MATHEMATICAL BIOSCIENCES
AND ENGINEERING
Volume **2**, Number **1**, January **2005**

http://www.mbejournal.org/

pp. **153–168**

THE EFFECTS OF AFFINITY MEDIATED CLONAL EXPANSION OF PREMIGRANT THYMOCYTES ON THE PERIPHERY T-CELL REPERTOIRE

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(Communicated by Yang Kuang)

Abstract. The immune system maintains a highly diverse T-cell repertoire, which is shaped by active interactions between developing thymocytes and endogenous peptide/MHC molecules through the principle of positive and negative selections. Detours et al. developed a quantitative model addressing key immunologic notions such as selection, alloreactivity, and self-restriction. The model was based on the assumption that the clone size is uniformly distributed in the naive T-cell repertoire. However, recent biological findings have indicated that the naive T-cell repertoire is highly skewed, due to the uneven proliferation of premigrant single-positive thymocytes. In this paper, the model is revised to include these new findings. The effects of the uneven clonal expansion are investigated in detail and their biological significance is discussed. It is found that the uneven clonal expansion can significantly enhance the self-MHC restriction, while avoiding decreasing the alloreactivity. The clonal expansion therefore appears to be an additional selection event, resulting in fine tuning of the repertoire. In this way, T-cells reaching the periphery pool can fulfill maximum competence: both high self-restriction and high alloreactivity.

1. **Introduction.** The thymus is the central organ for producing mature T-cells expressing a highly diverse repertoire of T-cell receptors (TCRs) [1]. This is achieved by active interactions between developing thymocytes and endogenous peptide/MHC molecules [30], through the principle of positive and negative selections. Positive selection works by promoting the selective survival and expansion of thymocytes that can bind self-peptide/MHC complexes with low affinity, while negative selection works by eliminating thymocytes whose TCRs recognize abundant peptide/MHC complexes in the thymus with high affinity. Thymocytes with TCRs unable to perceive self-peptide/MHC complexes die by neglect. In brief, there exists a narrow window $[K_P,\ K_N]$ for selecting thymocytes, which allows death by neglect of the useless ($z < K_P$), active elimination of the dangerous ($z > K_N$), and survival of the useful ($z\epsilon[K_P,\ K_N]$). Here, z denotes the affinity of a thymocyte for peptide/MHC complexes; $K_P(K_N)$ is the positive (negative) selection threshold. The selection is very stringent, and overall there are only about 3% of thymocytes

2000 *Mathematics Subject Classification.* 92B15.

Key words and phrases. clonal expansion, mathematical modeling, positive/negative selection, probability theory, T-cell development.

can fully mature [27] and leave the thymus. Also, K_N is the activation threshold for mature T-cells in the periphery.

Detours et al. developed a mathematical model for the affinity-driven T-cell repertoire selection on the basis of the probability theory [9-13]. The model relied on a minimal representation of MHC molecules, peptides, and TCRs that support the notions of affinity, ligand diversity, and ligand size. Positive and negative selection affinity thresholds were inferred from experimental estimates of the stringencies of the overall selection process. Mathematical expressions were given of key immunologic notions such as the alloreactivity level, the self-MHC restriction ratio, and the foreign antigen response frequency. Their results were in the range of experimental observations and thus supported the validity of the model.

In deriving mathematical expressions, the clone size was assumed uniformly distributed in the naive T-cell repertoire [9]. However, recent biological findings indicated that the naive T-cell repertoire is highly skewed, because of the uneven proliferation of premigrant single-positive (SP) thymocytes [5], the last physiologic event occurring in the thymic medulla [6, 22]. According to these findings, different monoclonal TCR transgenic SP thymocytes proliferated at different rates in a given MHC context; conversely, mature thymocytes expressing a given TCR, generated in mice of different MHC haplotypes, also showed different rates of proliferation. Thus, premigrant thymocyte expansion is TCR-mediated and correlates with the TCR affinity for self-peptide/MHC ligands. These new findings are at odds with the uniform clone size distribution assumed by Detours et al. and imply that their theory may need a revision.

In this paper, the effects of the uneven clonal expansion are investigated in detail and their biological significance is discussed. The paper is organized as follows. In section 2, the original Detours et al. model is recapitulated, centering on mathematical expressions of the alloreactivity level, the self-MHC restriction ratio, and the foreign antigen response frequency. In section 3, a new expression of $p_\eta(k)$ is given and compared with the original one. In sections 4 and 5, the model is further revised to include the affinity mediated proliferation of SP thymocytes, whose effects on the alloreactivity level, the self-MHC restriction ratio, and the foreign antigen response frequency are numerically investigated.

2. **Detours et al. model.**

The model was described in detail in [9-13] where the readers are referred to for the overall conceptual design, definitions, and derivations. Table 1 lists definitions of most important parameters. Key notions that are mostly related to the present paper are recapitulated in the following subsections.

2.1. **Foreign peptide response frequency.**

The foreign peptide response frequency is the fraction of postselection T-cell clones responding to a given foreign peptide presented by its carrier MHC. Since only one of the n_m self-MHCs can carry the peptide, this specific self-MHC is called fpMHC. The foreign peptide response frequency is given by

$$R = \sum_{z>K_N} [p_\eta * p_\theta](z), \tag{1}$$

where η and θ are the match scores of a selected TCR with fpMHC and the foreign peptide, respectively. The probability distribution of X ($X = \eta$ or θ) is p_X; $*$ is the convolution operator. Equation (1) implies that to activate a naive T-cell the total affinity $\eta + \theta$ must exceed the threshold K_N. Because the foreign peptide appears to the TCR as completely random, $p_\theta = p_I^{l_p}$ is simply the convolution of l_p uniform

probability distributions p_I (the peptide has on average l_p epitopes all contributing to the match score θ). However, $p_\eta \neq p_\gamma$ (see below for p_γ), since self-MHCs have selected the TCR and thus do not appear as completely random to the repertoire any more. It has the following expression:

$$p_\eta(k) = \frac{1}{n_m}\frac{1}{f} M_{\gamma,n_m}(k) \sum_{z=K_P-k}^{K_N-k} M_{\theta,n_p}(z) + \left(1 - \frac{1}{n_m}\right) \frac{1}{1-f_P} p_\gamma(k) \sum_{z=0}^{K_P-k} M_{\theta,n_p}(z), \tag{2}$$

where $p_\gamma = p_I^{l_m}$ is the convolution of l_m uniform probability distributions p_I (a MHC has on average l_m epitopes all contributing to the match score γ) and $M_{X,n}(\cdot)$ is the maximum of n independent random variables with identical distribution p_X. The mathematical expression of $M_{X,n}(\cdot)$ can be found in [7].

2.2. **Self-MHC restriction ratio.** The extent of self-MHC restriction has typically been estimated by comparing the effector activity against foreign peptides presented by self-MHCs with the activity against foreign peptides presented by foreign MHC [4, 28, 29, 31, 32]. The response frequency to a foreign peptide presented on self-MHCs, R, was given by Equation (1). The response frequency to a foreign peptide presented on foreign MHC, R_a, is given by

$$R_a = \sum_{z>K_N} [p_\gamma * p_\theta](z). \tag{3}$$

The reason p_γ is used instead of p_η is that foreign MHCs have never participated in the selection and appear as completely random to the postselection T-cell repertoire. The self-MHC restriction ratio is defined as

$$r = \frac{R}{R_a}. \tag{4}$$

2.3. **Alloreactivity.** The alloreactivity, a, is defined as the fraction of clones in the repertoire responding to foreign MHC haplotypes in combination with self-peptides. Although the peptides are self, the fact that they are presented by foreign MHCs indicate that they have never been presented by self-MHCs during selection (different MHCs present widely different peptides [9, 12]). Therefore, they still cannot be distinguished from random peptides by the postselection repertoire (they did not have the opportunity to be loaded and got acquainted with the repertoire). One thus obtains

$$a = \sum_{z>K_N} p_\omega(z) = f_P \cdot (1 - f_N), \tag{5}$$

where $p_\omega = M_{\delta,n_m}$ and δ is the maximal match score between a random TCR and a set of MHC-peptide complexes made out of a random MHC combined with n_p peptides ($p_\delta = p_\gamma * M_{\theta,n_p}$). It is worth noting the definition difference between a and R_a: a is on foreign MHCs and self-peptides, while R_a is on a random foreign MHC and a random foreign peptide.

3. **Derivation of $p_\eta(k)$.** Equation (2) was derived under the assumption that only one MHC has a match score large enough to mediate positive selection. As a result, the reasoning has been greatly simplified: the probability that a selected TCR is matched against an MHC that drove selection is $1/n_m$, and the probability that it is not is $1-1/n_m$. In addition, Equation (2) contains a minor flaw. To show this one sums up over k both sides of Equation (2), and obtains

$$\sum_k p_\eta(k) = \frac{1}{n_m}\sum_k p_\phi(k) + \left(1-\frac{1}{n_m}\right)\sum_k \frac{1}{1-f_P} p_\gamma(k) \sum_{z=0}^{K_P-k} M_{\theta,n_p}(z), \qquad (6)$$

where

$$p_\phi(k) = \frac{1}{f} M_{\gamma,n_m}(k) \sum_{z=K_P-k}^{K_N-k} M_{\theta,n_p}(z)$$

was defined in [9]. Since $\sum_k p_\eta(k) = \sum_k p_\phi(k) = 1$, Equation (6) reduces to

$$\sum_k p_\gamma(k) \sum_{z=0}^{K_P-k} M_{\theta,n_p}(z) = 1 - f_P. \qquad (7)$$

The left side of Equation (7) equals $\sum_{z<K_P} p_\delta(z)$, while the right side of Equation (7) equals $\sum_{z<K_P} p_\omega(z)$. However, $\sum_{z<K_P} p_\omega(z)$ approximately equals $\sum_{z<K_P} p_\delta(z)$ only when f_P (or f) is small. Therefore, Equation (2) cannot be completely correct. Indeed, my calculations indicate that $\sum_k p_\eta(k)$ obtained from Equation (2) deviates from 1 as f increases. In comparison, $\sum_k p_\eta(k)$ obtained from my derivation, Equation (12), keeps to 1 consistently (see Figure 1a). Although imperfect, Equation (2) is still a useful approximation, since under normal conditions it is indeed unlikely that more than one MHC has a match score large enough to mediate positive selection [9], and biologically reasonable f, ranging from 0.0019 to 0.03 [12], is indeed small. Figure 1b shows $p_\eta(k)$ obtained by Equations (2) and (12), with $f = 0.03$. One sees the difference is very small. For $f = 0.0075$, the difference is even smaller (not shown). In the following, I give another derivation of $p_\eta(k)$, which does not depend on any assumption or simplification. Although the reasoning seems to be a little complicated, the final expression has the same level of complexity as Equation (2), and the calculation is also quite easy. The derivation is as follows.

Among the n_m self-MHCs, only one can carry the foreign peptide. This specific MHC is called fpMHC to indicate that it is the carrier of the foreign peptide. Accordingly, the postselection repertoire can be partitioned into two groups: group U is selectable by fpMHC, and group V is not selectable by fpMHC. I use the word 'selectable' because the possibility (though practically very small) exists that more than one MHC selects a thymocyte. That is, group U includes those cells selected by many MHCs, and fpMHC must be one of them. Cells in group V were selected by MHC(MHCs) other than fpMHC. In Figure 2a, I represents the preselection repertoire (the whole repertoire); $U+V$ represents the postselection repertoire; u and v are subsets of groups U and V, respectively; and $u+v$ include all the selected T-cells whose match score with fpMHC is k. One sees that $p_\eta(k)$, the fraction of cells whose TCR-fpMHC match score being k over the selected repertoire, is exactly $(u+v)/(U+V)$. One further has

$$p_\eta(k) = \frac{u+v}{U+V} = \left(\frac{u}{I}+\frac{v}{I}\right) \Big/ \left(\frac{U+V}{I}\right). \qquad (8)$$

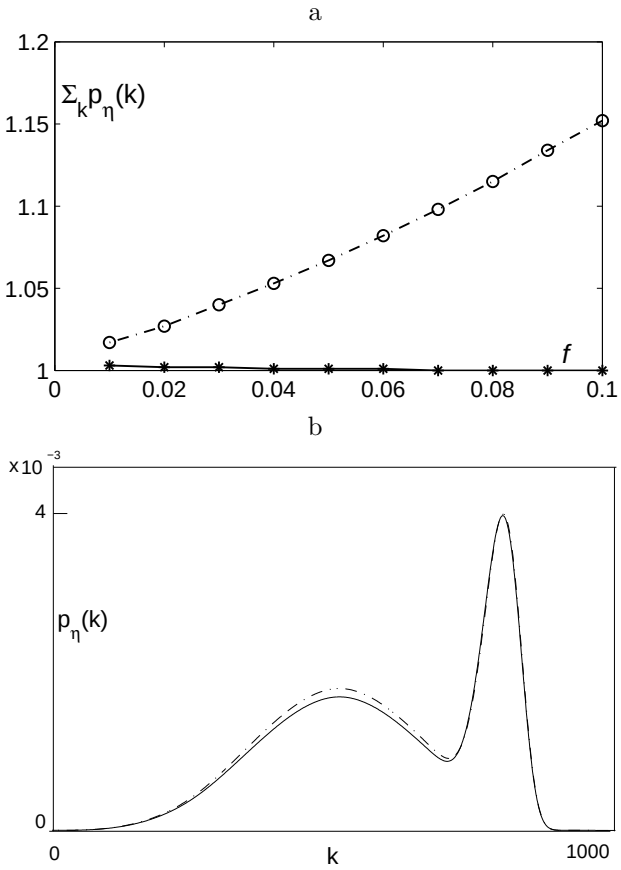

FIGURE 1. Comparison of $p_\eta(k)$ between the result of Detours et al. (dash-dotted line) as obtained from Equation (2) and my result (solid line) as obtained from Equation (12). (a) Deviation of $\sum_k p_\eta(k)$ from 1. The large deviation of the dash-dotted line implies that the result of Detours et al. is imperfect. (b) Shape of $p_\eta(k)$.

Here, u/I is given by

$$\frac{u}{I} = p_\gamma(k) \sum_{z=K_P-k}^{K_N-k} M_{\theta,n_p}(z) \sum_{z=0}^{K_N} M_{\delta,n_m-1}(z), \tag{9}$$

where $p_\gamma(k)$ is the probability of a random TCR-MHC match score being k. Multiplying $p_\gamma(k)$ with $\sum_{z=K_P-k}^{K_N-k} M_{\theta,n_p}$ yields the fraction of cells selectable by fpMHC. A further restriction $\sum_{z=0}^{K_N} M_{\delta,n_m-1}(z)$ is exclude those overly reactive with the other $n_m - 1$ MHCs (those with $z > K_N$). Then v/I is given by

$$\frac{v}{I} = p_\gamma(k) \sum_{z=0}^{K_P-k} M_{\theta,n_p}(z) \sum_{z=K_P}^{K_N} M_{\delta,n_m-1}(z). \tag{10}$$

Multiplying $p_\gamma(k)$ with $\sum_{z=0}^{K_P-k} M_{\theta,n_p}(z)$ yields the fraction of cells not selected by fpMHC. These cells were selected by the other n_m-1 MHCs and became members of the group V, for which $\sum_{z=K_P}^{K_N} M_{\delta,n_m-1}(z)$ must be multiplied. Here, $(U+V)/I$ is exactly f, the fraction of the postselection repertoire over the preselection repertoire;

$$\frac{U+V}{I} = f = \sum_{z=K_P}^{K_N} p_\omega(z). \tag{11}$$

Finally, one obtains

$$p_\eta(k) = \frac{p_\gamma(k)}{f}\Bigg(\sum_{z=K_P-k}^{K_N-k} M_{\theta,n_p}(z) \sum_{z=0}^{K_N} M_{\delta,n_m-1}(z) + \sum_{z=0}^{K_P-k} M_{\theta,n_p}(z) \sum_{z=K_P}^{K_N} M_{\delta,n_m-1}(z)\Bigg). \tag{12}$$

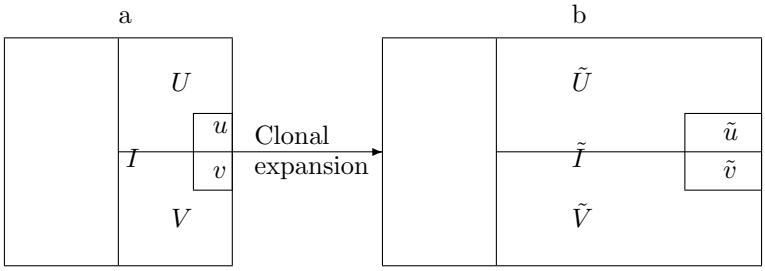

FIGURE 2. Effects of the clonal expansion on T-cell pools. (a) Pre-expansion T-cell pools. The whole repertoire is represented by I, U is the group of cells selectable by fpMHC, and V is the group of cells not selectable by fpMHC but selected by the other self-MHCs. Subsets of U and V, u and v, respectively, are groups of cells whose match score with fpMHC is k. (b) Post-expansion T-cell pool. Since non-selected cells do not proliferate, $\tilde{I}-(\tilde{U}+\tilde{V}) = I-(U+V)$. Other pools are all expanded.

4. **Effects of the affinity-mediated clonal expansion.** About 3% of T-cells produced in the thymus reach the periphery [26]. However, the fraction of clones, which the model deals with, and the fraction of cells differ because a significant portion of mature T-cells divide before emigrating to the periphery [15, 22, 25]. On average, a postselection cell goes through about two divisions, and hence each clone consists, on average, of four cells. If 3% of thymocytes survive selection, the actual fraction of clones is $f = 3 \times \frac{1}{4} = 0.75\%$ [9-13].

There are two special patterns of the clonal expansion. The first pattern assumes that the expansion is even: all selected cells divide twice with no skewing of the repertoire. This pattern is called the even expansion (E-expansion). E-expansion was assumed by Detours et al; in their model, the only effect of the expansion was to reduce f from 3% to 0.75%. After that the model can be treated as if

there were no expansion at all, since E-expansion, being even, does not change the proportionality of the repertoire.

Recent experimental findings have revealed that the second pattern, which is called the affinity-mediated expansion, or uneven expansion (U-expansion), is more practical. It was found that the proliferation rate of SP thymocytes is TCR-mediated and is correlated with the TCR affinity for self-peptide/MHC ligands during the selection process [5]. For example, TCR transgenic mice expressing $H-2^{b,b}$, $H-2^{b,k}$, or $H-2^{k,k}$ MHC haplotypes were studied. The 15-fold higher proliferation in $H-2^{k,k}$ mice correlates with the greater affinity of the TCR for k/k class II than for b/b class II MHC molecules. The $H-2^{k,b}$ mice have an intermediate proliferation rate because the TCR interacts with k/b class II molecules with an intermediate affinity. In summary, higher affinity cells receive more intense signals and expand faster, and lower affinity cells receive less signals and expand slower, while keeping the mean expansion fold as approximately 4. Therefore, the expansion fold g is a monotone increasing function of the affinity z, $g = g(z)$, satisfying

$$\int_{K_P}^{K_N} g(z)p_\omega(z)\,dz = 4\int_{K_P}^{K_N} p_\omega(z)\,dz. \tag{13}$$

Since prominent proliferation can be found only at the upper range of the selection window $[K_P, K_N]$, $g(z)$ may well be an exponentially increasing function: increase very slowly (rapidly) at the lower (upper) range of the selection window. Therefore, the power function

$$g(z) = 1 + \alpha_n(z - K_P)^n \tag{14}$$

is chosen, where α_n can be determined by substituting Equation (14) into Equation (13), yielding

$$\alpha_n = \frac{3\int_{K_P}^{K_N} p_\omega(z)\,dz}{\int_{K_P}^{K_N}(z-K_P)^n p_\omega(z)\,dz}. \tag{15}$$

Figure 3 shows function $g(z)$ obtained with $n = 0, 1, 3, 5, 7, 9$ and the other parameters in Table 1. Obviously, $n = 0$ degenerates to E-expansion.

In the following, effects of U-expansion on key immunologic notions are discussed. Notations shall be covered by a tilde in order to distinguish them from previous notations. For example, $\tilde{R}(R)$ is the foreign peptide response frequency under the condition of the U-expansion (E-expansion).

4.1. **Foreign peptide response frequency.** Foreign peptides, being foreign, appear as random ($p_\theta = p_I^{l_p}$) to any T-cell repertoire. Therefore, one has

$$\tilde{R} = \sum_{z>K_N} [p_{\tilde{\eta}} * p_\theta](z). \tag{16}$$

From Figure 2 one sees that

$$p_{\tilde{\eta}} = \frac{\tilde{u}+\tilde{v}}{\tilde{U}+\tilde{V}} = \left(\frac{\tilde{u}}{I} + \frac{\tilde{v}}{I}\right) \Big/ \frac{\tilde{U}+\tilde{V}}{I}, \tag{17}$$

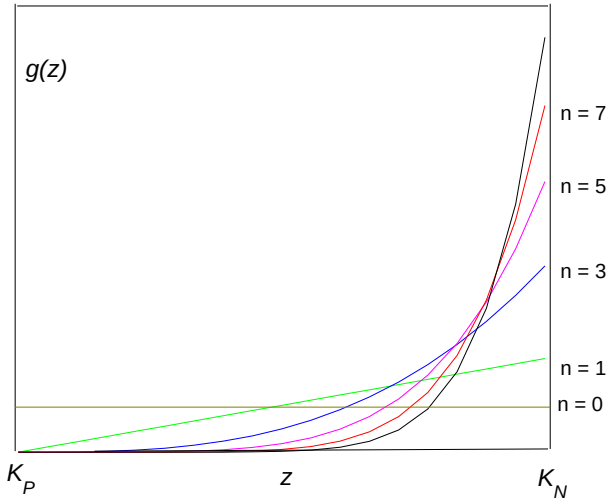

FIGURE 3. Fold of expansion as a power function of affinity z, $g = g(z)$. Different colors correspond to different powers used.

where

$$\frac{\tilde{u}}{I} = p_\gamma(k) \sum_{z_1=K_P-k}^{K_N-k} \sum_{z_2=0}^{K_N} M_{\theta,n_p}(z_1) M_{\delta,n_m-1}(z_2) g\left(max\{z_1+k, z_2\}\right)$$

$$= p_\gamma(k) \sum_{x=K_P}^{K_N} \sum_{z=0}^{x} M_{\theta,n_p}(x-k) M_{\delta,n_m-1}(z) g(x)$$

$$+ p_\gamma(k) \sum_{x=K_P}^{K_N} \sum_{z=x}^{K_N} M_{\theta,n_p}(x-k) M_{\delta,n_m-1}(z) g(z), \tag{18}$$

$$\frac{\tilde{v}}{I} = p_\gamma(k) \sum_{z_1=0}^{K_P-k} \sum_{z_2=K_P}^{K_N} M_{\theta,n_p}(z_1) M_{\delta,n_m-1}(z_2) g(z_2), \tag{19}$$

$$\frac{\tilde{U}+\tilde{V}}{I} = \sum_{z=K_P}^{K_N} p_\omega(z) g(z). \tag{20}$$

Here one sees that the function $g(\cdot)$ is modulated into $\tilde{u}/I$, $\tilde{v}/I$, and $(\tilde{u}+\tilde{v})/I$. Particularly in Equation (18), since the cells are selectable by fpMHC and many other MHCs, the proliferation rate is determined by the maximum of $z_1 + k$ (the affinity for the fpMHC/peptide complex) and z_2 (the maximum affinity for non-fpMHC/peptide complexes).

Incidently, for $n = 0$ the function $g(\cdot)$ equals 4 and Equation (17) reduces to Equation (12), as expected.

4.2. **Self-MHC restriction ratio.** Since R_a, the response frequency to a foreign peptide presented on foreign MHC, is not altered by U-expansion (see Equation (3), both p_γ and p_θ are not altered), one has

$$\tilde{r} = \frac{\tilde{R}}{R_a}. \tag{21}$$

4.3. **Alloreactivity.** U-expansion only increases total cell numbers and changes proportionality among TCR varieties, but it cannot create new varieties. Therefore, the argument of section 2.3 applies again: both foreign MHCs and the peptides they present appear as random to the repertoire. One thus has

$$\tilde{a} = a = f_P \cdot (1 - f_N). \tag{22}$$

5. **Numerical calculations.** Parameters in Table 1 constitute a standard example used throughout the works of Detours et al. [9-13], except n, which is set to be 5 in this section.

TABLE 1. One set of parameters used as a standard example

Name	Definition	Value
n_m	Number of MHC loci	3
n_p	Number of self-peptides	10^4
l_m	Number of MHC epitopes on average	4
l_p	Number of peptide epitopes on average	6
f	Fraction of selected T-cell clones	0.75%
f_N	Fraction of positively selected T-cell clones that survive negative selection	37%
$f_P = f/f_N$	Fraction of positively selected T-cell clones	2.03%
d_{max}	Discreteness of affinity	255
n	Power of function $g(z)$	5

With the parameter values of Table 1, $p_\eta(k)$ and $p_{\tilde{\eta}}(k)$ are obtained by Equations (12) and (17), respectively, and are shown in Figure 4. One sees that for this example, the uneven expansion only slightly skews $p_\eta(k)$ toward higher affinity values. By using Equations (1), (3), (4), (16), (21), and (22), $R = 1.30 \times 10^{-5}$, $R_a = 9.75 \times 10^{-7}$, $r = 13.33$, $\tilde{R} = 1.59 \times 10^{-5}$, $\tilde{r} = 16.29$, and $\tilde{a} = a = 0.0128$ are obtained. One sees that the slight change of $p_\eta(k)$ results in a small but significant (0.22-fold) increase of the self-MHC restriction ratio (namely, $\tilde{r}/r = 1.22$). In the following, $\tilde{r}/r$ is denoted by ξ.

By varying one parameter each time while keeping the others fixed as in Table 1, the calculation is repeated. The obtained ξ values are shown in Figures 5, 6, and 7.

Parameter ranges in [12, Table 1] were inferred from experimental data and encompassed a very wide array of biologically reasonable quantitative hypotheses. They are as follows: $n_m = \{3\}$, $n_p = \{10^2, 10^3, \ldots, 10^8\}$, $l_m = \{2, 3, \ldots, 8\}$, $l_p = \{4, 5, \ldots, 11\}$, $f = \{0.19\%, 0.37\%, 0.75\%, 1.5\%, 3\%\}$, $f_N = \{20\%, 37\%, 95\%\}$, and in total $1 \times 7 \times 7 \times 8 \times 5 \times 3 = 5,880$ combinations. A biological justification for these parameter ranges was given in [10]. The calculation is repeated for every one of these combinations with results obtained. In Figure 8, $r - \tilde{r}$ and $r - \xi$ relations are shown as stars for all the combinations. In Figure 8a all stars are over the bisector, which indicates that the affinity-mediated clonal expansion does enhance the quality (self-restriction) of periphery T-cells. The largest $\tilde{r}$ is 1034, which corresponds to the parameter set ($n_m = 3$, $n_p = 10^4$, $l_m = 5$, $l_p = 5$, $f = 0.19\%$, $f_N = 95\%$, $n = 5$). For E-expansion, this parameter set yields $r = 279$, which is 3.7-fold smaller. Figure 8b reveals an interesting relationship: ξ inversely correlates with r, by and large. This implies that the U-expansion is an adaptive

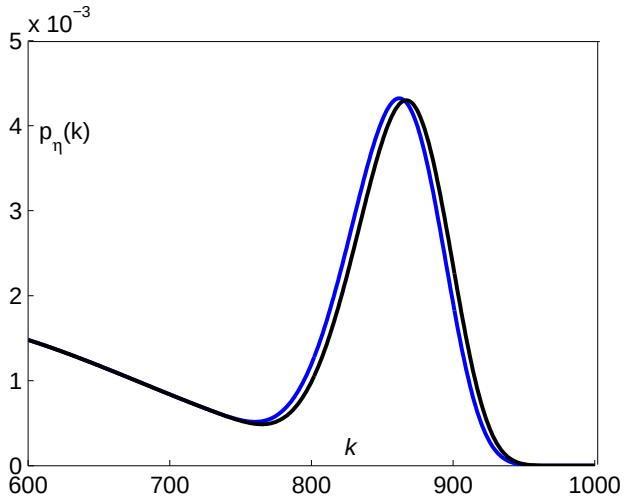

FIGURE 4. $p_\eta(k)$ (blue line) and $p_{\tilde{\eta}}(k)$ (black line) as obtained by Equations (12) and (17), respectively, with the parameter values of Table 1.

process: those combinations that have small r values enhance more drastically than combinations whose r values are already large. Figure 9 shows the histogram of ξ. For all the combinations, 2.3% increases more than 9-fold, 10.4% increases more than 4-fold, 27.3% increases more than onefold. Most (72.6%) combinations have fold increases in between 0 and 1. Averaged over all the 5880 combinations, $\langle r \rangle$, $\langle \tilde{r} \rangle$, and $\langle \xi \rangle$ are 23.7, 55.2, and 2.29, respectively.

6. **Discussion.** Both T-cell development and immune responses rest heavily on the interaction of clonally distinct T-cell receptors (TCRs) with cell-surface molecular complexes composed of major histocompatibility complex (MHC) molecules bound to foreign or to self-peptides on antigen-presenting cells (APCs). It is widely accepted that the interaction affinity plays a pivotal role in T-cell selection and activation (e.g., [16, 18, 21]), especially after the publication of the experimental results of Alam et al. [2].

On the basis of probability theory, Detours et al. developed a mathematical model that framed key immunologic notions, such as TCR affinity and alloreactivity, in rigorous quantitative terms. The results derived from the model largely agreed with experimental observations and thus supported the validity of the model. Most significantly, they found that self-MHC restriction and alloreactivity are not independent but are inversely correlated. For years, research on self-MHC restriction has been particularly intricate and rich in controversies [24], because results of restriction experiments had great discrepancies: both low and nearly absolute self-restriction levels were measured. Detours et al. realized its random nature and developed the quantitative model, by which parameters characterizing molecule structures (n_m, n_p), varieties (l_m, l_p), and stringency of positive/negative selections (f, f_N) were related to key immunologic notions, including the self-MHC restriction. They suggested that there is no need to reconcile high alloreactivity and absolute self-restriction, because self-restriction is not high on *average* [12].

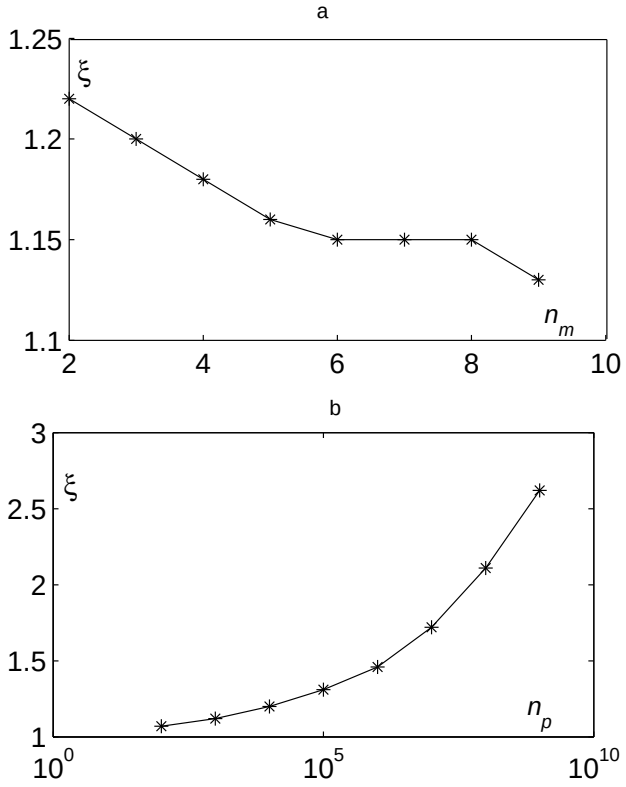

FIGURE 5. (a) ξ as a function of n_m or (b) n_p. Other parameter values are from Table 1.

However, the fact that self-MHC restriction is deemed as absolute in many textbooks [1, 3, 8, 14, 17, 19, 20, 23] implies that the value of r may be much higher than what Detours et al. have estimated.

In this paper, effects of skewed T-cell repertoire resulting from the affinity-mediated clonal expansion have been studied in detail, both theoretically and numerically. Postselection thymocytes undergo on average two divisions before leaving the thymus. According to recent experimental results, the proliferation rate is by no means uniform but correlates with the affinity of the TCR-MHC/peptide interaction. Clones selected by higher (lower) affinity interactions receive stronger (weaker) signals to promote expansion. In this way, T-cells reaching the peripheral pool might be more suited to responding to self-MHCs. By enumerating all the 5,880 parameter sets provided by Detours et al., significant improvement of self-MHC restriction is verified. The value of $\langle \tilde{r} \rangle$ is 55.2, which is much easier than $\langle r \rangle = 23.7$ to account for the high frequency of the absolute self-MHC restriction observed in practice. The affinity-mediated clonal expansion thus provides a mechanism to shorten the distance between the results of Detours et al. and medical practices. That is, according to the theory of Detours et al., both self-restriction and alloreactivity are not high on average, this is somewhat in contradiction with medical observations that both are high most of the time (that is why absolute self-restriction is often written into textbooks). Our results increase the self-restriction

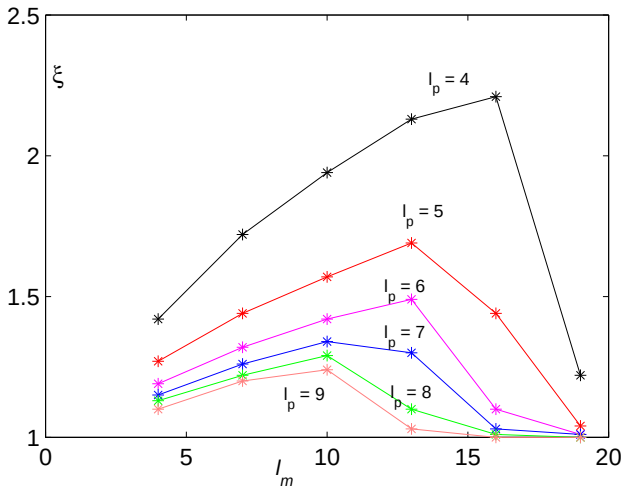

FIGURE 6. Values of ξ as a function of l_m. Different colors correspond to different l_p values. Other parameter values are from Table 1.

ratio and are thus more close to practice. Of the six key parameters, $n_m = 3$ is known; l_m, l_p, f, and f_N are all deterministic parameters (they are unique for the studied species). The reason we use multiple trial values are simply that we do not know their exact values. However, n_p, the number of self-peptides present in the thymus, is case dependent. If n_p is overly large, $M_{\theta,n_p}(k)$ (the probability of θ, the maximum match score between peptides and TCR being k) skews to larger k, which causes $p_\eta(k)$ to skew to smaller k. This will generate a repertoire of T-cells of small match score with self-MHCs (a small R). Such a repertoire is difficult to be activated and it may be easier for the infected pathogen to evade immune surveillance. On the contrary, if n_p is overly small, a repertoire of T-cells will be generated with large match score with self-MHCs (a large R). Such a repertoire is apt to be activated, and the animal may be more susceptible to get autoimmune diseases.

The positive/negative selection is completely determined by parameters n_m, n_p, l_m, l_p, f, and f_N. However, changing any one of them to increase the self-MHC restriction ratio $r = R/R_a$ will decrease the alloreactivity a most of the time, for R, R_a, and r are all controlled by any of these parameters and are much entangled. That is why according to the Detours et al. model, r and a cannot both be high. Therefore, within the context of the positive/negative selection, there is little space left for the immune system to optimize its parameters to achieve both high self-restriction and high alloreactivity. Fortunately, the immune system finds the way of the affinity-mediated clonal expansion, which only increases R (through $p_\eta(k)$) without affecting foreign MHC related quantities R_a and a. In this way, both high self-MHC restriction and high alloreactivity can be obtained to fulfill maximum immune competence.

The overall magnitude of the clonal expansion is fixed as fourfold, the same as the Detours et al. model, to allow a fair comparison. The parameter n controls the shape of the function $g(z)$ and finally determines how much the repertoire is skewed. As n increases, the task of expanding fourfold is taken on more and more by clones

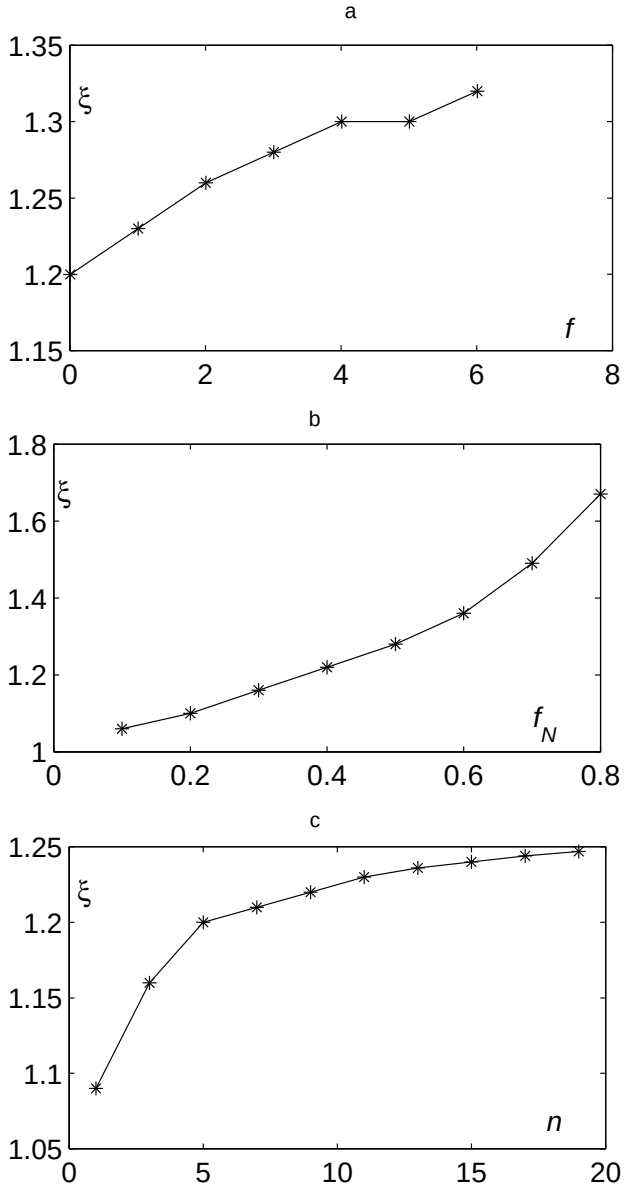

FIGURE 7. (a) ξ as a function of f, (b) f_N, or (c) n. Other parameter values are from Table 1.

of higher affinity values. For example, if $n = 9$, the lower $2/3$ range of the selection window hardly proliferates, while the upper $1/3$ range must proliferate tenfold to achieve the total fourfold expansion ($\frac{1}{3} \times 10 + \frac{2}{3} = 4$). As expected, a larger n is more favorable to enhancing the self-restriction (Fig. 7c). Interestingly, recent experimental results did indicate that only clones belonging to the upper range expand significantly [5]. The immune system has learned this and achieved such an efficiency probably through evolution. Proliferation of SP thymocytes therefore

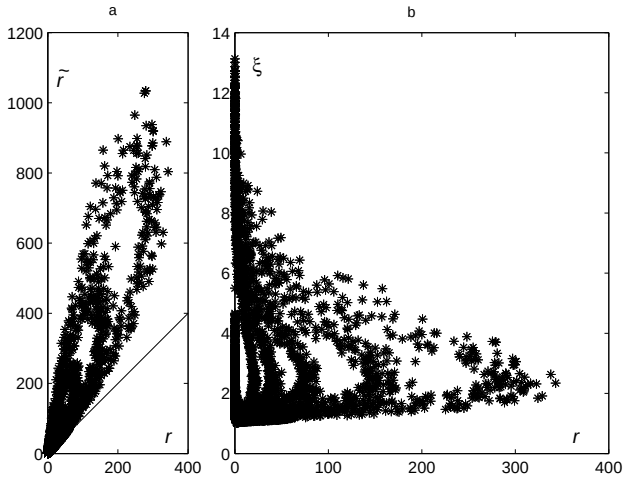

FIGURE 8. (a) $r - \tilde{r}$ and (b) $r - \xi$ relations shown as stars for all the 5,880 combinations.

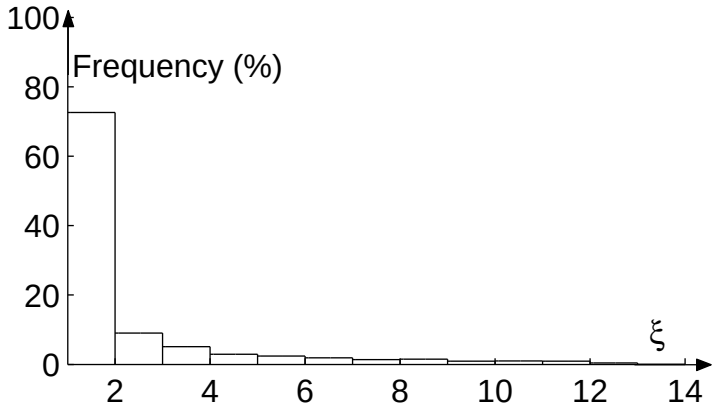

FIGURE 9. Histogram of ξ values.

appears to be an additional selection event, resulting in fine tuning of the repertoire of T-cells leaving the thymus, skewing this repertoire toward T-cell clones in the upper range of the selection window. In this way, T-cells reaching the periphery pool can fulfill maximum competence: both high self-restriction and high alloreactivity. In the present theoretical context, it is impossible to experimentally determine n and $g(z)$, since the affinity z, ranging from 0 to 255, is totally theoretical. To determine them by experiments, one has to find a practical way to measure the affinity; one good way of which is to use the technique of surface plasmon resonance [2]. By using this technique to measure the affinity and the flow cytometry to measure the cell proliferation, n and $g(z)$ can be determined.

Quantities in this paper are all statistical mean values. They do not represent the results of a specific biological or clinical experiment, but the mathematical expectation of that specific experiment. In other words, they represent the mean values

of a great number of individual experiments. For example, the self-restriction ratio, $r = R/R_a$, measures the degree of self-restriction of the studied T-cell repertoire. If 60% of T-cells respond to self-MHC and foreign peptide complex and 10% of T-cells respond to foreign-MHC and foreign peptide complex, r would be 6. However, different experiments use different foreign peptides and MHCs and yield different results (some even yield absolute self-restriction, because $R_a = 0$). Therefore, the mathematical expectation should be used to measure self-restriction. The increases of r reported in this paper only imply that in general the degree of self-MHC restriction is better than previously thought. For each individual experiment, the r value is very likely but not necessarily increases.

The present model suggests a preferential expansion of T-cells whose TCR affinity is large. Therefore, in the periphery, a larger portion of T-cells is close to the level of self-recognition than previously thought. This to some degree explains the mechanism of autoimmunity and the onset of autoimmune diseases. Following pathogen infection, T-cells are activated to eliminate the pathogen. However, because a large portion of T-cells has affinity values only slightly smaller than the self-reaction threshold, a slight perturbation (environmental variations, genetic mutations) may cause the immune system to attack itself. It should be noted that autoimmunity is a very complicated phenomenon whose mechanisms are largely unknown. The present theory, however, at least explains a mechanism that can increase the probability of the onset of autoimmune diseases.

The affinity plays a central role in this paper. To refine the model and for theoretical development, an affinity-structured continuous model, probably in the form of partial differential equations, should be constructed as soon as possible. Such a model is hopeful to reveal the detailed landscape of T-cell selection, proliferation, and maturation in the domain of affinity, with the aid from in vitro experiments.

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Received on July 14, 2004. Revised on October 12, 2004.

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