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Ping Ye and Denise E. Kirschner

The human thymus exports newly generated T cells to the periphery. As no markers have been identified for these recent thymic emigrants (RTE), it is presently impossible to measure human thymic output. T cell receptor excision circles (TREC) have been recently used to assess thymic output during both health and disease. Using a mathematical model, we quantify age-dependent changes both in the number of RTE generated per day and in TREC concentration during an 80-year lifespan. Through analyses, we demonstrate that RTE and peripheral T cell division have the same potential to affect TREC concentration at any age in healthy people. T cell death also influences TREC concentration, but to a lesser extent. During aging, our results indicate that thymic involution primarily induces an age-dependent decline in TREC concentrations within both CD4+ and CD8+ T cell populations. We further apply this model for studying TREC concentration during HIV-1 infection. Our analyses reveal that a decrease in thymic output is the major contributor to the decline in TREC concentration within CD4+ T cells, whereas both increased peripheral T cell division and decreased thymic output induce the decline in TREC concentration within CD8+ T cells. Therefore, we suggest that T cell turnover should be examined together with TREC concentration as a measure of RTE. If peripheral T cell division remains relatively unchanged, then TREC concentration indeed reflects thymic output. The Journal of Immunology, 2002, 169: 4968–4979.
periphery within RTE. Thus, TREC levels in the periphery could reflect RTE numbers. TREC are stable and are not duplicated during mitosis; therefore, TREC concentration is diluted out with each cell division. This explains why thymocytes have higher TREC concentrations as compared with peripheral blood T cells (11, 12), and why naive T cells have higher TREC concentrations than memory T cells (10).

TREC concentration has been widely used as a measurement of the number of RTE during HIV-1 infection and treatment, hematopoietic stem cell transplantation, and thymectomy (9, 10, 13–16). However, controversy exists as to whether TREC concentrations are a good marker for RTE, because TREC concentrations are also affected by peripheral T cell turnover events, such as T cell division and death (9, 10, 17). Different techniques are used to measure TREC concentrations, such as real-time PCR (10, 17), quantitative-competitive-PCR (9, 13), and PCR-ELISA (18). Measurement units of TREC concentration vary as well, including TREC per 10⁶ PBMCs (10), TREC per CD4⁵RA⁺ T cells (17), TREC per microgram DNA of T cells (9, 14), and TREC per 10⁵ CD4⁺ T cells (13). Together, these factors make it difficult to interpret and compare TREC data between studies.

It is not yet possible to experimentally differentiate the effects of thymic output and T cell turnover on TREC concentration in a quantitative way, because thymic output cannot be measured. To this end, we use mathematical modeling to predict whether TREC concentrations are a good marker of thymic function, as represented by the number of RTE. Our model captures age-dependent changes in thymopoiesis, RTE, and TREC levels. Using uncertainty and sensitivity analyses, we quantify the potential roles of thymic output, T cell division, T cell death, and intracellular TREC degradation in affecting TREC concentration at each specific age. We further define elements that contribute most to changes in TREC concentration that occur during aging and HIV-1 infection.

**Human Thymopoiesis Model**

To quantify age-dependent changes in thymic output per day (i.e., the number of RTE produced per day), we develop a model that describes human thymopoiesis. Bone marrow progenitor cells migrate to the thymus and differentiate into CD3⁻CD4⁻CD8⁻ triple-negative (TN) cells. TN cells then differentiate into CD3⁺CD4⁺CD8⁻ intrathymic T progenitor (ITTP) cells. ITTP cells give rise to the predominant thymocyte subset, CD3⁺CD4⁺CD8⁺ double-positive (DP) cells. After positive and negative selection, the surviving DP cells further differentiate into either CD3⁺CD4⁺CD8⁻ single-positive (SP4) or CD3⁺CD4⁺CD8⁺ single-positive (SP8) cells (19). A minor subpopulation of both SP4 and SP8 cells emigrates to the periphery to become RTE and joins the T cell pool (19). Fig. 1 presents a diagram of this process. The equations and parameters for the human thymopoiesis model are discussed in the Appendix.

**Quantification of human thymocytes and RTE**

Once model equations are derived that describe Fig. 1 (see Appendix), we then solve the system numerically. We present graphs for the corresponding simulations of this virtual model of human thymopoiesis. To build the model, we require a representation for thymic epithelial space (TES), which is the functional thymic tissue containing all thymocytes. Fig. 2A shows data for the maximal number of thymocytes calculated from the volume of the TES region (solid dots) and our best fit function for these data (solid line) during an 80-year lifespan (see Appendix).

Using this function for TES region, we can simulate the model behavior presented in Fig. 1. Our model results suggest that five thymocyte subsets increase in numbers to a maximum value at age 1 year and then decrease at a rate of ~5% per year (Fig. 2B). When comparing curve shapes in Fig. 2A and Fig. 2B, it can be seen that age-dependent changes in the average number of thymocytes is proportional to the maximal number of thymocytes in the TES region. This implies that involution quantitatively affects thymopoiesis. Shown in Fig. 2C is the simulation for the average number of RTE produced per day during an 80-year lifespan for an individual. The numbers of CD4⁺ and CD8⁺ RTE produced per day increase to their greatest values at the age of 1 year and then decline with age. Total thymocyte number has a 2-log decrease

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**FIGURE 1.** Model representing human thymopoiesis. Five thymocyte subsets are included, TN, ITTP, DP, SP4, and SP8 cells. Represented are thymocyte growth (γ) limited by the maximal number of thymocytes in thymic epithelial space (TES(t)), thymocyte differentiation (f), death (d), and emigration to the periphery as RTE (e, r) (i = 1, 2, 3, 4, 5; j = 1, 2, 3, 4). The definitions and values of parameters are given in the Appendix.
during 80 years. Correspondingly, the total number of RTE exported from the thymus per day declines exponentially by 2 orders of magnitude during 80 years (Fig. 2D).

The model predicts the observed ratio of SP4 to SP8 cells as 2, consistent with human data (19, 20). This ratio in the thymus determines the CD4<sup>+</sup>:CD8<sup>+</sup> T cell ratio in the blood, which ranges from 1.5 to 2.5 (21). Our model predicts that the maximum number of thymic emigrants at the age of one is 1.1 x 10<sup>9</sup> cells/day (Fig. 2D), which is comparable with another estimate of human thymic emigrants of 10<sup>9</sup>/day (22). Our virtual model also predicts that the ratio of thymic emigrants per day to total thymocyte numbers is 1.5%. This is consistent with experimental data ranging between 1 and 5% in mice (23). Thus, this model likely reflects the process of human thymopoiesis. More importantly, we quantify the number of RTE entering the periphery per day during the normal aging process, which cannot be measured experimentally (Fig. 2C).

**Human TREC Model**

The sjTREC concentration declines during aging and HIV-1 infection (9, 10, 17, 24). Some studies indicate that reduced thymic production of RTE contributes most to the decline of sjTREC concentration (9, 10, 24), whereas others suggest that increased cell division but not thymic dysfunction affects sjTREC concentration (17, 25). To address the question of whether the TREC concentration is mainly affected by thymic output or peripheral T cell turnover, we extended the thymopoiesis model to illustrate dynamic changes in sjTREC, the most ubiquitous human TREC species that is produced during TCR gene rearrangement, and the one that is most consistently measured experimentally. Fig. 3 presents the dynamics for sjTREC between the thymus and peripheral blood and lymphoid tissues (LT). Total T cell levels (including both naive and memory T cells) are influenced by thymic output of

**FIGURE 2.** Thymopoiesis model illustrates thymic involution during 80 years of life. **A,** Best fit function \( T_{es}(t) \) (solid line) for data (●) derived from the volume of TES region (2). **B,** Model solutions of five thymocyte subsets. **C,** Number of RTE exported from the thymus per day. **D,** Total thymocytes (sum of five thymocyte subsets in **B**) and total RTE exported per day (sum of CD4<sup>+</sup> and CD8<sup>+</sup> RTE in **C**) on a log scale.

**FIGURE 3.** Model representing human sjTREC dynamics. Total sjTREC levels are affected by thymic output to the periphery as RTE \((e_1, e_2)\), which contain sjTREC (sjTREC concentration within RTE \(c_1, c_2\)), T cell death \((\delta_1, \delta_2)\), and sjTREC degradation \((e_1, e_2)\). Total T cell levels are affected by thymic output of RTE \((e_1, e_2)\), T cell growth \((\gamma_1, \gamma_2)\), and T cell death \((\delta_1, \delta_2)\). The sjTREC concentration within T cells is affected by each event. For simplicity, sjTREC in SP cells and RTE are not drawn. The definitions and values of parameters are given in the Appendix.
RTE, T cell growth, and T cell death. sJTREC are generated within thymocytes and exported to the periphery within RTE. Thus, total sJTREC levels are determined by thymic output of sJTREC, T cell death, and intracellular sJTREC degradation. Studies of sJTREC levels in thymectomized humans and macaques indicated that there is no significant extrathymic source of cells containing sJTREC (9, 11). T cell death induces the loss of sJTREC within T cells, and this loss of sJTREC is proportional to the loss of T cells. As the sJTREC concentration is determined by both total sJTREC levels and total T cell levels, events that affect them will indirectly induce changes in sJTREC concentration, including thymic output, T cell growth, T cell death, and sJTREC degradation. Using our TREC model, we reveal how thymic output and T cell turnover affect sJTREC concentrations at each specific age and during the entire lifespan in healthy people, and explore the principal factors contributing to the decline in sJTREC concentrations during HIV-1 infection. The equations and parameters for the human TREC model are discussed in the Appendix.

Predicting total T cell numbers, total sJTREC levels, and sJTREC concentration

The dynamic changes of CD4⁺ and CD8⁺ T cells in peripheral blood/LT based on our simulations are shown in Fig. 4A. The initial increase of T cells is due to the large output of newly generated T cells from the thymus in the early years of life (see Fig. 2C). Then, T cell numbers continuously increase until age 30 years. After age 30 years, T cell numbers remain at a relatively stable level with a slow decline over time, as suggested by data (26, 27). Our simulation results of T cell dynamics are within the normal range calculated from human data on cell numbers per mm³ of blood, blood volume, and body weight (error bars in Fig. 4A) (26, 28–31). Increased peripheral T cell division maintains the 30-year increase and subsequent relative equilibrium in T cell numbers (data not shown). This is consistent with data from two animal models of older sooty mangabeys and mice that lack thymic function, indicating that most T cell production occurred in the periphery (27, 32).

Shown in Fig. 4B are changes in total sJTREC within both CD4⁺ and CD8⁺ T cells during 80 years. The level of total sJTREC in peripheral blood/LT reaches its maximum around 15 months of age and remains at that level for ~12 months. Then total sJTREC level decreases concomitant with the decline in number of RTE produced per day.

We simulate sJTREC concentration within CD4⁺ T cells by dividing total levels of sJTREC within CD4⁺ T cells with total CD4⁺ T cells. In experimental measurements (9), results were presented in units of sJTREC/1.5 × 10⁵ T cells. Hence we multiply our sJTREC/T cell results by 1.5 × 10⁵ (Fig. 4C, solid line). Similarly, we calculate the sJTREC concentration within CD8⁺ T cells (Fig. 4D, solid line). The sJTREC concentrations for both CD4⁺ and CD8⁺ T cells decline ~2 logs during an 80-year period.

Douek et al. (9) reported that sJTREC concentrations decline by 1–1.5 logs in both blood CD4⁺ and CD8⁺ T cells during the lifetime of an individual (in units of sJTREC/1.5 × 10⁵ T cells). Zhang et al. (10) measured sJTREC level (±1 circles) per 10⁶ PBMCs and found a total decrease of 2 logs over seven decades. To convert data from Ref. 10 into units of sJTREC/1.5 × 10⁵ T cells, we divide sJTREC/PBMCs with the ratios: total lymphocytes/PBMCs and T cells/total lymphocytes over time (26, 33, 34), and then multiply by 1.5 × 10⁵ T cells. sJTREC data from Douek et al. and Zhang et al. were both measured using blood samples. The number of T cells in blood represents ~2% of total T cells in the body (35–37). We scale the data sets appropriately by assuming that T cells in humans continuously circulate between blood and lymph, exchanging in the blood 48 times daily in healthy individuals (35), and that the levels of sJTREC per T cell in blood are similar to sJTREC per T cell in LT. Our model results of average sJTREC concentrations in blood/LT during aging are comparable with two experimental data sets from Douek et al. and Zhang et al. (Fig. 4, C and D). The slight differences are likely due to different PCR techniques, target cell populations, and unit conversions used for data sets.

FIGURE 4. Model simulations during 80 years for levels of total T cells, total sJTREC, and sJTREC concentrations. A, Total T cells in peripheral blood/LT. The normal range for T cells is calculated from data on cell number/mm³ blood, blood volume, and body weight (error bars in Fig. 4A) (26, 28–31). Increased peripheral T cell division maintains the 30-year increase and subsequent relative equilibrium in T cell numbers (data not shown). This is consistent with data from two animal models of older sooty mangabeys and mice that lack thymic function, indicating that most T cell production occurred in the periphery (27, 32).

B, Total sJTREC in peripheral blood/LT. C, sJTREC concentration within CD4⁺ T cells (in units of sJTREC/1.5 × 10⁵ cells). D, sJTREC concentration within CD8⁺ T cells (in units of sJTREC/1.5 × 10⁵ cells). The experimental data from Ref. 9 (△) represent individual cases, whereas the data from Ref. 10 (▲) are the median of multiple cases.
Elucidating principal factors contributing to TREC concentration in healthy people

The key to interpreting TREC data is to quantitatively distinguish between the effects of thymic output and T cell turnover on sjTREC concentration (38). Although T cell division and T cell death can be measured experimentally, RTE levels cannot. To explore how both thymic and peripheral events affect sjTREC concentration, we study the parameters that correspond to each of four events that determines the sjTREC concentration: thymic output; T cell division; T cell death; and sjTREC degradation. In this section, we address relative contributions of these four events to sjTREC concentration in healthy people, which will help interpret sjTREC data during T cell depletion conditions. To do this, we first examine how these four events affect sjTREC concentration at each specific age, and then we test how these four events combined induce sjTREC concentration decline during 80 years in healthy people.

sjTREC concentration dynamics at a given age. To address relative contributions to sjTREC concentration at a given age, we implement uncertainty and sensitivity analyses for four parameters: SP4 emigration rate; T cell division rate; T cell death rate; and sjTREC degradation rate (cf Ref. 39). Detailed methods for these analyses are discussed in the Appendix. We present results for one of the outcome variables, sjTREC concentration within CD4\(^+\) T cells.

We first perform the uncertainty analysis by varying each parameter independently. Shown in Fig. 5 are the changes in sjTREC concentration in response to individual variations of each parameter. The solid line represents the median level of sjTREC concentration when the parameter is given its baseline value. The range between upper and lower bounds represents the 95% confidence interval for the simulated median of sjTREC concentration. According to the 95% confidence interval, thymic emigration, T cell division, and sjTREC degradation affect sjTREC concentration to a greater extent than does T cell death, and this is true at any age over 80 years of life (p < 0.005). No significant differences are found between the effects of thymic output and T cell division (p > 0.05), and we assume the sjTREC half-life (degradation rate) is likely constant. Therefore, our results indicate that, at any age, when these events have similar magnitude changes, thymic output and peripheral T cell division can have an equal impact on sjTREC concentration, whereas T cell death affects it less.

We also perform the same uncertainty analyses for total sjTREC levels and total T cell levels. Our results indicate that decreased thymic output leads to a decrease in total sjTREC levels and total T cell levels, which is more evident early in life. Increased T cell division affects T cell levels, but not total sjTREC levels. Increased T cell death leads to a decrease in total sjTREC levels as well as T cell levels (data not shown).

The above experiments were performed by varying each of four events, thymic output, T cell division, T cell death, and sjTREC degradation, independently. What remains to be determined is the effect on sjTREC concentration when all four events are allowed to
vary in combination. This sensitivity analysis will assess the importance of these four events relative to each other for inducing variability in sjTREC outcomes. Model simulations of sjTREC concentrations for nine virtual patients show large variations and different shape curves (Fig. 6) arising from uncertainties in the four governing parameters. Differences in the values of four parameters may explain the considerable discrepancy observed from patient to patient and study to study (Fig. 4, C and D) (9, 10, 24).

To explore which events contribute most to the variations observed in sjTREC concentration, we perform the same experiment 10 times (with 9 samples each time as in Fig. 6) by simultaneously varying all 4 parameters within a large range. Parameter sensitivity is represented by the mean partial rank correlation coefficients (PRCC) between sjTREC concentration and each parameter: emigration rate of SP4 cells; T cell division rate; T cell death rate; and sjTREC degradation rate, respectively. We perform this calculation at different ages to assess whether certain parameters have greater or lesser effects at different times (Table I). The SP4 emigration rate, T cell division rate, T cell death rate, and sjTREC degradation rate are all significantly correlated with sjTREC concentration at any age (p < 0.05). The contributions of thymic output, T cell division, and death to sjTREC concentration gradually increase during aging, whereas sjTREC degradation remains constant. Thymic output, T cell division, and sjTREC degradation are more critical in regulating sjTREC concentration than is T cell death (p < 0.005). No significant difference is observed between the effects of thymic output and T cell division on sjTREC concentration (p > 0.05). If we assume the half-life of sjTREC is relatively constant, our analyses suggest that both thymic output and T cell division can strongly and equally affect sjTREC concentration at any age. These results are consistent with our previous findings when varying the parameters individually (Fig. 5). Similar results are also observed for sjTREC concentration within CD8⁺ T cells when varying these parameters (data not shown). This important finding distinguishes the relative roles of thymus and peripheral T cell turnover in regulating sjTREC concentration and provides a quantitative rule to characterize which events affect sjTREC concentration during disease states.

**sjTREC concentration dynamics during 80 years.** The uncertainty and sensitivity analyses characterized how thymus and T cell events can affect sjTREC concentration at each specific age. To assess the sjTREC concentration decline during the overall aging process, we simulate the sjTREC concentration within CD4⁺ T cells using either a constant thymic output, or a constant growth rate, or a constant death rate (values at age 1 year). As shown in Fig. 7, when fixing either the growth rate (dashed line) or the death rate (long dashed line) to be constant, simulations of sjTREC concentration have a similar trend of decline as the healthy controls (solid line). However, when thymic output is maintained at a fixed level, the resulting simulation of sjTREC concentration remains in a relatively steady state during 80 years (dotted line). This finding indicates that decreased output of RTE due to thymic involution predominantly elicits the decline of sjTREC concentration within CD4⁺ T cells during aging. This result is robust when we apply any constant values for thymic output, T cell growth rate, or T cell death rate (data not shown). Similar results are achieved for sjTREC concentration within CD8⁺ T cells (data not shown). Therefore, sjTREC concentration can represent thymic output for healthy people during the aging process.

**Elucidating principal factors contributing to TREC concentration decline during HIV-1 infection**

Our human thymopoiesis and TREC model provides a systematic way to quantitatively analyze how the thymus and peripheral T cell turnover contribute to changes in sjTREC concentration during T cell depletion situations, such as HIV-1 infection, hemopoietic stem cell transplantation preceded by chemoradiotherapy, thymectomy, and chemotherapy.

To apply our TREC model as a predictor of thymic function, we examine an HIV-1 infection scenario. HIV-1 disease is characterized by a gradual decline of CD4⁺ T cells, with an initial rise followed by a fall in CD8⁺ T cells during a 10-year period typically (40). sjTREC concentrations within both CD4⁺ and CD8⁺ T cells decrease during early HIV-1 infection (9, 13). Some studies suggest that the decline in sjTREC concentration results from a decrease in thymic output (9, 41). Evidence shows that HIV-1

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**Table I. PRCC between sjTREC concentration and each parameter**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>SP4 Emigration Rate (c₁)</th>
<th>T Cell Division Rate (γ₁)</th>
<th>T Cell Death Rate (δ₁)</th>
<th>sjTREC Degradation Rate (ε₁)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.73</td>
<td>-0.77</td>
<td>0.64</td>
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</tr>
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<tr>
<td>40</td>
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<td>-0.84</td>
</tr>
<tr>
<td>70</td>
<td>0.84</td>
<td>-0.90</td>
<td>0.73</td>
<td>-0.86</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.78 ± 0.05*</td>
<td>-0.82 ± 0.06*</td>
<td>0.67 ± 0.03</td>
<td>-0.85 ± 0.01*</td>
</tr>
</tbody>
</table>

* Parameter sensitivity is evaluated by PRCC. Data represent mean PRCC from 90 experiments, and each PRCC is significant at the 0.05 level.

* p < 0.05, compared with T cell death rate using absolute values (Student’s t test).

†, p > 0.05, compared with T cell division rate using absolute values (Student’s t test).
interrupts thymopoiesis by directly infecting CD4⁺ thymocytes and stromal cells, inducing the decline of thymic output (42, 43, 44). Other studies indicate that global immune activation induced by HIV-1 leads to increased cell division, causing the dilution of sjTREC concentration (17, 25).

Experimental studies suggest that the division rate of CD4⁺ T cells in patients with HIV-1 infection increases ~1- to 2-fold as compared with uninfected controls, whereas the division rate for CD8⁺ T cells increases ~7- to 8-fold as compared with uninfected controls (25, 45, 46, 47). Death rates of both CD4⁺ and CD8⁺ T cells increase ~3- to 4-fold during HIV-1 infection (45, 46). No quantitative data have been reported as to how RTE levels change during HIV-1 infection, due to the lack of a phenotypic marker for human RTE. This is the basic question that hinders distinguishing the relative role of the thymus in HIV-1 infection. However, our model provides a method to address this question.

As shown in Fig. 8, we simulate changes in sjTREC concentrations and T cell levels during early HIV-1 infection in a virtual group of people age 30 years. To achieve dynamics similar to those reported by clinical data for both T cells and sjTREC concentrations, our model predicts that thymic output of CD4⁺ RTEs must decline 10- to 15-fold, and thymic output of CD8⁺ RTEs must decline 1- to 6-fold. Experimental data show that HIV-1 directly infects CD4⁺ thymocytes and causes the SP4:SP8 ratio to invert from 2 to 0.5 (20, 42), suggesting that the decrease in CD4⁺ RTE is greater than that occurring in CD8⁺ RTE, which is consistent with our model predictions.

Our analyses reveal that although sjTREC concentrations within CD4⁺ and CD8⁺ T cells similarly decline during HIV-1 infection, factors that contribute to these phenomena differ according to changes in kinetics of thymic output predicted by our model and T cell turnover reported from literature. Because similar level changes in thymic output and T cell division elicit parallel changes in sjTREC concentration and similar level changes in T cell death induce much less of an effect to sjTREC concentration for a short period of time, our results suggest that decreased thymic output mainly induces the decline of CD4⁺ sjTREC concentration, whereas increased peripheral T cell division and decreased thymic output both contribute to the decline in CD8⁺ sjTREC concentration. Therefore, sjTREC concentration within CD4⁺ T cells is an adequate representation of RTE levels; however, sjTREC concentration within CD8⁺ T cells is not a useful representation of thymic output during HIV-1 infection.

**Discussion**

The number of RTE exported from the thymus per day is a critical representation of thymic function. This is particularly important for reconstitution of the immune system under conditions resulting in temporary or permanent T cell depletion. Measuring the number of RTE produced per day is an invaluable tool for studying establishment of the naive T cell repertoire, as well as for providing age-dependent changes in thymic contribution to the peripheral T cell pool under normal conditions.

Characterization of human RTE has been hampered by the lack of a marker that distinguishes them from long-lived naive T cells. A number of phenotypic markers have been used for the evaluation of naive T cells, with no clear understanding of which naive phenotype is best enriched for by RTE, including CD45RA⁻/CD45RO⁺, CD45RA⁻/CD62L⁻/CD27⁻/CD95low (48). One study using a phenotypic approach suggested that CD103-bearing naive CD8⁺ T cells are a subpopulation of human RTE (12). Another study indicated that cord blood naive T cells are human RTE (49). These identified RTE have rapid rates of apoptosis like thymocytes, and the presence of IL-7 maintains their survival and expansion in an Ag-independent manner.

Our first objective was to quantify age-dependent changes in total numbers of human RTE produced per day, which cannot be experimentally measured. We develop a model of human thymopoiesis to capture thymic involution. This is the first model for studying human thymopoiesis and quantifying the number of RTE exported per day, and our model is developed based mainly on

**FIGURE 8.** Simulation of changes in T cell levels and sjTREC concentrations during early HIV-1 infection in a virtual group age 30 years. The solid line represents simulation of healthy controls. The dotted line and error bars represent simulation of means and 95% confidence intervals of means, respectively, for HIV-1-infected people. For CD4⁺ T cells, thymic output is decreased ~10- to 15-fold, cell division is increased 1- to 2-fold, and cell death is increased ~3- to 4-fold, as compared with healthy controls. For CD8⁺ T cells, thymic output is decreased ~1- to 6-fold, cell division is increased ~7- to 8-fold, and cell death is increased ~3- to 4-fold, as compared with healthy controls. The unit for sjTREC concentration is sjTREC/1.5×10⁵ cells. A, Changes in CD4⁺ T cells. B, Changes in CD8⁺ T cells. C, Changes in sjTREC concentration within CD4⁺ T cells. D, Changes in sjTREC concentration within CD8⁺ T cells.
human experimental studies. Our results indicate that thymic output per day decreases exponentially by two orders of magnitude during 80 years (Fig. 2D). These are useful data for monitoring thymic function in healthy individuals, as well as providing a baseline comparison of thymic output during disease states.

We quantify thymic output as a rate measurement of the number of RTE produced per day. Currently, the longevity and migration patterns of human RTE are not known, rendering the calculation of total numbers of RTE in the peripheral blood/LT difficult. However, total RTE levels are mainly affected by thymic output per day. Thus, monitoring thymic output per day is a more direct and accurate estimation of thymic function as compared with total RTE numbers.

Recently, TREC concentration has been widely used to measure thymic output. Data suggest that TREC levels change during aging and disease (9, 10, 13, 14, 15, 17, 24, 41). However, it is still an open question as to whether TREC concentrations are mainly affected by thymic output or peripheral T cell events. Different techniques have been used to measure TREC levels, and the unit for measuring TREC levels varies from study to study, which can affect interpretation of TREC data (9, 10, 17, 18).

Our second aim was to verify whether TREC concentration is an accurate predictor of thymic function, represented by the number of RTE produced per day during aging and HIV-1 infection. We first simulate sjTREC concentrations as they change with age and validate our model with two experiment data sets. Using uncertainty and sensitivity analyses, we next quantify how key processes potentially control the dynamics of sjTREC concentration. Our results strongly suggest that thymic output and peripheral T cell division could equally affect sjTREC concentration at any age. T cell death contributes less to changes in sjTREC concentration than do thymic output and T cell division. We further demonstrate that sjTREC concentration is a good measurement for both CD4+ and CD8+ RTE in healthy people and for CD4+ RTE during HIV-1 infection. Our findings have implications for interpreting TREC data during other T cell depletion situations, such as hemopoietic stem cell transplantation, thymectomy, and chemotherapy. We propose that peripheral T cell division and death should be examined before considering TREC concentration as a representation of thymic function.

A previous modeling study (17) suggested that only T cell division determines changes in TREC concentrations during aging and HIV-1 infection. This result is based on the assumption that neither division of naive T cells nor intracellular degradation of TREC occurs. Although division rates of naive cells are relatively low, they are unlikely to be zero. Their results later show that naive T cell division does occur. Similarly, although TREC are stably maintained within T cells, they have a finite half-life, likely shorter than or close to the half-life of T cells; TREC in chickens have been shown to have a half-life of ~2 wk (50). Our model predicts that sjTREC degradation rate is 0.002/day, similar to the T cell death rate, for example. The previous study (17) further assumes that naive T cell numbers are proportional to thymic output, and thus TREC concentration is not a measurement of thymic output but of T cell division. However, data suggest that naive T cell numbers and thymic output are not proportional, given that a subset of naive T cells have a very long lifespan and a subset of memory T cells can revert back to naive phenotype (22).

Recent clinical data support the thymus as a key contributor to TREC concentration decline during aging and HIV-1 infection (38, 41). One study suggests that changes in peripheral T cell division do not adequately explain many observations of TREC concentrations, including similar ratios of CD4+ TREC to CD8+ TREC in HIV-1-infected patients and healthy controls, similar TREC responses for discordant responders and good responders to antiretroviral therapy, and increased TREC concentration despite increased proliferation of T cells immediately after antiretroviral therapy (41). Another study indicates that the TREC concentration indeed reflects thymic output during aging (38). This study further suggests that thymic output most likely affects TREC concentration within CD4+ T cells and that a combination of increased T cell division and decreased thymic output reduces TREC concentration within CD8+ T cells after HIV-1 infection (38). These results are consistent with our model predictions for TREC concentrations during both aging and HIV-1 infection scenarios.

Almost all TREC studies in humans are performed using peripheral blood. However, the majority of T cells (~98%) exist in the peripheral lymphoid system (35-37). Thus, quantification of TREC levels in different LT compartments would contribute greatly to our understanding of TREC dynamics. One study using a primate animal model observed that CD4+ and CD8+ T cells present in the lymph nodes contain more sjTREC than do peripheral blood T cells, suggesting that RTE can home into lymphoid tissues (11). Another study using a rat model observed that the percentages of RTE among T cell population were comparable in blood and all other LT compartments, indicating that rat RTE continuously migrate through blood and lymphoid organs as naive T cells (51). Our TREC model presents average sjTREC concentrations in peripheral blood/LT assuming that the sjTREC concentration in T cells within LT is similar to those in peripheral blood T cells. If the results from the primate animal model is applicable to humans, then our model simulations of the sjTREC concentration represent a lower boundary approximation.

Quantification of TREC concentration in two distinct pools of memory and naive T cells is an important next step. TREC concentration has been measured in the naive T cell pool (17). The differences in turnover rates of these T cell subclasses can further elucidate TREC dynamics. Currently, most experiments on TREC concentration are measured using CD4+ and CD8+ T cells (9, 11-13, 18, 38, 41, 48). Therefore, we can accurately build a model for TREC dynamics in these two compartments. Our model can be further adapted to explore TREC dynamics in memory and naive T cell pools when more data are available.

Overall, this study characterizes the relative roles of thymic output and periphery T cell turnover in regulating TREC concentrations. We propose that peripheral T cell division and death should be examined together with TREC concentration. Our model can be used as an integrated system for testing whether TREC concentration is an accurate marker for RTE levels in all situations by incorporating both T cell dynamics and TREC concentrations that are experimentally measured.

**Appendix**

**Human thymopoiesis model**

Defining mathematical notation for the terms shown in Fig. 1, $T(t)$ represents TN cells at time $t$, $I(t)$ represents ITTP cells, $D(t)$ represents DP cells, $S_4(t)$ represents SP4 cells and $S_8(t)$ represents SP8 cells. The mathematical representation describing the interaction of these five thymocyte subsets is based on a previous model of mouse thymocyte subsets (52). However, the thymopoiesis process is different in humans and mice (compare Refs. 19 and 23), and our model reflects these differences. We suppress the time dependence, $(t)$, in the variables for ease of readability, except where needed for emphasis:

$$\frac{dT}{dt} = s(t) + r_1 \left( 1 - \frac{z(t)}{T(t)} \right) T - f_I T - d_I T,$$

$$\frac{dI}{dt} = f_I T + r_2 \left( 1 - \frac{z(t)}{T(t)} \right) I - f_D I - d_D I,$$

$$\frac{dD}{dt} = f_D I + r_3 \left( 1 - \frac{z(t)}{T(t)} \right) D - f_D D - d_D D.$$
Evaluating the value of these two terms, our model provides a way to quantify

In division and death terms, variables and parameters defined in the thymopoiesis model

TREC concentrations are determined by total TREC dynamics and total T cell dynamics. Define $T_4$ and $T_5$ to represent total CD4$^+$ and CD8$^+$ T cells in both blood/LT, respectively, and $T_6$ and $T_7$ to represent total sjTREC levels within CD4$^+$ and CD8$^+$ T cells in both blood/LT, respectively. Then a model for sjTREC and T cell dynamics based on Fig. 3 is given by

$$\frac{dT_4}{dt} = e_1 S_4 + \delta_1 T_4 - \delta_2 T_5$$

(6)

$$\frac{dT_5}{dt} = e_2 S_5 + \delta_1 T_4 - \delta_2 T_5$$

(7)

$$\frac{dT_6}{dt} = c_1 e_1 S_4 - \delta_1 T_6 - \sigma_1 e_1$$

(8)

$$\frac{dT_7}{dt} = c_2 e_2 S_5 - \delta_1 T_7 - \sigma_2 e_2$$

(9)

The thymus provides newly generated T cells to the periphery (e$S_4$, e$S_5$), and the level of thymic output gradually declines during aging. Regenerative division of T cells (g$S_4$) helps to maintain peripheral T cell homeostasis during human growth, as a compensation to the involution of thymic function (27, 32). Death of T cells (d$S_4$, d$T_5$, d$T_6$, d$T_7$) occurs in a density-dependent fashion. In division and death terms, k$S_4$ and k$T_5$ represent the average numbers of total CD4$^+$ and total CD8$^+$ T cells in the peripheral blood/LT, respectively. Here we model T cell dynamics for healthy individuals during aging: thus T cell growth and death are not further induced by Ag and inflammatory signals.

Total sjTREC levels are mainly affected by three processes: input from the thymus (c$e_1 S_4$, c$e_2 S_5$), loss due to death of T cells (d$S_4$, d$T_5$, d$T_6$, d$T_7$), and loss due to degradation of sjTREC within T cells (e$T_6$, e$T_7$). The loss of TREC due to T cell death is proportional to T cell death.

We do not include T cell division and differentiation terms, because these two processes do not affect total sjTREC levels. With dynamics of both total sjTREC and total T cells, TREC concentrations can be simulated by dividing sjTREC by T cells.

### Parameter estimation

The variables and parameters used in the human thymopoiesis model Equations 1–5 are defined in Table II. The parameters are the rate coefficients for each process described. Values for variables and rates were estimated from the literature. We give weight to human data, followed by data generated by murine and in vitro systems. For rates for which poor or no data exist, we perform uncertainty and sensitivity analyses to explore the change in outcomes over a full range of possible values. We outline below how we estimated the parameters.

Although the thymus undergoes involution during aging, the distribution of thymocyte subsets that is seen in adults is similar to the distribution observed in fetuses (3). TREC levels per thymocyte in adults also appear to be the same in fetuses and newborns (3). Thymic stromal cells, which are associated with thymocyte development, remain present and active throughout life (53). These data suggest that the effect of aging on the thymus appears to be quantitative rather than qualitative. To describe thymic involution dynamics, we represent the maximal number of thymocytes in TES region, $T_{es}(t)$, and the bone marrow cell source, $s(t)$, as time-dependent functions:

$$T_{es}(t) = \frac{4.20 \times 10^9 \times \left(0.00285 e^{-0.0103 t} + 0.001358\right)}{1.94 \times 10^{11} - T_{es}(1\text{ yr}) \times 0.943^{t - 1\text{ yr}} + T_{es}(t)}$$

(10)

$$s(t) = 5 \times 10^4 \times \frac{t}{10^{11} - T_{es}(t)}$$

(11)

where $w(t) = 3.911 + 4.082(t/365) - 0.060(t/365)^2$ is the function for body weight. The body weight function is obtained by fitting data (28) using nonlinear least squares.

The lymphatic thymic tissue is composed of TES and perivascular space (2). The TES region, where thymopoiesis occurs, contains all of

Table II. Variables and parameters defined in the thymopoiesis model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Value$^a$</th>
<th>Unit</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T$</td>
<td>TN cells</td>
<td>$1.80 \times 10^9$</td>
<td>/thymus</td>
<td>3, 19, 58</td>
</tr>
<tr>
<td>$I$</td>
<td>ITTP cells</td>
<td>$1.29 \times 10^9$</td>
<td>/thymus</td>
<td>3, 19, 58</td>
</tr>
<tr>
<td>$D$</td>
<td>DP cells</td>
<td>$3.76 \times 10^8$</td>
<td>/thymus</td>
<td>3, 19, 58</td>
</tr>
<tr>
<td>$S_4$</td>
<td>SP4 cells</td>
<td>$7.21 \times 10^8$</td>
<td>/thymus</td>
<td>3, 19, 58</td>
</tr>
<tr>
<td>$S_5$</td>
<td>SP8 cells</td>
<td>$3.61 \times 10^8$</td>
<td>/thymus</td>
<td>3, 19, 58</td>
</tr>
<tr>
<td>Parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r_1$</td>
<td>Growth rate of TN cells</td>
<td>1.5</td>
<td>/day</td>
<td>23</td>
</tr>
<tr>
<td>$r_2$</td>
<td>Growth rate of ITTP cells</td>
<td>1.0</td>
<td>/day</td>
<td>23</td>
</tr>
<tr>
<td>$r_3$</td>
<td>Growth rate of DP cells</td>
<td>1.5</td>
<td>/day</td>
<td>23</td>
</tr>
<tr>
<td>$r_4$</td>
<td>Growth rate of SP4 cells</td>
<td>0.5</td>
<td>/day</td>
<td>23</td>
</tr>
<tr>
<td>$r_5$</td>
<td>Growth rate of SP8 cells</td>
<td>0.5</td>
<td>/day</td>
<td>23</td>
</tr>
<tr>
<td>$f_1$</td>
<td>Differentiation rate of TN cells into ITTP cells</td>
<td>0.3</td>
<td>/day</td>
<td>19</td>
</tr>
<tr>
<td>$f_2$</td>
<td>Differentiation rate of ITTP cells into DP cells</td>
<td>0.5</td>
<td>/day</td>
<td>19</td>
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<tr>
<td>$f_3$</td>
<td>Differentiation rate of DP cells into SP4 cells</td>
<td>0.04</td>
<td>/day</td>
<td>3, 59</td>
</tr>
<tr>
<td>$f_4$</td>
<td>Differentiation rate of DP cells into SP8 cells</td>
<td>0.02</td>
<td>/day</td>
<td>3, 59</td>
</tr>
<tr>
<td>$e_1$</td>
<td>Emigration rate of SP4 cells</td>
<td>0.07</td>
<td>/day</td>
<td>7</td>
</tr>
<tr>
<td>$e_2$</td>
<td>Emigration rate of SP8 cells</td>
<td>0.07</td>
<td>/day</td>
<td>7</td>
</tr>
<tr>
<td>$d_1$</td>
<td>Death rate of TN cells</td>
<td>0.27</td>
<td>/day</td>
<td>23, estimated</td>
</tr>
<tr>
<td>$d_2$</td>
<td>Death rate of ITTP cells</td>
<td>0.17</td>
<td>/day</td>
<td>23, estimated</td>
</tr>
<tr>
<td>$d_3$</td>
<td>Death rate of DP cells</td>
<td>0.33</td>
<td>/day</td>
<td>23, estimated</td>
</tr>
<tr>
<td>$d_4$</td>
<td>Death rate of SP4 cells</td>
<td>0.26</td>
<td>/day</td>
<td>23, estimated</td>
</tr>
<tr>
<td>$d_5$</td>
<td>Death rate of SP8 cells</td>
<td>0.26</td>
<td>/day</td>
<td>23, estimated</td>
</tr>
</tbody>
</table>

$^a$ Values for variables are initial values.

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by guest on April 21, 2017 http://www.jimmunol.org/ Downloaded from
the thymocytes. A precise correlation has been shown between the number of RTE and functional thymic tissue in chickens (5). Here we assume that the volume of the TES region represents the maximum allowable number of thymocytes, i.e., the carrying capacity of the thymus for total thymocytes. The TES region reaches its highest level of 21.8 cm\(^3\) by the age of one (2), containing maximally 10\(^{11}\) thymocytes (54). We use the ratio 10\(^{11}\) thymocytes/21.8 cm\(^3\) TES volume to calculate the maximal number of thymocytes from the TES volume (Fig. 2c). The values for the TES volume are obtained by subtracting the mean volume of perivascular space from the mean volume of lymphatic thymic tissue by corresponding age groups from a stereological evaluation of 136 human thymuses (2). The maximal number of thymocytes, \(T_{th}(t)\), can be obtained by a best fit function using nonlinear least squares (Equation 10 for 1 year < \(t\)). The thymic involution rate is 5.66% per year after the first year of life. During the first year of life, the function defining the maximal number of thymocytes is derived from the calculation of thymus weight (Equation 10 for 0 \(<\ t \leq 1\ \text{year}\) (55).

Bone marrow shows no loss of function with age (54); however, the recovery of normal T cell subsets after bone marrow transplantation depends on thymic function (56, 57). On the basis of this evidence, we assume that the number of source cells from bone marrow utilized by the thymus is determined by the number of available thymocytes. Thus, we track this change in cell source, \(s(t)\) (Equation 11), as a function of \(T_{th}(t)\). The seeding rate of progenitor cells into TN cells is 5 \(10^4\) per mouse (58). The maximal number of thymocytes, \(T_{th}(t)\), can be obtained by a best fit function using nonlinear least squares (Equation 10 for 1 year < \(t\)). The thymic involution rate is 5.66% per year after the first year of life. During the first year of life, the function defining the maximal number of thymocytes is derived from the calculation of thymus weight (Equation 10 for 0 \(<\ t \leq 1\ \text{year}\) (55).

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The initial value of total thymocytes is 75% of maximal number of thymocytes, implying that one-third of total thymocytes are produced each day. This is derived from a calculation of growth rates, which is consistent with experimental reports (23, 58). The percentages of TN, ITTP, DP, SP4, and SP8 cells are 3.5, 2.5, 73, 14, and 7%, respectively, from studies of human thymic specimens (3, 19). Thus, the initial values for the five thymocyte subsets are calculated by multiplying 75% of maximal number of thymocytes at birth by the corresponding subset percentage. This allows for up to a 25% increase in thymocyte number.

Growth rates of thymocytes have been measured experimentally in the range of 0.5–1.5/day in mice (23). Generally, immature thymocytes have greater potential for expansion, whereas mature thymocytes grow relatively slowly (23). We estimate growth rates of TN, ITTP, DP, SP4, and SP8 cells to be 1.5/day, 1.0/day, 1.5/day, 0.5/day, and 0.5/day, respectively.

It takes 3 days on average for TN cells to differentiate into ITTP cells (19); thus, the differentiation rate for TN cells is 0.33/day. Similarly, the differentiation rate for ITTP cells is calculated as 0.53/day (19). Approximately 5% of DP cells survive positive and negative selection to become SP cells (59), and the ratio of SP4/SP8 equals 2 (3); therefore, we estimate the differentiation rates of SP4 and SP8 cells to be 0.04/day and 0.02/day, respectively. Newly formed SP cells spend an average of 14 days in the thymus before emigration into the periphery as observed in a murine thymus system (7). Hence emigration rates of SP4 and SP8 cells are estimated as 0.07/day.

Although no available data exist for death rates of subsets of human thymocytes, the average lifespan of mouse thymocytes has been estimated to be 3 days (23). If we assume that the thymus is in steady state over a short time, then the maximal number of thymocytes and cell source are constant and Equations 1–5 can be set to 0 (i.e., no change in the cell rates). Based on that assumption, the death rates for the five subsets of thymocytes can be calculated to be 0.27/day, 0.17/day, 0.33/day, 0.26/day, and 0.26/day, for TN, ITTP, DP, SP4, and SP8 cells, respectively.

The variables and parameters used in the human TREC model Equations 6–9 are defined in Table III. The average numbers of CD4\(^+\) and CD8\(^+\) T cells in the blood linearly increase during the first 30 years of life and then reach a relative steady state, considering age-dependent changes in cell number per mm\(^3\) blood, blood volume, and body weight (26, 28–31). We assume that the number of T cells in the LT have a pattern similar to that in the blood during human growth. Thus, the functions for the average numbers of total CD4\(^+\) and total CD8\(^+\) T cells in the peripheral blood/LT are as follows:

\[
k_d(t) = \begin{cases} 
4.33 \times 10^{10} \times t & \text{for } 0 \leq t \leq 30 \text{ years} \\
3.7 \times 10^{10} \times t & \text{for } 30 \text{ years } < t
\end{cases}
\]

(12)

Conflicting data exist regarding the lifespan of T cells. The half-life of T cell subgroups are likely different as well. For example, naive T cells may live longer than memory T cells (45, 46). Literature suggests that the T cell death rate ranges from 0.0001/day to 0.014/day (45, 46, 60). Because the death rate in this model is the average death rate for the total CD4\(^+\) T cell population or for the total CD8\(^+\) T cell population, and the half-life for CD4\(^+\) and CD8\(^+\) T cells is similar (45, 46), we choose the value 0.002/day for both death rates of CD4\(^+\) and CD8\(^+\) T cell populations, respectively. Similarly, the division rate of T cells has been estimated within the range of 0.0007/day to 0.01/day (45, 60). We choose the value 0.002/day for both CD4\(^+\) and CD8\(^+\) T cell division rates. Half-saturation constants \(s_1\) and \(s_2\) are estimated from the model simulation as 10\(^8\).

There were approximately 200,000 sjTREC per 150,000 CD4\(^+\) and CD8\(^+\) T cells (100% naïve type) observed in cord blood (9). Three to four cell divisions occur between rearrangements that produce sjTREC and sjTREC (9). Thus, on average 200,000sjTREC = 17677.67 sjTREC exist within 150,000 CD4\(^+\) and CD8\(^+\) T cells in cord blood. The concentration of sjTREC within cord blood T cells is calculated to be 17677/150,000 = 0.118/cell. The sjTREC concentration in cord blood CD4\(^+\) and CD8\(^+\) T cell division rates. Half-saturation constants \(s_1\) and \(s_2\) are estimated from the model simulation as 10\(^8\).

Table III. Variables and parameters defined in the TREC model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Value(^a)</th>
<th>Unit</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_{th})</td>
<td>Total CD4(^+) T cells</td>
<td>4.33 \times 10^{10}</td>
<td>/blood and LT</td>
<td>28, 29, 30</td>
</tr>
<tr>
<td>(T_{th})</td>
<td>Total CD8(^+) T cells</td>
<td>1.80 \times 10^{10}</td>
<td>/blood and LT</td>
<td>28, 29, 30</td>
</tr>
<tr>
<td>(r_t)</td>
<td>Total sjTREC within CD4(^+) T cells</td>
<td>5.11 \times 10^9</td>
<td>/blood and LT</td>
<td>9, 28, 29, 30</td>
</tr>
<tr>
<td>(r_t)</td>
<td>Total sjTREC within CD8(^+) T cells</td>
<td>2.12 \times 10^9</td>
<td>/blood and LT</td>
<td>9, 28, 29, 30</td>
</tr>
<tr>
<td>Parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(y_1)</td>
<td>Growth rate of CD4(^+) T cells</td>
<td>0.002</td>
<td>/day</td>
<td>45, 60</td>
</tr>
<tr>
<td>(y_2)</td>
<td>Growth rate of CD8(^+) T cells</td>
<td>0.002</td>
<td>/day</td>
<td>45, 60</td>
</tr>
<tr>
<td>(\delta_1)</td>
<td>Death rate of CD4(^+) cells</td>
<td>0.002</td>
<td>/day</td>
<td>45, 46, 60</td>
</tr>
<tr>
<td>(\delta_2)</td>
<td>Death rate of CD8(^+) cells</td>
<td>0.002</td>
<td>/day</td>
<td>45, 46, 60</td>
</tr>
<tr>
<td>(s_1)</td>
<td>Half-saturation constant for CD4(^+) T cells on growth rate</td>
<td>1 \times 10^{10}</td>
<td>cell</td>
<td>Estimated</td>
</tr>
<tr>
<td>(s_2)</td>
<td>Half-saturation constant for CD8(^+) T cells on growth rate</td>
<td>1 \times 10^{10}</td>
<td>cell</td>
<td>Estimated</td>
</tr>
<tr>
<td>(e_1)</td>
<td>Degradation rate of sjTREC within CD4(^+) T cells</td>
<td>0.002</td>
<td>/day</td>
<td>50, estimated</td>
</tr>
<tr>
<td>(e_2)</td>
<td>Degradation rate of sjTREC within CD8(^+) T cells</td>
<td>0.002</td>
<td>/day</td>
<td>50, estimated</td>
</tr>
<tr>
<td>(c_1)</td>
<td>sjTREC concentration within CD4(^+) RTE</td>
<td>0.118</td>
<td>sjTREC/cell</td>
<td>9</td>
</tr>
<tr>
<td>(c_2)</td>
<td>sjTREC concentration within CD8(^+) RTE</td>
<td>0.118</td>
<td>sjTREC/cell</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^a\) Values for variables are initial values.
by varying the sjTREC degradation rates for CD4+ and CD8+ T cells. Finally, we use the value of 0.002/day as the sjTREC degradation rate.

The initial conditions (at birth) for total CD4+ and CD8+ T cells are 4.33 × 10^10 and 1.80 × 10^10, respectively (28–30). The initial condition for total sjTREC levels within CD4+ T cells is 5.109 × 10^11, calculated by multiplying total CD4+ T cell number at birth by 0.118 sjTREC/cell (TREC concentration in RTE). Similarly, 2.124 × 10^11 has been calculated as the initial condition for total sjTREC level within CD8+ T cells.

Once the model equations are derived and the parameter values are estimated, we then solve the system of nonlinear ordinary differential equations using an appropriate numerical method. We solve the system in two separate computational programs to ensure accuracy.

Uncertainty and sensitivity analysis

We use a Latin Hypercube Sampling scheme and PRCC (of Ref. 39) for uncertainty and sensitivity analyses, respectively. Briefly, for the uncertainty analysis, a random sample of each of the parameters to be tested is generated from a list of parameter ranges and distributions. In our case, this sampling is done 90 times. The values in each column of the matrix generated from the sampling are randomly chosen values for a given parameter from a widely defined, evenly partitioned range of values. The sets of values in each row of the matrix are then used in 90 independent simulations. These values provide a range of results for each solution curve generated, evenly partitioned range of values. The sets of values generated from the sampling are randomly chosen values for a given parameter range. In our case, this uncertainty analysis, a random sample of each of the parameters to be tested is generated from a list of parameter ranges and distributions. In our case, this sampling is done 90 times. The values in each column of the matrix generated from the sampling are randomly chosen values for a given parameter from a widely defined, evenly partitioned range of values. The sets of values in each row of the matrix are then used in 90 independent simulations. These values provide a range of results for each solution curve generated, evenly partitioned range of values. The sets of values generated from the sampling are randomly chosen values for a given parameter range.

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10. DiRita, David Markovitz, and Athena Kourtis and Seema Bajaria for help-


20. DiRita, David Markovitz, and Athena Kourtis and Seema Bajaria for help-


