naive T-cell division is, at least partly, spurious.[37] Increased activation of the immune system in healthy Ethiopians could also account for their low naive $CD4^+$ T-cell counts and for their increased naive T-cell division rates.[42] Second, increased activation at low naive $CD4^+$ T-cell counts would explain why the division rate of naive $CD8^+$ is negatively correlated with the naive $CD4^+$ T-cell count[40,41] (as if CD4 homeostasis were driving $CD8^+$ naive T-cell division). Third, the high naive T-cell division rates in HIV-1 patients with low naive $CD4^+$ T-cell counts drop rapidly during anti-viral therapy reducing viral levels, and hence reducing immune activation, while the naive T-cell counts hardly recover.[41]

Summarising, the observed high fractions of $Ki67^+$ naive T-cells in the blood when naive $CD4^+$ T-cell counts are low (i.e. less than 100 cells/μl) together with the fact that this correlates with decreased TREC levels in these cells[42] prove that such aberrant circumstances trigger division of naive $CD4^+$ T-cells in the periphery. The nature of the signals driving this division remains enigmatic however.

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Discussion following lecture of R.J. de Boer

J. Vandenbroucke: Just to be provocative, although I am fond of mathematics I would like to know what is the necessity of mathematics? When they develop mathematic models, mathematicians first make an intuitive jump and then try to prove that they are correct. You probably also made intuitive jumps first, by performing extreme thought experiments. For example, what will happen if we shut off the thymus? You probably knew in advance what would happen, because you knew the explanation. The explanation was that the longevity of the cells is so high that one would not see any effect. So I suspect that you read that paper by Douek et al.[1] and immediately knew that the authors were wrong, because you made the jump from your extreme thought experiment and you modelled it. Not to belittle what you have done, but this is to show people who maybe are not mathematically inclined that you can start with an extreme thought experiment about cells and end up making a model of it.

R. de Boer: Basically, I agree. The model is just the tool to support our thinking. So we do thought experiments, but we do better thought experiments because we have the model as a sort of back-up.

Levin: The point that Dr Vandenbroucke raises is well taken, but experimentalists have the same problem. As a friend of mine put it, "I would not have seen it, if I had not already believed it." I think Dr de Boer said, "Look, there is an alternative explanation for these observations." The authors would probably say no, because they believe in their interpretation. This kind of thought experiment however enables you to consider the alternatives. Mathematics makes it such that you cannot mess around. Another benefit of mathematical modelling is that you have to say precisely what you are doing.

Van Wijngaarden: Many years ago, attempts were made to cure AIDS patients by implanting thymic tissue. If I understand you correctly, you are giving a kind of explanation why that does not work, is that correct?

R. de Boer: You are taking things a little further than I said. I said there is currently no evidence that thymic production in HIV patients is more impaired than in other people in middle life. You could argue that it makes sense to give HIV patients additional thymic tissue exactly for the reasons Dr Wodarz has been giving, because you want to expand the repertoire of cells able to respond to the virus. The implanting experiments you refer to were a failure, because in the presence of HIV these cells are rapidly infected. Maybe now again with Dr Wodarz' results, you could help CD4 response to develop by giving a thymus graft.

Van der Meer: Perhaps you would model that first before doing such an experiment?

R. de Boer: Yes, I would always model it first. I would have to use a totally different model, because now we are talking about repertoires and until now I have been talking only about total T-cell numbers.

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