The Contribution of the Thymus to the Recovery of Peripheral Naive T-Cell Numbers During Antiretroviral Treatment for HIV Infection

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Abstract: The quantitative contribution of the thymus to the maintenance of peripheral populations of naive T cells is poorly understood. Several new lines of evidence indicate that thymic activity continues into adulthood, albeit at lower levels than in early life, and that this is important for a range of lymphopenic disorders. A measure of thymic activity that is often used is the quantification of T-cell receptor excision circles (TRECs). It has been shown that TREC levels decline after infection with HIV-1 and that they recover to above normal levels after antiretroviral treatment. The reasons for the latter observation are unknown. Here we quantitatively explore different possible causes for supranormal levels of TREC per cell and show that the small total number of cells involved in reconstituting the TREC+ T-cell pool of HIV-1–infected patients suffices to explain the observation. Even the expected small thymic outputs into a strongly depleted naive T-cell peripheral pool lead to a slow transient of elevated levels of TREC per cell. The main biological lesson from our quantitative modeling approach is that middle-aged human thymi continue to produce naive T cells and that this production can be demonstrated by tracking the increase of total TREC numbers (rather than the TREC content).

Key Words: HIV, thymus, TREC, mathematical models

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INTRODUCTION

The quantitative contribution of the thymus to the maintenance of peripheral populations of naive T cells is poorly understood but is known to decline with age. Thymic activity into adulthood has recently been shown to be important for reconstituting the peripheral naive T-cell pool and for restoring repertoire diversity after lymphopenic conditions, such as HIV infection and certain cancer therapies.[1–4] New quantitative assays based on measuring T-cell receptor excision circles (TRECs), which are formed during T-cell ontogeny in the thymus, have been developed to identify recent thymic emigrant T cells in the periphery.[5,6] These assays may allow a better characterization of the role of thymic output in reconstituting naive T cells with increasing age, after bone marrow transplantation, and during antiretroviral therapy for HIV infection.

Several studies have shown that T cells in HIV-infected subjects have a lower TREC content (ie, TREC per cell) compared with age-matched uninfected individuals.[5,7–9] TREC measurements between individuals are variable, however, and there is a substantial overlap between the TREC content of HIV patients and age-matched controls.[9] Different studies have shown that antiretroviral treatment leads to an increase in TREC+ cells,[5,10–12] and at least one report indicated that after therapy with successful suppression of viremia, HIV-infected people can have higher TREC content in their naive T cells than normal subjects (ie, "supranormal" levels of TREC), and this occurs before the naive T-cell numbers have recovered.[11] Several other studies suggest a similar pattern of supranormal TREC contents, which is reviewed below in "Experimental Data." However, there is no consensus on how to interpret the supranormal TREC contents during highly active antiretroviral therapy (HAART).

We and others have shown that the precise interpretation of TREC data depends heavily on the assumed dynamics of naive T cells and recent thymic emigrants (RTEs) in particular.[8,13,14] Notwithstanding these difficulties, several putative mechanisms have been put forth to explain the supranormal levels of TREC content upon successful suppression of viremia by HAART.[10,11,15] One possibility is that there are different subpopulations of naive T cells, with different TREC content. For example, Kimmig et al[16] have shown that a subpopulation of naive CD4+ T cells expressing the CD31 marker is enriched for TREC and suggested that these could be true RTEs. However, CD31 cannot be a unique marker for RTEs, because in human adults thymic output should be low, and a major fraction of naive T cells is CD31 +.[16–18] One study showed that the repertoire diversity of CD31− naive T cells is much lower than what one would expect for a true naive repertoire,[18] suggesting that CD31− naive T cells have been selected to clonally expand, although

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another study showed that the CD31− naive T-cell repertoire is diverse.[17] Finally, the TREC contents of CD31+ and CD31− naive T cells decline in parallel,[17] demonstrating that CD31+ naive T cells also divide. Other experiments, measuring the regulation of thymic output after grafting extra thymii into mice, have indicated that RTEs are a subpopulation of the naive compartment with dynamics that are different from the established peripheral naive T-cell pool.[19,20] It has been proposed[11,15] that this RTE population is responsible for the rapid increase in TREC content mentioned above if most of the TRECs reside in a relatively small subpopulation of RTEs with distinct dynamics.[21]

Another possibility is that the subpopulations of TREC+ and TREC– cells have different trafficking properties resulting in a different spatial distribution between blood and lymph nodes.[22,23] Because TREC+ naive T cell should on average be less mature than TREC−naive T cells, one could understand that TREC+ naive T cells preferentially home to lymphoid tissues. Because HAART induces a redistribution of T cells from lymph nodes to blood, suggested to be the main source of the early increase in memory CD4+ T cells in the blood,[24–29] HAART could affect TREC+ and TREC− cells in different ways, leading to increase in TREC content in the periphery during therapy.[11,22] A third possibility has been implicated by Di Mascio et al,[10] who interpreted their longitudinal data using a mathematical model. In this way, they suggested that quantitative differences in the normalization of the model parameters (ie, death, proliferation, and activation rates) during HAART explain the dynamics of TREC versus naive cells. Finally, other processes may be at play, including abnormal recovery of inflammation status and compromised or altered signals, such as cytokine production or major histocompatibility complex class II interactions.[11]

Our objective here is to systematically present a quantitative analysis of the hypotheses of redistribution and the putative 2 populations in explaining the evidence for supranormal TREC per cell. We also analyze a new hypothesis that we believe explains most of the observations in a more parsimonious way. Our major finding is that even low thymic production, of a few new TREC+ naive T cells, suffices to explain the observations, given that total naive T-cell numbers are severely depleted. The most important implications of this are that the size of the naive T-cell pool plays a major role in the TREC dynamics, that the thymus remains functional in these adult human patients, and that thymic output can be measured by the recovery of the peripheral TREC+ T-cell populations.

RESULTS

Experimental Data

An important experimental observation that came to light recently is that in the periphery TRECs increase faster than naive T cells do upon HAART treatment of HIV-1–infected individuals. Several groups have explicitly made this observation.[10,11] At least one detailed study has shown that not only TRECs in naive cells increase with treatment but also that TREC per cell in successfully treated patients achieved levels higher than in age-matched uninfected controls.[11] Adding the information on TREC totals (TREC per μL), we depict the data from this study in Figure 1. Taking age explicitly into consideration, naive T-cell numbers in treated patients remain significantly below normal ($P = 0.0001$) whereas the TREC total normalizes ($P = 0.71$). As a consequence, the TREC content is supranormal in treated patients ($P = 0.016$) after controlling for age. Indeed, in these well-suppressed HIV-infected patients, who are on therapy for a median of 20 months, the median TREC content was 0.026 TREC-naive T cell whereas in age-matched uninfected controls, the corresponding median was almost 10-fold lower, that is, a frequency of 0.0027 TREC-naive T cell.

The Harris et al[11] study had a cross-sectional population design. In a more recent longitudinal study,[10] 23 HIV-infected patients were followed from the start of HAART up to 42 months posttreatment initiation. For each patient, naive T-cell numbers, total TREC, and TREC content were measured at least on 3 occasions: baseline, 5–8 months posttreatment start (median 6 months), and 13–42 months after HAART (median 18 months). The total number of TRECs recovered faster than the naive T cells, that is, a ~4-fold increase versus a ~2-fold increase, respectively, over the 18-month period. This implied a very fast increase in TREC content that reached a mean of more than 0.2 per naive T cell at the 6-month time point.[10] This study did not include age-matched controls, but such a high TREC content is expected to be supranormal,[9] although their patients had unusually high number of TREC per cell before treatment.

It is important to note that several other studies indicate similar longitudinal trends. For example, in a study of TREC and HIV infection, longitudinal data were presented for a number of individual patients during treatment.[5] In at least some patients studied, the TREC content in naive T cells increases much faster than naive T-cell numbers (eg, patients A36, C25, D29, E29) whereas other patients show similar behavior for those 2 quantities. In another study where TREC content was analyzed in peripheral blood mononuclear cells (PBMC), chronically infected patients starting at low levels of TREC content (<0.0022 TREC per PBMC) had large increases in this variable, reaching apparent supranormal values by 9–12 months after the start of treatment (see Figure 4 in Zhang et al[9]).

This "meta-analysis" of current data on TREC dynamics during treatment of HIV+ patients suggests that TREC contents often, but not always, become supranormal. Analyzing different hypotheses to interpret these experimental data, we seek for an explanation for the phenomenon of supranormal TREC levels and for the fact that this phenomenon is not always observed.

Interpretation of the Experimental Data

Redistribution

One possible explanation for the supranormal TREC content is the preferential release of TREC+ cells (over TREC− cells) from the lymph nodes during treatment. Indeed, some evidence indicates that TREC+ cells are entrapped in lymphoid tissue during HIV infection[23] and that they "are rapidly and selectively released into circulation with antiretroviral treatment."[22] Experimental evidence indicates that

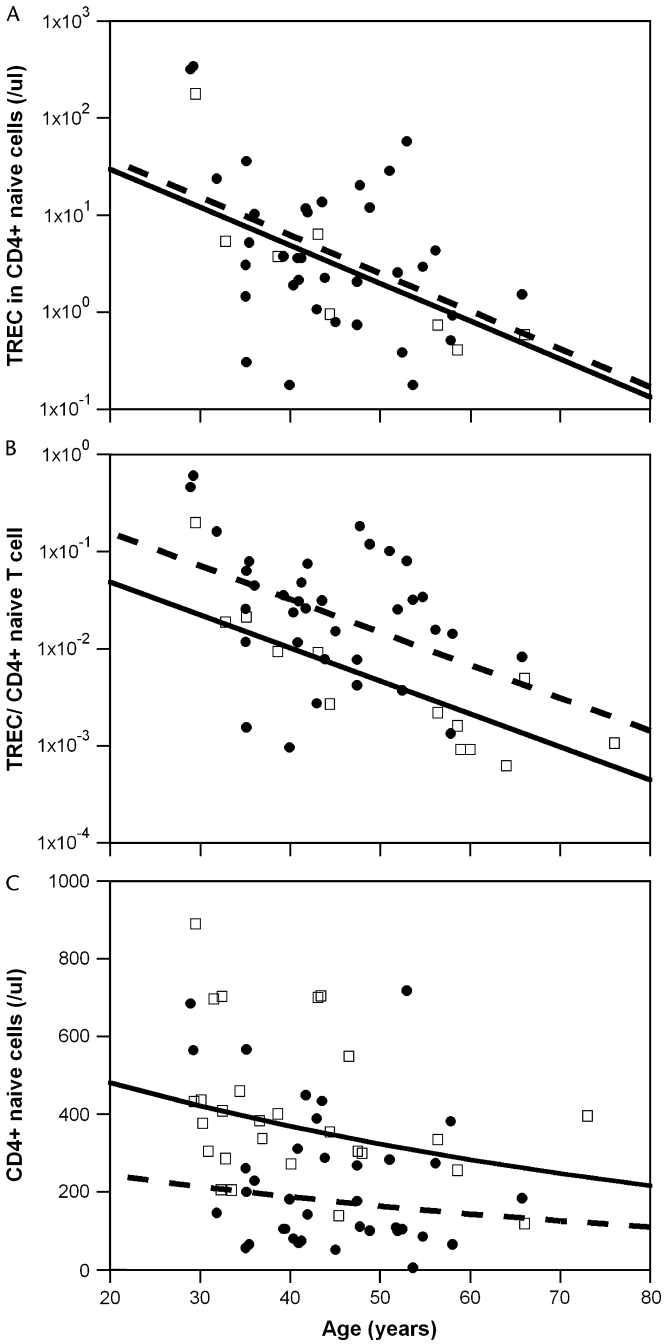

FIGURE 1. Change with age in (A) TREC total (per microliter), (B) TREC content (per naive T cell), and (C) naive T cells in the CD4+ population. The ● and □ represent data from HIV-infected and HIV-uninfected subjects, respectively. The straight lines show the best regression through the data for uninfected (solid line) and infected (dashed) subjects. There are no significant differences between the slopes of the lines in any panel, but there is an increased TREC content in HIV-infected subjects, when corrected for age ($P = 0.016$). Data from Harris et al.[11]

the TREC content of CD4+ T cells is higher in lymph tissue (LT) than in peripheral blood, both in healthy controls (1.38 cjTRECs/CD4+ T cell in LT vs 0.96 cjTRECs/CD4+ T cell in the blood) and during HIV infection (0.55 cjTRECs/CD4+ T cell vs 0.27 cjTRECs/CD4+ T cell, respectively)[23] (Fig. 2). Note that this study reports cjTREC rather than the more usual sjTREC, which explains the higher values, and that both types of TREC decline with age almost in parallel.[5] During HIV infection, there is a greater bias for retention of TREC+ cells in the LTs because in uninfected individuals the ratio of TREC content in LT over that in PBMC is $1.38/0.96 \approx 3/2$, which does not reach statistical significance, and in infected subjects, this ratio has increased to $0.55/0.27 \approx 2/1$, which is a significant difference between content in LT and blood.[23]

However, there are 2 problems with the "Redistribution" explanation. First, one would expect that therapy-induced redistribution would tend to reestablish the preinfection relationship between the LT and PBMC TREC content. Thus, the TREC content in the periphery would go from 1/2 of that in the LT to the normal 2/3 of the TREC content in the LT. But because the TREC content in LT is lower than normal, the predicted TREC content in PBMC after therapy would also be lower than normal, that is, $2/3 \times 0.55 = 0.37$ cjTRECs/CD4+ T cell (Fig. 2), even in the best case scenario where redistribution to the periphery does not lead to appreciable loss of TREC content in the LT. By redistribution, it is difficult to obtain supranormal TREC content in the periphery when the source of these cells, that is, the LT, has a lower than normal TREC content. It could be argued that TREC+ CD4+ T cells are released much earlier from the LT into the periphery than TREC− CD4+ T cells[22] but then a second problem becomes apparent. Redistribution acts on a short-time scale of weeks, and this is why it has been invoked as the mechanism for the early increase in CD4+ T cells after HAART.[24–29] However, the increase to supranormal levels of TREC content is observed even after 20 months of therapy,[11] and it seems unlikely that the

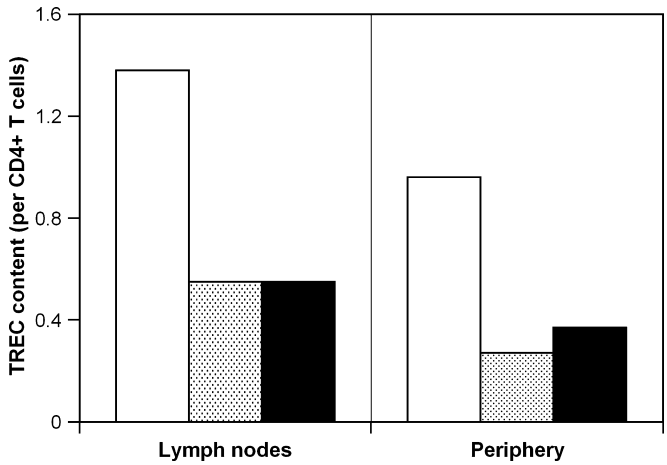

FIGURE 2. Comparison of TREC content in lymph nodes and periphery. Experimentally measured cjTREC per CD4+ T-cell for uninfected (white) and infected individuals (shaded bars) from Nokta et al.[23] Expected cjTREC content for treated individuals (black bars) if treatment leads to reestablishment of the relative proportion of TREC content between lymph nodes and periphery, that is, the ratio between the black bars is the same as between the white bars.

difference in the timescale at which the distribution of TREC+ and TREC− CD4 T cells normalizes is so large.

Two Subpopulations of Naive T Cells

Another hypothesis for explaining the transient supranormal TREC content during HAART is that naive T cells are composed of 2 subpopulations, that is, RTEs and truly naive T cells, and that most TRECs reside in the RTE subpopulation, which has much faster dynamics.[11,15,16,20] A rapid and increased recruitment of RTE into the naive T-cell repertoire after the start of antiretroviral treatment is thought to explain the observed supranormal TREC content.

To analyze this possibility in more detail, we developed a population dynamical model for these subpopulations (see Appendix). We considered an RTE population (R) and a truly naive population (N). Together these populations form the experimentally observed naive phenotype T-cell population (T) (either naive CD4+ or CD8+ T cells). We also considered the populations of TREC-positive cells among the RTEs and truly naive T cells, T_R and T_N, respectively. RTEs are produced in the thymus, and after their arrival in the periphery, they either die or become recruited into the truly naive T-cell pool. In this model, we deliberately do not include T-cell redistribution[12] because we analyzed the impact of redistribution by itself above, and the present model serves to separately analyze the effect of 2 naive populations. Thus, we are studying each of these effects in turn.

Calculating the steady state of this model demonstrated an important counterintuitive result (see Appendix). Increasing the recruitment rate of RTE into the truly naive population actually "decreases" the steady-state TREC content of the whole naive T-cell pool. Although the total TREC numbers at steady state increased due to increased recruitment of RTE, the total naive T-cell population increased at least as much. Thus, increasing RTE recruitment reduces the average TREC "content" of naive T cells.

One criticism of this model is that we expect that homeostatic mechanisms are in operation to keep the naive T-cell population at regulated levels. Indeed, earlier work has shown that predictions about TREC content may crucially depend on the inclusion of homeostasis in the model.[8,12] To test the robustness of our counterintuitive result, we included homeostasis on the truly naive population in the model (see Appendix). Incorporation of RTE into the naive T-cell pool probably depends on the size of this pool, such that when truly naive T cells are depleted, the fraction of RTE incorporated into the long-lived population increases. Current experimental evidence[19] suggests that the size of the RTE pool itself is independent of peripheral homeostatic mechanisms and depends mostly on the magnitude of the thymic output. Accordingly, in our model, the size of the RTE population is independent of the fraction of RTE recruited. Homeostasis could also regulate the proliferation and/or death rates of truly naive T cells, which has similar effects on naive T-cell numbers and their TREC content.[30]

The steady states of this model where RTE recruitment and the rate of naive T-cell division are homeostatically regulated (ie, both increase when naive T-cell numbers decrease) are shown as a function of the maximal rate of RTE recruitment in Figure 3. Importantly, increasing the maximal rate of RTE recruitment decreases the TREC content (Fig. 3B), which confirms the analytical result for the nonhomeostatic version of the model. Note that the actual recruitment, f/H, does increase when the maximum rate of recruitment, f, increases (Fig. 3B). The explanation for this counterintuitive result is the same as before, even though increasing RTE recruitment leads to more TRECs, it leads to even more truly naive T cells. And indeed naive T cells increase faster than total TREC (Fig. 3A), resulting in an overall decrease in TREC content. We have repeated these analyses for a model with homeostasis on RTE recruitment and naive T-cell death, d_N, and a fixed naive T-cell division rate, p. The results obtained in terms of the effect of increased recruitment were very similar (not shown). Overall, these equilibrium results suggest that even in a homeostatic model, increased recruitment of RTEs into the truly naive T-cell population cannot explain the long-term supranormal TREC content as seen in HIV-1–treated patients who are well suppressed for almost 2 years.[11]

Thymic Output

Here we would like to propose a new hypothesis for the supranormal TREC content based on the crucial aspect that

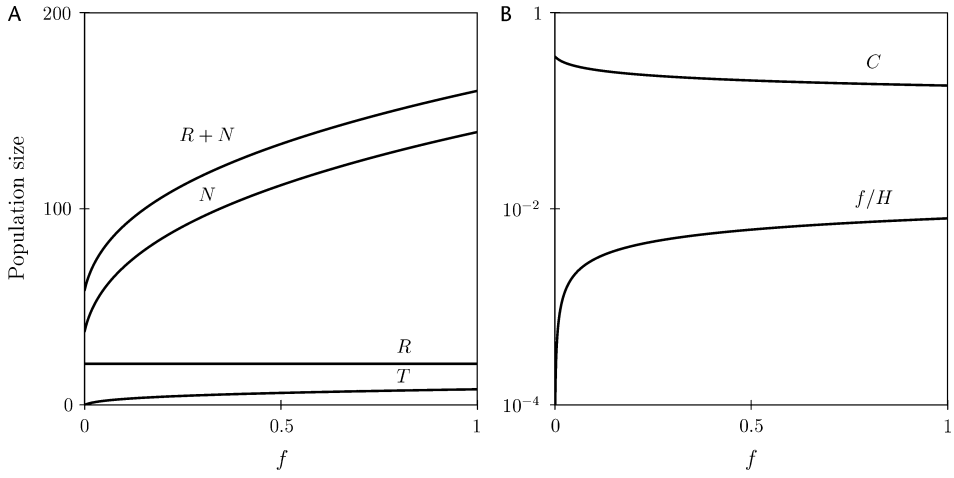

FIGURE 3. Steady states of the RTE model (see Appendix) as a function of the RTE recruitment rate f. We allow for homeostasis on RTE recruitment, f, and naive T-cell renewal, p, by dividing these rates by $H = 1 + (N/h)^2$. Panel (A) shows the effect of RTE recruitment on the total number of naive T cells ($R + N$), on truly naive T cells (N) and RTEs (R), and on the total number of TRECs (T). Panel (B) shows that the TREC content per naive T cell, C, goes down when the actual RTE recruitment, f/H, goes up. Parameters: c = 16, h = 12.5 cells, p = 0.01 per day, f = 0.1, d_N = 0.001 per day, d_R = 1/21 per day, and σ = 1 cell per day.

recovery to supranormal levels involves small numbers of TREC+ cells. First, let us consider an illustrative example of this phenomenon (Table 1): a typical 39-year-old uninfected individual may have ~400 naive T cells/μL and 0.0094 TREC/naive T cell, corresponding to an average of only 3.8 TREC+ cells/μL (data from a real individual in Harris et al[11]). Assume that during infection, that is, until the start of treatment, the patient has lost half of his/her naive T cells (to ~200/μL) and, to explain the lower TREC content, has lost most of his/her TREC+ cells (eg, to 1/μL) leading to 0.005 TREC/naive cell. Then, after ~20 months of successful treatment, this patient may have recovered to ~230 naive T cells/μL and 0.0452 TRECs/naive T cell (data taken from another, age-matched, real individual from Harris et al[11]). This means that during treatment, the patient's TREC total has approached 10 TREC+ cells/μL. If all these new naive T cells come from the thymus, this would imply that during the 20 months of treatment the thymus has had an output of (at least) 30 cells/μL, of which 9 (30%) were TREC+. These numbers correspond to a thymic output of 0.05 cells/μL/d (a total output of $\sim 1.2 \times 10^7$ cells/d), which is perfectly consistent with the very slow turnover of human naive T cells, and readily explain the supranormal value of TREC content observed in this patient.

The calculations in the previous paragraph serve only to illustrate the point that a small output from the thymus, within the range of that expected for adults, suffices to explain supranormal levels of TREC. To analyze this scenario in more detail, we developed a simple mathematical model, with just 1 population of naive T cells, some of which are TREC+ and some are TREC− (Fig. 4). (This model differs from the above because here we do not consider RTE, again to study the different hypothesis separately.) In this model, new naive T cells are produced by the thymus, and once in the periphery, these cells may proliferate or be lost due to activation and/or death. Choosing reasonable values for these parameters (Fig. 4), we demonstrate that starting from very low numbers of total TREC and naive T cells in the periphery, it is easy to achieve supranormal TREC content during therapy (Fig. 4A). Moreover, these supranormal values are achieved relatively quickly and stay elevated for considerable periods of time. This happens because of the small absolute numbers involved (only ~3 TREC+ cells/μL are produced by the thymus over the period of 2 years, consistent with a thymic output of ~0.03 cells/μL/d as above) and occurs despite the fact that the majority of new cells produced by the thymus and by proliferation are TREC negative. In agreement with the data, the recovery of naive T cells is much slower than that of the TRECs, and naive T-cell numbers remain well below normal levels after 2 years of successful therapy (Fig. 4B).

The parameters in Figure 4 were tuned to obtain a steady state corresponding to the healthy controls in the Harris et al[11] data. Similar results to those depicted in Figure 4 are obtained in the much simpler case where we ignore proliferation and death during the recovery phase, that is, setting $p = d_N = 0$ such that both the TREC+ cells and the total number of naive T cells increase linearly with time. Indeed, our results are quite robust to the set of parameters used.

Another feature that is explained by this "low TREC numbers" phenomenon is that the TREC content reaches higher levels for those patients starting from a lower nadir of naive CD4+ T cells (see the different lines in Fig. 4A), which is a natural explanation for the corresponding experimental observation.[11] We plot the experimental data,[11] adding the information on TREC totals, for those individuals with naive T-cell counts <400 cells/μL in Figure 5 [patients with higher, ie, normal, counts are either not expected to have experienced a large depletion during infection—eg, a short untreated infection—or to have recovered for longer to nearly normal levels of naive T cells (see below)]. The data in this figure are consistent with our simple mathematical model: patients with the lowest naive T-cell counts have the highest TREC content (Fig. 5A) but have similar TREC numbers (Fig. 5B). The TREC content of patients with medium to normal naive CD4+ T-cell counts overlaps substantially with those of the healthy controls (Fig. 5C), and the difference between these 2 groups is marginally significant, largely due to a few patients with very high TREC content. Summarizing, this suggests that the supranormal TREC content is just a consequence of the dynamics of low TREC and T-cell numbers. Moreover, in patients treated early, with larger numbers of CD4+ T cells before treatment, we do not expect to see supranormal TREC content even after successful therapy (see dashed dotted line in Fig. 4A).

After a long period of successful treatment and viral load suppression, our model will approach a steady state with normal naive T-cell numbers and a normal TREC content. This implies that after several years the TREC content will decrease from its supranormal levels to approach its normal levels (not shown). How high TREC content gets and how long it stays elevated depend on the initial conditions and the specific parameters chosen for the model, which obviously differs from patient to patient. This would give rise to the wide range of TREC contents seen in the data.

TABLE 1. Illustrative Example of Changes in TREC Content for a Normal Individual After Infection With HIV-1 Followed by Treatment*

	Naive Cells	TREC/Cell	TREC/μL
Uninfected	400	0.0094	3.8
HIV-1 infected	200	0.005	1.0
HIV-1 treated	230	0.045	10

*The data for the uninfected and treated individuals are from Harris et al[11] whereas the HIV-1 infection case is hypothetical.

DISCUSSION

The supranormal TREC content observed in treated HIV-1–infected individuals[10,11] is readily explained by the very simple argument that the release of a few TREC+ naive T cells from the thymus into a strongly depleted, largely TREC−, naive T-cell population transiently allows for a very high TREC content. Here we have shown that this transient of supranormal TREC content easily extends over a period of more than 2 years and that TRECs recover much faster than the

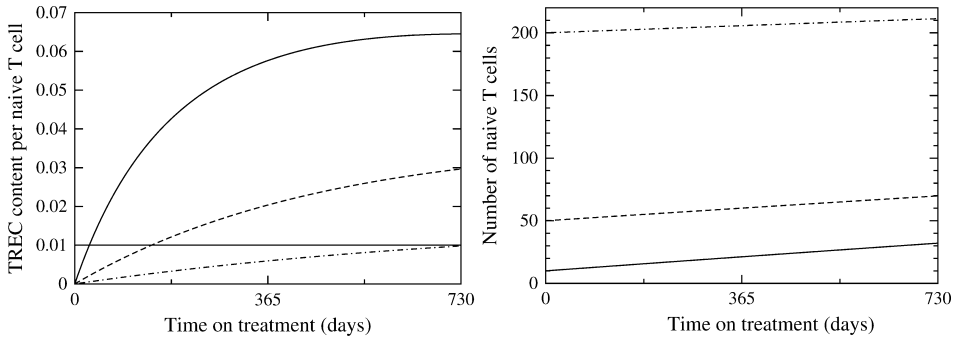

FIGURE 4. Supranormal TREC content during naive T-cell recovery induced by the thymus. We consider the following model for TREC+ naive T cells, N^+, and total naive T cells, N: $\frac{dN^+}{dt} = \alpha\sigma - d_N N^+$ and $\frac{dN}{dt} = \sigma + (p - d_N)N$ Thus, $C = N^+/N$ is the TREC content per naive T cell. Considering a human adult, the thymic output, σ, should be small. We assumed a thymic production of $\sigma = 0.032$ cell/μL/d corresponding to a fractional output of less than 10^{-4} per day (taking a count of 400 CD4+ T cells/μL for a normal individual[11]). The parameter α was set to 1/8 to represent the TREC content of RTE (eg, cord blood cells).[31] Because naive T cells are long lived, we give them a half-life of 2 years by setting $d_N = 0.001$ per day, and if naive T cell numbers ultimately approach a normal steady state of N = 400 cells after the transient recovery phase, then one can solve that $P = 0.00092$ per day. At the steady state, the normal TREC content is $C = 0.01$ per cell [see the horizontal line in Panel (A)]. For simplicity, we start with no TREC+ cells (ie, $N^+(0) = 0$). The 3 lines correspond to 3 initial population sizes: n = 10 (solid line), n = 50 (dashed line), and n = 200 (dash dotted line).

naive T-cell count. The most important biological lesson that we learn from this is that these data do not provide evidence for, or against, a dynamically separate RTE population containing most of the TRECs but do provide evidence for de novo production of new naive T cells by the thymus in middle-aged HIV patients under antiretroviral treatment. Moreover, our simple quantitative reasoning also explains why the largest increases in TREC content are usually seen in patients with lower naive CD4+ T-cell counts before therapy.[9,11,12] The same thymic output into a smaller naive T-cell population naturally leads to higher TREC content.

The highest TREC content that our hypothesis can account for is that of RTE, which is estimated to be around 0.11 TREC/cell[31] and which is one or two orders of magnitude above the normal levels of 0.001–0.01 TRECs/naive T cell.[9] Interestingly, some individuals, both normal and HIV-1–infected subjects, have higher TREC content of up to 0.61 TREC/cell.[11] Such a high TREC content could either be measurement noise, as TREC data tend to be variable, or might indicate an early exit of RTE from the thymus after only 1 or 2 divisions post the formation of the signal-joint TREC.

To test whether increased recruitment of RTEs during therapy could explain the supranormal TREC content, we developed a model to analyze the dynamics of RTEs, as a separate population from the truly naive T cells. We found that increased recruitment per se cannot explain that observation because it is expected to decrease the equilibrium TREC content. The fact that we do not need a small rapid subpopulation of RTE containing most of the TRECs to explain the supranormal TREC contents in treated HIV patients[11,15] should not be taken as evidence against the notion that RTEs form a dynamically distinct subpopulation. Indeed, in our RTE model [Eqs. (1) and (2)], we can observe similar supranormal TREC contents as depicted in Figure 4 if we consider similar cases of strongly depleted naive T-cell populations with small thymic outputs of TREC+ RTE (not shown). The mechanism underlying these increased TREC contents is not the increased recruitment rate of RTE, because that would give the opposite results, but simply the incorporation of a limited number of TREC+ RTEs into a small pool of largely TREC− naive T cells. In contrast, with our explanation of the supranormal TREC levels, the previous suggestion that most of the TRECs in the peripheral blood reside in a small subpopulation of RTE[15] does loose most of its evidence. Moreover, TREC data obtained after thymectomy in monkeys argue against this because one would expect a very fast early decline in total TREC after thymectomy, which is not seen in the data.[31,32]

In the longitudinal study of Di Mascio et al,[10] TREC totals per microliter also increased faster than naive T cells over approximately 18 months of treatment. Comparing naive CD4+ with naive CD8+ T cells, the recovery rates were somewhat faster in the CD4+ T cells. This could be taken as evidence in favor of our notion that most of the recovery is due to thymic production because CD4+ cells typically outnumber CD8+ cells in the single-positive thymocyte population. In the same article,[10] the TREC content of the CD4+ naive T cells increased from 0.1 to more than 0.2 TRECs/naive T cell whereas that of the CD8+ cells increased from 0.075 to 0.1 TRECs/naive T cell after approximately 6 months of treatment. The higher TREC content in the CD4+ T cells is readily explained by the faster recovery of the TREC totals and the lower nadir in the naive CD4+ T cells as compared with the CD8+ T cells. Thus, these longitudinal data seem in agreement with our interpretation and therefore do not require the more complicated interpretation of differences in the normalization of proliferation, death, and the recruitment rates of naive T cells into memory cells.[10]

Summarizing, the main biological lesson from our quantitative modeling approach is that middle-aged human thymi continue to produce naive T cells and that this production can be demonstrated by tracking the increase of total TREC numbers (rather than the TREC content). A true quantification of thymic output from TREC data remains difficult because TREC+ naive T cells may die and/or be recruited into clonal expansion and contraction by antigenic stimulation. Recovery rates of TREC+ naive T cells can therefore only provide a lower bound on the thymic output.

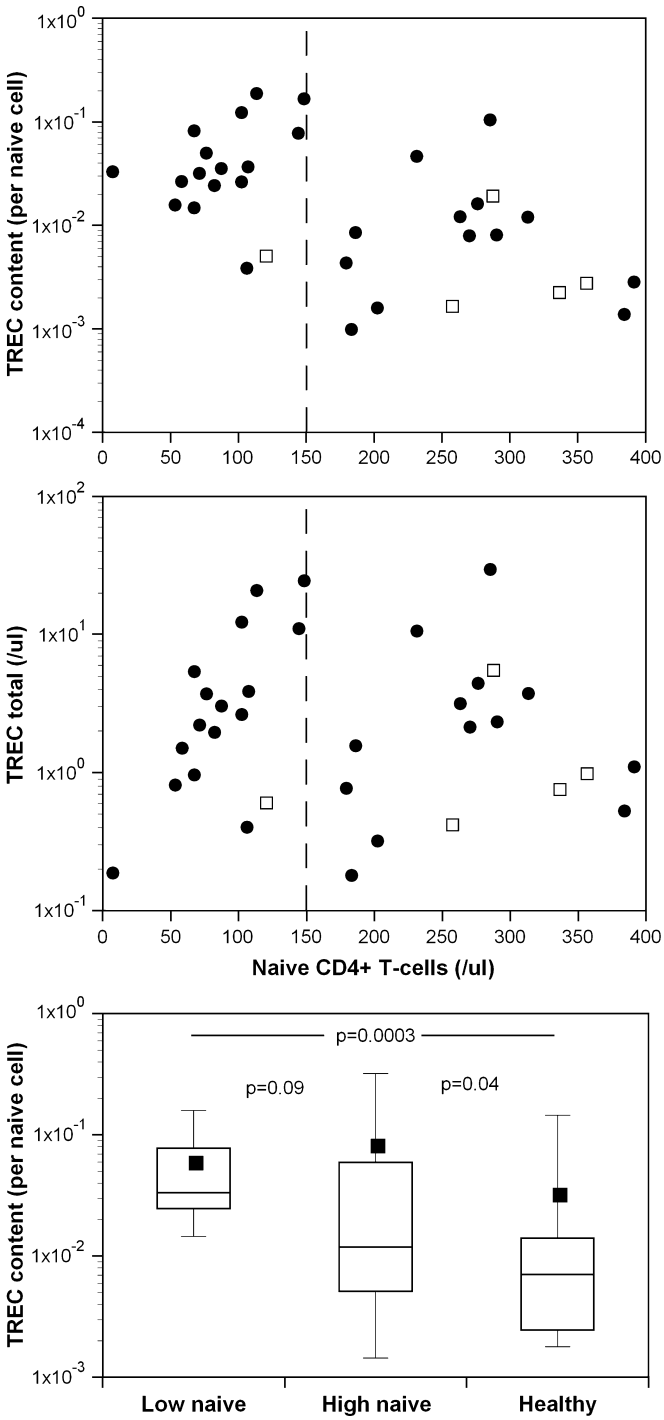

FIGURE 5. TREC levels versus naive T-cell numbers. (A) For treated infected individuals (●) with low naive T-cell counts (<150 cells/μL, indicated by the vertical dashed line), the TREC content is higher than for individuals with higher naive T-cell numbers or for uninfected individuals (□) whereas (B) TREC total is similar for a wide range of naive T-cell counts, including both infected (●) and uninfected individuals (□). In panel (C), we present box plots of the TREC content for 3 groups, infected individuals with less than 150 cells/μL (n = 16), infected individuals with more than 150 cells/μL (n = 19), and uninfected individuals (n = 8) (data from Harris et al[11]).

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APPENDIX

We consider an RTE population (R) and a truly naive population (N) and together these populations form the experimentally observed naive phenotype T-cell population (T) (either CD4+ or CD8+ T cells). We also consider the populations of TREC-positive cells among the RTE and truly naive, T_R and T_N, respectively. With these populations, we write the following model

$$\begin{aligned} \frac{dR}{dt} &= \sigma - d_R R \\ \frac{dN}{dt} &= cfd_R R + (p - d_N)N, \end{aligned} \tag{1}$$

where σ is the thymic output in cells per day, d_R is the daily loss rate of RTE, either by incorporation into the truly naive pool (of a fraction $f \leq 1$) or by death of the RTE, c is a term that allows for expansion of RTE as they incorporate into the naive pool (there is no expansion if $c = 1$), p and d_N are the proliferation and loss rates of the truly naive T cells. Here we assume that RTEs only proliferate when they are recruited into the naive pool. We now write the corresponding equations for the TREC-positive cells in those 2 compartments. They are

$$\begin{aligned} \frac{dT_R}{dt} &= \alpha\sigma - d_R T_R \\ \frac{dT_N}{dt} &= fd_R T_R - d_N T_N, \end{aligned} \tag{2}$$

where α is the proportion of thymic emigrants that are TREC positive. This is a simplified version of previous models developed by us and others.[8,10,12] Here we do not include redistribution from tissue because we are interested in analyzing the different hypotheses for supranormal levels of TREC content separately: 2 populations of naive cells versus redistribution from tissues.

At steady state, equations (1) and (2) yield

$$\begin{aligned} \bar{R} &= \frac{\sigma}{d_R} \text{ and } \bar{N} = \frac{cf\sigma}{d_N - p} \\ \bar{T}_R &= \frac{\alpha\sigma}{d_R} \text{ and } \bar{T}_N = \frac{\alpha f\sigma}{d_N}, \end{aligned} \tag{3}$$

for naive total cells and TREC+ cells, respectively. The TREC content (TREC per cell) of the observable naive population is $(T_R + T_N)/(R + N)$, which is easily calculated to be

$$\bar{C} = \alpha \frac{\frac{fd_R}{d_N} + 1}{\frac{cfd_R}{d_N - p} + 1} \tag{4}$$

And the total TREC in the naive T-cell population is

$$\bar{T}_R + \bar{T}_N = \frac{\sigma}{d_R} + \frac{f\sigma}{d_N} \tag{5}$$

One can see from equation (4) that increasing the recruitment of RTE, f, decreases the TREC content. This effect is observed both if we consider an expansion model ($c > 1, p = 0$) or a proliferation model ($c = 1, p > 0$). In fact, inspection of equation (4), for the equilibrium of TREC content, demonstrates that increasing f always leads to a decrease in C, as long as either $p > 0$ or $c > 1$. This occurs because, when f increases, the expression $cfd_R/(d_N - p)$ in the denominator always increases more than the expression fd_R/d_N in the numerator. On the other hand, increases in f do lead to increases in TREC total, but these increases are only proportional to the amount of TREC residing in the truly naive population. That is, if 90% of the naive T-cell TRECs reside in the RTE population and only 10% in the truly naive subpopulation, then increasing recruitment, f, 10-fold would only approximately double the measured TREC total.

We also study similar models that include homeostatic mechanisms of maintenance of the total naive pool. In these, we assume that homeostasis only works on the truly naive T cells and not on the RTE population.[19,20] Homeostasis is implemented by dividing p and f, in the equations above, by $1 + (N/h)^2$. That is, the larger N, the smaller the proliferation rate and/or the fraction of cells incorporated into the truly naive pool.

The existence of these 2 populations makes a difference for the dynamics of TREC, only if there is an imbalance in the proportion of TREC residing in each and/or there is a difference between the dynamics of these populations. This last property is reflected in the different loss rates for these 2 populations, d_R and d_N. Studies in mice[19,20] suggested that RTEs have a fairly rapid turnover compared with truly naive T cells, that is, an expected life span of approximately 3 weeks. We therefore assume that $d_R \gg d_N$ and use d_R = 1/21 per day. The dynamical importance of the RTE population depends on its size, that is, what fraction it represents of the total naive population, $\bar{R}/(\bar{R} + \bar{N})$, and on the fraction of TREC residing in RTE, that is, $\bar{T}_R/(\bar{T}_R + \bar{T}_N)$ As an example, by choosing the following parameters: $c = 16$, $p = 0.01$ per day, $f = 0.1$, $d_N = 0.001$ per day, $d_R = 1/21$ per day, $\sigma = 1$ cells per day, and $h = 12.5$, the fraction of RTE in the total naive population at steady state is 23% and the fraction of TREC in RTE is 87%. The former was tuned to have good agreement with the experimental data[19,20] and the latter was chosen to maximize the effect of RTE on TREC.[15]

