Cells of the mature $\alpha\beta$ T cell repertoire arise from the development in the thymus of bone marrow precursors (thymocytes). $\alpha\beta$ T cell maturation is characterized by the expression of thousands of copies of identical $\alpha\beta$ T cell receptors and the CD4 and/or CD8 co-receptors on the surface of thymocytes. The maturation stages of a thymocyte are: (1) double negative (DN) (TCR$^{-}$, CD4$^{-}$ and CD8$^{-}$), (2) double positive (DP) (TCR$^{+}$, CD4$^{+}$ and CD8$^{+}$), and (3) single positive (SP) (TCR$^{+}$, CD4$^{+}$ or CD8$^{+}$). Thymic antigen presenting cells provide the appropriate micro-architecture for the maturation of thymocytes, which “sense” the signalizing environment via their randomly generated TCRs. Thymic development is characterized by (i) an extremely low success rate, and (ii) the selection of a functional and self-tolerant T cell repertoire. In this paper, we combine recent experimental data and mathematical modeling to study the selection events that take place in the thymus after the DN stage. The stable steady state of the model for the pre-DP, post-DP and SP populations is identified with the experimentally measured cell counts from 5.5- to 17-week-old mice. We make use of residence times in the cortex and the medulla for the different populations, as well as recently reported asymmetric death rates for CD4 and CD8 SP thymocytes. We estimate that 65.8% of pre-DP thymocytes undergo death by neglect. In the post-DP compartment, 91.7% undergo death by negative selection, 4.7% become CD4 SP, and 3.6% become CD8 SP. Death by negative selection in the medulla removes 8.6% of CD4 SP and 32.1% of CD8 SP thymocytes. Approximately 46.3% of CD4 SP and 27% of CD8 SP thymocytes divide before dying or exiting the thymus.

Keywords: thymocytes, negative selection, positive selection, death by neglect, mathematical model, steady state
Exposure to tissue-restricted antigens allows for further deletion of T cells specific for self-antigens they may encounter in the periphery. Finally, those cells that have been positively selected, yet have avoided negative selection, will mature and migrate to the periphery (11).

Previous efforts to develop mathematical models of thymic selection have been based on deterministic approaches or cellular automata simulations. These studies have shown the importance of (i) thymic antigen diversity on the size of the selected T cell repertoire (12), (ii) death rates for the more differentiated thymocyte subsets (13), (iii) thymocyte proliferation and residence times (14), (iv) epithelial networks for thymocyte development and migration (15), (v) thymocyte competition for antigen (16), (vi) self-pMHC complexes expressed on dendritic cells (DCs) (17), (vii) receptor–ligand binding affinity (18), and (viii) a sharp threshold in TCR-ligand binding affinity that defines the boundary between negative and positive selection (19). Recent work by Ribeiro and Perelson (20) supports the need to develop appropriate mathematical models to interpret T cell receptor excision circles (or TREC) data, which are used to quantify thymic export (20). Sinclair et al. in Ref. (21) bring together experimental immunology with mathematical modeling to conclude that CD8 precursor thymocytes are more susceptible to death than CD4 precursors. This asymmetry in the death rates underlies the experimentally observed CD4:CD8 T cell ratio in the periphery.

Previous experimental studies have tried to determine the number of cells going through positive and negative selection in the thymus. However, reports estimating the relative number of cells undergoing negative selection compared to positive selection have been widely variable. Some find that very few cells undergo negative selection; others find that two times more cells undergo negative selection than positive selection (22–25). In this report, we develop a deterministic mathematical model of T cell development in the thymus. Some of us recently published a report where we used a novel approach (Bim−/−Nur77GFP mice) that allowed us to calculate the number of cells undergoing positive and negative selection (26). Using previously published data on the relative life-span of DP and SP cells, we estimated the hourly rate of both positive and negative selection (26). In this manuscript, we make use of (i) a subset of this experimental data, and (ii) the asymmetric death rates observed for CD4 and CD8 precursor thymocytes (21), to develop two mathematical models that will enable us to estimate selection rates in the cortex and the medulla, and provide a quantitative measure for the stringency of thymic selection. The first model (see Section 2.1) allows the identification of the following parameters: DN thymocyte influx into the cortex, pre-DP and post-DP death rates, and pre-DP and post-DP differentiation rates. Under the assumption of asymmetric death rates for the CD4 and CD8 SP thymocytes (21), we extend the first model to provide estimates for the following medullary rates (see Section 2.2): CD4 and CD8 SP death, proliferation, and maturation rates.

### 2. MATERIALS AND METHODS

#### 2.1. A FIRST MODEL OF THYMIC DEVELOPMENT AFTER THE DN STAGE

In this section, we introduce a deterministic model of thymocyte development after the DN stage. This first model will be required to calibrate the parameter values of the second model introduced in Section 2.2. In particular, and as described in Section 3.2, the first model allows the identification of parameter values for the following rates: $\phi$, $\mu_1$, $\mu_2$, $\phi_1$, and $\phi_2$.

This mathematical model makes use of a data set obtained from the analysis of eight C57BL/6 wild type and Bim deficient mice (average age 9 weeks), that express a Nur77GFP reporter and Bim deficiency were novel modifications that allowed us to quantify cells that normally would be deleted by strong TCR signaling. In the mathematical model, we consider the following thymocyte populations: $n_1$, the population of pre-selection DP thymocytes (double positive), that are TCR$\beta^{low}$ and CD69$^{low}$ (26), $n_2$, the population of post-selection DP thymocytes, that are TCR$\beta^+$ and CD69$^{high}$ (26), and $n_3$, the population of mature SP (single positive) thymocytes.

We assume that DN thymocytes differentiate to become pre-selection DP thymocytes with rate (cells/day) $\phi$. We further assume that after the DN stage, thymocyte cell fate is determined by the TCR signal, which a given thymocyte has received. Sinclair et al. used CFSE labeling to show that there is no proliferation at the post-DP stage (see Figure A1 of their manuscript) (21). Stritesky et al. looked at proliferation in the post-DP pool with BrdU labeling, and found no evidence (26). We have, thus, only included proliferation in the SP thymocyte population (21, 26). The three populations, $n_1$, $n_2$, and $n_3$, are involved in the following selection events in the cortex and the medulla (see Figure 1):

- $\emptyset \rightarrow n_1$ – flux of DN thymocytes into compartment $n_1$,
- $n_1 \xrightarrow{\phi} n_2$ – differentiation from pre-DP ($n_1$) to post-DP ($n_2$) thymocytes induced by TCR signal,
- $n_1 \xrightarrow{\mu_1} n_3$ – death by neglect of pre-DP thymocytes due to lack of (or weak) TCR signal,
- $n_2 \xrightarrow{\phi} n_3$ – differentiation from post-DP ($n_2$) to SP ($n_3$) thymocytes sustained by intermediate TCR signal,
- $n_2 \xrightarrow{\mu_2} n_3$ – apoptosis of post-DP ($n_2$) thymocytes due to strong TCR signal,
- $n_3 \xrightarrow{\lambda_3} n_3$ – proliferation of SP thymocytes ($n_3$) in the medulla, and
- $n_3 \xrightarrow{\lambda_3} n_3$ – apoptosis of SP ($n_3$) thymocytes due to strong TCR signal.

The time evolution of the three populations can be described by the following set of ordinary differential equations (ODEs), which are based on the selection events described above:

\[
\begin{align*}
\frac{dn_1}{dt} &= \phi - \phi_1 n_1 - \mu_1 n_1 , \\
\frac{dn_2}{dt} &= \phi_1 n_1 - \phi_2 n_2 - \mu_2 n_2 , \\
\frac{dn_3}{dt} &= \phi_2 n_2 - \phi_3 n_3 - \mu_3 n_3 + \lambda_3 n_3 .
\end{align*}
\]

These equations can be used to study the dynamics of thymic selection and to estimate the rates of thymocyte maturation and death.
We conclude this section with the analytical solution of the system of ODEs [equation (1)], given initial conditions, which provides a unique steady state, if and only if
\[
\beta_3 = \psi_3 + \mu_3 - \lambda_3 > 0,
\]
so that we have \(n_3^* > 0\). In order to study the linear stability of the steady state, we calculate \(A\), the Jacobian matrix of equation (1), as follows:
\[
A = \begin{pmatrix}
-(\psi_1 + \mu_1) & 0 & 0 \\
-\psi_2 & -(\psi_2 + \mu_2) & 0 \\
0 & \psi_2 & -(\psi_3 + \mu_3 - \lambda_3)
\end{pmatrix}.
\]

This unique steady state exists if and only if \(\beta_1 = -(\psi_1 + \mu_1), \beta_2 = -(\psi_2 + \mu_2), \beta_3 = -(\psi_3 + \mu_3 - \lambda_3)\).

Therefore, the steady state [equation (2)] is stable, if and only if, \(\psi_3 + \mu_3 - \lambda_3 > 0\), which is also the condition for its existence. We conclude this section with the analytical solution of the system of ODEs [equation (1)], given initial conditions, which provides the time evolution of the three thymocyte populations:
\[
n_1(t) = n_1^* + n_1(0) e^{-(\psi_1+\mu_1)t},
\]
\[
n_2(t) = n_2^* + \frac{n_2(0) \psi_1}{(\psi_2 + \mu_2) - (\psi_1 + \mu_1)} e^{-(\psi_1+\mu_1)t} + \frac{n_2(0) \psi_2}{(\psi_3 + \mu_3 - \lambda_3) - (\psi_2 + \mu_2)} e^{-(\psi_2+\mu_2)t} + \frac{n_2(0) \psi_3}{(\psi_3 + \mu_3 - \lambda_3) - (\psi_1 + \mu_1)} e^{-(\psi_3+\mu_1)t},
\]
\[
n_3(t) = n_3^* + \frac{n_3(0) \psi_1}{(\psi_2 + \mu_2) - (\psi_3 + \mu_3 - \lambda_3)} e^{-(\psi_1+\mu_1)t} + \frac{n_3(0) \psi_2}{(\psi_3 + \mu_3 - \lambda_3) - (\psi_2 + \mu_2)} e^{-(\psi_2+\mu_2)t} + \frac{n_3(0) \psi_3}{(\psi_3 + \mu_3 - \lambda_3) - (\psi_1 + \mu_1)} e^{-(\psi_3+\mu_1)t},
\]

where \(n_1(0), n_2(0), n_3(0)\) represent the initial conditions for the thymocyte populations. Note that in the late time limit, that is, if \(t \to +\infty\) and \(\psi_3 + \mu_3 - \lambda_3 > 0\), then \(n_1(t) \to n_1^*, n_2(t) \to n_2^*\) and \(n_3(t) \to n_3^*\) as it is the unique stable steady state.

2.2. A second model of thymic development after the DN stage: CD4 and CD8 SP thymocytes

As described in Section 2.1, the first deterministic model will allow us to calibrate some of the parameters of a more comprehensive model, which we now introduce. We subdivide the SP thymocyte population in two classes: CD4 SP and CD8 SP thymocytes. This is an extension of the model introduced in the previous section, and is motivated by the fact that experimentally, SP thymocytes express either the CD4 or the CD8 co-receptor. We now have four different thymocyte populations to consider: \(n_1\), the population of pre-selection DP (double positive) thymocytes, \(n_2\), the population of post-selection DP thymocytes, \(n_3\), the population of mature CD4+ SP (single positive) thymocytes, and \(n_4\), the population of mature CD8+ SP (single positive) thymocytes.

As described in the previous section, we assume that DN thymocytes differentiate to become pre-selection DP thymocytes with rate (cells/day) \(\phi\), and that after the DN stage, thymocyte cell fate is determined by the TCR signal, which a given thymocyte has received. Thus, the four populations, \(n_1, n_2, n_3, n_4\), and \(n_8\), with \(n_3 = n_4 + n_8\), are involved in the following selection events in the cortex and the medulla (see Figure 2):

- \(\psi_1 \to n_1\) flux of DN thymocytes into compartment \(n_1\),
- \(n_1 \xrightarrow{\psi_1} n_2\) differentiation from pre-DP (\(n_1\)) to post-DP (\(n_2\)) thymocytes induced by TCR signal,
The parameters and thymocyte populations of the first and second model are related by the following equations:

\[
\begin{align*}
    \frac{d\phi}{dt} &= \phi - \frac{n_1}{n_4} n_1 - \mu_1 n_1, \\
    \frac{d\phi}{dt} &= \phi_1 n_1 - (\phi_4 + \phi_8) n_2 - \mu_2 n_2, \\
    \frac{d\phi}{dt} &= \phi_4 n_2 - \phi_4 n_4 - \mu_4 n_4 + \lambda_4 n_4, \\
    \frac{d\phi}{dt} &= \phi_8 n_2 - \phi_8 n_8 - \mu_8 n_8 + \lambda_8 n_8.
\end{align*}
\]

The time evolution of the four populations can be described by the following set of ODEs:

\[
\begin{align*}
    n_1(t) &= n_1^i + n_1(0) e^{-(\phi_1 + \mu_1)t}, \\
    n_2(t) &= n_2^i + n_2(0) \frac{\phi_1}{(\phi_4 + \phi_8 + \mu_2)} e^{-(\phi_4 + \phi_8 + \mu_2)t} + n_2(0) e^{-(\phi_4 + \phi_8 + \mu_2)t},
\end{align*}
\]

We are interested in studying the steady state of these populations, as the experimental data correspond to population cell numbers in the four stages (pre-DP, post-DP, CD4 SP, and CD8 SP) for a steady state thymus (26). The steady state of the system of equations [equation (6)] is given by:

\[
\begin{align*}
    n_1^* &= \frac{\phi_1}{\phi_4 + \phi_8 + \mu_2}, \\
    n_2^* &= \frac{n_1^* \phi_1}{\phi_4 + \phi_8 + \mu_2}, \\
    n_4^* &= \frac{n_1^* \phi_1}{\phi_4 + \mu_4 - \lambda_4}, \\
    n_8^* &= \frac{n_1^* \phi_1}{\phi_8 + \mu_8 - \lambda_8}.
\end{align*}
\]

This unique steady state exists if and only if \(\xi_4 + \mu_4 - \lambda_4 > 0\) and \(\xi_8 + \mu_8 - \lambda_8 > 0\), so that we guarantee \(n_2^* > 0\) and \(n_8^* > 0\). In order to study the linear stability of the steady state, we calculate the Jacobian matrix of equation (6), as follows:

\[
B = \begin{pmatrix}
-\phi_1 & 0 & 0 & 0 \\
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]

\[
B = \begin{pmatrix}
-\phi_4 - \phi_8 - \mu_2 & 0 & 0 & 0 \\
0 & -\phi_4 - \mu_4 - \lambda_4 & 0 & 0 \\
0 & 0 & -\phi_8 - \mu_8 - \lambda_8 & 0
\end{pmatrix}
\]
Table 1 | Experimental steady state thymocyte cell counts for the wild type pre-DP, post-DP, CD4 SP, and CD8 SP populations.

| Mouse | \( n^*_1 \) (pre-DP) (cells) | \( n^*_2 \) (post-DP) (cells) | \( n^*_3 \) (SP) (cells) | \( n^*_4 \) (SP CD4) (cells) | \( n^*_5 \) (SP CD8) (cells) |
|-------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1     | 82.58 \times 10^6 | 9.30 \times 10^6 | 18.36 \times 10^6 | 13.85 \times 10^6 | 4.51 \times 10^6 |
| 2     | 142.19 \times 10^6 | 19.94 \times 10^6 | 26.20 \times 10^6 | 18.73 \times 10^6 | 7.46 \times 10^6 |
| 3     | 89.00 \times 10^6  | 5.98 \times 10^6  | 15.98 \times 10^6 | 11.88 \times 10^6 | 4.10 \times 10^6 |
| 4     | 29.32 \times 10^6  | 2.09 \times 10^6  | 5.61 \times 10^6  | 4.40 \times 10^6  | 1.21 \times 10^6 |
| 5     | 29.32 \times 10^6  | 2.09 \times 10^6  | 5.61 \times 10^6  | 4.40 \times 10^6  | 1.21 \times 10^6 |
| 6     | 51.26 \times 10^6  | 5.93 \times 10^6  | 9.01 \times 10^6  | 6.85 \times 10^6  | 2.16 \times 10^6 |
| 7     | 64.48 \times 10^6  | 6.81 \times 10^6  | 11.64 \times 10^6 | 9.03 \times 10^6  | 2.61 \times 10^6 |
| 8     | 218.94 \times 10^6 | 15.42 \times 10^6 | 40.20 \times 10^6 | 29.46 \times 10^6 | 10.74 \times 10^6 |
| Mean  | 88.39 \times 10^6  | 8.45 \times 10^6  | 16.57 \times 10^6 | 12.33 \times 10^6 | 4.25 \times 10^6 |
| Standard deviation | 60.11 \times 10^6  | 5.89 \times 10^6  | 11.05 \times 10^6 | 7.94 \times 10^6  | 3.12 \times 10^6 |

The bold font highlights the mean and the standard deviation from the individual mice data.
Given $a_1$, we can then estimate $\varphi_1$ from the equation $\varphi_1 = \frac{a_1}{\tau_1}$.

5. Given $\tau_1$, $\varphi_1$, and the fact that $\tau_1 = \frac{1}{\mu_1 + \varphi_1}$, we can obtain an estimate for $\mu_1$.

6. We are now left with three remaining parameters: $\varphi_2$, $\mu_2$, and $\lambda_3$. Given the experimental constraints on $\tau_1$, $\tau_2$, and $\tau_3$, we assume that the average time to proliferate, $1/\lambda_3$, cannot be $<7$ days. Therefore, we consider $\lambda_3$ to be constrained in the interval $[1/7, \tau_3^{-1}]$, with time measured in days. We sample equally spaced values for $\lambda_3$, and for each value, we compute $\varphi_2 = \frac{\varphi_1}{n_1^i(\varphi_1 + \mu_1 + \lambda_3)}$. The ratio $a_2 = \frac{n_1^i}{n_2^i}$ is computed using the linear regression method described above (see Figure 3). In this way, we obtain an estimate for $\varphi_2$. We note that the $p$-values for $a_1$ and $a_2$ are given by $7.57 \times 10^{-3}$ and $6.85 \times 10^{-3}$, respectively (both smaller than the significance level $\alpha = 0.05$).

7. Given $\tau_2$, $\varphi_2$, and the fact that $\tau_2 = \frac{1}{\mu_2 + \varphi_2}$, we can obtain an estimate for $\mu_2$.

8. From steps 6 and 7 above, we have generated (a table of) values for $\varphi_2$ and $\mu_2$, given a fiducial value for $\lambda_3$ in the interval $[1/7, \tau_3^{-1}]$. The mice considered in the experimental study are 5.5–17 weeks old, and their thymus is in steady state (26). Thus, we expect that the parameter values can only be accepted if the corresponding system of ODEs attains steady state by 3 weeks. Therefore, we only accept parameter values which provide thymocyte cell counts at time $t = 21$ days that are within one standard deviation from the experimentally determined values (see Table 1). That is, we impose for the given parameter set that the mathematically predicted value $n_i(t = 21$ days) belongs to the interval $n_i^\pm \xi_i$, with $i = 1, 2, 3$, and where $n_i^\pm$ is the (experimental) mean number of cells in compartment $i$, and $\xi_i$ is the (experimental) standard deviation in compartment $i$, as given in Table 1.

We obtain the following parameter values:

- $\phi = 35.350 \times 10^6$ cells/day, $\mu_1 = 0.263$ day$^{-1}$, $\mu_3 = 0.099$ day$^{-1}$, $\varphi_1 = 0.137$ day$^{-1}$, $\varphi_3 = 0.151$ day$^{-1}$, and $\lambda_3 \in [1.295, 1.443]$ day$^{-1}$, $\varphi_2 \in [0.050, 0.198]$ day$^{-1}$, $\lambda_3 \in [0.143, 0.250]$ day$^{-1}$.

These parameters imply the following thymic selection rates:

3.1.1. Death rates

$9.7 \times 10^5$ cells/h die by neglect in compartment 1 ($\mu_1 n_1^i$), $4.8 \times 10^5$ cells/h die by negative selection in compartment 2 ($\mu_2 n_2^i$), and $6.9 \times 10^4$ cells/h die by negative selection in compartment 3 ($\mu_3 n_3^i$).

3.1.2. Differentiation rates

$5.0 \times 10^5$ cells/h are positively selected in compartment 1, that is, become post-DP from pre-DP ($\varphi_1 n_1^i$), $4.4 \times 10^5$ cells/h are positively selected in compartment 2, that is, become SP from post-DP ($\varphi_2 n_2^i$), and $1.0 \times 10^5$ cells/h leave compartment 3 to go to the periphery ($\varphi_3 n_3^i$).

3.1.3. Proliferation rate

$1.3 \times 10^5$ cells/h proliferate in compartment 3 ($\lambda_3 n_3^i$).

We have also computed the stringency of thymic selection, which we define as given by the ratio:

$$\frac{\varphi_3 n_3^i}{\phi} = 6.79\%.$$

Finally, we have computed the (per cell) probability to die, given that the cell is in compartment $i$ ($i = 1, 2, 3$), as well as the (per cell) probability to proliferate in the medulla. We have obtained:

$$p_1 = \frac{\mu_1}{\mu_1 + \varphi_1} = 65.8\%,$$

$$p_2 = \frac{\mu_2}{\mu_2 + \varphi_2} = 91.7\%,$$

$$p_3 = \frac{\lambda_3}{\mu_3 + \varphi_3 + \lambda_3} = 22.9\%,$$

$$Q_i = \frac{\lambda_i}{\mu_i + \varphi_i + \lambda_i} = 42.2\%.$$

3.2. Parameter estimation for the second model (means)

In this section, we make use of previously published experimental data (26) that provide thymocyte cell counts for the four subsets considered: pre-DPs, post-DPs, CD4 SPs, and CD8 SPs. We only make use of the experimental data for the wild type mice. The data will allow us to provide experimental estimates for the steady state thymocyte cell counts: $n_1^i, n_2^i, n_3^i, n_4^i$. As described in Section 3.1, we also need residence times for each population subset, $\tau_i$, with $i = 1, 2, 4, 8$. If we make use of the model (see Section 2.2), the residence time in compartment $i$ can be expressed as:

$$\tau_i = \frac{1}{\varphi_i + \mu_i}, \quad \text{for } i \in \{1, 2\}, \quad \text{and}$$

$$\tau_i = \frac{1}{\mu_i + \xi_i}, \quad \text{for } i \in \{4, 8\}.$$

Recent experimental data provide support for asymmetric death rates in the CD4 and CD8 SP compartments (21). The estimated death rates for CD4 and CD8 SP thymocytes are $\mu_4 = 0.04$ day$^{-1}$ and $\mu_8 = 0.11$ day$^{-1}$. We also make use of the estimates derived in Section 3.1 for $\phi, \mu_1, \mu_2, \varphi_1$, and $\varphi_2$. Finally,

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1Private communication from Ben Seldon and Andy Yates.
the average residence times in each compartment, as described in Section 3.1, are given by:

\[
\tau_1 = 60 \text{ h} = 2.5 \text{ days}, \quad \tau_2 = 16 \text{ h} = 0.67 \text{ days}, \\
\tau_4 = 96 \text{ h} = 4 \text{ days}, \quad \tau_8 = 96 \text{ h} = 4 \text{ days}.
\]

In order to derive estimates for the model parameters, we have carried out the following steps:

1. Given \(\tau_4, \mu_4\), and the fact that \(\tau_4 = \frac{1}{\mu_4 + \xi_4}\), we can obtain an estimate for \(\xi_4\).
2. In the same way, given \(\tau_8, \mu_8\), and the fact that \(\tau_8 = \frac{1}{\mu_8 + \xi_8}\), we can obtain an estimate for \(\xi_8\).
3. We are now left with four remaining parameters: \(\phi_4, \phi_8, \lambda_4\), and \(\lambda_8\). We know that \(\phi_2 = \phi_4 + \phi_8\). We sample \(\phi_4\) in the interval \([0, \phi_2]\), where \(\phi_2\) is the mean value of the interval obtained in Section 2.1, and for each fiducial value for \(\phi_4\), we compute the corresponding value for \(\phi_8\).
4. Given \(\tau_4, \phi_4\), and the fact that \(n_4^* = \frac{n_4^0 \phi_4}{\tau_4 + \phi_4}\), we can compute the fraction \(a_3 = \frac{n_4^*}{n_4^0}\) by linear regression (see Figure 4), and thus obtain an estimate for \(\lambda_4\). Note that we will reject values of \(\lambda_4\) that imply the proliferation time is larger than 7 days (see Section 3.1).
5. In Section 3.1, we obtained an estimate for the mean of \(\lambda_3\), and we know that \(\lambda_4 n_4^* + \lambda_8 n_8^* = \lambda_3 n_3^*\). As before, we can compute the fractions \(a_4 = \frac{n_4^*}{n_4^0}\) and \(a_5 = \frac{n_8^*}{n_8^0}\) by linear regression (see Figure 4), and thus obtain an estimate for \(\lambda_8\). Note that we will reject values of \(\lambda_8\) that imply the proliferation time is larger than 7 days (see Section 3.1). We note that the \(p\)-values for \(a_3, a_4,\) and \(a_5\) are given by \(8.43 \times 10^{-3}, 3.33 \times 10^{-7},\) and \(4.56 \times 10^{-8}\), respectively (smaller than the significance level).
6. From steps 3, 4, and 5 above, we have generated (a table of) values for \(\phi_8, \lambda_4,\) and \(\lambda_8\), given a fiducial value for \(\phi_4\) in the interval \([0, \phi_2]\). As discussed in Section 3.1, we only accept parameter values which provide thymocyte cell counts at time \(t = 21\) days that are within one standard deviation from the experimentally determined values (see Table 1).

We obtain the following parameter values:

\[
\mu_4 = 0.04 \text{ day}^{-1}, \quad \mu_8 = 0.11 \text{ day}^{-1}, \\
\xi_4 = 0.21 \text{ day}^{-1}, \quad \xi_8 = 0.14 \text{ day}^{-1},
\]

and

\[
\phi_4 = 0.070 \text{ day}^{-1}, \quad \phi_8 = 0.054 \text{ day}^{-1},
\]

\[
\lambda_4 = 0.216 \text{ day}^{-1}, \quad \lambda_8 = 0.093 \text{ day}^{-1}.
\]

These parameters imply the following thymic selection rates:

### 3.2. Death rates

2.05 \times 10^4 \text{ cells/h die by negative selection in compartment 4} (\mu_4 n_4^*) and 1.95 \times 10^4 \text{ cells/h die by negative selection in compartment 8} (\mu_8 n_8^*).

### 3.2.2. Differentiation rates

2.50 \times 10^4 \text{ cells/h are CD4 positively selected in compartment 2, that is, become CD4 SP from post-DP} (\phi_4 n_4^*), 1.90 \times 10^4 \text{ cells/h are CD8 positively selected in compartment 2, that is, become CD8 SP from post-DP} (\phi_8 n_8^*), 1.08 \times 10^5 \text{ cells/h leave compartment 4 to go to the periphery} (\xi_4 n_4^*), and 2.48 \times 10^4 \text{ cells/h leave compartment 8 to go to the periphery} (\xi_8 n_8^*).

### 3.2.3. Proliferation rates

1.10 \times 10^4 \text{ cells/h proliferate in compartment 4} (\lambda_4 n_4^*), and 1.60 \times 10^4 \text{ cells/h proliferate in compartment 8} (\lambda_8 n_8^*).

![FIGURE 4](www.frontiersin.org) Linear regression plots for the second model.
We can compute the stringency of thymic selection, defined by the ratio:

$$\frac{J_4 \alpha^2 + \xi_5 \eta_9}{\phi} = 8.96\%.$$  

We can provide an estimate for the cortical positive selection probabilities, that is the (per post-DP cell) probability to become a CD4 SP or a CD8 SP, and the probability to be negatively selected in the cortex. We have obtained:

$$s_1 = \frac{\varphi_4}{\mu_2 + \varphi_4 + \varphi_8} = 4.7\%,$$

$$s_2 = \frac{\varphi_8}{\mu_2 + \varphi_4 + \varphi_8} = 3.6\%,$$

$$p_2 = \frac{\mu_2}{\mu_2 + \varphi_4 + \varphi_8} = 91.7\%.$$  

Finally, we have computed the (per cell) probability to die, given that the cell is in compartment $i$, as well as the (per cell) probability to proliferate in the medulla. We obtain:

$$p_4 = \frac{\varphi_4}{\mu_4 + \xi_4 + \lambda_4} = 8.6\%,$$

$$q_4 = \frac{\lambda_4}{\mu_4 + \xi_4 + \lambda_4} = 46.3\%,$$

$$p_8 = \frac{\mu_8}{\mu_8 + \xi_8 + \lambda_8} = 32.1\%,$$

$$q_8 = \frac{\lambda_8}{\mu_8 + \xi_8 + \lambda_8} = 27.0\%.$$  

These probabilities imply that the probability to exit the thymus as a mature CD8 thymocyte (that has already reached the medulla) is given by

$$\frac{100}{100 - (8.6 + 46.3) \%} = 45.1\%,$$

and the probability to exit as a mature CD4 thymocyte (that has already reached the medulla) is given by

$$\frac{100}{100 - (32.1 + 27.0) \%} = 40.9\%.$$  

### 3.3. Sensitivity Analysis

In this section, we explore the sensitivity of the parameters to perturbations in the experimental data. For the first model, the experimental data are given in terms of the following eight quantities:

$$\theta = (\tau_1, \tau_2, \tau_3, \phi_{out}, \omega_1, \omega_2, \theta_3, \tilde{n}_1),$$

where $a_1, a_2$ are the coefficients of the linear regression of $\frac{n_7}{n_9}$ and $\frac{n_8}{n_3}$, respectively, and $\tilde{n}_1$ is the experimental mean value of $n_1$.

We perturb each entry of the vector $\theta$ by adding and subtracting 10% of its value. Therefore, we now have two values for $\theta_i$ equal to $\theta_i + \frac{1}{2} \theta_i$ and $\theta_i - \frac{1}{2} \theta_i$. Consequently, we have $2^8$ sets of $\theta_i$ which will be used to compute the corresponding model parameters as described in Section 3.1. Parameter values will only be accepted if they provide a stable solution before $t = 21$ days.

For the second model, the experimental data is given in terms of the following seven quantities:

$$\theta = (\tau_4, \tau_8, \mu_4, \mu_8, \omega_3, \omega_4, \omega_5),$$

where $a_3, a_4, a_5$ are the coefficients of the linear regression of $\frac{n_7}{n_9}, \frac{n_8}{n_3}$, and $\frac{n_9}{n_7}$, respectively. We have made use of the means of the following parameters of the first model: $\phi, \varphi_1, \mu_1, \varphi_2, \mu_2, \lambda_3$.  

### Table 2 | Means, 95% trimmed and minimum–maximum intervals of the model parameters.

| Parameter | Mean value | 95% Trimmed interval | Minimum–maximum interval range |
|-----------|------------|----------------------|--------------------------------|
| $\phi$    | $35.86 \times 10^0$ | $(35.65, 36.06)$ | $(28.93, 43.21 \times 10^0)$ |
| $\varphi_1$ | $0.139 \text{ day}^{-1}$ | $(0.129, 0.140) \text{ day}^{-1}$ | $(0.112, 0.167) \text{ day}^{-1}$ |
| $\varphi_2$ | $0.136 \text{ day}^{-1}$ | $(0.134, 0.139) \text{ day}^{-1}$ | $(0.041, 0.274) \text{ day}^{-1}$ |
| $\varphi_4$ | $0.140 \text{ day}^{-1}$ | $(0.136, 0.145) \text{ day}^{-1}$ | $(0.060, 0.264) \text{ day}^{-1}$ |
| $\varphi_8$ | $0.134 \text{ day}^{-1}$ | $(0.129, 0.138) \text{ day}^{-1}$ | $(0.010, 0.214) \text{ day}^{-1}$ |
| $\mu_1$   | $0.265 \text{ day}^{-1}$ | $(0.263, 0.267) \text{ day}^{-1}$ | $(0.196, 0.333) \text{ day}^{-1}$ |
| $\mu_2$   | $1.372 \text{ day}^{-1}$ | $(1.365, 1.378) \text{ day}^{-1}$ | $(1.083, 1.618) \text{ day}^{-1}$ |
| $\mu_4$   | $0.040 \text{ day}^{-1}$ | $n/a$ | $(0.036, 0.044) \text{ day}^{-1}$ |
| $\mu_8$   | $0.110 \text{ day}^{-1}$ | $n/a$ | $(0.099, 0.121) \text{ day}^{-1}$ |
| $\lambda_4$ | $0.181 \text{ day}^{-1}$ | $(0.179, 0.184) \text{ day}^{-1}$ | $(0.116, 0.226) \text{ day}^{-1}$ |
| $\lambda_8$ | $0.085 \text{ day}^{-1}$ | $(0.080, 0.090) \text{ day}^{-1}$ | $(0.078, 0.092) \text{ day}^{-1}$ |
| $\xi_4$   | $0.231 \text{ day}^{-1}$ | $(0.230, 0.233) \text{ day}^{-1}$ | $(0.229, 0.233) \text{ day}^{-1}$ |
| $\xi_8$   | $0.152 \text{ day}^{-1}$ | $(0.150, 0.154) \text{ day}^{-1}$ | $(0.149, 0.155) \text{ day}^{-1}$ |

We perturb each entry of the vector $\theta$ as described above. Therefore, we have $2^7 n_{\theta}$ sets of $\theta$, with $n_{\theta}$, the number of different values considered for $\varphi_2$ in the interval $(0, \varphi_2)$. Parameter values will only be accepted if they provide a stable solution before $t = 21$ days.

The results of the sensitivity analysis, with 95% trimmed intervals and minimum–maximum interval ranges, are given in Table 2.

### 3.4. Variability in the Selection Rates

The (trimmed and minimum–maximum) intervals derived in Section 3.3 allow us to estimate the variability in the different selection rates discussed in Sections 3.1 and 3.2. For example, given variations in the parameters, the corresponding variations in the selection rates can be shown to be:

$$\Delta p_i = \frac{1}{(\mu_1 + \varphi_1)^2} (\varphi_1 \Delta \mu_1 + \mu_1 \Delta \varphi_1) \quad \text{for} \quad i = 1, 2,$$

$$\Delta p_3 = \frac{1}{(\mu_3 + \varphi_3 + \lambda_3)^3} [((\varphi_3 + \lambda_3) \Delta \mu_3 + \mu_3 \Delta \varphi_3 + \mu_3 \Delta \lambda_3),$$

$$\Delta q_3 = \frac{1}{(\mu_3 + \varphi_3 + \lambda_3)^3} [\lambda_3 \Delta \mu_3 + \lambda_3 \Delta \varphi_3 + (\mu_3 + \varphi_3) \Delta \lambda_3,$$

$$\Delta s_i = \frac{1}{(\mu_2 + \varphi_2 + \lambda_2)^2} [\varphi_2 \Delta \mu_2 + \varphi_2 \Delta \varphi_2 + (\mu_2 + \varphi_2) \Delta \lambda_2,$$

$$\Delta p_i = \frac{1}{(\mu_1 + \xi_1 + \lambda_1)^2} [\mu_1 \Delta \xi_1 + \mu_1 \Delta \lambda_1,$$

$$+ (\xi_1 + \lambda_1) \Delta \mu_1] \quad \text{for} \quad i = 4, 8,$$

$$+ (\xi_1 + \lambda_1) \Delta \mu_1] \quad \text{for} \quad i = 4, 8,$$

$$+ (\xi_1 + \lambda_1) \Delta \mu_1] \quad \text{for} \quad i = 4, 8,$$

$$+ (\xi_1 + \lambda_1) \Delta \mu_1] \quad \text{for} \quad i = 4, 8,$$

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$$+ (\xi_1 + \lambda_1) \Delta \mu_1] \quad \text{for} \quad i = 4, 8,$$

$$+ (\xi_1 + \lambda_1) \Delta \mu_1] \quad \text{for} \quad i = 4, 8.$$
\[ \Delta q_i = \frac{1}{(\mu_i + \xi + \lambda_i)^2} \{ \lambda_i \Delta \xi_i + \lambda_i \Delta \mu_i + (\xi_i + \mu_i) \Delta \lambda_i \} \quad \text{for} \quad i = 1, 2, 3, 4, 5, 6, 7, 8. \]  

We present in Table 3 the variability of the selection rates.

### 4. DISCUSSION

We have brought together experimental data with a mathematical compartment model [similar to other progression models of CD4 and CD8 T cell development (13, 14, 18, 21, 33)] to provide estimates for the selection events that take place in the thymus. We have made use of a range of experimental data: (i) steady state thymocyte cell counts (26), mean residence times in each compartment (27–29), and thymocyte cell counts (26), mean residence times in each compartment estimates for the selection events that take place in the thymus.

We have recently addressed the temporal dynamics of thymic selection, as it was rescued by Bim deficiency (26). Sinclair et al. (14) obtained estimates of thymic selection rates, using an experimental procedure that temporarily blocks thymic output and a mathematical model in which rates of transit from compartment to compartment depend on the number of cell divisions. Their model can capture the thymic “conveyor belt” (34, 35) scheme, but requires more differential equations and more parameters than equation (6). Despite the differences between their theoretical and experimental models and ours, similar estimates for thymic selection rates are found. For example, we estimate that 1.2 million post-DP become CD4 SP thymocytes per day and 0.5 million post-DP become CD8 SP thymocytes per day; their estimates are 0.9 and 0.2 million, respectively. Finally, we estimate that 2.6 million CD4 SP thymocytes per day and 0.6 million CD8 SP thymocytes per day exit the thymus. Their estimates are 2.4 and 0.5 million, respectively.

Further attempts to quantify thymic selection rates making use of mathematical models also include those of Faro et al. (12). The mathematical model developed by Faro and collaborators does not include time dynamics, but describes the relationship between the number of selected ligands and the probability of selection of a given thymocyte. Thomas-Vaslin et al. (14) obtained estimates of thymic selection rates, using an experimental procedure that temporarily blocks thymic output and a mathematical model in which rates of transit from compartment to compartment depend on the number of cell divisions. Their model can capture the thymic “conveyor belt” (34, 35) scheme, but requires more differential equations and more parameters than equation (6). Despite the differences between their theoretical and experimental models and ours, similar estimates for thymic selection rates are found. For example, we estimate that 1.2 million post-DP become CD4 SP thymocytes per day and 0.5 million post-DP become CD8 SP thymocytes per day; their estimates are 0.9 and 0.2 million, respectively. Finally, we estimate that 2.6 million CD4 SP thymocytes per day and 0.6 million CD8 SP thymocytes per day exit the thymus. Their estimates are 2.4 and 0.5 million, respectively.

Our estimates of how many CD4 and CD8 SP thymocytes survive and exit the thymus reflect the skewed CD4:CD8 SP thymocyte ratio observed in C57BL/6 mice, which is approximately 4:1 (36). This ratio is similar to the reported CD4:CD8 ratio of recent thymic emigrants (37), and raises the question of what accounts for the CD4 bias. While we were able to determine death and differentiation rates for both CD4 and CD8 SP thymocytes (see Table 2), our experimental approach did not allow us to determine what fraction of the post-DP pool was MHC class I versus II restricted. Therefore, we could not address the issue of when and how the CD4:CD8 bias becomes established. The approach of Sinclair et al., which used MHC class I and class II deficiency, allowed them to address this question. Their data suggest that the skewed CD4:CD8 ratio reflects asymmetry in post-selection DP death rates, rather than more efficient positive selection of CD4 compared to CD8 thymocytes (21). Yet, the parameter estimation allows us to compare the following different CD4:CD8 ratios (see

| Rate | Initial Value (%) | After Perturbation (%) | \( \Delta \) Value (%) | \( \Delta \) Min–max (%) |
|------|-------------------|------------------------|------------------------|------------------------|
| \( p_1 \) | 65.8 | 65.66 | ±0.76 | ±20.55 |
| \( p_2 \) | 91.7 | 90.98 | ±0.49 | ±1728 |
| \( p_3 \) | 22.91 | 24.07 | ±0.90 | ±28.22 |
| \( q_4 \) | 42.24 | 41.74 | ±0.99 | ±30.53 |
| \( q_5 \) | 4.69 | 8.51 | ±0.80 | ±15.16 |
| \( q_6 \) | 3.61 | 8.13 | ±0.79 | ±15.03 |
| \( q_7 \) | 8.59 | 8.84 | ±0.37 | ±4.91 |
| \( q_8 \) | 46.29 | 40.07 | ±1.29 | ±20.49 |
| \( q_9 \) | 32.12 | 31.71 | ±2.25 | ±35.37 |
| \( q_{10} \) | 27.0 | 24.5 | ±3.58 | ±64.81 |

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Section 3.2): (i) the CD4:CD8 ratio of positive selection in the post-DP pool (differentiation from post-DP to either CD4 SP or CD8 SP) is given by $\frac{\phi_4}{\phi_8} \approx 5:4$, (ii) the CD4:CD8 ratio in the SP pool is given by $\frac{n^*_4}{n^*_8} \approx 3:1$, and (iii) the CD4:CD8 ratio of positive selection in the SP pool (differentiation from SP to peripheral early thymic emigrants) is given by $\frac{\xi_4}{\xi_8} \approx 4:1$. Our observations indicate that the CD4 bias is progressively established, as the thymocytes mature from the post-DP stage until the exit of the SP stage to migrate to the periphery.

Our mathematical analysis has also allowed us to estimate the stringency of thymic selection, defined by:

$$\sigma = \frac{\xi_4 n^*_4 + \xi_8 n^*_8}{\phi} = 8.96\%,$$

that is, the ratio between the number of thymocytes per unit time that exit the thymus and the number of thymocytes per unit time that enter the pre-DP stage. The sensitivity analysis described in Section 3.3 allows us to provide a value of $\Delta \sigma = 0.2\%$, where we have made use of the minimum–maximum interval ranges (see fourth column of Table 2). A different measure of stringency could be based on the probability of a cell surviving the maturation process. In our notation, this would correspond to the following:

$$(1 - p_1) \times (1 - p_2) \times (1 - p_3) = 2.19\%.$$

We note that this measure of stringency is the probability of not dying in any of the three compartments considered in the model (pre-DP, post-DP, and SP). As discussed in Appendix A.1, and given that in the SP pool, thymocytes may proliferate, there is a need to consider this special case. Our estimates suggest that a population of $10^3$ pre-DP thymocytes will yield 69 CD4 and 25 CD8 SP thymocytes that leave the medulla to get incorporated into the peripheral naive T cell pool (see details in Appendix A.1).

The sensitivity analysis (see Section 3.3) and the variability of the selection rates derived from it (see Section 3.4) give us the confidence to conclude, that our parameter estimation is robust. We are aware that the experimental data we have made use of [steady state thymocyte cell counts (26)] do not provide the exquisite time resolution described in Ref. (21). However, the supporting mathematical model described in Section 2.2, allows us to obtain the time evolution of the thymocyte populations, once the parameters have been estimated. In Figure 5, we plot the time evolution of the total number of cells in each compartment of the mathematical model: pre-DP, post-DP, CD4 SP, and CD8 SP thymocytes. We start with no cells at time zero, $n_i(t=0) = 0$ for $i = 1, 2, 4, 8$. Trajectories have been plotted for a period of 6 weeks and have been computed for every permutation of the parameter set presented in Table 2. The subset of parameters shared with the simple model ($\phi, \psi, \mu_1, \mu_2$), were fixed at their mean values. Thus, 548 distinct parameter sets were generated. The system of equations (6) was solved using a fourth order Runge–Kutta method (Python source code).

![Figure 5](image-url)
The approaches introduced in this paper have shed some light on the probabilities and timescales that characterize cellular fate in the thymus after the DN stage. We plan to generalize the mathematical model introduced here, making use of experimental data for the strength of TCR binding in Nur77^{+}CD4^{-}CD8^{-} mice (26), to investigate issues such as the death rate in the post-DP pool and the CD4:CD8 ratio. Our model assumes that all progenitors in a particular pool behave with identical kinetics, i.e., move through the various stages of selection at the same rate. Future model refinements will come from consideration of the heterogeneity of the pools, which are known to include cells that will become iNKT cells, regulatory T cells, and intraepithelial lymphocytes (2). It is also possible that progenitors of the same general class move through the selection process with different kinetics (34). The models introduced here can serve as a first step to study human thymic selection, although comprehensive data on human thymic subsets, their sizes, and residence times are not yet available. It would be of great interest to apply the model to data on thymic subsets and cellularity in children, keeping in mind that residence times of human subsets may differ from murine ones (38). Finally, we note that we have not mentioned the relevance of cytokines, such as IL-7, during thymic development. Some differences have already been described for the role of IL-7R in human versus mouse T cell development (38, 39). We hope in the near future to combine mechanistic mathematical models of IL-7 and IL-7R (40) with the T cell development model introduced here to address these issues.

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Appendix

A.1. Stringency of Thymic Selection: A Stochastic Model

In this section, we present the details that allow us to compute the stringency of thymic selection for the mathematical model considered in Section 2.2.

Let us assume that at time \( t = 0 \), there exists a single T cell in a given compartment. In our case, the compartment can be the pre-DR, post-DR, or SP (either CD4 SP or CD8 SP) stages. The cell, at any time, may die (with rate, \( \mu \)), divide (with rate, \( \lambda \)), and produce two daughter cells, or leave the compartment (with rate, \( \xi \)) to enter a different compartment. The waiting times for each event are assumed to be exponentially distributed, and daughter cells are assumed to behave identically to the initial single cell. We introduce the bivariate Markov process \( \{X(t), Y(t)\}_{t \geq 0} \), where \( X(t) \) is the number of cells in the compartment at time \( t \), and \( Y(t) \) is the number of cells which have left the compartment (to enter a different compartment). Our aim is to calculate the expected number of cells (and variance) that leave the compartment.

State probabilities for the Markov process are defined as follows (41)

\[
p(x,y,t) = \text{Prob}[X(t) = x, Y(t) = y | X(0) = 1, Y(0) = 0],
\]

(A1)

and transition probabilities for this process are defined as follows (41)

\[
p(x,y,z,t) = \text{Prob}[X(t + \Delta t) = w, Y(t + \Delta t) = z | X(t) = x, Y(t) = y]
\]

\[
= \begin{cases} 
\lambda x \Delta t + o(\Delta t), & \text{if } w = x + 1, z = y, \\
\mu x \Delta t + o(\Delta t), & \text{if } w = x - 1, z = y, \\
\xi x \Delta t + o(\Delta t), & \text{if } w = x - 1, z = y + 1, \\
1 - (\lambda + \mu + \xi)x \Delta t + o(\Delta t), & \text{if } w = x, z = y, \\
o(\Delta t), & \text{if } w, z \text{ otherwise.}
\end{cases}
\]

The Kolmogorov (or master) equation for this process is given by (41)

\[
\frac{d p(x,y,t)}{dt} = \lambda (x-1)p(x-1,y,t) + \mu (x+1)p(x+1,y,t) \\
+ \xi (x+1)p(x+1,y-1,t) - (\lambda + \mu + \xi) x p(x,y,t).
\]

(A2)

Let \( m_X(t) \) be the expected number of cells in the compartment under consideration, and \( m_Y(t) \) be the expected number of cells which have left the compartment. Similarly, let \( m_{XX}(t) \) be the expectation of the random variable \( X(t)^2 \), \( m_{XY}(t) \) be the expectation of the random variable \( X(t)Y(t) \), and \( m_{YY}(t) \) be the expectation of the random variable \( Y(t)^2 \). Then, making use of the probability generating function technique (41), we derive the time evolution for the first two moments of the system:

\[
\frac{d m_X(t)}{dt} = (\lambda - \mu - \xi) m_X(t),
\]

(A4)

\[
\frac{d m_Y(t)}{dt} = \xi m_X(t),
\]

(A5)

\[
\frac{d m_{XX}(t)}{dt} = 2(\lambda - \mu - \xi) m_{XX}(t) + (\lambda - \mu - \xi) m_X(t),
\]

(A6)

\[
\frac{d m_{XY}(t)}{dt} = (\lambda - \mu - \xi) m_{XY}(t) + \xi [m_{XX}(t) - m_X(t)],
\]

(A7)

\[
\frac{d m_{YY}(t)}{dt} = \xi [2m_{XY}(t) + m_X(t)].
\]

(A8)

Given that we start with a single cell, the expected number of cells at time \( t \) is given by

\[
m_X(t) = e^{(\lambda - \mu - \xi)t}.
\]

(A9)

Under the restriction \( \lambda < \mu + \xi \), the expected number of cells tends to zero as \( t \to +\infty \). This implies that all cells from the single T cell progenitor either die or leave the compartment for sufficiently large times. The expected number of cells which leave the compartment is given by

\[
m_Y(t) = \frac{\xi}{\mu + \xi - \lambda} \left[ 1 - e^{(\lambda - \mu - \xi)t} \right].
\]

(A10)

As \( t \to +\infty \), the expected number of cells to leave the compartment can be shown to be

\[
\lim_{t \to +\infty} m_Y(t) = \frac{\xi}{\mu + \xi - \lambda}.
\]

(A11)

We now solve the remaining ODEs equations (A6–A8), to find

\[
m_{YY}(t) = \frac{2\lambda \xi^2}{(\lambda - \mu - \xi)^2} \left[ \frac{1}{2} e^{(\lambda - \mu - \xi)t} - e^{(\lambda - \mu - \xi)t} \right]
\]

\[
- \frac{4\lambda \xi^2}{(\lambda - \mu - \xi)^2} \left( t - \frac{1}{\lambda - \mu - \xi} \right) e^{(\lambda - \mu - \xi)t}
\]

\[
+ \frac{\xi}{\lambda - \mu - \xi} e^{(\lambda - \mu - \xi)t} - \frac{2\lambda \xi^2}{(\lambda - \mu - \xi)^3}
\]

\[
- \frac{\xi}{\mu + \xi - \lambda}.
\]

(A12)

It, therefore, follows that the random variable \( Y(t) \), which represents the number of cells leaving the compartment under consideration, has the following variance (in the limit \( t \to +\infty \))

\[
\sigma_Y^2 = \lim_{t \to +\infty} \left[ m_{YY}(t) - m_Y(t)^2 \right] = \frac{2\lambda \xi^2}{(\mu + \xi - \lambda)^3} + \frac{\xi}{\mu + \xi - \lambda}
\]

\[
- \frac{\xi^2}{(\mu + \xi - \lambda)^2}.
\]

(A13)

A.2. Stringency of Thymic Selection in the Four Compartment Model

The previous example can easily (but laboriously) be extended to the mathematical model introduced in Section 2.2.

We may evaluate the expected number of, for example, CD4+ T cells produced by a single pre-DR progenitor (or more generally \( N \) pre-DR progenitors). We only present the time evolution of the moment generating function. The deterministic equations describing the mean and variance of the numbers of cells in each
compartment (pre-DP, post-DP, SP CD4, and SP CD8) are left for
the reader to derive. When counting the number of CD4\(^+\) T cells
leaving the thymus, the moment generating function satisfies the
following partial differential equation

\[
\frac{\partial M}{\partial t} = \mu_1 (e^{-\theta_1} - 1) \frac{\partial M}{\partial \theta_1} + \varphi_1 (e^{-\theta_1} e^{\theta_2} - 1) \frac{\partial M}{\partial \theta_1} + (\mu_2 + \varphi_2) (e^{-\theta_2} - 1) \frac{\partial M}{\partial \theta_2} + \varphi_4 (e^{-\theta_2} e^{\theta_4} - 1) \frac{\partial M}{\partial \theta_2} + \mu_4 (e^{-\theta_4} - 1) \frac{\partial M}{\partial \theta_4} + \lambda_4 (e^{\theta_4} - 1) \frac{\partial M}{\partial \theta_4} + \xi_4 (e^{-\theta_4} e^{\theta_8} - 1) \frac{\partial M}{\partial \theta_4}. \tag{A14}
\]

The symmetry of the mathematical model implies that an
equivalent equation for the number of CD8\(^+\) T cells leaving the
thymus can be obtained by interchanging the indexes 4 and 8.
For our derived parameter set, the previous equation allows us to
conclude that the expected number of CD4\(^+\) T cells, a single thy-
mocyte in the pre-DP compartment produces, is 0.069 (standard
deviation 0.96), whereas the expected number of CD8\(^+\) T cells
which leave the thymus is 0.025 (standard deviation 0.59).
To put this into perspective, a population of \(10^3\) pre-DP thy-
mocytes is expected to produce 69 CD4\(^+\) T cells which leave the
thymus (standard deviation 66), and 25 CD8\(^+\) T cells (standard
deviation 41). More generally, a population of \(N\) pre-DP thymo-
cytes is expected to produce 0.069\(N\) CD4\(^+\) T cells and 0.025\(N\)
CD8\(^+\) T cells.