

The Effects of Age, Thymectomy, and HIV Infection on α and β TCR Excision Circles in Naive T Cells¹

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Due to homeostasis total naive T cell numbers remain fairly constant over life despite a gradual involution of the thymus. The contribution of the thymus to maintaining naive T cell pools is typically measured with TCR excision circles (TRECs) that are formed in thymocytes. The mechanisms underlying thymic involution are poorly understood. Some data suggest that thymocytes undergo fewer divisions in old (small) than young (large) thymi, and other data suggest that the number of TRECs per thymocyte is independent of age. If thymic involution were associated with a decreased number of divisions of the thymocytes, this would markedly complicate the interpretation of TREC data. To study this we develop a mathematical model in which the division rate of thymocytes decreases with increasing age. We describe the dilution of TRECs formed during the arrangement of both chains of the TCR by division of thymocytes, recent thymic emigrants, and mature naive T cells. The model behavior is complicated as TREC contents in naive T cells can increase with age due to decreased dilution in the thymus. Because our model is consistent with current data on the effects of age and thymectomy on TRECs in peripheral T cells, we conclude that aging may well affect thymocyte division, which markedly complicates the interpretation of TREC data. It is possible, but more difficult, to let the model be consistent with the rapid changes in α and β TRECs observed shortly after HIV infection. *The Journal of Immunology*, 2006, 177: 4391–4401.

T cell receptor excision circles (TRECs)⁴ are stable circular DNA fragments that are excised during TCR formation in the developing T lymphocyte in the thymus (1–3). In this process, several rearrangement events take place in a particular order (4). During the triple-negative thymocyte stage, the β -chain of the TCR is rearranged and the $D\beta J\beta$ -TREC is excised. Several divisions later, at the double-negative stage, the α -chain is rearranged and the signal-joint (sj) TREC is formed, and again, three to four divisions later, the coding-joint TREC is formed (2). TRECs are not replicated during cell division and are passed on to one of the two daughter cells. TREC measurements are typically expressed as the number of TRECs per cell and are called the average TREC content. Measuring the total number of TRECs in a population of cells is a much better measure of thymic output (5, 6) but requires estimates of total cell numbers.

Naive T cells originate from cells emigrating from the thymus, and the total amount of productive thymic tissue decreases ~100-fold with increasing age (7). Because TRECs are formed in the thymus, they seem to be an excellent tool to measure the contribution of the thymus to the peripheral T cell pool (2, 8). TRECs have been measured in PBMCs, CD4⁺, and CD8⁺ T cells, and in their naive and memory subpopulations. The average sj-TREC content in the blood of healthy human volunteers decreases >10-

fold in individuals of increasing age (2, 8–13). First, it was thought that this decline in TREC content was a direct result of the age-related decline in thymic output (2). However, mathematical modeling demonstrated that decreased thymic production is certainly required, but is not sufficient (14, 15). The average TREC content can decline only with decreasing thymic output in the presence of homeostasis compensating for the decreased thymic output (14, 15). Homeostatically increased division rates (14), and/or expected life spans (15), of naive T cells, dilute the TREC contents. Otherwise, decreased thymic output decreases TREC numbers and cell numbers to a similar extent, and TREC contents remain unaffected.

The mechanisms underlying thymic involution are poorly understood (16). The study of Jamieson et al. (8) suggested that thymus tissue remains functional in the elderly, and that there is a similar level of TCR rearrangement because the sj-TREC content of thymocytes remains independent of age. This would suggest that in old thymi there is just a smaller total number of thymocytes, and that each thymocyte undergoes the same conveyor belt program with the same number of divisions as thymocytes in young thymi. These data (8) seem to contradict earlier data demonstrating that Con A-stimulated thymocytes from old thymi incorporated less BrdU than thymocytes from young thymi (17, 18). Dion et al. (12) studying $D\beta J\beta$ -TRECs and sj- α -TRECs in the blood found that the β -TREC content is fairly independent of age, while the sj/ β -TREC ratio decreased >10-fold with increasing age. Because this ratio is not influenced by peripheral changes in division and death, this also suggested a decreased number of cell divisions between the β and α rearrangements with increasing age (12). The interpretation of TREC data becomes much more complicated when the number of thymocyte divisions depends on age because TREC contents are expected to increase with age if there is less dilution in the thymus. We therefore developed a novel mathematical model to learn what to expect if thymic involution were associated with fewer divisions (12, 17, 18), and to study whether this can be reconciled with the age-independent sj-TREC content of thymocytes (8).

Dion et al. (12) also stressed the importance of recent thymic emigrants (RTEs) as a population with a much higher turnover

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⁴ Abbreviations used in this paper: TREC, TCR excision circle; sj, signal-joint; RTE, recent thymic emigrant.

than truly naive T cells, and a much higher TREC content. If most of the TRECs were located in a small subpopulation of the naive T cells, one would be able to explain rapid changes in TREC contents in the presence of slow changes in naive T cell numbers (12, 13, 19). The hypothesis of a separate RTE population with high turnover is supported by Berzins et al. (20), who found that the introduction of extra thymic lobes in mice led to an approximately linear increase in the size of the total naive cell pool that was equivalent to the amount of RTEs produced by the extra lobes in three weeks. Additionally, data from Boursalian et al. (21) show that RTEs are immature and undergo further differentiation. We study the impact of RTEs on TREC contents of the peripheral naive T cell pool by including such a separate population in our model. Following the suggestion of Berzins et al. (20), RTEs in our model are not under the homeostatic control of the naive T cells, and are short-lived. We assume that their recruitment into the pool of truly naive T cells depends on the total size of that pool.

The last objective in this study is to examine the effect of HIV infection on T cell kinetics in our model. In humans, naive $CD4^+$ and $CD8^+$ T cells are depleted during HIV infection (22). The mechanism that underlies this depletion is still unknown. Naive $CD4^+$ T cells may be lost directly by infection, and/or by hyperactivation, and thus be transferred to the memory pool (23, 24). Infection of naive $CD4^+$ T cells becomes most pronounced at later stages of disease after the R5 to X4 phenotype switch of the virus (25). However, HIV can also infect $CD4^+$ thymocytes, and thereby reduce thymic output of naive T cells (2, 26). With our model we attempt to provide more insight in the effects of these mechanisms on decreasing total naive T cell numbers and their TRECs.

Materials and Methods

The mathematical model describes $TCR\beta^+$ thymocytes that have rearranged the β -chain of the TCR and that die, divide, and mature into a later class of $TCR\alpha\beta^+$ thymocytes that have also rearranged the α -chain of the TCR, and that also die, divide, and mature to ultimately leave the thymus to become an RTE, and possibly a truly naive T cell (Fig. 1). The first $TCR\beta^+$ thymocytes may or may not contain a β -TREC, and divide at an age-dependent rate, ρ_i , die at rate δ_b , and mature to the $TCR\alpha\beta^+$ thymocytes at rate μ_b (Fig. 1). Because these $TCR\beta^+$ thymocytes may contain a β -TREC they are called B_i in the model, where the subscript i denotes the number of divisions these cells have completed (to later compute their average TREC content (see below)). A similar model applies to the $TCR\alpha\beta^+$ thymocytes, which are called S_j in the model, because they have formed the sj-TREC. Because these cells are subjected to positive and negative selection we give them a higher death rate δ_s . Their maturation into RTE is incorporated as an efflux rate ϵ . Thymocytes at the $TCR\alpha\beta^+$ stage exit from the thymus at a fixed rate ϵ (and after completing κ divisions). The thymocyte division rate, ρ_i , decreases exponentially with age.

The population biology of RTEs is poorly understood. Probably only a small fraction is ultimately incorporated in the naive T cell repertoire (12, 19, 20). The model proposed here is analogous to clonal expansion. Those RTEs receiving sufficient stimulation from self, food and/or foreign peptides after leaving the thymus are rescued at rate μ_r , and undergo a fixed number of divisions, n , at rate ρ_r , and die slowly at rate δ_N (Fig. 1). Their clonal progeny ultimately settles in the naive repertoire. The likelihood, μ_r , that RTEs undergo this clonal expansion to end up in the truly naive repertoire decreases when the size of the truly naive T cell pool increases, according to a simple sigmoidal function. Those RTE that are not rescued die rapidly at rate δ_r (Fig. 1). Because RTEs are not fully differentiated when they leave the thymus (21), we do not allow them to be primed by Ag. Only truly naive cells are primed at rate α .

Truly naive T cells appear by the last division of the RTE, die slowly at a rate δ_N , are primed by Ag at rate α , and have a density dependent renewal ρ_N (Fig. 1). At low naive T cell densities naive T cells maintain themselves by renewal; at high densities the renewal rate decreases by another sigmoid function similar to the μ_r of RTEs. These two sigmoid functions account for the homeostasis in the model: at decreased naive T cell densities there is an increased settlement of RTEs in the naive repertoire, and there is increased renewal of the naive T cells. Because there are no data available on this, we made a conservative choice and have parameterized these sigmoid functions such that the RTE homeostatic response is initiated first

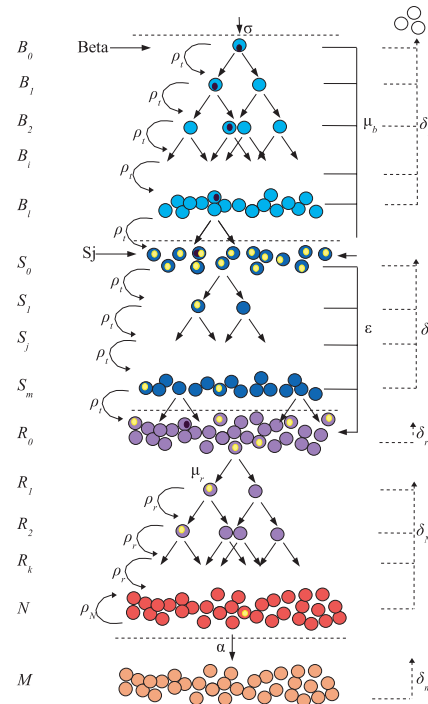


FIGURE 1. Graphical representation of the model. The light blue cells at the top are the $TCR\beta^+$ thymocytes, B_i , maturing into dark blue $TCR\alpha\beta^+$ thymocytes, S_j , which maintain the purple RTE, R_i , that may become red truly naive T cells, N , and in the unlikely event of priming by Ag (α), these become the orange memory cells, M . Black circles denote β -TRECs and yellow circles denote sj-TRECs. The parameters ρ , δ , and μ represent division, death, and maturation rates, respectively. The parameter ϵ denotes the rate of efflux from the thymus.

(i.e., at a higher naive T cell density). This is conservative because it reduces naive T cell renewal, i.e., TREC dilution, and increases RTE survival, i.e., TREC supply, somewhat. Focusing on TRECs, we model memory T cells as a population with a fixed size. To compute their TREC content we have to define a death rate δ_M (Fig. 1). Below we provide the differential equations defining the model, and show how TREC totals and TREC contents can be calculated. Note that the model is not a perfect conveyor belt, because maturation and emigration do not strictly depend on the number of divisions the cells have completed. In a perfect conveyor belt model, the TREC dilution becomes independent of the thymocyte division rate, because cells have to complete the required number of divisions before they move to the subsequent compartment.

Mathematical model

The model starts at the $TCR\beta^+$ stage at which the β -TREC has formed (Fig. 1). Thymocytes in this stage are called B_0 , and with every division they move to the next division number, B_i . When the $TCR\alpha$ -chain is rearranged and the sj TREC is formed the $TCR\alpha\beta^+$ thymocytes move to the S_0 population. For the $TCR\beta^+$ thymocytes, we write:

$$\frac{dB_0}{dt} = \sigma - (\rho_i + \delta_b + \mu_b)B_0, \quad (1)$$

$$\frac{dB_i}{dt} = 2\rho_i B_{i-1} - (\rho_i + \delta_b + \mu_b)B_i, \quad (2)$$

for $i = 1, 2, \dots, l$. Here, σ is the source of thymocytes that have successfully rearranged the β -chain of the TCR, and ρ_i is the time-dependent division rate:

$$\rho_i = \rho_0 e^{-\lambda i}. \quad (3)$$

For simplicity, it is assumed that all $TCR\beta^+$ cells that have divided $> l$

times generate a sj-TREC and enter the S_0 compartment of the $\text{TCR}\alpha\beta^+$ thymocytes:

$$\frac{dS_0}{dt} = \sum \mu_b B_i + 2\rho_r B_l - (\rho_r + \delta_s + \varepsilon) S_0, \quad (4)$$

$$\frac{dS_j}{dt} = 2\rho_r S_{j-1} - (\rho_r + \delta_s + \varepsilon) S_j, \quad (5)$$

for $j = 1, 2, \dots, m$. The ε terms denote the exit of $\text{TCR}\alpha\beta^+$ thymocytes from the thymus, after which they become RTE. Again, we assume that cells maximally complete m divisions before they exit from the thymus, i.e., for RTE, R_j , and truly naive T cells, N , we write:

$$\frac{dR_0}{dt} = \sum \varepsilon S_j + 2\rho_r S_m - (\delta_r + \mu_r) R_0, \quad (6)$$

$$\frac{dR_1}{dt} = 2\mu_r R_0 - (\rho_r + \delta_N) R_1, \quad (7)$$

$$\frac{dR_k}{dt} = 2\rho_r R_{k-1} - (\rho_r + \delta_N) R_k, \quad (8)$$

$$\frac{dN}{dt} = 2\rho_r R_n - (\delta_N + \alpha) N + \rho_N N, \quad (9)$$

for $k = 2, 3, \dots, n$. Recruitment of RTE into clonal expansion, and of naive T cells into renewal, is modeled with sigmoid Hill functions:

$$\mu_r = \frac{\mu_{r0}}{1 + (N/g)^2} \quad \text{and} \quad \rho_N = \frac{\rho_{N0}}{1 + (N/h)^2} \quad (10)$$

that have their maxima, μ_{r0} and ρ_{N0} , when $N = 0$, and are half-maximal at $N = g$ and $N = h$, respectively. The fraction $\mu_r/(\mu_r + \delta_r)$ of the R_0 cells are rescued from death and become naive T cells after a fixed number of divisions. Summarizing, the B and S populations are thymocytes, and the R and N sum up to the naive T cells. Memory T cells are modeled as a population of a fixed size M .

TRECs

There are two ways to keep track of the average number of TRECs in each of these populations. One could split every differential equation given above into two, one for the TREC^+ cells and another one for the TREC^- cells (5). Instead, we keep track of the total number of TRECs in each cell type and compute the average TREC content later. This gives identical results (data not shown), but requires fewer equations. Additionally, some cells may express two TRECs when the TCR genes on both chromosomes are productively rearranged (27). In our model, this appears as a parameter for the expected initial number of TRECs per cell. This parameter linearly scales the total number of TRECs in the population (15).

The following two blocks of equations follow naturally from the equations listed above, i.e., no further assumptions are required. The only new parameter that needs to be introduced is the death rate of memory T cells, δ_M , and the two scaling factors c_B and c_S for the initial number of TRECs per cell. Using the \hat{B} symbol for the total number of β -TRECs in each subpopulation, we write:

$$\frac{d\hat{B}_B}{dt} = c_B \sigma - (\delta_b + \mu_b) \hat{B}_B - \frac{\rho_r B_l}{2^l}, \quad (11)$$

$$\frac{d\hat{B}_{S_0}}{dt} = \mu_b \hat{B}_B + \frac{\rho_r B_l}{2^l} - (\rho_r + \delta_s + \varepsilon) \hat{B}_{S_0}, \quad (12)$$

$$\frac{d\hat{B}_{S_j}}{dt} = \rho_r \hat{B}_{S_{j-1}} - (\rho_r + \delta_s + \varepsilon) \hat{B}_{S_j}, \quad (13)$$

$$\frac{d\hat{B}_{R_0}}{dt} = \sum \varepsilon \hat{B}_j + \rho_r \hat{B}_{S_m} - (\delta_r + \mu_r) \hat{B}_{R_0}, \quad (14)$$

$$\frac{d\hat{B}_{R_1}}{dt} = \mu_r \hat{B}_{R_0} - (\rho_r + \delta_N) \hat{B}_{R_1}, \quad (15)$$

$$\frac{d\hat{B}_{R_k}}{dt} = \rho_r \hat{B}_{R_{k-1}} - (\rho_r + \delta_N) \hat{B}_{R_k}, \quad (16)$$

$$\frac{d\hat{B}_N}{dt} = \rho_r \hat{B}_{R_n} - (\delta_N + \alpha) \hat{B}_N, \quad (17)$$

$$\frac{d\hat{B}_M}{dt} = \alpha \hat{B}_N - \delta_m \hat{B}_M. \quad (18)$$

β -TRECs are expected to be present in every thymocyte having rearranged the β -chain(s), and entering the model as a B_0 cell. Because of multiple rearrangements each cell can have up to two β -TRECs (27). In the model, β -TRECs are therefore formed at rate $c_B \sigma$, where c_B is the expected number of β -TRECs of a cell having completed the β -chain rearrangement. β -TRECs can only disappear from the $\text{TCR}\alpha\beta^+$ population when cells die or mature to $\text{TCR}\alpha\beta^+$ thymocytes, and not when they proliferate. Because all division stages in the $\text{TCR}\alpha\beta^+$ population have the same death and maturation rate, we can write a single ODE for the total TRECs in the $\text{TCR}\alpha\beta^+$ thymocytes (Eq. 11). For the last stage, which moves into $\text{TCR}\alpha\beta^+$ thymocytes at rate $\rho_r B_l$, we have to write a separate term, acknowledging that one loses an average of 2^{-1} TRECs when one B_l cell moves to the S_0 compartment. It is no longer possible to have one differential equation for the total number of TRECs in $\text{TCR}\alpha\beta^+$ thymocytes, because the fraction of S_0 cells containing a β -TREC is changing over time (unlike the fixed fraction, c_B , in the $\text{TCR}\alpha\beta^+$ thymocytes). From Eq. 12 onward, we copy the corresponding equations for the cell density, omitting the factor two in the division terms because TRECs do not duplicate.

For the total number of sj-TRECs, we derive a very similar model:

$$\frac{d\hat{S}_S}{dt} = c_S \sum \mu_b B_i + 2c_S \rho_r B_l - (\delta_s + \varepsilon) \hat{S}_S - \frac{\rho_r S_m}{2^m}, \quad (19)$$

$$\frac{d\hat{S}_{R_0}}{dt} = \varepsilon \hat{S}_S + \frac{\rho_r S_m}{2^m} - (\delta_r + \mu_r) \hat{S}_{R_0}, \quad (20)$$

$$\frac{d\hat{S}_{R_1}}{dt} = \mu_r \hat{S}_{R_0} - (\rho_r + \delta_N) \hat{S}_{R_1}, \quad (21)$$

$$\frac{d\hat{S}_{R_k}}{dt} = \rho_r \hat{S}_{R_{k-1}} - (\rho_r + \delta_N) \hat{S}_{R_k}, \quad (22)$$

$$\frac{d\hat{S}_N}{dt} = \rho_r \hat{S}_{R_n} - (\delta_N + \alpha) \hat{S}_N, \quad (23)$$

$$\frac{d\hat{S}_M}{dt} = \alpha \hat{S}_N - \delta_m \hat{S}_M. \quad (24)$$

Sj-TRECs are formed in all cells arriving at the S_0 stage. Writing for these TRECs an expected initial content of c_S , we can write a single equation (Eq. 19) for the total number of sj-TRECs in the $\text{TCR}\alpha\beta^+$ thymocytes. Again, we need a special term, and the 2^{-m} correction, for the S_m cells exiting the thymus by division. For the later stages, we have to write separate equations.

The TREC content of any subpopulation can be calculated by dividing the TREC total by the corresponding population density. Intracellular degradation of TRECs has not been included in our model because TRECs can still be found decades after thymectomy (2), suggesting that the intracellular degradation rate is small. The model was coded in the C programming language using the variable time step Runge-Kutta integrator provided by Press et al. (28).

Parameters

The parameter values (Table I) are partly based on experimental and theoretical estimates, and partly had to be chosen as a best guess. We have manually tuned free parameter values to get a good correspondence to the data. Because our aim is to test whether decreased thymocyte division rates can be compatible with the current TREC data, this tuning seems reasonable. We have aimed for parameter settings where the RTEs form a small subpopulation (29) containing many TRECs. Other parameter combinations might be able to give the same results in terms of thymic output, and the decrease in the number of divisions between β and sj-TREC formation. Steinmann et al. (7) showed that the involution of the thymus is $\sim 0.05 \text{ y}^{-1}$, which is about 100-fold over a human lifetime. The thymic output of a healthy 30-year-old adult was estimated to be $\sim 10^8$ cells per day (30). Little is known what happens within the thymus, but Dion et al. (12) estimated that the sj/ β TREC ratio in the blood decreases >10 -fold between 20 and 70 years of age. To obtain such a decrease in this ratio, and the observed decrease in thymic output, the thymocyte proliferation rate, ρ_r , is reduced at a rate $\lambda = 5 \times 10^{-5} \text{ d}^{-1}$, i.e., the proliferation rate halves in ~ 40 years. The initial thymocyte proliferation rate was assumed to be $\rho_{r0} = 0.6 \text{ d}^{-1}$, and the daily production of $\text{TCR}\alpha\beta^+$ thymocytes was set to $\sigma = 4.48 \times 10^8$ cell per day, which in combination delivered the measured thymic output of 10^8 cells per day at the age of 30 (30).

The initial TREC contents, c_B and c_S , only scale the total number of TRECs in the model, and have no effect on the dynamics (15). Without loss of generality they can therefore be set to one. Their actual values should

Table I. *Parameter values in the model*

Interpretation	Value
Thymocyte proliferation rate	$\rho_t = 0.6e^{-5 \times 10^{-5t}} \text{ d}^{-1}$
TCR β^+ thymocyte production rate	$\sigma = 4.48 \times 10^8 \text{ cells d}^{-1}$
TCR β^+ thymocyte death rate	$\delta_b = 0.01 \text{ d}^{-1}$
TCR β^+ thymocyte maturation rate	$\mu_b = 0.15 \text{ d}^{-1}$
Maximum number of divisions at TCR β^+ stage	$l = 10$
TCR $\alpha\beta^+$ thymocyte death rate	$\delta_s = 0.1 \text{ d}^{-1}$
Maximum number of divisions at TCR $\alpha\beta^+$ stage	$m = 3$
RTE proliferation rate	$\rho_r = 0.25 \text{ d}^{-1}$
RTE death rate	$\delta_r = 0.05 \text{ d}^{-1}$
Maximum RTE maturation rate	$\mu_{r0} = 0.05 \text{ or } 0.01 \text{ d}^{-1}$
Saturation constant RTE maturation	$g = 5 \times 10^{10} \text{ cells}$
Number of divisions at RTE stage	$n + 1 = 3 \text{ or } n + 1 = 7$
Naive T cell death rate	$\delta_N = 0.005 \text{ d}^{-1}$
Maximum naive T cell proliferation rate	$\rho_{N0} = 0.1 \text{ d}^{-1}$
Saturation constant naive T cell proliferation	$h = 2.5 \times 10^{10} \text{ cells}$
Naive T cell priming rate	$\alpha = 10^{-5} \text{ d}^{-1}$
Memory T cell death rate	$\delta_M = 0.02 \text{ d}^{-1}$
Scaling factors for initial TRECs per cell	$c_B = c_S = 1$

also be of order magnitude one. Because most secondary β -chain rearrangements will not produce the same β -TREC one expects that $c_B \approx 1$. Additionally, despite the lack of allelic exclusion for the α -chains (27, 31), Douek et al. (2), estimate that only two-thirds of the rearrangements produce a TREC. This would argue that $c_S = 1.33$.

The death rate of the TCR β^+ thymocytes was assumed to be low and was set to $\delta_b = 0.01 \text{ d}^{-1}$. The chance for such a cell to form a sj-TREC was assumed to be much higher, i.e., $\mu_b = 0.15 \text{ d}^{-1}$, which means that only a small fraction of the TCR β^+ thymocytes dies. The number of divisions at the TCR β^+ stage determines the β /sj-TREC ratio. Setting the maximum to $l = 10$ divisions was sufficient to explain the observed change of this ratio with age. TCR $\alpha\beta^+$ thymocytes undergo positive and negative selection and have been given a 10-fold higher death rate $\delta_s = 0.1 \text{ d}^{-1}$. Because the sj-TREC content of RTE in the human cord blood is 0.118 TRECs/cell (8, 32), we estimated that TCR $\alpha\beta^+$ thymocytes undergo a maximum of $m = 3$ divisions, which is in perfect agreement with earlier estimates (33, 34). The division rate of TCR $\alpha\beta^+$ thymocytes was made equal to that of TCR β^+ thymocytes.

Berzins et al. (20) estimated that RTEs in mice have a life-expectancy of ~ 20 days. In the absence of estimates in humans we set $\delta_r = 0.05 \text{ d}^{-1}$. Setting the maximum rate at which RTEs are expanded into truly naive cells to the same $\mu_{r0} = 0.05 \text{ d}^{-1}$, we obtain that in lymphopenic conditions half of the RTEs are recruited into the naive pool. This recruitment drops rapidly when there are more than $g = 5 \times 10^{10}$ naive T cells, i.e., in a normal human adult with $\sim 10^{11}$ naive T cells, $\sim 1\%$ of the RTEs is recruited. The proliferation rate of RTEs was assumed to be lower than the thymocyte proliferation rate, i.e., $\rho_r = 0.25 \text{ d}^{-1}$. RTEs undergo $n + 1$ divisions and setting $n = 2$ we obtain that every RTE that is recruited forms a clone of $2^3 = 8$ truly naive T cells. The death rate of the recruited RTEs is assumed to be similar to that of the truly naive cells, i.e., $\delta_N = 0.005 \text{ d}^{-1}$, which is in the same range as measured in monkeys by Arron et al. (35).

In HIV patients with very low CD4 $^+$ T cell counts, 10% of the naive T cells could be in division (14). Assuming that cell division of naive T cells takes about a day, the maximum renewal rate of naive T cells was therefore set to $\rho_{N0} = 0.1 \text{ d}^{-1}$. This renewal rate drops below its half-maximal value when there are more than $h = 2.5 \times 10^{10}$ naive T cells, i.e., when cell numbers drop, the homeostatic response of increased RTE recruitment takes precedence over the increased renewal. In a healthy human adult, with $\sim 10^{11}$ naive CD4 $^+$ T cells, the proliferation rate is $\sim \rho_N = 0.006 \text{ d}^{-1}$. The rate at which naive cells are primed by Ag should be very low. Setting

$\alpha = 10^{-5} \text{ d}^{-1}$, we obtain that half of the naive cells fail to see their specific Ag during life. The turnover rate of memory T cells is known to be higher than that of naive T cells, and assuming an expected life span of 50 days we set $\delta_M = 0.02 \text{ d}^{-1}$.

Some simulations were performed with another parameter setting. In cases where we study a large impact of RTE, we let the RTE complete $n + 1 = 7$ divisions and deliver a clone of $2^7 = 128$ truly naive cells. To compensate for this, the maximum RTE recruitment rate was reduced to $\mu_{r0} = 0.01 \text{ d}^{-1}$.

Results

Experimental data

TREC measurements typically have considerable measurement noise. The variation in published data on the decline of sj-TREC content in CD4 $^+$ T cells with age is summarized in Fig. 2. Thymocytes have ~ 0.25 sj-TRECs per cell, which is ~ 2 -fold higher than the sj-TREC content of human cord blood lymphocytes (8). Importantly, the sj-TREC content of thymocytes is not declining with age (8). The sj-TREC content in naive CD4 $^+$ T cells at age 20 (i.e., 0.1 sj-TRECs cell $^{-1}$) (13) is higher than that in unsorted CD4 $^+$ T cells (Fig. 2), because naive T cells have a higher sj-TREC content than memory T cells. In the extreme case where memory cells contain no sj-TRECs, the expected difference between the TREC content of naive and total T cells would be 2-fold (assuming a 1:1 naive:memory ratio). This is approximately right for the data of Douek et al. (2), but the data of Nobile et al. (11) and Dion et al. (12) have a somewhat lower sj-TREC content at age 20 (Fig. 2). The ~ 100 -fold decline of the sj-TREC content in naive CD4 $^+$ T cells (13) is larger than that of any other study (Fig. 2). There is no good explanation for this, because the percentage of naive T cells decreases with increasing age, which would give a faster decline of the sj-TREC content in total CD4 $^+$ T cells than in naive CD4 $^+$ T cells. The sj-TREC content of CD3 $^+$ T cells and/or PBMCs starts lower, which was to be expected, and decline at a rate within the range of the total CD4 $^+$ T cells (Fig. 2).

Given the measurement noise typical for measuring sj-TREC levels, it is admirable that Dion et al. (12) were able to repeatedly measure β -TRECs (that are present at 100-fold lower concentrations). They report a constant level of 10^{-4} β -TRECs per CD4 $^+$ T cell in healthy controls between 20 and 70 years old. Because the sj-TREC content declines >10 -fold (Fig. 2), the sj/ β -TREC ratio

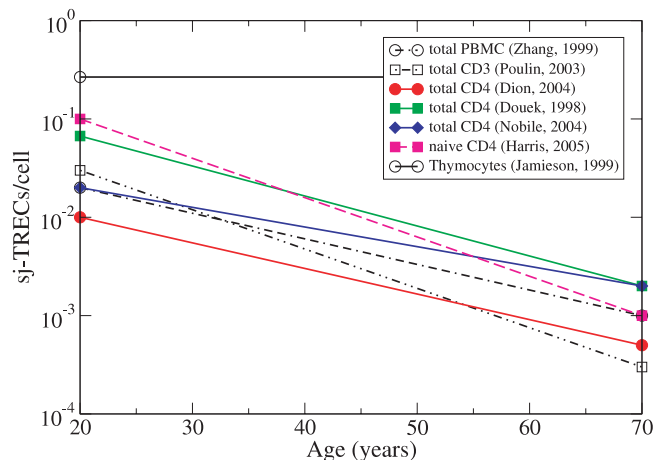


FIGURE 2. The decline in the sj-TREC content in CD4 $^+$ T cells with age. The figure combines various previous publications by reading the sj-TREC content at ages 20 and 70 from the figures in the papers, and re-expressing that as the number of sj-TRECs per cell. The lines represent the sj-TREC content per PBMC (9), per CD3 $^+$ T cell (10), per CD4 $^+$ T cell (2, 11, 12), per naive CD4 $^+$ T cell (13), and per thymocyte (8).

decreases >10 -fold from age 20 to 70 years. This ratio is completely determined by the number of divisions of $\text{TCR}\beta^+$ thymocytes before rearranging the α -chain, because after that both sj and β -TRECs change in the same way. Thus, these data suggest that the number of thymocyte divisions decreases with increasing age (12).

Model behavior

By numerical simulation of the mathematical model, with the parameters described in *Materials and Methods*, we aim to get better insight in the behavior of the population sizes, the TREC totals, and the TREC content, of the various subpopulations of the model (Fig. 3). The only effect of age in our model is the reduction of the thymocyte division rate, and as a consequence the total thymic output. The total number of naive T cells is $\sim 10^{11}$ cells (30), which declines marginally in 80 years and is in the model almost entirely composed of truly naive T cells because the RTEs form a much smaller subpopulation (Fig. 3a) (29). The RTE pool decreases proportionally to the thymic output, which decreases >100 -fold and is close to the previously estimated 10^8 cells d^{-1} at age 30 (30). Because the naive T cell population rapidly fills up by renewal, we find that, even at very young age, only 10% of the total naive T cells is an RTE. At age 30, $<2\%$ of the naives are an RTE, which seems realistic because the contribution of the thymus to the naive T cell pool should be small at this age.

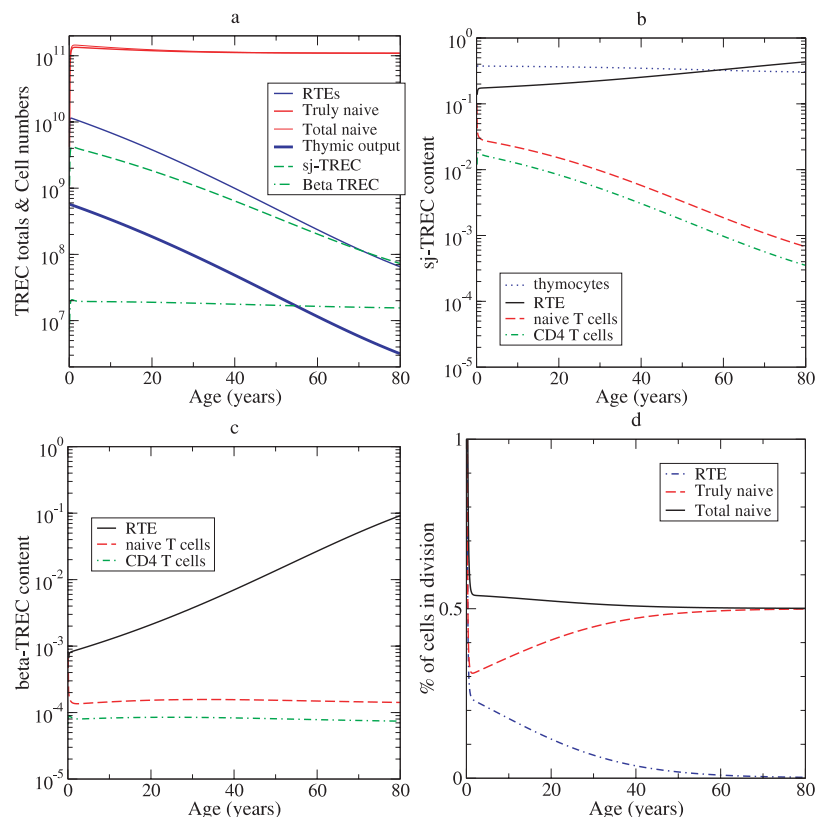
The absolute number of β -TRECs that is formed, $c_B\sigma$, does not change with age, and is not influenced by division. The total number of β -TRECs in the periphery therefore remains fairly constant (Fig. 3a) and decreases somewhat because a larger fraction of the thymocytes dies when their division rate decreases. In contrast, the absolute number of sj-TRECs that is formed is decreasing with increasing age. Because the α -chain of the TCR is rearranged much later than the β -chain, the number of sj-TRECs depends much stronger on the total number of divisions in the thymus than

the number of β -TRECs. Thus, the total number of sj-TRECs decreases and remains almost proportionally to the thymic output (Fig. 3a). Because the β -TREC numbers stay constant, the decline of the sj/ β -TREC ratio also resembles the decline of the thymic output. In the model, the sj/ β -TREC ratio is ~ 200 at birth and ~ 5 at 80 years of age (data not shown), which is in good agreement with the data of Dion et al. (12).

The average sj-TREC content in RTEs is markedly higher than that in total naive cells (Fig. 3b). This is due to the clonal expansion involved in the recruitment of RTEs into the naive repertoire and due to the dilution of TRECs in the truly naive T cells by renewal. The sj-TREC content in RTEs is increasing due to the decreased division in the thymus. Nevertheless, the sj-TREC content in naive T cells (and in total T cells) declines about a 50-fold (Fig. 3b). Apparently, the dilution of TRECs by renewal of truly naive T cells suffices to compensate for the increased TREC content of RTE with age, and we still obtain the experimentally observed TREC decline in naive T cells (Fig. 2), which is less than the decline in thymic output (Fig. 3a). Another reason why the degree at which sj-TREC contents decline in naive T cells is less than the decline in thymic output is that the recruitment of RTEs into the naive repertoire is increasing with age as a homeostatic response to the lower thymic output.

Importantly, the sj-TREC content of thymocytes hardly changes with age (Fig. 3b). This is in excellent agreement with the data of Jamieson et al. (8) (see Fig. 2), who conclude that adult thymocytes have a similar degree of TCR rearrangement. However, the core assumption of our model is that the number of thymocyte divisions decreases markedly with increasing age, and in our model this leads to a 100-fold decrease of thymic output. Given that the number of thymocyte divisions decreases, one would a priori expect their sj-TREC content to increase (like that in the RTEs; Fig. 3b). Instead, the sj-TREC content of thymocytes decreases somewhat (Fig. 3b), because the total number of sj-TRECs

FIGURE 3. The effect of age on total cell numbers and total TRECs (a), on sj-TREC contents in RTEs, in total naive T cells, and in total T cells (b), on β -TREC contents in the same subpopulations (c), and the fraction of naive T cells in division (d). TREC totals are summed over all peripheral T cell populations (i.e., RTE, naive, and memory T cells).



that is formed also decreases. The limited number of thymocyte divisions after the rearrangement of the α -chain, the decreased number of sj-TRECs formed, together with the decreased total number of $\text{TCR}\beta^+$ and $\text{TCR}\alpha\beta^+$ thymocytes, balances out and gives a fairly constant sj-TREC content in thymocytes. The TREC content of thymocytes can become lower than that of RTEs because it is averaged over all thymocytes, including the vast majority which have not rearranged the α -chain, and the fraction of sj-TREC⁺ thymocytes decreases with increasing age.

Fig. 3c shows the β -TREC content of RTEs, total naive T cells, and total T cells. Due to the age-related decrease in the number of intrathymic divisions, the β -TREC content of RTEs increases markedly with age. This increase is so large that the peripheral renewal that was responsible for the decrease of the sj-TREC content in total naive T cells, can no longer compensate, and the β -TREC content of naive (and total) T cells remains fairly constant (Fig. 3c). Qualitatively, this is in good agreement with Dion et al. (12), who found no significant change in the β -TREC content of total CD4^+ cells. Obviously, for other parameter values, the β -TREC content of truly naive T cells can be made to increase or decrease with age. Our main result is that the experimental observation of constant β -TREC contents in peripheral T cells (12) are obtained with this model for reasonable parameter values.

A problem with density dependent naive T cell renewal is that one expects the highest division rates at old age (15), which is in disagreement with the data (36, 37). This problem is solved in our model because the number of RTEs decreases with increasing age (Fig. 3d). As a consequence, the fraction of dividing cells in the total naive population remains approximately constant, while the fraction of dividing truly naive T cells is increasing with age due to homeostasis (Fig. 3d).

Summarizing, despite the fact that the core assumption by Dion et al. (12), that “thymic involution is associated with decreased division of thymocytes,” seemed to contradict the data of Jamieson et al. (8), the model resolves this contradiction, and its behavior is in good qualitative agreement with current data on T cell population sizes and TREC contents. This does not prove that thymic involution is associated with fewer divisions, but demonstrates that this is perfectly possible.

Thymectomy

Given that our model at least qualitatively agrees with data, we use it to learn what to expect for T cell population sizes and their TRECs after thymectomy. In our model, we simulate thymectomy by removing the thymus output at age 30 (i.e., by setting $\varepsilon = \rho_t = 0$; Fig. 1). In our model, thymectomy leads to a rapid depletion of the RTE pool, due to their short expected life span (Fig. 4a). The total naive pool remains relatively stable because the contribution of the RTEs was small anyway at this age, and because truly naive T cells maintain themselves by slow renewal. In this example, 5 years after thymectomy, only 10% of the original naive T cell number is lost. In the model, the total naive population size after thymectomy always approaches the same steady state.

Total TREC numbers drop somewhat due to rapid loss of RTE, and then approach a slow decline reflecting the slow death of truly naive T cells (Fig. 4a). Because cell numbers stay more or less constant after thymectomy, the TREC content of the naive cells declines at the same rate as the total TREC numbers (Fig. 4, b and c). The fact that thymectomy gives a mild increase in the rate at which the TREC content declines is in agreement with the data on thymectomy in macaques (35). Finally, note that the peripheral events have the same effect on sj and β -TRECs (cf Fig. 4, b and c), i.e., the ratio between the two becomes fixed after thymectomy (data not shown).

Dion et al. (12) observed rapid changes in TREC contents and TREC numbers during the first months of HIV infection and postulated that this was due to the loss of a small population of RTE containing most of the TRECs. There is a rapid initial decline in the TRECs in Fig. 4, but this is too small to be observable. To test the conjecture of Dion et al. (12), we can increase the difference in the TREC content of RTEs and truly naive T cells in our model by increasing the number of RTE divisions (Fig. 4, d–f). Confirming the conjecture (12), we indeed find a sharp decline in both total TREC numbers (Fig. 4d) and TREC content (Fig. 4, e and f) directly after thymectomy. Total naive T cell numbers hardly decline because the RTE population was small to begin with. Summarizing, for sufficiently large differences in the TREC content of RTEs and truly naive T cells, we can explain rapid changes in the TRECs while the naive T cell population size remains more or less constant (12). However, it remains an open question whether such a large difference in the TREC content is realistic, e.g., in Fig. 4, d and f, one RTE expands into a clone of $2^7 = 128$ naive T cells. Because the second down-slope of the sj-TRECs in naive T cells is completely determined by their death rate, the TRECs would be maintained for longer when the death rate, δ_N , were slower (e.g., due to homeostasis increasing the expected life-spans). This is important because we have a fairly rapid loss of the sj-TREC content after thymectomy (Fig. 4), and given the lack of data on thymectomized human adults, it remains unclear whether or not this is realistic (6).

Human immunodeficiency virus

The mechanisms underlying CD4^+ T cell depletion during HIV infection remain unclear and may involve direct infection, reduced thymus output, and hyperactivation (23, 24). The decreased sj/ β -TREC ratio in HIV⁺ patients suggested that HIV decreases the number of cell divisions of thymocytes (12). Using our model, we investigate what to expect if this were true, and the division rate in the thymus, ρ_t , would suddenly be decreased at an adult age by HIV infection. A 2-fold reduction of the thymocyte division rate in a 30-year-old adult leads to a >10-fold reduction in the thymic output, which is followed by a similar reduction in the RTE population (Fig. 5a). Due to homeostasis, the total number of naive cells stays fairly constant. Decreasing the number of intrathymic divisions decreases the number of sj-TRECs that are formed in the thymus. Note that the sj-TREC content of RTEs is increased after infection (because $\text{TCR}\alpha\beta^+$ thymocytes divide less), but that the average sj-TREC content of the total naive pool decreases due to the reduced output of RTEs (Fig. 5b). The formation of β -TRECs is not affected by reducing cell division in the thymus, and total β -TREC numbers remain constant (Fig. 5a). The β -TREC content of RTEs increases ~ 10 -fold in half a year because there is less dilution at the $\text{TCR}\beta^+$ and $\text{TCR}\alpha\beta^+$ thymocyte stages (Fig. 5c). This marked increase of the β -TREC content in RTEs masks the lower input of RTEs into the naive T cell pool, such that the β -TREC content of the naive T cells is hardly affected (Fig. 5c).

These results are inconsistent with observations of Dion et al. (12), who found that the β -TREC content of PBMCs increased 3.5-fold within 6 mo after HIV infection, while their sj-TREC content decreased 2-fold. Due to the longevity of naive T cells, we find slow changes in the TRECs in the total naive pool (Fig. 5, a–c). One can increase the turnover of naive T cells by allowing for hyperactivation in the model (6, 14). Assuming that HIV infection also leads to increased priming of naive T cells, we increased the α parameter in the bottom row of Fig. 5. This form of hyperactivation leads to an almost 2-fold reduction in naive T cell numbers (Fig. 5d). The TREC dynamics change very little by adding hyperactivation (Fig. 5, e and f) because the increased priming

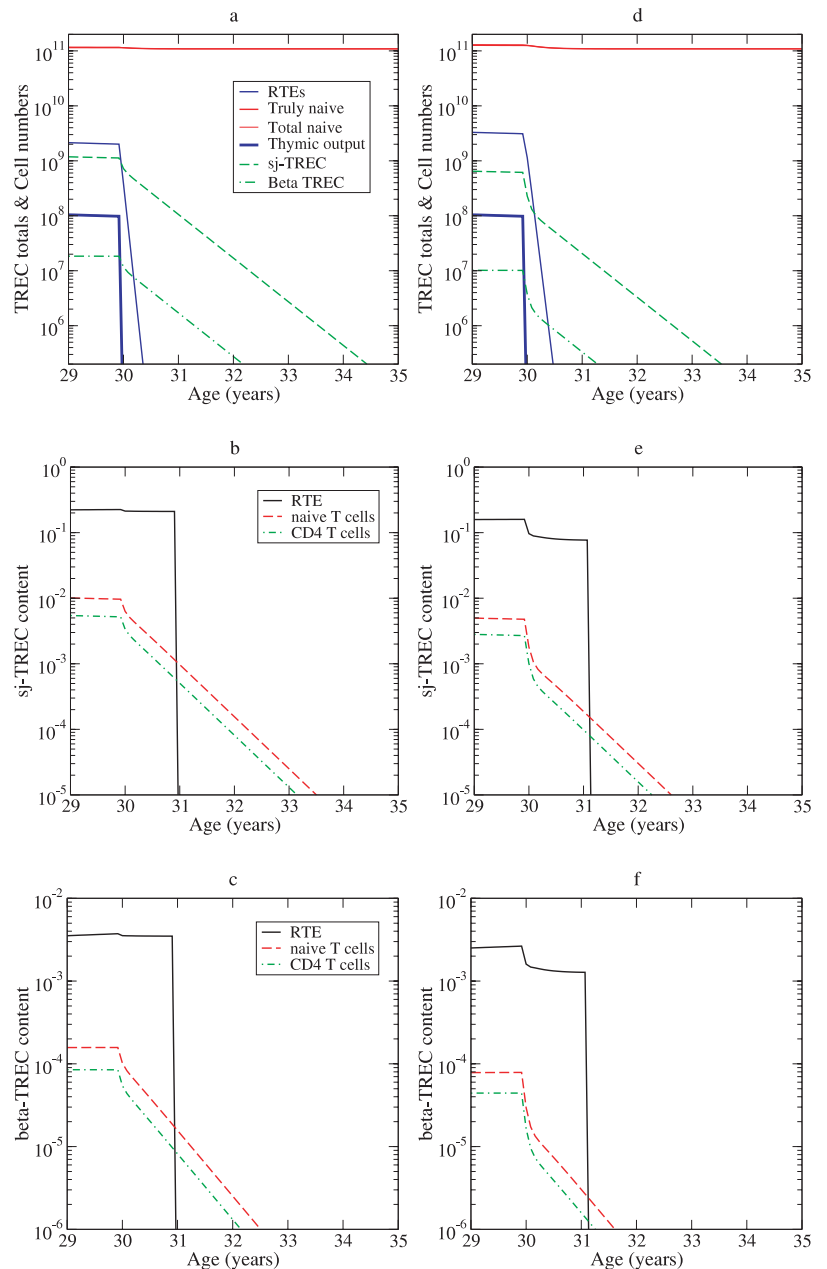


FIGURE 4. The effect of thymectomy in a model. RTEs divide three times in *a–c* and seven times in *d–f*. Thymectomy is performed at age 30, and the three panels show the total population sizes (*a*), the sj-TREC content (*b*), and the β -TREC content (*c*). Note the 100-fold difference in the scale of the vertical axis in *b* and *c*.

increases TREC contents due to higher recruitment of RTEs, while the larger renewal due to the homeostatic response decreases TREC contents by dilution (6, 14), and these effects balance out. There is a small increase in the β -TREC content of naive cells, which is caused by the drop in naive T cell numbers, and hence a larger relative contribution of the RTEs.

Next, consider the alternative model with the large difference in the TREC content of RTEs and naive T cells (Fig. 6). The rapid drop of the RTEs now leads to a sharper initial drop in total sj-TREC numbers (Fig. 6*a*). The sj-TREC content of RTEs decreases temporarily because the fraction of RTEs in the R_0 stage decreased compared with that in later RTE stages (Fig. 6*b*). Later, the sj-TREC content of RTEs increases due to the decreased dilution in the thymus. Although we find more rapid changes in this alternative model, we fail to find large increases in the β -TREC content of the naive cells.

Finally, we again allow for hyperactivation by increasing the priming rate of naive T cells. This hardly affects population sizes

and TREC totals, apart from the rapid drop in naive T cell numbers (Fig. 6*d*). Because of this the β -TREC content of the naive T cell pool increases. When truly naive T cell numbers drop by hyperactivation, the relative fraction of RTEs increases, which increases the β -TREC content of the whole pool (Fig. 6*f*). Thus, the increase in the naive β -TREC content is explained by an increase in the β -TREC content of RTEs, and a shift in the relative contribution of the two subpopulations. Although Dion et al. (12) found a larger increase in the β -TRECs, our results are at least in qualitative agreement with these observations.

Discussion

This work confirms May’s (38) thesis that “mathematical models help one to think clearly.” The dynamics of the two types of TRECs in the various T cell populations are intuitively extremely difficult to predict. The better understanding of the changes in the TRECs observed in the model helped us to explain those observed

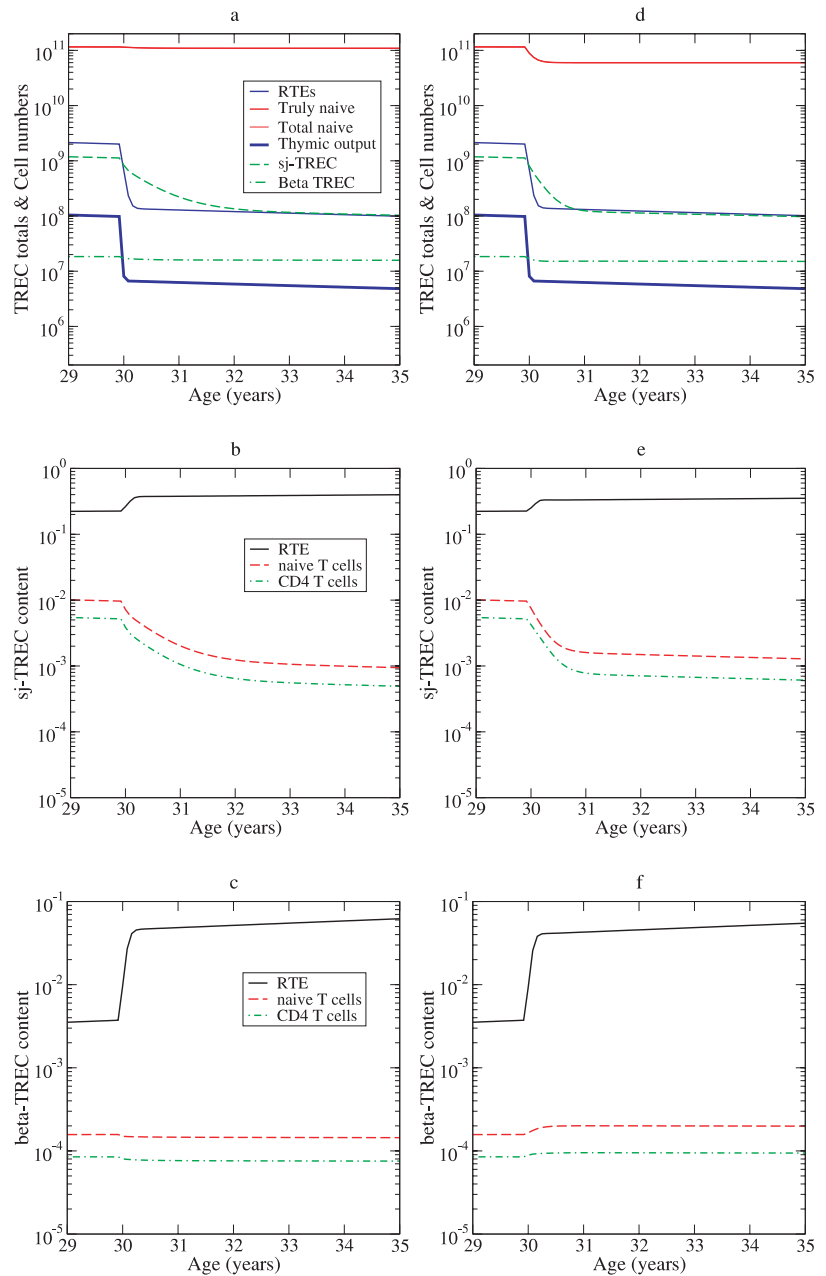


FIGURE 5. The effect of HIV infection in a model assuming three RTE divisions. In *a–c*, we consider a 2-fold reduction of the thymocyte division rate at age 30. In *d–f*, we add on hyperactivation by setting $\alpha = 0.01 \text{ d}^{-1}$.

in experiments. Our overall question was whether decreased thymocyte division as the mechanism for decreased thymic production (12) is consistent with current data, and the results have answered this question positively. The mechanisms underlying thymic involution are incompletely understood (16). The fact that the data suggesting that thymocyte division decreases with increasing age (12, 17, 18) are perfectly compatible with current TREC data suggests that decreasing thymocyte division rates may indeed play a role in thymic involution. Unfortunately, this makes the interpretation of TREC data even more difficult (6).

The prime motivation for assuming age-dependent thymocyte division rates was the observed decrease in the sj/ β -TREC ratio in the blood (12), and the data demonstrating a reduction of thymocyte division with increasing age (17, 18). Because β -TRECs are formed before the sj-TRECs, their dilution is influenced more strongly by changes in thymocyte division. The same argument would apply to the coding joint TRECs (cj-TRECs) that are formed a few divisions later than the sj-TREC (2). The cj/sj-TREC

ratio should therefore also decrease with increasing age. This is in disagreement with the data of Douek et al. (2), but in agreement with relatively rapid decline of the cj-TREC content reported by Al-Harhi et al. (39). Additional studies seem required to test this further. Due to the decreased thymic output there is more truly naive T cell renewal at old age in our model. Data suggest that the fraction of Ki67⁺ naive T cells is not increasing with age (36, 37). Splitting the naive T cells into RTEs that divide frequently and truly naive T cells that normally divide infrequently, we found that the fraction of dividing naive T cells is more or less constant. This was not possible in our earlier model (15).

We could alternatively have made the more classical assumption, and explain decreased thymus production by decreasing the influx, σ , of progenitor cells (16, 40, 41). In that model the TREC content of TCR $\alpha\beta^+$ and TCR β^+ thymocytes, and the overall sj/ β -TREC ratio would be completely independent of age, because cells on average complete the same number of divisions in the thymus. The β and sj-TREC content of peripheral T cells would

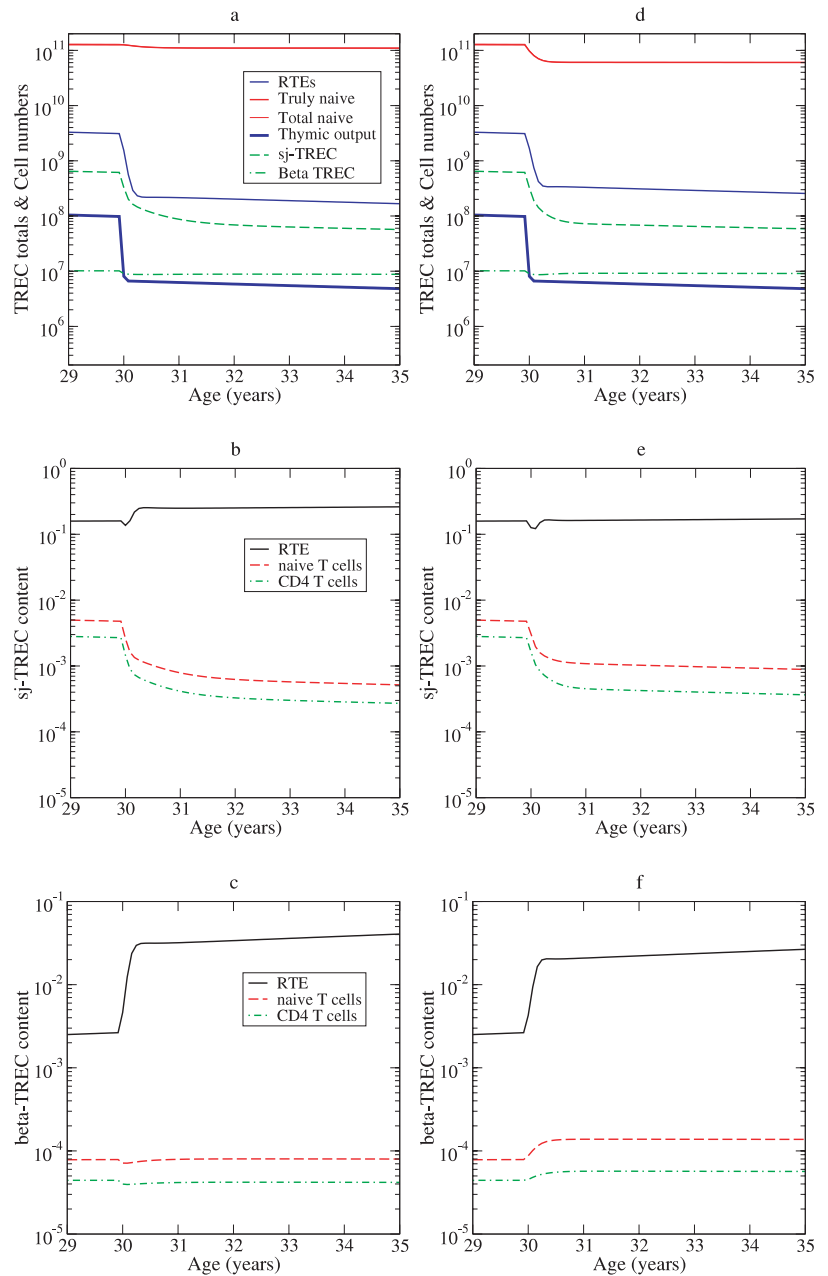


FIGURE 6. The effect of HIV infection in a model assuming seven RTE divisions. In *a–c*, we consider a 2-fold reduction of the thymocyte division rate at age 30. In *d–f*, we add on hyperactivation by setting $\alpha = 0.01 \text{ d}^{-1}$.

decline normally due to the combination of reduced thymic output and peripheral dilution by division (14, 15). Other parameters that could depend on age are the fraction of productive rearrangements (c_B and c_S) and/or the thymocyte death rates (δ) could change with age. Decreasing c_B or c_S will linearly affect the β -TRECs and sj-TRECs, respectively. Increasing the thymocyte death rate will qualitatively have a similar effect as decreasing the proliferation rate, because both lead to lower output and fewer divisions at the $\text{TCR}\alpha\beta^+$ stage. We have not studied the above mechanisms extensively, because there is as yet no data supporting these effects, but one could use our model to study any of these putative mechanisms. Another complicating factor we have omitted from the model is the possible feedback from peripheral T cell numbers on thymic production, via the presence of mature T cells in the thymus (42, 43), and/or the peripheral consumption of IL-7 (16). Although increased thymic production helps to explain increased TREC contents in HIV patients during therapy (12, 13, 44), this interpretation remains controversial because there is no corresponding increase in naive T cell numbers (6, 13, 19, 44).

Thymectomy at age 30 has little effect on naive T cell numbers in our model, and increases the loss of TRECs (Fig. 4). There is very little solid evidence on the effects of thymectomy on naive T cell numbers in human adults, but there is some evidence supporting the increased loss of TRECs after adult thymectomy (2, 45). Thymectomy in young children has a strong impact on the number of naive T cells and their TREC content (46, 47). Whether or not the loss of TRECs after thymectomy is biphasic depends on the difference in TREC contents between RTE and truly naive T cells (Fig. 4), and a rapid loss of RTE can account for a rapid loss of TRECs. However, in the absence of good markers for RTEs, this remains hypothetical, and data from thymectomized monkeys (35) and human adults (45) show little evidence of a biphasic decline in TREC numbers (6) (R. M. Ribeiro and R. J. de Boer, manuscript in preparation).

Similarly, the model can in principle account for the observed (rapid) changes in β - and sj-TREC content after HIV infection, by allowing for large (rapid) shifts in the relative contribution of RTEs and truly naive T cells to the total population. However,

there is no good evidence supporting such rapid changes. Increased recruitment of RTEs into the naive compartment reduces the average death rate of cells containing TRECs and will increase the TREC totals, but not necessarily the TREC content (R. M. Ribeiro and R. J. de Boer, manuscript in preparation). In our model we have implemented hyperactivation as the increased priming of naive T cells. Increasing the loss rate of cells increases the TREC content of that population, because on average, less naive T cells become old completing several cell divisions (6, 14, 15). The reason why the sj-TREC content nevertheless goes down is the increased renewal by homeostasis. Increased division rates of naive T cells during HIV infection are probably also due to increased cytokine levels (13, 14, 48–50). Apparently, the increased division of naive T cells fails to compensate for their increased priming, and CD4⁺ and CD8⁺ naive T cell numbers are slowly eroded over the course of HIV infection (22, 51, 52).

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Disclosures

The authors have no financial conflict of interest.

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