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Quantifying Thymic Export: Combining Models of Naive T Cell Proliferation and TCR Excision Circle Dynamics Gives an Explicit Measure of Thymic Output

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Understanding T cell homeostasis requires knowledge of the export rate of new T cells from the thymus, a rate that has been surprisingly difficult to estimate. TCR excision circle (TREC) content has been used as a proxy for thymic export, but this quantity is influenced by cell division and loss of naive T cells and is not a direct measure of thymic export. We present in this study a method for quantifying thymic export in humans by combining two simple mathematical models. One uses Ki67 data to calculate the rate of peripheral naive T cell production, whereas the other tracks the dynamics of TRECs. Combining these models allows the contributions of the thymus and cell division to the daily production rate of T cells to be disentangled. The method is illustrated with published data on Ki67 expression and TRECs within naive CD4+ T cells in healthy individuals. We obtain a quantitative estimate for thymic export as a function of age from birth to 20 years. The export rate of T cells from the thymus follows three distinct phases, as follows: an increase from birth to a peak at 1 year, followed by rapid involution until ~8 years, and then a more gradual decline until 20 years. The rate of involution shown by our model is compatible with independent estimates of thymic function predicted by thymic epithelial space. Our method allows noninvasive estimation of thymic output on an individual basis and may provide a means of assessing the role of the thymus in diseases such as HIV.

The thymus is the primary source of naive T cells and plays a key role in establishing and maintaining the peripheral T cell pool. In children, the T cell compartment grows continuously with age from birth to adulthood. Cell numbers then remain approximately stable throughout adult life. During the first 20 years of life, the thymus is known to involute, and its output is supplemented by division within the existing peripheral naive T cell pool (1–6). However, the absolute and relative contributions of the thymus to the peripheral naive T cell pool and how these contributions change with age are difficult to measure. Despite a wide array of immunological markers, imaging techniques, and histological studies, we still lack a direct quantitative measure of thymic export and are unable to answer some basic questions about the contribution of the thymus to lymphocyte homeostasis in health and in disease.

In humans, much of our knowledge of the thymus comes from biopsy studies. Steinmann et al. (1) showed that the thymus reaches its maximum volume by 1 year of age and then remains constant, but the relative size of the intrathymic compartments changes substantially with age. The thymic epithelial space (TES)1 involves by 70% over the first 20 years, accompanied by a simultaneous expansion of the perivascular space and connective and adipose tissue. Because the TES is the main site of thymopoiesis and the majority of thymocytes are found in this region, we can infer that the functionality of the thymus involutes continuously with age. Previous studies of thymic function have assumed that the rate of export of cells to the periphery is proportional to the volume of the TES (5, 6), but it is not obvious that there is a direct correlation between these two variables. The TES is a site of extensive expansion and selection in which less than 5% of cells survive the development process and any minor changes in the rate of production or loss of cells may have a large impact on the rate of export (7–9). In fact, although the cellular density of thymic tissue declines with age, it does not directly parallel the changes in the TES (10).

In vivo isotope labeling has been useful for quantifying turnover in the naive CD4+ T cell population in adults (11, 12), but the approach cannot readily be used to quantify thymic export and the rate of involution. The method does not allow cells dividing in the periphery to be distinguished from those that divided in the thymus and emigrated to the periphery during the period of administration of the label. It is also difficult to justify the use of this labeling and sampling procedure in young children.

TCR excision circles (TRECs), the cell surface marker CD314 and, more recently, protein tyrosine kinase 7 (PTK7) expression by peripheral T cells have been used as surrogate markers for thymic export. TRECs are stable, nonreplicative extrachromosomal circles of DNA excised during TCR gene rearrangement (13–15). TRECs

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1 Abbreviations used in this paper: TES, thymic epithelial space; PTK7, protein tyrosine kinase 7; sj, signal-joint; TREC, TCR excision circle.
are produced in the thymus and can be measured in peripheral CD4⁺ and CD8⁺ T cells. CD31 identifies a subset of naive cells that is significantly enriched with TRECs (16), PTK7 is expressed at high levels on mature CD4⁺CD8⁻ thymocytes and a subset of naive CD31⁺CD4⁺ T cells that is highly enriched for TRECs (17). Both the TREC content and the proportion of recent thymic emigrants within the naive pool, defined by CD31 or PTK7 expression, are known to decline with age (13, 16 –20). However, these measures are determined by a combination of thymic export, cell division, and longevity of thymic emigrants and cannot therefore give a strict measure of the decline in thymic export (3, 5, 19, 21–23).

Ki67 is a nuclear cell cycle marker that is expressed only in proliferating cells from late stage G1, and is rapidly degraded on exit from cycle (24, 25). Ki67 expression coincides with the deterministic phase of cell cycle (24 –28), and thus provides a marker of exit from cycle (24, 25). Ki67 expression coincides with the de-
migation out of the system. A simple dynamical model can be used to describe how total numbers of resting, \( X(t) \), and dividing, \( Y(t) \), naive CD\( ^4 \) T cells change with time, as follows:

\[
\frac{dX(t)}{dt} = \theta(t) + 2r(t)Y(t) - (a(t) + \deltaX(t))X(t),
\]

\[
\frac{dY(t)}{dt} = a(t)X(t) - (r(t) + \deltaY(t))Y(t),
\]

where cells enter the resting naive cell compartment from the thymus at rate \( \theta(t) \) (cells day\(^{-1}\)), resting cells enter cell cycle at rate \( a(t) \), and are lost irreversibly from the naive resting pool at rate \( \deltaX(t) \). The dividing population has its own rate of cell loss \( \deltaY(t) \), and cells revert to the resting state at rate \( r(t) \). All rates are in units of days\(^{-1}\). We assume that upon completion of cell cycle, two daughter cells will return to the naive resting pool.

In this model of the population dynamics, the total number of naive cells is equal to the sum of the resting and dividing compartments, and we have the following relationship:

\[
\frac{dN(t)}{dt} = \frac{dx(t) + Y(t)}{dt}
\]

Combining equations 1, 5, and 6, we obtain the following:

\[
\theta(t) + \rho(t)N(t) - \delta(t)N(t) = \theta(t) + r(t)Y(t) - \deltaX(t)X(t) - \deltaY(t)Y(t),
\]

where total loss of naive cells, \( \delta(t)N(t) \), is equal to the combined loss from the resting and dividing compartment, \( \deltaX(t)X(t) + \deltaY(t)Y(t) \). We infer from equation 8 that the number of cells added to the naive population through cell division in the periphery each day, \( \rho(t)N(t) \), is equal to \( r(t)Y(t) \), where the rate of reversion of dividing cells to the resting pool, \( r(t) \), can be interpreted as the inverse of average time spent expressing Ki67 during one complete cell cycle, \( 1/\Delta \) (an alternative modeling approach gives a comparable result and is described in Appendices, Part 3): contribution of peripheral cell division (cells/day) = \( \rho(t)N(t) = r(t)Y(t) \sim Y(t)/\Delta \) (total number cells expressing Ki67/duration of Ki67 expression).

Ki67 is expressed in late stage G\(_1\), S, G\(_2\), and M phase of cell cycle (24, 25). The majority of variability in cell cycle duration is thought to arise from the length of G\(_1\), whereas the period of Ki67 expression coincides with the more deterministic B-phase of cell cycle, which is thought to be associated with a sequence of known physiological events of conserved length (26–28, 35). Gett and Hodgkin (36) determined the average division time of stimulated naive CD\( ^4 \) T cells to be 12.4 h (±1.0 h). We argue that this reflects the minimum time taken for a naive cell to divide, and hence complete the deterministic phase of cell cycle, and let \( \Delta \) be constant with mean 0.52 (day\(^{-1}\)). This is likely to be an upper bound on \( \Delta \), because it also includes some time in interphase.

We estimate the total number of cells produced through cell division per day by combining data for Ki67 expression as a fraction of naive CD\( ^4 \) T cells, \( \gamma(t) \), with predicted total body naive cell numbers, \( N(t) \):

\[
\text{Contribution of peripheral cell division (cells/day)} = \rho(t)N(t) = \frac{\gamma(t)N(t)}{0.02\Delta}, \tag{9}
\]

FIGURE 1. A simple model of naive CD\( ^4 \) T cell dynamics. The naive population is divided into two compartments according to cell cycle status: at rest or dividing, in which Ki67 expression is used as a surrogate marker for dividing cells.

Thymic Export
\( \theta(t) \)

Resting Naive Cells

\( (X(t)) \)

Transition in to cell cycle

\( (a(t)) \)

Dividing Naive Cells

\( (Y(t)) \)

2 daughter cells return to rest

\( \delta(t) \)

\( \deltaX(t) \)

\( \deltaY(t) \)

\( \Delta \)

FIGURE 2. A. Fraction of naive CD\( ^4 \) T cells expressing Ki67 (data taken from Hazenberg et al. (29) and Douek et al. (4)) fitted to an exponential decay; 0.02 exp(−0.1 t) (t measured in years). B. Estimated number of naive CD\( ^4 \) T cells generated by peripheral division per day. Mean (solid line), 2.5 and 97.5% quantiles (dashed lines).

An expression for thymic export

Combining equations 4 and 9, we obtain an explicit expression for thymic export as a function of age by combining the distributions of the three components. This procedure is outlined in Appendices, Part 4, and described in detail elsewhere (6).

\[
\text{Thymic export (cells day}^{-1}\text{)} = \theta(t) = \frac{\gamma(t)N(t)\tau}{c - \tau},\tag{10}\]

where \( \gamma(t) \) is the fraction of naive CD\( ^4 \) T cells expressing Ki67 such that \( Y(t) = \gamma(t)N(t) \) (Fig. 2A, data taken from studies by Hazenberg et al. (29) and Douek et al. (4)). \( r(t) \) is the naive CD\( ^4 \) T cell count per unit volume of blood (37), and \( V(t) \) is the predicted total blood volume at age \( t \). We estimate blood volume using a relationship between blood volume and body weight described by Linderkamp et al. (38), where standard body weight was estimated using standard Centers for Disease Control and Prevention growth data (39). We assume that lymphocytes in the blood account for 2% of total body lymphocytes (40), and that Ki67 expression is homogenous between blood and lymph nodes. The latter assumption is given some support by Fleury et al. (41), who found that the percentage of CD\( ^4 \) T cells expressing Ki67 in healthy individuals was 1.06% in the blood compared with 0.75% in the lymph nodes.

We obtain the 2.5 and 97.5 percentiles of the population distribution of the total daily contribution of peripheral division to the naive CD\( ^4 \) T cell pool as a function of age by combining the distributions of the three components. This procedure is outlined in Appendices, Part 4, and described in detail elsewhere (6).

Results

Quantifying peripheral postthymic production as a function of age

We consider data for Ki67 expression in the naive CD\( ^4 \) T cell population over ages of 0–30 years from two healthy cohorts (4, 29). These studies show that Ki67 expression declines with age (Fig. 2A). We find that the fraction of naive cells expressing Ki67

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fits an exponential decay function, \( a \exp(-ct) \) (age \( t \) in years), where \( a = 0.02 \) (0.012, 0.03) and \( c = 0.1 \) (0.03, 0.28) (95% confidence intervals in parentheses).

The total number of dividing naive CD4\(^+\) T cells as a function of age, \( Y(t) \), was estimated by combining this decay function with our estimate of total peripheral naive CD4\(^+\) T cell numbers. This was used in equation 9 to calculate the expected total number of cells added to the naive population each day through peripheral cell division, between ages 0 and 20 years (Fig. 2B). Although the proportion of naive CD4\(^+\) T cells expressing Ki67 declines monotonically with age, peak production in terms of absolute numbers of cells added to the naive pool each day occurs at age 1 year. We predict mean peripheral naive CD4\(^+\) T cell production to be 2.8 (0.8, 6.9) \times 10^8\) cells per day at age 1, dropping to 6.8 (0.2, 41) \times 10^8\) cells per day in individuals aged 20 (parentheses indicate estimated 2.5 and 97.5 percentiles at the population level). This corresponds to a continuous decline in the rate of division; 3.7% of the naive CD4\(^+\) T cell population is dividing per day at birth, and this drops to 0.5% by age 20. The rate of decline in naive T cell production is comparable to estimates from previous modeling studies: from 2% at birth to 0.2% at age 20 (6). It is also in accord with estimates of naive production rates from deuterated glucose-labeling studies in young adults of 0.2% (11).

**Quantifying thymic export as a function of age**

We use Ki67 expression and TREC content within the naive CD4\(^+\) T cell population to obtain an explicit expression of thymic output (equation 10) and present a continuous estimate for thymic export from birth to age 20 years (Fig. 3). The life history of a healthy thymus appears to comprise three distinct stages: the rate of thymic export increases during the first year of life, rapidly diminishes between the ages of 1 and 8 years, and then slowly declines from about age 8 years onward. This trend directly follows the age dependence of peripheral T cell production by proliferation (Fig. 3B) and is a consequence of the growth of the naive compartment with age while maintaining constant TREC content within the naive pool (4).

Using this approach, we predict that at birth the average thymus will export 6.9 \times 10^8\) CD4\(^+\) T cells per day (1.3 \times 10^8, 2.5 \times 10^9\), a rate that doubles during the first 12 mo of life to yield a daily export of \( \sim 1.4 \times 10^9\) CD4\(^+\) T cells per day at 1 year (2.7 \times 10^9, 5 \times 10^9\). This is consistent with histological studies showing that the human thymus continues to grow during the first year of life, reaching maximal volume at 1 year (1) and peak cellular density at 9 mo (10, 42). This is not altogether surprising because this period coincides with a time of rapid growth of the entire body and maturation of the immune system along with the decline in maternal Ab protection.

Thymic export declines in a biphasic manner from 1 to 20 years, consistent with the decline predicted by histological studies (1) (Fig. 3B). There is a rapid contraction from \( \sim 1 \) to 8 years corresponding to an average decline in output of 12% per year, which is somewhat faster than the involution predicted by changes in the volume of TES with age: where average annual rate of involution, \( x \), is estimated by \( \theta(t) = \theta(t_0)(1 - x)^t\). Following on from this rapid decay, there is a slower involution phase from \( \sim 8 \) years onward that follows the involution of TES volume more closely. During this phase, thymic output drops from 5.6 (0.7, 25) \times 10^9\) to just under 3.5 (0.1, 27) \times 10^9\) CD4\(^+\) T cells per day by age of 20, corresponding to an average involution rate of 4% per year.

**Discussion**

Using mathematical modeling to analyze cell population dynamics, we are able to obtain a quantitative estimate for thymic export using naive CD4\(^+\) T cell count, TREC density, and Ki67 expression within the naive population. We show that the number of cells exported by the thymus per day doubles during the first year of life and then declines in a biphasic manner throughout childhood; the most extensive decline in production is observed between the ages of 1 and 8. In previous work (6), we modeled the density of TREC within the naive population to infer a relationship between the relative contributions of thymic export and peripheral cell division to the naive CD4\(^+\) T cell population. We investigated changes in naive production and loss with age on the assumption that thymic export follows a monotonically decreasing function fitted by Steinmann et al. (1), based on the involution of the TES. It is not necessarily clear that TES volume directly correlates to the number of cells exported to the periphery each day. In addition, assessing TES volume requires careful study of biopsied thymic material and is not a particularly tractable technique for assessing thymic export on an individual basis. In the present study, we use Ki67 expression to quantify peripheral postthymic production and apply this to a model of TREC to obtain an independent estimate of thymic export.

We predict that the median combined production of naive CD4\(^+\) T cells through cell division and thymic export will be \( \sim 1.0 \times 10^9\) cells per day at the age of 20. This is comparable to estimates of total production in young healthy volunteers of 5.7 \times 10^9 naive CD4\(^+\) T cells per day obtained by deuterated glucose labeling (2) and 1.3 \times 10^9 naive CD4\(^+\) T cells per day as measured by in vivo heavy water labeling studies (12). At present there are currently no direct quantitative measures of thymic export alone, although we can compare our estimates of thymic output during involution from 1 to 20 years with the rate at which the thymic epithelial space shrinks with age. If we allow for the growth of thymic volume to 1 year, we find that the pattern of thymic involution predicted by the TES (1) correlates to our expected thymic output (Fig. 3B).

**FIGURE 3.** A. Estimated rate of thymic export of CD4\(^+\) T cells (cells/day): mean (solid line) and 2.5 and 97.5% quantiles (dashed lines). B. Thymic involution predicted by mean thymic export (solid line) and relative volume of thymic epithelial space allowing for growth during first year of life, scaled to maximal volume (dashed line) (1).

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To model TREC$s within the peripheral naive CD4$^{+}$ T cell population, we require an estimate of the TREC concentration in thymic emigrants, $c$. Previous studies suggest that this is stable with age from 0 to 60 years (13, 30), but the value of this concentration cannot be directly measured and is a source of uncertainty in our estimates. We argue that $c$ is approximately equal to 0.25 based on the sTREC content of recent thymic emigrants (CD31 expressing naive CD4$^{+}$ T cells) in neonates (20) and the observation that three divisions occur within the thymus following the formation of sTREC$s (13). We are able to determine an upper bound for the TREC concentration in thymic emigrants from the sTREC concentrations measured in CD4$^{+}$ single-positive thymocytes (0.6) (43); this is a conservative estimate because proliferation is known to occur at the single-positive stage of thymocyte development (44). The parameter $c$ directly influences our estimates for thymic export, although the scaling is independent of age; taking the maximal value of $c = 0.6$ gives a 3-fold lower estimate for thymic export than our estimate of 0.25.

Another potential source of uncertainty in our estimates as discussed above is the duration of Ki67 expression. This period coincides with the deterministic B-phase of cell cycle, and we assume that it is fixed at 12 h (36). Alternative experimental estimates for the duration of the cell cycle in CD4$^{+}$ T cells vary from 12 to 20 h (45). This parameter scales our estimates for both peripheral production and thymic export and is something that could be more accurately determined by experiment. This does not influence the predicted rate of decline in production with age; however, taking an upper and lower bound on Ki67 expression to be 20 and 12 h leads to predicted thymic export of $2.1 \times 10^{9}$ and $3.5 \times 10^{9}$ cells per day at age 20, respectively.

Residual expression of Ki67 on thymic emigrants is a possibility. However, the extent to which this will influence our estimates for peripheral production is limited by the rapid loss of Ki67 upon exit from cell cycle (25); even if all cells exit the thymus immediately on completion of cell cycle, a $t_{1/2}$ of 1 h means that Ki67 expression resulting from thymic emigrants would still be negligible compared with expression resulting from peripheral division.

We have described a method for explicitly quantifying thymic export using parameters that can be directly measured from peripheral blood. When modeling in vivo lymphocyte dynamics, there is always a need to translate the parameters that we observe in peripheral blood to the lymphatic system. In this study, we assume that the systems are homogeneous. It has been shown that there is no significant difference between TREC concentrations in lymph nodes as compared with peripheral blood in healthy individuals (46), and Ki67 expression has also been observed to be similar in both compartments (47).

To estimate total body cell numbers, we assume that at any given time only 2% of lymphocytes will be found in the blood. The decline in the frequency of naive T cells in blood with age is well established (37, 48), although there exists significant variation between individuals. This interindividual variation is magnified when we calculate the size of the lymphatic compartment, and it accounts for most of the variance that we predict at the population level.

If our understanding of normal thymic export in health is limited, then the role of the thymus in disease is even less understood. Premature involution of thymic volume is associated with rapid disease progression in HIV-infected individuals (49), but it is not known whether this reduction in thymic volume reflects reduced thymic export. Older HIV-infected individuals show increases in thymic volume compared with age-matched healthy controls (50). A better measure of the number of cells exported by the thymus is required to properly understand the role of the thymus in disease progression and T cell reconstitution following antiretroviral therapy. TREC levels are decreased in HIV-infected individuals (4), but this may be associated with increased rates of peripheral division rather than decreased thymic output (3, 51). The thymus will play a role in determining the rate of depletion of CD4$^{+}$ T cells in both children and adults, and will also contribute to the nature of T cell reconstitution occurring following chemotherapy, hematopoietic stem cell transplant, thymic implant, or treatment with antiretroviral therapy in HIV. In this study, we have presented a method to measure thymic export using peripheral TREC and Ki67 expression data in healthy individuals, as a step toward quantifying the role of the thymus in HIV disease progression.

Appendices

Part 1. Modeling change in TREC$s per cell with time

We let $\pi(t)$, the per cell TREC content at time $t$, equal $T(t)/N(t)$ and consider the derivative of $\pi(t)$, as follows:

$$\frac{d\pi(t)}{dt} = \frac{\frac{dT(t)}{dt} - \frac{dN(t)}{dt}}{N(t)^2}.$$  

Using the derivatives of $N(t)$ and $T(t)$ as determined by equations 1 and 2, we obtain equation 3 in the manuscript, as follows:

$$\frac{d\pi(t)}{dt} = \frac{N(t)(\theta(t) - \delta(t)N(t)) - T(t)(\theta(t) + \rho(t)N(t) - \delta(t)N(t))}{N(t)^2} = \frac{\theta(t)}{N(t)}c - \pi(t) - \rho(t)\pi(t).$$  

Part 2. Validate assumption that TREC$s per cell do not change to age 20

From equations 4 and 9, we obtain an expression for thymic export in terms of the size of the total naive pool, $N(t)$, the proportion of dividing cells, $\gamma(t)$, and the rate at which the average TREC content per naive cell changes with time, as follows:

$$\theta(t) = \left(\frac{\gamma(t)\sigma(t)}{\Delta} + \frac{d\pi(t)}{dt}\right)\frac{N(t)}{c - \pi(t)}.$$  

where $c$ is the average TREC content of thymocytes leaving the thymus and $\Delta$ is average length of Ki67 expression, both of which are assumed to be constant. We considered the TREC content per naive CD4$^{+}$ T cell as measured in healthy children by Douek and colleagues (4) and found no significant change in this age range. A number of studies have now suggested that the average per cell TREC content is conserved during the first two decades of life (31, 32). So, we let $\pi(t)$, the number of TREC$s per naive CD4$^{+}$ T cell, be constant with mean 0.08 ± 0.01 (SEM).

Although we observe no significant change in $\pi(t)$ with age, there is considerable variation between individuals, so we consider $\pi(t)$ fitted to a linear decay and find the 95% confidence interval of the slope, or equivalently $d\pi/dt$, to be $-1.7 \times 10^{-5} \pm 2 \times 10^{-5}$. We justify the assumption that $\pi(t) = 0$ by considering that minimal Ki67 expression within the naive CD4$^{+}$ T cell population occurs at age 20 and is of the order 0.2%; we combine this with estimates for $\Delta (=0.5)$ (36), and $\theta(=0.08)$ (4) to numerically approximate Min[$\gamma(t)\pi(\Delta) = 3 \times 10^{-4}$]. We estimate Max[$\pi(t)$ from the 95% confidence interval as described above to be of the order $1.7 \times 10^{-3}$, so $\pi(t)$ is ~20-fold smaller than the first term, $\gamma(t)\pi(\Delta)$, in equation 14. This difference is even more pronounced in younger children, in which up to 2% of naive cells are dividing at any given
time, and \( \tau(t) \) is greater than 100-fold smaller than the first term; hence, we ignore \( \tau(t) \) and equation 14 becomes the following:

\[
\theta(t) = \frac{y(t)N(t)\tau}{\Delta(c - \tau)}.
\] (15)

In Fig. 4, we calculate thymic export assuming that TREC frequency in the naive CD4\(^+\) T cell pool declines continuously, from 0.1 at birth to 0.001 at age 80 (dashed line), compared with thymic export calculated assuming the value of TREC per naive cell is 0.08 and remains constant until age 20 (solid line).

Part 3. Alternative modeling approach to estimate contribution of peripheral cell division

Smith and Martin (26) proposed a model of cell cycle divided into two phases, as follows: a stochastic A-phase of variable length, representing the G\(_1\) stage of cell cycle, followed by a more deterministic B-phase including the S, G\(_2\), and M stages of cycle. Ki67 is a cell cycle marker that is expressed from late stage G\(_1\) and is rapidly degraded postmitosis (24, 25), and hence, provides a fairly good measure of the number of cells in B phase. We adapt this model to interpret Ki67 expression within the naive CD4\(^+\) T cell population.

We estimate that cells take \( \Delta_B \) days to complete B-phase, in which we assume that the time taken is relatively conserved, irrespective of cell type or age. Gett and Hodgkin (36) determined the average division time for a naive CD4\(^+\) T cell to be 12.4 (±1.0 h).

We assume that resting cells (Ki67\(^-\)) in an individual of age \( t \) transition into B-phase at a rate \( a(t) \). This is modeled as exponential processes, and so \( 1/a(t) \) is approximately the expected time to entry to B-phase for a given resting (Ki67\(^-\)) naive CD4\(^+\) T cell. Define \( S(t) \) to be the number of naive cells produced through cell division in the periphery each day. Cells produced between time \( t \) and \( t - 1 \) must enter the B-phase of cycle between \( t - 1 - \Delta_B \) and \( t - \Delta_B \) as follows:

\[
S(t) = \int_{t-\Delta_B}^{t-\Delta_B-1} N(i)a(i)e^{-\gamma \Delta_s} di,
\] (16)

where a cell takes \( \Delta_B \) days to complete B-phase, \( N(i) \) is the size of the total naive cell population, \( a(i) \) is defined previously, and time is measured in units of days. At any point in time, \( i \), we suppose that \( N(i)a(i) \) cells will enter the deterministic phase of cell cycle, and we estimate the probability of successfully surviving B-phase and completing mitosis as \( \exp(-\gamma \Delta_B) \), where \( \gamma \) is the death rate of naive cells in cycle. We assume that \( N(i) \) and \( a(i) \) do not change significantly over the period of a few days, and let them be \( N(t) \) and \( a(t) \), respectively; hence, we integrate over a constant and can express the number of naive cells produced through division in the periphery each day as the following:

\[
S(t) = N(t)a(t)e^{-\gamma \Delta_B}.
\] (17)

Using the same approach, a related expression can be derived for the number of cells that express Ki67 at any given time, \( Y(t) \):

\[
Y(t) = \int_{t-\Delta}^{t} N(i)a(i)e^{-\gamma (t-i)} di,
\] (18)

where \( \Delta \) is the duration of Ki67 expression. As before, we assume \( N(t) \) and \( a(t) \) are constant over the period of a few days, and hence, we find that the number of naive cells expressing Ki67 at any given time, \( t \), can be formulated as follows:

\[
Y(t) = \frac{N(t)a(t)}{\gamma} (1 - e^{-\gamma t}).
\] (19)

We wish to find an expression for total cell production per day in terms of Ki67 expression. Studies suggest that the duration of Ki67 expression, \( \Delta \), will be approximately equal to the duration of B-phase (24–26), \( \Delta_B \); hence, we derive the following expression for cell production per day from equations 17 and 19:

\[
S(t) = \frac{Y(t)\gamma}{e^{\gamma t} - 1} \approx \frac{Y(t)}{\Delta + \frac{\gamma \Delta^2}{2} + O\left[\frac{\gamma^2 \Delta^3}{3!}\right]}
\] (20)

where \( \gamma \) is the rate of loss of naive cells from cycle. We have no estimate for this term from the literature; however, we do know that it must be between 0 and 1. We ignore the last terms because they are at least 50-fold smaller than \( \Delta \); \( \Delta \approx 0.5 \) (days) and \( \gamma < 1 \) implies that \( \gamma^2 \Delta^3/3! < \Delta/48 \).

Applying this definition of peripheral production to equation 4 we find that:

\[
\theta(t) = \frac{y(t)N(t)\tau}{e^{\gamma t} - 1} \approx \frac{y(t)N(t)\tau}{\Delta + \frac{\gamma \Delta^2}{2} (c - \tau)}
\] (21)

We have no experimental estimate for \( \gamma \); dividing cells represent such a small proportion of the overall naive pool that the rate of loss from this subpopulation may well be significantly higher than the average rate of loss from the bulk naive CD4\(^+\) T cell population. In Fig. 4, we calculate thymic export using equation 21 for the extreme values of \( \gamma \) and find that the predicted output is fairly
robust to the value of $\gamma$. Using this approach to model Ki67 expression gives comparable estimates for thymic export as the approach described previously in Materials and Methods (compare Figs. 3 and 5).

Part 4. Modeling distribution of thymic export function

We have modeled the distribution of total body naive cells, $N(t)$, and TREC content per naive cell, $\tau(t)$, in previous work (6).

We use a simple exponential decay model, $y(t) = a \exp(-ct)$ (measured in years) to describe the experimentally observed frequency of naive CD4+ T cells in blood that are Ki67+ as a function of age. $R$ is used to determine the least-square estimates of the model parameters. We construct a distribution for $y(t)$ by considering the variation in the predicted parameters; $a$ is normally distributed with mean 0.019 and variance 0.005, $c$ has a mean of 0.099 (95% confidence interval: 0.026, 0.271) and is best fit to a lognormal distribution with parameters $\mu = -2.45$ and $\sigma = 0.56$.

Average naive cell cycle duration, $\Delta$, is assumed to be normally distributed with mean 0.52 days and variance 0.05 (36).

Thymic export, $\theta(t)$, is a nonlinear function of $N(t)$, $\tau(t)$, $\Delta$, and $\tau(t)$, and so we use a Monte Carlo approach to construct an approximate, empirical distribution for $\theta(t)$, as described previously (6).

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Disclosures

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References


