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Competitive control of the self-renewing T cell repertoire

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Abstract

We develop a mathematical model for the self-renewing part of the T cell repertoire. Assuming that self-renewing T cells have to be stimulated by immunogenic MHC–peptide complexes presented on the surfaces of antigen-presenting cells, we derive a model of T cell growth in which competition for MHC–peptide complexes limits T cell clone sizes and regulates the total number of self-renewing T cells in the animal. We show that for a sufficient diversity and/or degree of cross-reactivity, the total T cell number hardly depends upon the diversity of the T cell repertoire or the diversity of the set of presented peptides. Conversely, for repertoires of lower diversity and/or cross-reactivity, steady-state total T cell numbers may be limited by the diversity of the T cells. This provides a possible explanation for the limited repertoire expansion in some, but not all, mouse T cell re-constitution experiments. We suggest that the competitive interactions described by our model underlie the normal T cells numbers observed in transgenic mice, germ-free mice and various knockout mice.

Introduction

The peripheral T cell repertoire is maintained by production of virgin cells in the thymus and by self-renewal of activated cells in the periphery. The mature T cell repertoire consists of a relatively constant number of cells distributed over an enormously large number of T cell clones with different antigen specificities. The early expansion of the mouse T cell repertoire during the first 3 weeks of post-natal development is largely due to the output of virgin cells from the thymus (1). For the $CD8^+$ T cell repertoire of 12-week-old mice it has recently been suggested that the pools of virgin and of self-renewing cells are regulated independently, each constituting about half the peripheral T cell repertoire (2). Other data, however, suggest that T cell self-renewal is down-regulated in the presence of a functional thymus and that self-renewal predominates only in the absence of a thymic output of virgin cells (3). Data from TCR transgenic mice suggest that the T cell self-renewal process relies on conventional MHC-restricted antigen-driven expansion (4). Virgin T cells tend to be long-lived resting cells (2,5), whereas part of the self-renewing T cell pool have a shorter lifespan and maintain themselves by cell division (5,6). Memory and virgin T cells have different re-circulation pathways (7) and different requirements for stimulation by antigen (8).

In this paper we devise a model for the regulation of the pool of self-renewing T cells. The model is based upon competitive binding of T cells to ligands expressed on MHC molecules. For reasons of simplicity and clarity, and because the experimental data are contradictory, we ignore the impact of the virgin repertoire on the repertoire of self-renewing cells. The central questions addressed in this paper are the maintenance of diversity in the self-renewing repertoire, and the relation between repertoire diversity and total T cell numbers. The pool of self-renewing T cells is known to attain an equilibrium size. Mice reconstituted with increasing numbers of mature T cells attain peripheral T cell populations of similar size (9). TCR transgenic mice, i.e. mice with few T cell clones, attain a total T cell population size that is similar to that of normal mice (6). Surprisingly, in other T cell re-constitution experiments the steady-state total T cell level seems to be determined by the number of T cells the mice were inoculated with (3,10). Our model suggests as a possible explanation for these contradictory results that the steady-state T cell numbers depend on the cross-reactivity and the diversity of the T cells used to reconstitute the mice.

Mice reconstituted with peripheral T cells attain normal $CD4^+/CD8^+$ ratios that are independent of the $CD4^+/CD8^+$ ratio of the injected cells (9). The same is true for transgenic mice producing few $CD4^+$ T cells (9). The self-renewing $CD4^+$

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and $CD8^+$ compartments also seem to compete with one another, however. In the absence of either the $CD4^+$ or the $CD8^+$ subset, the remaining subset compensates so that a normal number of total T cells is attained. This compensatory phenomenon has been described by anti-CD4 or anti-CD8 antibody treatment (9,11), in CD4 knockout mice (12,13), in MHC class I (14) and class II (15) knockout mice, and in AIDS patients with depressed CD4 counts (16). This is sometimes referred to as the 'blind T cell homeostasis hypothesis', which states that a constant number of T cells is maintained without regard to $CD4^+$ or $CD8^+$ phenotype (11,13,16,17). An ecological interpretation of these experiments is that activated $CD4^+$ and $CD8^+$ cells compete for the same immunological 'niche'. By filling this niche the normal total number of T cells is attained.

In our model the diversity of the T cell repertoire is maintained by the diversity of the ligands stimulating self-renewing T cells to proliferate. A recent study suggested that total $CD4^+$ T cell numbers in the lymph nodes of H2-M-deficient mice, which fail to normally present a diverse set of peptides, are 30–50% of that of normal mice (18). From this study it is difficult to establish whether these T cells are maintaining themselves by self-renewal. The independent regulation of the virgin and the self-renewing compartment of T cells (2) could explain the 50% total T cell level by a complete absence of $CD4^+$ T cell self-renewal in the H2-M-deficient mice. Consistent with this are the findings that total $CD4^+$ T cell numbers in the thymus were not as reduced and that most of the $CD4^+$ T cells expressed a naive phenotype (18).

For B cells, similar ecological views were expressed when it was shown that self-reactive B cells are excluded from the repertoire of recirculating B cells by cellular competition for niches in lymphoid follicles (19). Additionally, B cell repertoire selection was studied in mice reconstituted with various sets of B cell progenitors (20). The results show that normal B cell numbers are attained whatever the potential diversity and that more diverse subsets expand at the cost of the less diverse subsets (20,21). Further, these experimental results could be fit by ecologically motivated models that include immigration from the bone marrow, competition for resources in the periphery and rapid death of cells that fail to obtain resources (21). Similar results have recently been obtained with $CD8^+$ T cells: diverse repertoires outcompete transgenic repertoires of lower diversity and irrespective of diversity normal total T cell numbers are attained (22). Additionally, it was shown that the competitive abilities of a repertoire correlates with peripheral T cell activation and division (22), suggesting that more stimulatory resources are available for the more diverse repertoires.

We have recently postulated that the immunological niches that T cells are competing for are the immunogenic peptides presented on antigen-presenting cells (APC) (23,24). This notion was formally developed by deriving mathematical models for the growth of T cells [see also (25,26) for similar derivations]. The models resemble ecological models for competitive population growth (23,24). The different immunogenic peptides form different ecological resources, and due to the principle of competitive exclusion (27), every resource (i.e. MHC–peptide combination) can maintain maximally one T cell specificity. Additionally, we derived that the maximum clone size is limited by the MHC–peptide concentration (23,25). This is what ecologists call a carrying capacity.

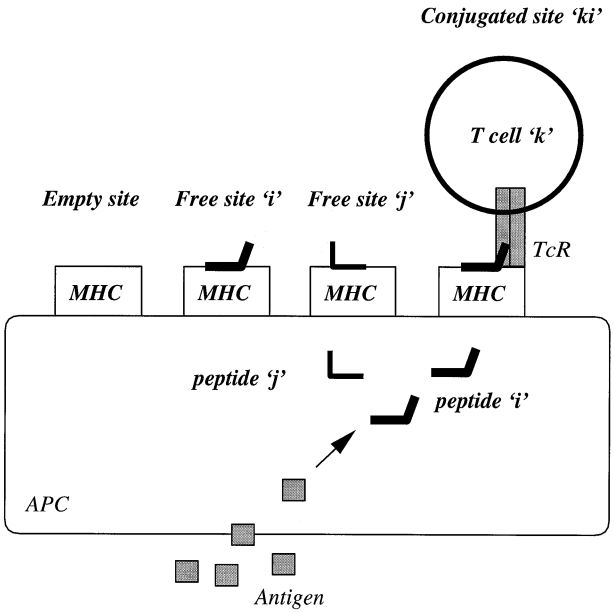


Fig. 1. Antigen is taken up and degraded into peptides, which are then presented on MHC molecules on the surface of an APC. In our model, a T cell binds to a 'site' on an APC. A site can be 'empty', i.e. present no peptide or not enough peptide to simulate a T cell, or it can be 'presenting', i.e. present enough peptide to stimulate a T cell. An empty site is assumed not to bind any T cell, while a presenting site can bind a T cell. A site presenting peptide *i* is called a site of type *i*. Presenting sites are either free or have a T cell bound. In our model very few of the sites remain empty.

In this paper we extend these results by studying the competitive regulation of the T cell repertoire and of total T cell numbers. By computer simulation we study how the total number of T cells varies as a function of the diversity of the T cell repertoire, of the diversity of the peptides and of the T cell cross-reactivity.

Model

We consider an immune system in which many T cell clones are stimulated to divide by many different immunogenic peptides presented on APC. It is assumed that the T cells have different affinities for the different MHC–peptide complexes. We define a 'site' as a place where a T cell can potentially bind an APC (see Fig. 1). Binding is presumed to be determined by interactions between the TCR and the MHC–peptide complexes within the site. At any given time, a site can be either 'empty', i.e. presenting no peptide or not enough peptide to stimulate a T cell, or it can be 'presenting', i.e. presenting enough peptide to stimulate a T cell. For simplicity, we assume that a site can only present one peptide at a stimulatory concentration. Clearly, this is an over simplification. However, because an APC in our model has many sites, each of which can be presenting a different peptide, we believe this model captures the essence of antigen presentation by APC.

We use the peptide that a site is presenting as a label.

Thus, S_j, a site of type j presents peptide of type j. A site that is presenting may be 'free' or it may have a T cell bound. A site cannot bind another T cell until the bound T cell dissociates and the site becomes free. Thus, T cells compete with one another for APC presenting sites. This comprises the first part of our model. We have previously derived differential equations to describe the kinetics of T cell–APC site interactions (23–25).

The second part of the model considers the binding of peptides to the MHC molecules within APC sites. We assume peptides compete with one another for MHC binding. For our parameters this competition is so intense that almost all MHC molecules present a peptide and this thus generates APC with almost no empty sites. In the third part of the model we define an affinity matrix that determines the interactions between T cell clones and the MHC–peptide complexes within a site. Each clone is identified by the one and only MHC–peptide complex for which it has maximum affinity.

T cell growth

We previously developed several models for T cell growth on the basis of simple schemes for T cell activation (24,25). According to these schemes a T cell, T_i, binds a free site, F_j, forming a conjugate, C_{ij} (see Fig. 1). When this conjugate dissociates the site becomes free and the T cell may or may not be stimulated to divide into two daughter cells. A simple example is

$$T_i + F_j \underset{k_{d,ij}}{\overset{k_b}{\rightleftarrows}} C_{ij} \overset{k_p}{\rightarrow} 2T_i + F_j, \tag{1}$$

where the constants k_b, $k_{d,ij}$ and k_p define the rates for T cell binding, dissociation and activation/proliferation respectively.

For simplicity, we assume that the affinity is largely determined by the dissociation rate $k_{d,ij}$. Thus, defining a molecular binding force $0 \leqslant \alpha_{ij} \leqslant 1$ [see (23) for a detailed discussion], we assume that the dissociation rate is inversely related to this binding force, i.e. $k_{d,ij} = k_d/\alpha_{ij}$. Thus low affinity T cells can have very fast off-rates.

Scheme (1) is similar to that of an enzyme–substrate reaction. Thus, we introduce a constant, equivalent to the inverse of a Michaelis–Menten constant, which we call an 'effective affinity', and define as

$$K_{ij} = \frac{k_b}{k_d/\alpha_{ij} + k_p} = \frac{\alpha_{ij}\, k_b}{k_d + \alpha_{ij}\, k_p}. \tag{2a}$$

The effective affinity increases with α_{ij} and reaches its maximum

$$K_{max} = \frac{k_b}{k_d + k_p}, \tag{2b}$$

when $\alpha = 1$. In our previous model all three rate constants depended on the interaction strength α_{ij}; this also resulted in a similar saturated relationship between K_{ij} and α_{ij} (23).

Pursuing the analogy of scheme (1) with that of an enzymatic reaction, it can be simplified by a conventional Michaelis–Menten assumption. Defining S_j as the total number of sites presenting the MHC–peptide complex j, we derive in Appendix A that the concentration of free sites F_j under the Michaelis–Menten assumption is

$$F_j = \frac{S_j}{1 + \sum_i K_{ij}\, T_i}, \tag{3}$$

where K_{ij} is defined by Eq. (2). This says that the availability of free sites decreases as a function of *all* T cells recognizing this particular MHC–peptide combination. This is the basis of the T cell competition.

Using the Michaelis–Menten formulation allows one to translate scheme (1) into a T cell growth function of the form

$$\frac{dT_i}{dt} = k_p\, T_i \sum_j K_{ij}\, F_j - dT_i, \tag{4}$$

where F_j is given by Eq. (3). This is derived in Appendix A. The term dT_i has been added to scheme (1) to account for the death rate of the T cells.

Equation (4) suggests that each type of free site, F_j (presenting MHC–peptide complex j), functions as an independent 'resource' upon which T cells may grow. For each type of peptide-presenting site, the growth of any T cell recognizing this site is down-regulated by the avidity weighted sum of all T cells recognizing this site (Eq. 3).

MHC–peptide complexes and the generation of presenting sites

As a simple model for the generation of presenting sites, we consider that peptides, P_i, interact with empty sites, E, thus forming a site, S_i, presenting MHC–peptide complex i (see Fig. 1). Considering different peptides i, with affinity κ_i, we propose the following simple scheme

$$P_i + E \rightleftarrows S_i,$$

where P_i is the concentration of peptide i. Assuming that the loading of sites by peptide is at equilibrium

$$S_i = \kappa_i\, P_i\, E. \tag{5}$$

Clearly, this is a great simplification that ignores the generation of peptides by antigen processing and the synthesis and turnover of MHC molecules. However, the model captures the relevant feature that the number of sites loaded with peptide i is proportional to the concentration of the peptide and its affinity for MHC. Because sites on APC are either empty, E, or present one of the peptides i, S_i, we can write a conservation equation for S_T, the total number of sites on APC, i.e.

$$E = S_T - \sum_i S_i. \tag{6}$$

Because a professional APC can bind several T cells at a time (28) the total number of sites is generally assumed to be larger than the total number of APC.

From Eqs (5) and (6) one can derive that the concentration of site i should obey

$$S_i = S_T \frac{\kappa_i\, P_i}{1 + \sum_j \kappa_j\, P_j}. \tag{7}$$

This says that peptides compete with one another for getting presented and that each peptide is presented proportional to κ, its affinity for MHC. Substituting S_i, given by Eq. (7), into the T cell growth model of Eq. (4) we inter-relate parts one and two of our model.

Parameters

Our model contains a large number, say n, of different peptides, P_j and T cell clones T_i. For each T cell clone T_i we assume that there is exactly one MHC–peptide combination, i.e. type of site, that it recognizes with maximum affinity and possibly many others which it recognizes with lower affinity. Since in our model the effective affinity K_{ij} is a saturation function of the molecular binding force α_{ij} (see Eq. 2), we set the interactions in our model by an $n \times n$ matrix of binding forces α_{ij}.

Setting all diagonal elements of this matrix to one, i.e. $\alpha_{ii} = 1$ for all i, we say that clone i always recognizes MHC–peptide i with maximum affinity K_{max}. Each clone is thus identified by the MHC–peptide combination it recognizes with maximum affinity. To model cross-reactivity, we assume that each clone recognizes on average c other MHC–peptides, i.e. presenting sites, with randomly chosen affinities. We thus randomly set a subset of the off-diagonal elements α_{ij} to random numbers between 0 and 1. These random numbers are drawn from a uniform distribution such that $0 < \alpha_{ij} < 1$.

The number of APC sits is scaled such that for every peptide there is on average one site available for its presentation, i.e. we set $S_T = n$. The peptide concentrations are also scaled between 0 and 1. For each available peptide we randomly draw from a uniform distribution a number between 0 and 1 defining the fixed $\kappa_i P_i$ term in Eq. (7). For our parameters there will be hardly any empty sites. By Eq. (7), the average concentration of presenting sites is $S_i = 0.5n/(1 + 0.5n)$ sites, which for the high diversity that we typically consider, i.e. for large n, can be approximated by $S_i \sim 1$. Since a T cell clone, on average, interacts with c different MHC–peptide combinations, the total concentration of presenting sites a T cell is expected to interact with is $cS_i \sim c$.

Assume that, in the absence of competition, a T cell will bind to any of these sites on a time scale of 10 min (24), i.e. ~144 day^{-1} for all sites, or $k_b = 144/c$ day^{-1} site^{-1}. We previously estimated that a high-affinity T cell–APC conjugate breaks apart on a time scale of ~1 h, which we divided into $k_d = 4$ day^{-1} and $k_p = 20$ day^{-1} (24). For the maximum affinity we thus obtain $K_{max} = 6/c$ site^{-1}. Having set the rate constants and having drawn the random α_{ij} elements we can calculate for all clones the effective affinity K_{ij}. Note that because $K_{max} = 6/c$, the effective affinity K_{ij} is always inversely related to the cross-reactivity c. An advantage of this is that the total T cell density, i.e. the total biomass, remains independent of the cross-reactivity c.

In the model we distinguish 'expressed' and 'actual' repertoires. The expressed repertoire is the number of T cell clones present in our system at time 0. The actual repertoire is the repertoire that results after clonal competition. We introduce a parameter P_C, the probability that a given T cell clone is present in the expressed repertoire. We vary the diversity of the expressed repertoire 100-fold by varying this probability from $P_C = 0.01$ to $P_C = 1$. The initial T cell clone sizes are small randomly chosen positive numbers. We also define a parameter P_P for the probability that a given peptide is present in our system. The peptides remain present at randomly chosen fixed concentrations.

Results

Carrying capacity

We have previously shown that the maximum population size that a T cell clone in this model can attain is naturally limited by the availability of MHC–peptide complexes (more specifically by the affinity weighted concentration of the presenting sites this clone is interacting with (23,24). This can mostly easily be seen by considering the interaction between one T cell clone and one type of presenting site with a particular effective affinity K. For such a system one can drop the subscripts and rewrite Eq. (4) as

$$\frac{dT}{dt} = \frac{k_p K S T}{1 + K T} - dT, \qquad (9)$$

which has equilibria at $T = 0$ and $T = k_p S/d - 1/K$.

This equation for T cell growth has been employed previously by ecologists for modeling competitive population growth (29). For a sufficiently large and fixed value of S [i.e. $S > d/(k_p K)$], this model says the T cell clone will expand to a non-zero equilibrium size which ecologists call the carrying capacity (23,24). If the stimulating MHC–peptide sites are not present in sufficient quantity the T cell clone will become extinct. Immunologically this tells us that, if T cells are stimulated by peptides which persist in the immune system at a high enough fixed concentration, the corresponding T cell clones will persist. Thus, T cell populations are homeostatically controlled by competitive antigenic stimulation. Note that Carneiro and co-workers have employed related concepts of a 'niche' and its 'driving capacity' in the context of T_h1 and T_h2 differentiation (30). Here niche and carrying capacity are defined more strictly in terms of resource competition, i.e. in terms of the MHC–peptide complexes, the T cell populations are competing for.

Affinity selection

One of the most basic results from theoretical ecology is that species feeding from one and the same resource are *a priori* not expected to co-exist. This is the principle of 'competitive exclusion' (27). We have demonstrated previously that the same principle holds for the T cell growth model developed here (23,24). Competitive exclusion is demonstrated by studying an immune system with an expressed T cell repertoire that is much larger than the actual diversity of peptides in the system. Figure 2 depicts a numerical simulation of our model with $n = 200$ different T cell clones with two MHC–peptide combinations. We have set the cross-reactivity to $c = 20$, so that each clone on average interacts with 10% of the peptides. This simulation illustrates various results.

First, because each peptide is expected to evoke an initial immune response of 20 different T cell clones, the primary response has a wide diversity. This broad response quickly narrows down to a few clones having high affinity for the two

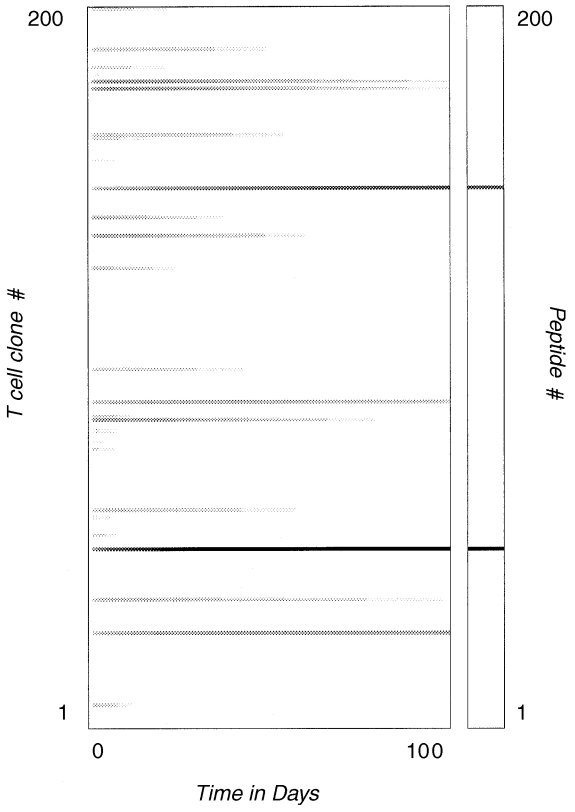


Fig. 2. Competitive exclusion illustrated by the immune response to two different MHC-peptide combinations. The panel on the right shows the location, i.e. the number, and the concentration of the two peptides, encoded by a gray-scale. The concentration of the lower peptide (i.e. number $i = 50$) is one, i.e. $\kappa_i P_i = 1$, and that of the upper one (i.e. number $i = 150$) is $\kappa_i P_i = 0.5$. The concentrations of the T cell clones are depicted in the panel on the left. Initially there are $n = 200$ T cell clones with a potential connectivity of $c = 20$, i.e. each T cell recognizes an average of 20 different MHC–peptide complexes. The T cell clones are plotted as a function of time with gray-scales indicating the clone's size. For the clones, the gray-scale is linear between white (i.e. $T_i = 0$) and black (i.e. $T_i = 1000$). Most clones disappear whereas clones 50 and 150 increase according to the concentration of the corresponding MHC–peptide combinations. Ultimately all clones beside these two maximum-affinity clones will disappear (not shown).

peptides. Ultimately, i.e. after ~ 200 days, only the two clones having the maximum affinity for the peptides survive. All others are excluded from the actual repertoire. Thus, ultimately, each peptide (i.e. each resource) maintains only a single clone and selects the clone having maximum affinity.

Second, the competition begins when the system has filled up. The gray-scales in Fig. 2 depict the clone sizes. The process of competitive exclusion starts when most clones have expanded several orders of magnitude. It is natural for any competitive process to become important only when the system gets crowded. Empirical data supporting this were recently reported in B cell competition experiments (20). Third, the two peptides differ in effective concentration, i.e. in $\kappa_i P_i$. The lower numbered peptide, which is available at the highest level, ultimately maintains the largest T cell population. Thus we see that the T cell clone sizes are indeed limited by the peptide concentration.

Fourth, the process of competitive exclusion may take long. The repertoire at day 100 consists of the two maximum affinity clones plus two clones with sub-maximal affinity. The affinity of the latter may differ only marginally from the maximum affinity or they may be responding to both peptides with sub-maximal affinity (see below). The time scale of the exclusion process is also influenced by the lifespan for T cells: the longer the lifetime the slower the exclusion. We have chosen a short lifespan of the T cells, $d = 1\ \text{day}^{-1}$, i.e. ~1 day, for two reasons; it speeds the simulations and because the cells in the self-renewing pool may be subject to activation-induced cell death (31) a short lifespan may be biologically reasonable.

It is important to keep in mind that the competitive exclusion principle is an equilibrium result, and that transiently the diversity may remain much higher. Since no natural immune system will ever be in equilibrium, much of its diversity could indeed be transient. Long-lived (memory) T cells could be responsible for high diversity over long periods of time.

For the case of a single peptide, the immunological interpretation of Fig. 2 is that the model accounts for the natural selection of the highest affinity clone. For B cells affinity selection is well established (32). The affinity selection depicted in Fig. 2 occurs in the absence of somatic mutation. Empirical data on affinity selection in T cells are scarce [see (23) for an overview]. Recently antigen-driven selection of T cells was demonstrated by comparing the *in vivo* TCR usage of primary and memory T cells (33). This suggested selection for T cells expressing greater specificity for the MHC–peptide complex.

For the case of a response to multiple peptides, which is depicted in Fig. 2, the situation is more complex. Because each peptide in the figure is expected to stimulate 20 T cell clones, i.e. 10% of the clones, we expect 1% of the clones to respond to both peptides. In Appendix B we derive that in our model such 'cross-reactive' clones will out-compete the two maximum-affinity clones whenever the sum of the two cross-reactive affinities exceeds the maximum affinity. Thus, in our model, clones may win the competition process by being cross-reactive, i.e. by having several sub-maximal affinity interactions. For other initial conditions we have observed in our simulations that only one T cell clone survives the selection process for the two peptides in Fig. 2. This clone interacted with both peptides and hence received greater stimulation.

T cell diversity and the total T cells

Experimental data demonstrate that lymphocyte repertoires of limited diversity attain a similar total number of T cells as repertoires of high diversity (6,20,21). These findings have been interpreted to say that 'control of lymphocyte numbers is probably independent of cell specificity' (6).

Our model suggests that this is true under some circumstances but not others. Our main assumption is that the self-renewing T cell repertoire is maintained by stimulatory interactions with immunogenic peptides. Because each peptide–MHC combination forms a resource maintaining maximally one T cell specificity, we have to consider a diverse repertoire of different MHC–peptide complexes for studying

a diverse T cell repertoire. Thus, in Fig. 3 we simulate a system with $n = 400$ different MHC–peptide complexes and clones. We study the effect of changing the diversity of the expressed T cell repertoire: the panels vary from the presence of 100% of the T cell clones in Fig. 3(a) to the presence of just 1% of the clones in Fig. 3(c). All peptides are present at fixed and random concentrations ($0 < \kappa_i P_i < 1$). For the cross-reactivity we assume each clone can on average interact with 40 peptides (i.e. $c = 40$).

With respect to the diversity of the actual repertoire we observe that in Fig. 3(a) the expressed repertoire of 400 clones is reduced to an actual repertoire of ~100 clones. Thus most clones are removed by the competitive exclusion process. For a lower diversity of the expressed repertoire this is no longer true: in Fig. 3(b and c) most clones of the expressed repertoire are maintained in the actual repertoire. Decreasing the diversity of the expressed repertoire, reduces the competition and allows for a smaller decrease of the diversity of the actual repertoire. Hence, competitive exclusion is the first process buffering the response of the total biomass to changes in T cell diversity.

Secondly, by the gray-scales, we observe that the individual clone sizes become larger when the diversity of the actual repertoire decreases. The three clones in Fig. 3(c) are individually much larger than the 100 clones in Fig. 3(a). The obvious reason for this is that in a diverse actual repertoire there is more competition between the clones, i.e. the niche-overlap increases with actual repertoire diversity. This is the second process buffering the response of the total biomass to changes in T cell diversity. We plot the total biomass of Fig. 3(a–c) as a function of T cell diversity, i.e. of P_C, by the heavy lines in Fig. 4.

The buffering of the total biomass strongly depends on the T cell cross-reactivity c. We here develop a rule of thumb for the 'total stimulation' of the actual repertoire. The total biomass is limited by the total amount of resources that are maintaining T cell proliferation. In our model we have n such resources, i.e. n MHC–peptide combinations. The expressed repertoire has a diversity of nP_C clones. Because each clone interacts on average with c resources, the total repertoire is maintained by maximally cnP_C resources. When this number becomes less than the number of resources available, i.e. when $cnP_C < n$ or $cP_C < 1$, the repertoire as a whole fails to utilize part of the resources and hence attains a lower total biomass. This explains the somewhat, i.e. 4-fold, lower total biomass of the repertoire with $P_C = 0.01$ (Figs 3d and 4) because $cP_C = 0.4 < 1$. Note that this rule of thumb, i.e. $cP_C < 1$, is independent of the number of clones n. Thus, we expect these results to apply also to an immune system with many more clones. In Fig. 5 we set $n = 1000$ to study the combined effects of T cell diversity, peptide diversity and cross-reactivity, and indeed find results similar to those depicted in Fig. 4 for $n = 400$. Summarizing, as long as $cP_C > 1$, we expect the total biomass to remain independent of the T cell diversity nP_C. This rule of thumb is also in agreement with recent ecological data suggesting that increasing the diversity of an ecosystem increases the system's total biomass (34). In grasslands plant productivity and resource utilization increased with increasing plant species richness (34). In other words, if increasing T cell diversity increases the total amount of resources utilized by the system, the total biomass should indeed increase.

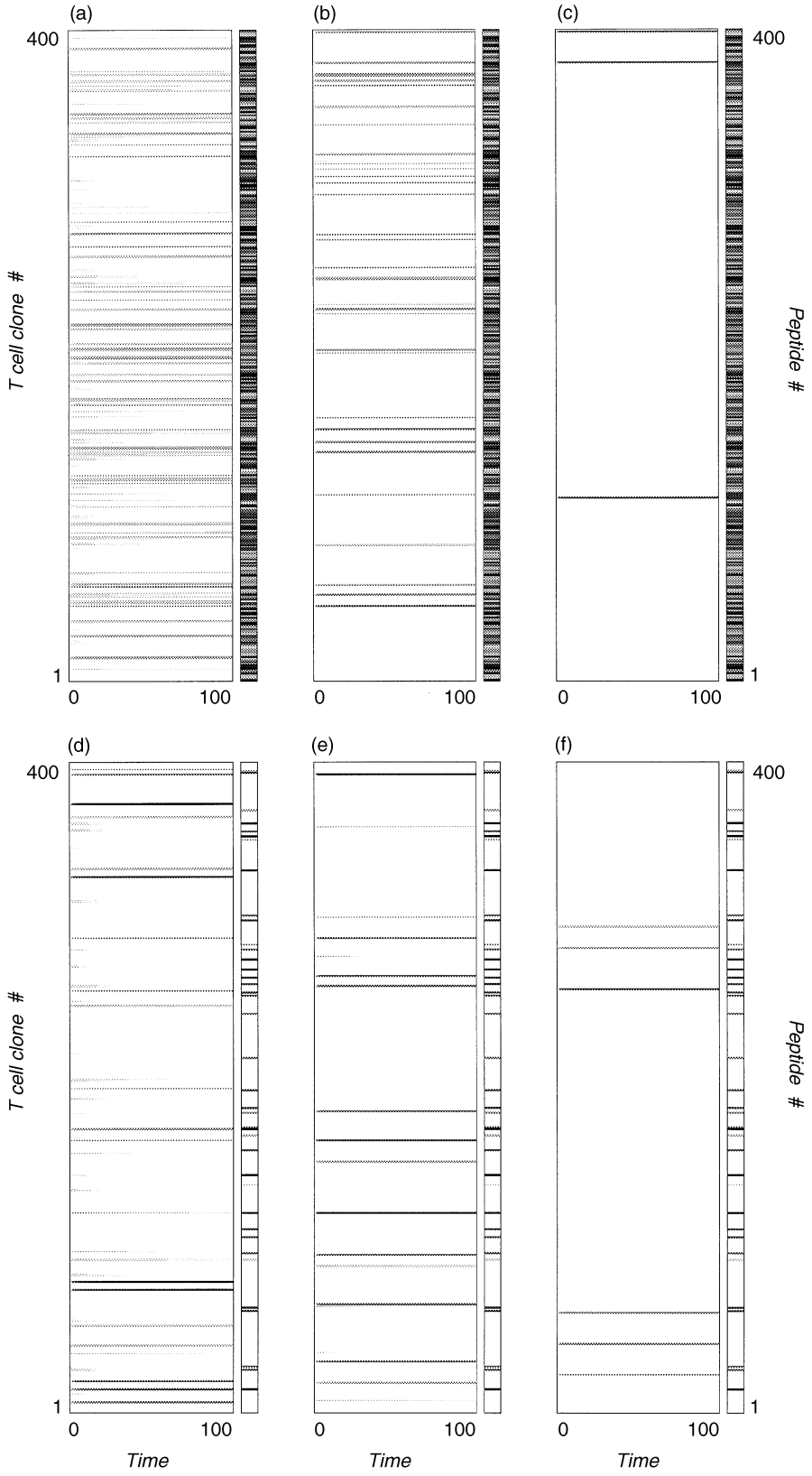
To explain the differences in the individual clones sizes in Fig. 3, we develop a rule of thumb for the niche-overlap. This is achieved by calculating the number of other T cell clones any clone is expected to interact with. First, the average number of resources for a T cell is c MHC–peptide combinations. Second, for each resource the T cell is competing with the $(c - 1)P_C$ other T cell clones that are also expected to have an affinity for this particular resource. Thus the niche-overlap per resource can be defined as $1 + (c - 1)P_C$ clones, where we add one for the overlap with the clone itself. The total stimulation of a T cell should thus be proportional to the sum of all resources it utilizes divided by the overlap per resource, i.e. to $c/[1 + (c - 1)P_C]$, which for large cross-reactivity, i.e. $c \gg 1$, can be approximated by $1/P_C$. Thus, for sufficiently large cross-reactivity, decreasing T cell diversity increases the stimulatory field of an individual clone. This explains why decreasing P_C from Fig. 5(a) to 5(c) increases the individual clone sizes. These notions for the niche overlap are again independent of the system size n and should hence carry over to the real immune system. In a large system, i.e. $n \gg c$, each clone can interact with an exceedingly small fraction of all MHC–peptides combinations, i.e. $c/n \to 0$ and can nevertheless have a significant niche overlap of $(c - 1)P_C$ other clones per resource.

Peptide diversity and total T cells

The principle of competitive exclusion dictates that the clonal diversity of the actual repertoire is limited by the diversity of the peptide repertoire. By studying H2-M-deficient mice it has been possible to design experimental systems with a limited peptide diversity (18). We have seen above, however, that if the only effect of reducing peptide diversity is to reduce the T cell diversity, this need not significantly influence total T cell numbers. Thus we explicitly investigate the effect of peptide diversity by simulating our model with only 10% of the MHC–peptide combinations (i.e. for $P_P = 0.1$, see Fig. 3d–f).

Comparing the simulation data depicted in Fig. 4 we see that limiting the peptide diversity to $P_P n = 40$ MHC–peptide combinations (dashed line) hardly influences total T cell numbers in our model. However, by reducing the diversity of

Fig. 3. Repertoire selection as a function of clonal diversity and peptide diversity. The organization of each panel is similar to that in Fig. 2. The system consists of $n = 400$ clones with an average cross-reactivity of $c = 40$ MHC–peptide combinations per clone. In panels (a–c) all $n = 400$ peptides are present at fixed random concentrations, i.e. $0 < \kappa_i P_i < 1$; in panels (d–f) we set $P_P = 0.1$ such that just 10% of the peptides is present. We change the diversity of the potential T cell repertoire from $P_C = 1$ (a and d) to $P_C = 0.1$ (b and e), to $P_C = 0.01$ (c and f). Thus on the left all T cell clones are present and on the right 1% of all T cell clones are present. See the text for the interpretation of the results.


(a)
(b)
(c)
(d)
(e)
(f)
400
1
0
100
T cell clone #
Peptide #
Time

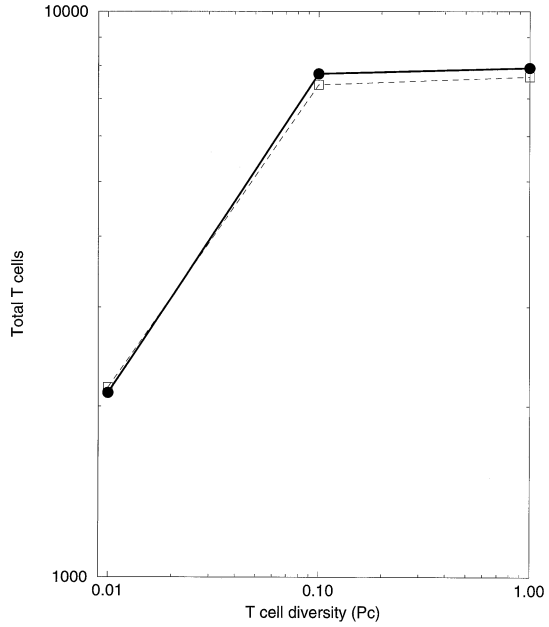


Fig. 4. Total T cell number as a function of T cell diversity. The total biomass of the six repertoires shown in Fig. 3 is depicted as a function of the T cell diversity (i.e. the probability P_C for the presence of a clone). The heavy line with solid circles denotes the T cell total for the case where all peptides are present (i.e. $P_P = 1$). The dashed lines denotes the case where 10% of the peptides are present (i.e. $P_P = 0.1$). The total biomass barely differs between the two cases.

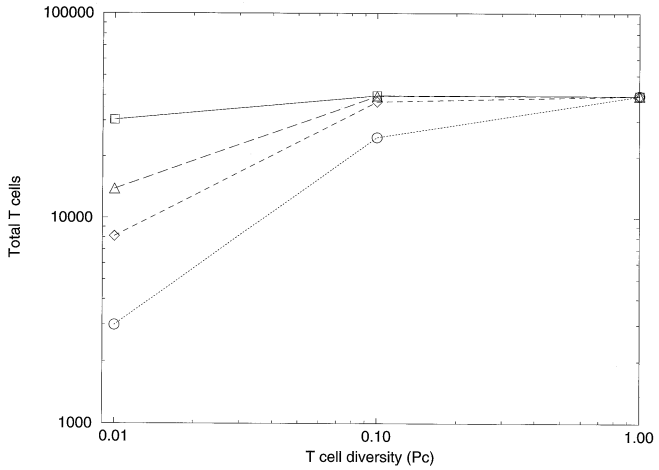


Fig. 5. Total T cell number as a function of T cell cross-reactivity. For a system with $n = 1000$ clones and $n = 1000$ peptides, we plot total T cell number as a function of T cell diversity for a cross-reactivity $c = 100$ (squares, solid line), $c = 50$ (triangles, long-dashed line), $c = 25$ (diamonds, dashed line) and $c = 10$ (circles, dotted line). When the connectivity is high the total T cell pool remains constant despite drastic changes in the T cell repertoire diversity.

peptides we get fewer clones, each of larger average size. This can be checked by comparing Fig. 3(a) with 3(d): for a lower presence of the peptides we obtain fewer clones in the actual repertoire (i.e. 113 versus 32) but the individual clone sizes tend to be larger (i.e. the lines are darker in Fig. 3d). The fact that the total biomass does not depend on the peptide diversity is due to the competition between the peptides for becoming presented. When the peptide diversity is lower, the peptides that are presented attain higher concentrations (by Eq. 7) and hence maintain larger T cell clones (see Eq. 9).

Cross-reactivity and total T cells

We study the impact of the T cell cross-reactivity on our results by simulating a system of $n = 1000$ T cell clones and $n = 1000$ MHC–peptide combinations for $c = 100$, 50, 25 and 10 connections per clone (Fig. 5). The results confirm the conclusions drawn above. As long as $cP_C > 1$, i.e. as long as almost all resources contribute to the maintenance of the repertoire, the total biomass remains fairly independent of the clonal diversity (see the solid line depicting $c = 100$). For our lowest cross-reactivity, i.e. $c = 10$ or 1% of the MHC–peptide combinations, a 100-fold reduction of the diversity reduces the total biomass ~10-fold.

Extrapolating to a typical mouse system size of $n = 10^6$ clones, we thus see that the maximum reduction in T cell diversity which fails to influence total T cell numbers is directly proportional to the T cell cross-reactivity c by our rule of thumb $cP_C > 1$. For instance, reducing the diversity of the expressed repertoire 10^4-fold is expected to affect total T cell numbers whenever the cross-reactivity is smaller than $c = 10^4$. Thus the validity of our results is limited by the cross-reactivity. When the cross-reactivity is low one could argue that this indicates that there should also be a global regulation of total T cell numbers (see the Discussion).

Repertoire selection

In Fig. 3(d) there are more T cell clones in the expressed repertoire than there are different MHC–peptide complexes. Hence we observe that the actual repertoire narrows by competitive exclusion. The clones that are ultimately maintained in the repertoire are not selected for having high affinity with one particular peptide, however. Most of the clones in Fig. 3(d) have maximal affinity for a peptide that is absent, i.e. most of the clones are maintained by sub-maximal interaction with various MHC–peptide combinations.

This is reminiscent of the empirical data suggesting that the T cell repertoire of self-renewing (memory) clones persisting after antigenic stimulation is very cross-reactive (8,40). For anti-viral immune responses it was found that the primary killer responses to serologically different viruses had low (i.e. 1%) cross-reactivity, whereas in secondary responses the cross-reactivity was over 20% (40). These data have been interpreted to say that long-term T cell memory is maintained by cross-reactivities because memory T cells can respond to many antigens that are not available to virgin cells (8).

The process of competitive exclusion in our model is not selecting for the clones with maximum affinity for particular MHC–peptide combinations. Instead, our model results suggest that self-renewing (memory) T cell clones might indeed have specificities for several peptides. Appendix B demonstrates that in a system with several resources, clones with several sub-maximal interactions can out-compete clones with fewer maximum-affinity interactions.

Discussion

In our model T cell clones are maintained only by specific antigenic stimulation. One would thus expect total T cell numbers to depend strongly on the diversity of the immunostimulatory ligands and the diversity of the T cell repertoire. However, we have demonstrated that for a sufficiently diverse and/or cross-reactive T cell repertoire, the total number of T cells at steady-state is largely independent of these factors. Thus, mouse T cell re-constitution experiments suggesting that the total number of T cells is independent of either diversity or the inoculum size (6,9,20,22) can be accounted for by our model if the diversity and/or the cross-reactivity remains sufficiently large. Work by Selin and collaborators suggests that this might be the case in that memory T cells, which are used to reconstitute the mice, can be extremely cross-reactive (40). Up to 25% of the $CD8^+$ lymphocyte response to a novel virus was reported to come from a memory population that was evoked by a heterologous virus given earlier.

The contradictory fact that in other re-constitution experiments T cell expansion is limited (35) and that total number of T cells at steady state seem to be determined by the inoculum size (3,10) can be accounted for in our model by arguing that in these experiments T cell diversity is limiting. Because one typically transfers millions of donor T cells, one would have to argue however that only a small, randomly selected, fraction of the donor T cells can expand in the recipient. Data showing that initially only a small fraction of the transferred T cells can be recovered in the recipient (10) provide some support for this argument.

Different steady-state total T cell numbers are attained in mice that receive different levels of irradiation (36). This can also be accounted for in our model by assuming that at high levels of irradiation T cell repertoire diversity becomes a limiting factor. These data are otherwise difficult to explain because the proliferative capacity of the irradiated T cells was indistinguishable from that of normal T cells and because normal T cells expand perfectly well in the irradiated mice (36).

Experiments on cytotoxic T lymphocyte (CTL) memory have suggested that self-renewal of memory cells plays an insignificant role in the maintenance of CTL memory. For example, Hou *et al.* (37) transferred immune spleen cells into MHC class I-deficient mice. In both normal controls and the MHC class I-deficient mice CTL precursor frequencies remained elevated for almost 6 months (37). In other studies, however, a significant fraction of the $CD8^+$ memory cells display an activated phenotype (37) and/or are cycling (38). Additionally, recent follow-up experiments suggest that for the maintenance of their memory function the transferred T cells require class I-restricted re-stimulation (39). Likewise, in monoclonal mice, memory $CD8^+$ T cells specific for the HY male antigen can be maintained in the absence of antigen but require the presence of MHC class I molecules (41). Further experiments of this kind, including experiments with mice kept in germ-free conditions so that cross-reactions with food antigens and/or intestinal bacteria are excluded, need to be done. Such experiments will not only provide clues on the nature of the stimulatory ligands maintaining self-renewing T cells, but might also provide estimates for the lifespan of T cells in the absence of self-renewal.

The B and T cell competition experiments discussed in our Introduction (20–22) suggest that diverse lymphocyte repertoires have a competitive advantage over non-diverse (e.g. TCR transgenic) repertoires. These results strongly support our notion of local competition for specific resources. Diverse repertoires are supposedly stimulated by a greater variety of antigens and would therefore have a smaller average niche-overlap than the non-diverse repertoires have. Indeed it is shown that the competitive ability of a repertoire correlates with activation and proliferation (22).

Despite our demonstration of T cell population control by specific stimulation, it could nevertheless be the case that the total T cell number is determined by non-specific factors. We have previously called this 'global' competition (42); it could involve competition for space, lymphokines (43), etc., as long as the resource being competed for is independent of T cell specificity. However, when all the competition is global, the clone proliferating most rapidly is expected to outcompete all others (42). Thus, while global competition can control the T cell population size, it cannot simultaneously account for repertoire diversity. Models with both 'local' (i.e. antigen specific) and global control can account for both T cell diversity and control of total population size. In fact, in our model the total number of APC provides a global factor regulating total T cell numbers. Activation of APC and B cells during immune responses may transiently increase the total number of available APC sites and hence transiently increase total T cell numbers.

Global competition might also account for the observed compensatory behavior of the $CD4^+$ and $CD8^+$ T cells. In the introduction we reviewed data on the competitive out-growth of $CD4^+$ and $CD8^+$ T cells. When both subsets are present normal CD4/CD8 ratios are attained (9), when one subset is absent the other compensates for this by expanding until normal T cell numbers are attained (9,12,14,15). Because $CD4^+$ and $CD8^+$ T cells interact with different classes of MHC molecules, and possibly even with different types of APC, it seems unlikely that they compete for the same sites on the APC. Assuming that the proliferation rates k_p and/or the death rates d are a function of total T cell numbers, i.e. CD4 plus CD8 T cells, we may combine local with global competition and account for blind CD4/CD8 homeostasis.

In our model MHC–peptide complexes act as a resource for T cells. One of the most basic results from theoretical ecology is that species feeding from one and the same resource are *a priori* not expected to co-exist. This is the principle of 'competitive exclusion' (26). The different immunogenic peptides form different ecological resources and due to competitive exclusion every resource (i.e. MHC–peptide combination) can maintain maximally one T cell specificity. Throughout this paper we have avoided the question of whether the peptides maintaining the T cell repertoire are foreign or self-peptides. Our results, stated simply, say that to maintain a diverse, equilibrium T cell repertoire one requires an at least equally diverse repertoire of immunogenic peptides. For this reason we previously suggested that it could be the high diversity of self-antigens that maintain the high diversity of the T cell repertoire (23). The fact that germ-free mice have normal T cell numbers (53,54) suggests that the foreign peptides do not play an important role in maintaining

the self-renewing T cell repertoire. Additionally, because memory T cells are extremely cross-reactivity (40), one could argue that their self-renewal relies on a wide variety of ligands, including self-peptides.

Recent data from the human immune system are creating further confusion on the issue of T cell renewal and thymic output. The recovery of peripheral $CD4^+$ T cells in adult patients who have received chemotherapy occurs on a very slow time scale of more than a year (44). Additionally, the recovery is (i) faster in young patients who presumably have a more functional thymus, (ii) associated with thymic enlargement and (iii) correlates with the appearance of cells expressing the CD45RA marker of virgin T cells (44). Hence, it appears that in these patients $CD4^+$ T cell self-renewal plays hardly any role and that the recovery is largely due to thymic output, and is hence impaired in adult patients. Similar data were recently described for multiple sclerosis patients treated with anti-CD4 antibodies (45). The recovery of the $CD4^+$ T cell count in the peripheral blood following withdrawal of the anti-CD4 treatment was very slow. Surprisingly, virgin and memory T cells seemed to recover at a similar rate. Another method of assessing cell division in humans involves measuring telomere length. Telomeres shorten 50–100 nucleotides per division. A similar rate of decrease in telomere length with age was observed in memory and virgin T cells (46). Elsewhere we formally show that these findings cannot be interpreted to say that memory and naive T cells are cycling at similar rates (De Boer and Noest, submitted). Additionally, calculations based on these data suggest that T cell renewal rates are extremely slow, i.e. with an average of 0.3 divisions per year (46). Other data also suggests that turnover rates and the rates of self-renewal are very slow in human memory T cells (47).

Another mechanism of competitive exclusion between T clones has recently been proposed in a model of cytotoxic T lymphocytes eliminating virus-infected cells (48). According to this mechanism, the clone that has the highest affinity for the antigen reduces the antigen concentration to such low levels that the other clones can no longer be stimulated. This form of competition between the CTL clones is 'indirect' and works via the antigen concentration. In contrast, the competitive exclusion process derived here works by 'direct' competition between T cell clones. Direct competition has the desirable property that it also applies to situations in which the antigen remains present at approximately constant levels, e.g. for self-antigens and for foreign antigens persisting in the immune system (39,49).

Our results are equilibrium results: competitive exclusion may take an extremely long time to be evident when clones differ marginally in affinity for the antigen they are competing for or when cells are long-lived. Additionally, from ecology it is well known that more than one competitor can be maintained on a single resource when the dynamics are oscillatory (50) or in the presence of external noise (51) or in the presence of additional limiting factors for the competitors (52). All of these possibilities could also apply to immune systems. Thus, our results can only provide a baseline understanding for the consequences of competitive control. Nevertheless, our theoretical results lead to new interpretations of experimental results that were previously contradictory and unexplained, and which intuitively seemed to deny the importance of antigenic stimulation in the competitive control of T cell numbers.

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Abbreviations

APC	antigen-presenting cell
CTL	cytotoxic T lymphocyte

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Appendix A

Scheme (1) can be simplified by a conventional Michaelis–Menten quasi-steady-state assumption. For the T cell site conjugates we write a quasi-steady-state equation

$$\frac{dC_{ij}}{dt} = k_b\, T_i\, F_j - (k_{d,ij} + k_p)C_{ij} = 0$$

$$\text{or} \qquad C_{ij} = K_{ij}\, T_i\, F_j\,. \tag{A.1a,b}$$

where the free site variable F_j is defined by the conservation equation

$$F_j = S_j - \sum_i C_{ij}\,, \tag{A.2}$$

which says that the concentration of free sites j equals the total minus all conjugates of site j with any of the T cells i. Substitution of Eq. (A.1b) into the conservation equation (A.2) gives

$$F_j = \frac{S_j}{1 + \sum_i K_{ij}\, T_i}\,, \tag{A.3}$$

which is written as Eq. (3) in the text.

Following scheme (1) we write for the T cells

$$\frac{dT_i}{dt} = -k_b \sum_j T_i F_j + \sum_j (k_{d,ij} + 2k_p) C_{ij} . \quad (A.4)$$

Since at quasi-steady state $dC_{ij}/dt = 0$ we can add the right hand sides of Eq. (A.1a) for all i and j to (A.4) to obtain

$$\frac{dT_i}{dt} = \sum_j k_p C_{ij} = k_p T_i \sum_j K_{ij} F_j = k_p T_i \sum_j \frac{K_{ij} S_j}{1 + \sum_l k_{lj} T_l} , \quad (A.5)$$

which by adding a T cell death term, yields Eq. (4) in the text.

From Eq. (A.5) we see that every MHC–peptide combination F_j forms an independent resource contributing additively to the proliferation. Because the resource F_j decreases with the number of T cells interacting with it (Eq. A.3), we 'naturally' obtain a proliferation term with competition between T cells.

Appendix B

Cross-reactive clones can out-compete mono-specific clones. Consider two clones T_1 and T_2 that each interact with maximum affinity with MHC–peptide presenting sites S_1 and S_2 respectively, i.e. $K_{11} = K_{22} = K_{max}$. A third clone T_3 interacts with both sites with lower affinity, i.e. we set $0 < K_{31}$, $K_{32} < K_{max}$. We study whether the cross-reactive clone T_3 can invade the equilibrium situation of clones T_1 and T_2.

For this situation the model of Eq. (4) simplifies to

$$\frac{dT_1}{dt} = T_1(k_p K_{max} F_1 - d) = 0, \quad (B.1)$$

$$\frac{dT_2}{dt} = T_2(k_p K_{max} F_2 - d) = 0, \quad (B.2)$$

where the free site concentrations are

$$F_1 = \frac{S_1}{1 + K_{max} T_1 + K_{31} T_3} \text{ and } \quad F_2 = \frac{S_2}{1 + K_{max} T_2 + K_{32} T_3} . \quad (B3)$$

Solving Eqs (B.1) and (B.2) we obtain

$$F_1 = F_2 = d/(k_p K_{max}). \quad (B.4)$$

The invasion criterion means that the cross-reactive clone increases, i.e. that

$$\frac{dT_3}{dt} = T_3 (K_p K_{31} F_1 + k_p K_{32} F_2 - d) > 0. \quad (B5)$$

Substituting Eq. (B.4) into condition (B.5) we obtain

$$K_{31} + K_{21} > K_{max} , \quad (B.6)$$

i.e. when the sum of the affinities of the cross-reactive clone exceeds the maximum affinity of the mono-specific clones, the cross-reactive clone will still grow when the mono-specific clones are already in equilibrium. When clone T_3 increases, the available free sites, i.e. F_1 and F_2, will decrease. Hence the growth rate of clones T_1 and T_2 becomes negative. Ultimately clone T_3 will replace clones T_1 and T_2.