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T Cell Repertoires and Competitive Exclusion

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Self-renewal is generally thought to play a major role in the maintenance of the T-cell repertoire. Here we develop a set of mathematical models for T-cell activation by peptides on antigen presenting cells (APCs). We show that competition between T cells is inherent to the processes involved in T cells binding APCs. We prove that for each dominant peptide only one T-cell clone can ultimately survive the competition. This is analogous to a classical result from theoretical ecology known as the principle of "competitive exclusion". These findings allow for three main results. First, competitive exclusion during an immune response to antigen implies that the clone(s) with the highest affinity for the dominant peptide(s) will outcompete all others. This allows for a form of "affinity selection". Second, the competition for binding antigen gives rise to regulation of T-cell numbers within a single clone. This allows for a regulated form of T-cell memory when T cells are continuously activated by a persisting antigen. Third, competitive exclusion implies that for each peptide only one T-cell specificity can be maintained in the repertoire. If the T-cell repertoire is largely maintained owing to cross-reactivities with various antigens, competitive exclusion means that the diversity of the T-cell repertoire is limited by the number of antigens stimulating the system. If the cross-reactivities were to involve activation by self antigens this would confirm an earlier result suggesting that the T-cell repertoire is diverse owing to the diversity of the self environment.

Introduction

The mouse T-cell repertoire consists of the order of 10^8 T cells most likely distributed over the order of 10^6–10^7 different clones (cf. Pannetier *et al.*, 1993). Since the cells comprising this repertoire have a lifespan that is much shorter than that of a mouse (Freitas & Rocha, 1993) it is clear that the total number of T cells is maintained dynamically. T cells can be generated in the thymus from precursors or they can be generated by self-renewal of established T cells in the periphery. Recent evidence indicates that the size of the T-cell population is controlled at the level of total T-cell numbers (Freitas & Rocha, 1993). Mice attain a similar total number of T cells when they are transplanted with many thymus lobes (Wallis *et al.*, 1979), when either the CD4 or the CD8 subset is suppressed or knocked out (Rocha *et al.*, 1989; Zijlstra *et al.*, 1990; Cosgrove *et al.*, 1990; Rahemtulla *et al.*, 1991) or when the repertoire is composed of very few specificities (Freitas & Rocha, 1993). It is well known that the enormous diversity of the T-cell repertoire is *generated* by V(D)J recombination (Chien *et al.*, 1984). How diversity can be *maintained* in the periphery is the question studied in this paper.

If the T-cell repertoire is largely maintained by self-renewal we face the problem that the clone that proliferates most rapidly is expected to outcompete all others. Ultimately this would limit the diversity to a single specificity. This competition problem does not arise when the T-cell repertoire is largely maintained by the source of novel cells from the thymus. However, because the thymus regresses around puberty, which probably reduces the thymic output, and because thymectomized mice and humans remain

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immunologically competent, it seems very likely that in the adult immune system the T-cell pool is largely maintained by self-renewal (Rocha *et al.*, 1989; Beverly, 1990).

Self-renewal relies on activation of T cells and cell division. Although the rate of proliferation may be controlled by various non-specific factors such as growth factors and hormones, the signal to proliferate most likely requires specific binding of the T-cell receptor to some antigen. This activation signal could either be due to cross-reactivities with self antigens or to simulation by foreign antigens resident in the system (Rock & Benacerraf, 1984; Stutman, 1986; Rocha *et al.*, 1989; Beverly, 1990). In order to account for immunological memory, foreign antigens may indeed persist for a long time (Gray & Matzinger, 1991). Thus, the clone receiving the best antigenic signals is expected to outcompete all others.

This argument leads to an "ecological" view of the T-cell repertoire. Populations, i.e. T-cell clones, competing for resources, namely, access to antigens, may or may not find a niche for survival in the system. The problem sketched above in fact corresponds to one of the most fundamental concepts of theoretical ecology. Gause (1934) formulated it as the principle of "competitive exclusion": two species with similar ecology cannot live together in the same place. In other words, species can only co-exist *in equilibrium* if they use different resources (Lack, 1954; Hutchinson, 1957; MacArthur & Levins, 1964; MacArthur, 1970; May, 1974; Levin, 1970; Kaplan & Yorke, 1977; Armstrong & McGehee, 1980). Applied to a T-cell repertoire in equilibrium, this suggests that diversity can only be maintained if clones use different resources, i.e. see different antigens.

In this paper we develop general models for T-cell population dynamics for which we prove this suggestion to be correct. For each peptide presented at densities high enough to stimulate T cells, the immune system can maximally sustain one T-cell specificity. This allows for three immunological interpretations. First, competitive exclusion implies a form of affinity maturation which does not involve hypermutation or any other explicit mechanism beyond the competition for binding antigen. Following Gray (1993), we use the term "affinity selection" for this emergent process. Second, the competition for binding antigen prevents infinite T-cell growth when cells are continuously activated. This allows for a regulated form of T-cell memory when antigens remain present for a long time (Gray & Matzinger, 1991; McLean, 1992). Third, we will argue that one explanation for the maintenance of the diversity of the T-cell repertoire is the diversity of self antigens. In order to account for a diversity of, say, 10^6 clones one requires of the order of 10^6 different peptides. If T cells are maintained owing to cross-reactivities with self antigens we confirm an earlier claim that the immune system is diverse because the self environment is diverse (De Boer & Perelson, 1993).

COMPETITIVE EXCLUSION

We consider systems in which there are many antigens present, each of which when processed will give rise to a few dominant peptides complexed to major histocompatibility complex (MHC) proteins on the surface of antigen presenting cells (APCs). We assume that T cells bind to the peptide forming a conjugate which ultimately breaks up with the T cell, either becoming activated, if enough of its antigen specific receptors interact with high enough affinity to the presented peptides, or the T cell dissociates without becoming activated.

From theoretical ecology we know that species can only co-exist if they exploit different recources, i.e. by "niche" differentiation. Since T-cell clones are also competing for resources, namely, peptides presented on APCs, we expect that the principle of competitive exclusion will hold in the immune system. One complication is that T-cell clones may have a source of novel cells from the thymus. We therefore study competitive exclusion by disallowing such a source, i.e. by considering a T-cell repertoire that is maintained totally by self renewal in the periphery.

For systems in which there are n T-cell clones, T_i, $i = 1, 2, \ldots, n$, competing for m possible peptides, P_j, $j = 1, 2, \ldots, m$ will derive a set of models for T-cell equations of the following general form:

$$\dot{T}_i = T_i\left(\sum_{j=1}^{m} k_{ij}^a K_{ij}\alpha_j - d_T\right), \tag{1}$$

where k_{ij}^a and K_{ij} are parameters defining the affinity of T_i for P_j, and α_j is a function that is model dependent but in general will depend on the concentration of peptide j and the population levels of the various T-cell clones. In our simplest model for the interaction between T cells and peptides

$$\alpha_j \equiv \frac{P_j}{1 + \sum_{i=1}^{n} K_{ij}T_i}. \tag{2}$$

We call this the "ecological" model. Equation (1) and variants of it will be derived in detail below. However, what is important is that in equilibrium all clones i have to satisfy $\dot{T}_i = 0$, and thus for each $i, i = 1, \ldots, n$,

$$\bar{T}_i = 0, \tag{3a}$$

or

$$\sum_{j=1}^{m} k_{ij}^{a} K_{ij} \alpha_j = d_T, \tag{3b}$$

where an overbar is used to denote an equilibrium solution. When considered for all values of i, eqn (3b) is a linear system of n equations in m unknowns, α_j, $j = 1, \ldots, m$. We are interested in the case in which $n \geqslant m$, i.e. there are more T-cell clones than peptides.

For this case, in which there are more equations than unknowns, a generic system can maximally give rise to m solutions for α_j. MacArthur & Levins (1964) have phrased this idea of generic behavior by the statement that it is "infinitely unlikely" that n planes drawn in a m-dimensional space will intersect in a point. Generally, at most m equations from (3b) can be simultaneously satisfied. Thus, for at least $(n - m)$ clones, $T_i = 0$. Hence, at most m clones can be non-zero, i.e. can be maintained in an equilibrium repertoire. Dismissing the degenerate problems in which the products $k_{ij}^{a} K_{ij}$ of some clones are a linear combination of those of other clones as "infinitely unlikely", we attain the main conclusion of this paper: *in equilibrium* the diversity of the T-cell repertoire is less than or equal to the number of antigens residing (i.e. persisting) in the system. This is analogous to the earlier ecological results saying that, in equilibrium, an ecosystem based upon n different resources can maximally sustain n different species competing for these resources (Lack, 1954; Hutchinson, 1957; MacArthur & Levins, 1964; MacArthur, 1970). Co-existence under non-equilibrium conditions has been discussed in the ecological literature (Levin, 1970; May, 1974; Kaplan & Yorke, 1977; Armstrong & McGehee, 1980), and the relevance of such considerations to the present model is commented on in the Discussion.

Affinity selection

The simplest immunological implication of our principle of competitive exclusion is a form of affinity maturation in the absence of hypermutation. During an immune response several peptides may get presented and for each peptide we expect several T-cell clones to respond. The principle of competitive exclusion implies that for each distinct peptide the clone that has the highest affinity for this peptide will outcompete all other clones responding to the same peptide. To see this, consider the case of a single peptide at concentration P. Let k_i^a and K_i be parameters defining the affinity of clone i for the peptide. Then from eqns (1) and (2)

$$\dot{T}_i = T_i(\beta_i - d_T), \tag{4}$$

where $\beta_i = k_i^a K_i P/(1 + \Sigma_{l=1}^{n} K_l T_l)$. Assume that clone 1 has the largest value of β, i.e. $\beta_1 > \beta_i$, $i = 2, 3, \ldots, n$, and that at equilibrium $\bar{T}_1 \neq 0$. Then, at equilibrium, where $\dot{T}_1 = 0$, $\beta_1 = d_T$. For clones $2, 3, \ldots, n$, $\beta_i < d_T$ and therefore for each of these clones $\dot{T}_i < 0$. Thus, clones $2, 3, \ldots, n$ will decrease in size until they are eliminated. Note that this is the only equilibrium that is possible in which all clones have not gone extinct. If, for example, we assumed clone 2 reached equilibrium with $\bar{T}_2 \neq 0$ and $\beta_2 = d_T$, then because $\beta_1 > \beta_2 = d_T$, $\dot{T}_1 > 0$. Thus, clone 1 would continuously increase in size, contradicting the assumption that this was an equilibrium. Notice that β_i is proportional to $k_i^a K_i$, i.e. to the affinity of clone i for peptide. Thus, owing to the competition for peptide, the average affinity will increase and the clonal diversity will decrease during the course of the response. We use the term "affinity selection" for this process. (The usual term "affinity maturation" has too much connotation with hypermutation.)

There seem to be little or no data on affinity selection among T cells. MacDonald *et al.* (1981) showed that cytotoxic T lymphocytes (CTL) obtained from mice primed with allogenic cells *in vivo* differed dramatically in affinity from those obtained by *in vitro* stimulation of naive CTL precursors. The simplest explanation for such affinity differences may be the preferential stimulation *in vivo* of high-affinity cells (Sherman & Maleckar, 1988). Gammon *et al.* (1990) show that T cells recognizing certain determinants of lysozyme outgrow others in culture. For the *in vivo* situation there is one additional speculative piece of evidence. The diversity of the T-cell repertoire in multiple sclerosis lesions seems to decrease in time, i.e. the repertoire diversity decreases in the transition from active to chronic lesions (Wucherpfennig *et al.*, 1992). In chronic lesions the repertoire is restricted to just a few clones (Oksenberg *et al.*, 1990). A possible explanation for the latter observation is selective outgrowth, i.e. affinity selection.

Extinction

From eqn (3) it is obvious that one possible equilibrium solution is $\bar{T}_i = 0$ for all i, i.e. all clones go extinct. This can occur if there is not enough peptide to stimulate T-cell growth in the periphery. For example, in eqn (4), β_1 is larger than β_i, $i = 2, \ldots, n$. Thus, if $\beta_1 < d_T$, then $dT_i/dt < 0$ for all i, and hence eventually all clones will go extinct. For

this to occur it is sufficient if $k_1^a K_1 P < d_T$, i.e. if $P < d_T/(k_1^a K_1)$.

An Ecological Model

We now show how models of the form of eqn (1) can be derived. As a first model, consider T-cell clones, T_i, that may bind to peptides freely available on the surface of APCs. We call these "free" peptides, F_j (Fig. 1). The binding of T cell i to free peptide j, with rate constant k_{ij}^b, results in T-cell–peptide–MHC complex C_{ij}. These complexes dissociate with rate constant k_{ij}^d, or give rise to an activated T cell, T_i^*, with rate constant k_{ij}^a. Activated T cells, T_i^*, are assumed to proliferate, yielding two non-activated daughter cells T_i, at per capita rate k_p. The rates of binding, dissociation, and activation may depend on the affinity of the T cell for the presented peptide.

This is depicted in the following scheme

$$T_i + F_j \underset{k_{ij}^d}{\overset{k_{ij}^b}{\rightleftarrows}} C_{ij} \xrightarrow{k_{ij}^a} T_i^* + F_j$$

and

$$T_i^* \xrightarrow{k_p} 2T_i. \tag{5}$$

For the complexes C_{ij} we write the quasi-steady-state equation

$$\dot{C}_{ij} = k_{ij}^b T_i F_j - C_{ij}(k_{ij}^d + k_{ij}^a) = 0, \tag{6}$$

where $i = 1, 2, \ldots, n$ and $j = 1, 2, \ldots, m$. This gives

$$C_{ij} = K_{ij} T_i F_j, \tag{7a}$$

where

$$K_{ij} \equiv \frac{k_{ij}^b}{k_{ij}^d + k_{ij}^a}. \tag{7b}$$

At any given time, presented peptides are either freely available on the surface of APCs or are bound to T-cell receptors, i.e. in complexes. Assume for the moment that each T cell binds a single peptide.

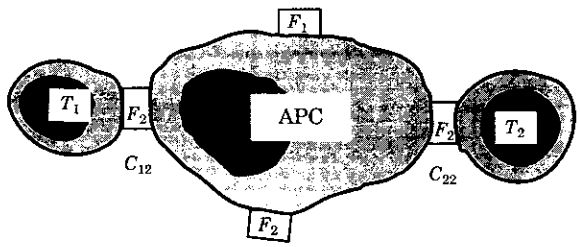

FIG. 1. Schematic illustration of an APC with peptides bound to MHC molecules on its surface. An MHC loaded with peptide j can be free (F_j) or be bound by T cell i and hence in the form of a complex (C_{ij}).

Then

$$P_j = F_j + \sum_i^n C_{ij}. \tag{8}$$

In the two APC models that we develop below we relax this constraint by considering "sites" at which T cells may bind the APCs. Substituting eqn (7a) we write

$$P_j = F_j\left(1 + \sum_i^n K_{ij} T_i\right), \tag{9a}$$

or

$$F_j = \frac{P_j}{1 + \sum_i^n K_{ij} T_i}, \tag{9b}$$

which says that the availability of peptides to bind decreases as a function of all T cells recognizing this peptide. For the activated T cells we write another quasi-steady-state equation

$$\dot{T}_i^* = \sum_j^m k_{ij}^a C_{ij} - k_p T_i^* = 0, \tag{10a}$$

hence

$$T_i^* = \frac{1}{k_p} \sum_j^m k_{ij}^a C_{ij}. \tag{10b}$$

For the T cells we write

$$\dot{T}_i = s - \sum_j^m k_{ij}^b T_i F_j + \sum_j^m k_{ij}^d C_{ij} + 2k_p T_i^* - d_T T_i, \tag{11}$$

where s is the source of T cells from the thymus and d_T is the rate of T cell turnover. Since $\dot{C}_{ij} = 0$ we may add eqn (6) to eqn (11). Further, we substitute eqns (7a) and (10b) to obtain

$$\dot{T}_i = s + T_i\left(\sum_j^m k_{ij}^a K_{ij} F_j - d_T\right), \tag{12}$$

which says that T-cell proliferation is proportional to F_j, the number of free-presented peptides. Finally, we substitute eqn (9b) to obtain an equation equivalent to eqn (1) except that it has a source

$$\dot{T}_i = s + T_i\left(\sum_j^m \frac{k_{ij}^a K_{ij} P_j}{1 + \sum_l^n K_{lj} T_l} - d_T\right). \tag{13}$$

Each peptide functions as an independent "resource" upon which T cells may grow. For each peptide, T-cell growth is regulated by the affinity weighted sum of all T cells recognizing this peptide. Thus, increasing the number of T cells that recognize each presented peptide or their affinity decreases the per capita T-cell growth rate.

For the case of one T-cell specificity with one peptide, i.e. $n = m = 1$, eqn (13) has the same form as the model of Fishman & Perelson (1993), which was based upon a more realistic description of the process involved in antigen presentation. Additionally, in the absence of a source, i.e. $s = 0$, we obtain a population growth equation

$$\dot{T} = T\left(\frac{k_a P}{1/K + T} - d_T\right), \tag{14}$$

where $T \equiv T_1$, $P \equiv P_1$, $k_a \equiv k_{11}^a$, and $K \equiv K_{ij}$. Equation (14) is similar to the models for the density dependent growth of populations devised by Maynard Smith & Slatkin (1973) and by Hassell (1975). One T-cell clone growing in response to a fixed peptide concentration will attain an equilibrium population density

$$\bar{T} = \frac{k_a P}{d_T} - \frac{1}{K}, \tag{15}$$

which in ecology is called the "carrying capacity". Thus, in response to continuous simulation by a fixed antigen concentration a T-cell population does not grow infinitely but rather attains an equilibrium density, wherein proliferation balances death. Further, if $P < d_T/(k_a K)$, there is too little "resource" to sustain the T-cell population: the only equilibrium solution is $\bar{T} = 0$.

ANTIGEN

The peptides forming the resources for T-cell activation are derived from antigenic molecules following the processing and presentation of the molecules by APCs. We will not model the processes involved in antigen processing, instead we make the plausible assumption that the concentration of peptide P_j derived from antigen A_j is proportional to the antigen concentration, i.e.

$$P_j = cA_j. \tag{16}$$

Thus, if the antigen concentration is fixed, as may be the case for certain self antigens, the peptide concentration will be constant.

To treat foreign antigens, we will assume that they are pathogens that can grow and generate an immune response. To model this we propose a phenomenological equation in which antigens grow logistically and are eliminated at a rate proportional to the number of T cells recognizing them. Thus, we assume

$$\dot{A}_j = rA_j(1 - A_j) - A_j \sum_i^n k_{ij}^e T_i, \tag{17}$$

where k_{ij}^e is the rate elimination of antigen j owing to T cells in clone i. In our model of T-cell growth, eqn (1), we need the concentration of peptide P_j. By an appropriate choice of the proportionality constant c in eqn (16), the maximum concentration of antigen, which appears in the logistic term, can be chosen to be one without loss of generality. The solutions of eqn (17) are

$$\bar{A}_j = 0, \tag{18a}$$

and

$$\bar{A}_j = 1 - \frac{1}{r}\sum_i^n k_{ij}^e T_i. \tag{18b}$$

Any antigen for which the latter solution is positive may persist in the system. Such an antigen will account for memory because it maintains the simulation of *one of the clones* responding to it.

Stability

To study T-cell memory we consider the equilibrium in which one antigen maintains the proliferation of one T-cell clone. For simplicity, we assume $s \approx 0$. For proliferating T cells this is reasonable because for each clone the production of naive T cells in the thymus is small. We rewrite eqns (13) and (17) as

$$\dot{T} = T\left(\frac{k_a K A}{1 + KT} - d_T\right), \tag{19a}$$

and

$$\dot{A} = rA(1 - A) - k_e AT, \tag{19b}$$

where $k_a \equiv ck_{11}^a$, $k_e \equiv k_{11}^e$, and we have omitted the subscripts for the population numbers. Since the maximum concentration of the antigen is one, the maximum per capita growth rate of the T cells is $k_a K - d_T$. Thus, proliferation of the T cells implies the parameter constraint

$$\rho \equiv k_a K - d_T > 0. \tag{20}$$

The non-trivial equilibrium of eqns (19a, b) corresponds to

$$\bar{T} = \frac{r\rho}{K(k_a k_e + d_T r)}, \tag{21a}$$

and

$$\bar{A} = \frac{d_T(k_e + Kr)}{K(k_a k_e + d_T r)}. \tag{21b}$$

The Jacobian matrix evaluated at this equilibrium is

$$\mathbf{J} = \begin{bmatrix} -\dfrac{r\rho d_T}{k_a(k_e + Kr)} & \dfrac{r\rho}{k_e + Kr} \\ -\dfrac{k_e d_T(k_e + Kr)}{K(k_a k_e + d_T r)} & -\dfrac{r d_T(k_e + Kr)}{K(k_a k_e + d_T r)} \end{bmatrix}. \quad (22)$$

This equilibrium is locally stable because $\mathrm{tr}\,\mathbf{J} < 0$ and $\det \mathbf{J} > 0$.

The system (19a, b) has a second equilibrium, $\bar{T} = 0$, $\bar{A} = 1$. The Jacobian matrix evaluated at this equilibrium is

$$\mathbf{J} = \begin{pmatrix} \rho & 0 \\ -k_e & -r \end{pmatrix}. \quad (23)$$

Since $\det \mathbf{J} < 0$, this equilibrium is unstable. Hence if there is a T-cell clone which can recognize the antigen, the T-cell clone will not go extinct.

From this analysis we can conclude that when we allow for net T-cell proliferation, i.e. when $\rho > 0$, a growing antigen will persist in an equilibrium in which it sustains T-cell proliferation. This equilibrium is stable and exists for any parameter setting consistent with $\rho > 0$.

T cell memory

Recent experimental data suggest that T-cell memory is dependent on the persistence of antigen in the system (Gray & Matzinger, 1991). The persisting antigen is supposed to allow for stimulation and renewal of the T cells specific for this antigen. Experiments on the lifetimes of naive and memory T cells support this idea because the data indicate that memory T cells have a shorter lifespan than naive T cells (Michie *et al.*, 1992). Thus T-cell memory may rely on continuous restimulation and proliferation of T cells via retained antigen (or peptides).

Our contribution to these findings is that the intrinsic competition process accounts for regulation of the continued proliferation. Thus, if the concentration of the persisting antigen is low the antigen specific T-cell clone will remain small despite its continuous activation. Previous models for T-cell memory relied on direct regulation of T-cell numbers by T cells (McLean, 1992; Schweitzer *et al.*, 1992). Additionally, the competitive exclusion principle indicates that for each persisting peptide only one T-cell specificity can be maintained in the repertoire.

Note that our results only apply to growing antigens. Non-growing antigens, for example, proteins injected by immunologists, can be described by

$$\dot{A}_j = -rA_j - A_j \sum_i^n k_{ij}^e T_i. \quad (24)$$

This cannot give rise to a non-trivial equilibrium. Such a system can only transiently account for memory by antigen persistence. If the decay of antigen at low concentrations is sufficiently slow, the system may be approximated by eqn (14) in which we describe the T-cell response to a fixed peptide concentration. Such a system has a T-cell equilibrium as long as the peptide concentration is sufficiently large, i.e. $P > d_T K/k_a$.

Affinity

In deriving eqn (1) we introduced the parameter

$$K_{ij} \equiv \frac{k_{ij}^b}{k_{ij}^d + k_{ij}^a}, \quad (7b)$$

which plays the role of an effective affinity in the model. However, suppose that there is some true binding strength κ_{ij} based upon molecular forces between the receptor of clone T_i and a peptide P_j present on a particular MHC molecule. For convenience, we scale these strengths such that $0 \leqslant \kappa_{ij} \leqslant 1$. The different rate constants k_{ij} can then be written as some function of κ_{ij}. As an example we here use linear functions. Thus we write

$$k_{ij}^b = k_b \kappa_{ij}, \quad k_{ij}^a = k_a \kappa_{ij}, \quad k_{ij}^d = k_d(1 - \kappa_{ij}), \quad (25)$$

where k_b, k_a, and k_d are the maximal binding, activation, and dissociation rate constants for a T-cell receptor having a maximal molecular match with a given MHC–peptide complex. Hence

$$K_{ij} = \frac{k_b \kappa_{ij}}{k_d + (k_a - k_d)\kappa_{ij}}. \quad (26)$$

Making the reasonable parameter choice $k_a > k_d$, K_{ij} is strictly positive and has the form of a Michaelis–Menten saturation function in κ_{ij}.

APC Based Models

The general scheme of T-cell activation in which a T cell becomes activated by interacting with peptides presented on an APC is much more complicated than we have assumed in the ecological model. By formulating two more realistic models, we investigate whether our main conclusion, i.e. competitive exclusion, hinges upon our simplifying assumptions. The APC models differ from the ecological one in that there is no conservation of peptides; rather there is conservation of "antigen presentation sites" on

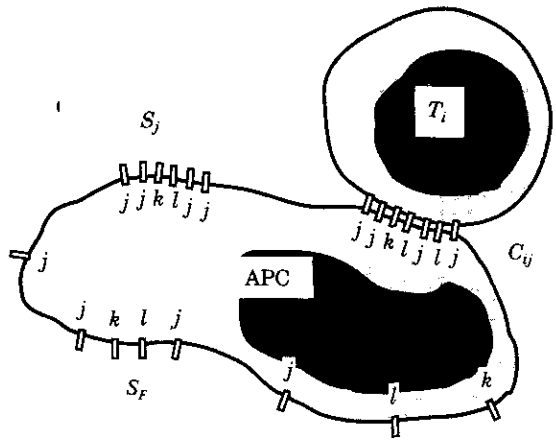

FIG. 2. Sites on an APC denote regions on the APC surface where T cells can bind. A site can be free, S_F, or loaded with peptide j at sufficient density to allow T-cell binding but not have a T cell bound, S_j, or it can be loaded with peptide j and have T cell i bound, thus forming a complex (C_{ij}).

APCs, and there is competition between peptides for getting presented on sites of the APCs.

To simplify the description of T-cell–APC interaction, we assume as in Fishman & Perelson (1993), that T cells interact with the APC over a portion of its surface, which we call a *site* (Fig. 2). Here we assume that sites can be in any of three states. First, a site can be free (S_F), i.e. bound with no peptide or bound with not enough peptide of any single type to enable specific binding and activation of a T cell. Second, a site (S_j) can be presenting peptide j in high enough concentration to enable some T cells to bind and become activated but no T cell may be bound to the site. Third, a site presenting antigen j in high concentration may be filled with T cell i interacting with the peptide. This we call a complex (C_{ij}).

We again consider a system in which there are n T-cell clones, T_i, $i = 1, 2, \ldots, n$, competing for sites on APCs in which one of m possible peptides, P_j, $j = 1, 2, \ldots, m$, can be presented. T cells T_i bind to sites S_j on APCs to form a complex C_{ij}. These complexes dissociate or give rise to an activated T cell, T_i^*. As in the ecological model, the rates of these three processes may depend on the affinity of the T cell for the presented peptide.

For our first "APC" model, consider an activated T cell, T_i^*, that divides at a rate k_p giving rise to two T_i cells. This is depicted in the following scheme

$$T_i + S_j \underset{k_{ij}^d}{\overset{k_{ij}^b}{\rightleftharpoons}} C_{ij} \xrightarrow{k_{ij}^a} T_i^* + S_j, \tag{27a}$$

$$T_i^* \xrightarrow{k_p} 2T_i, \tag{27b}$$

$$P_j + S_F \xrightarrow{k_j^u} S_j \xrightarrow{d_S} S_F, \tag{27c}$$

where k_j^u is the rate at which sites become loaded with peptide j at sufficient density to stimulate a T cell and d_S is the rate of turnover of MHC–peptide complexes. The rate constant k_j^u-encompasses both the rate of processing an antigen and the rate of presenting peptide j.

In Appendix A we write a conservation equation for the total number of sites S_T, we make quasi-steady-state assumptions for the complexes C_{ij}, the sites S_j, and the activated T cells T_i^*, and obtain the T-cell population dynamic equation

$$\dot{T}_i = s + T_i\left(\sum_j^m k_{ij}^a K_{ij} S_j - d_T\right), \tag{28}$$

where K_{ij} is defined by eqn (7b). This says that the rate of T-cell proliferation is proportional to a weighted sum of the S_j, the number of sites presenting peptides. Further, in Appendix A, we derive the following expression for S_j

$$S_j = \frac{S_T K_j^u P_j}{1 + \sum_i^n \sum_j^m K_j^u K_{ij} T_i P_j + \sum_j^m K_j^u P_j}. \tag{29}$$

where $K_j^u \equiv k_j^u / d_S$. This says that S_j, the number of sites presenting peptide P_j, is a saturating function of the free peptide concentration. Thus, according to this model the per capita T-cell proliferation rate is a saturating function of the concentration of antigen.

This is further explored in Fig. 3. In panel (a) we plot S_j as a function of the peptide concentration P_j in the presence and absence of T cells under the condition in which the uptake rate constant k_j^u is the same for all peptides j. When this is the case, we let $K^u \equiv k_j^u / d_S$. Both curves show the saturation effect. According to the data of Adorini *et al.* (1989) and Lorenz *et al.* (1990) the horizontal axis should correspond to a protein concentration in the 0·1–10 μM range. Since the availability of free sites is a decreasing function of the T-cell concentration [see eqn (29)] we plot S_j for $T_i = 0$ and for $T_i = 1$ [see Fig. 3(a)]. Most of the experimental data measure total T-cell proliferation, i.e. thymidine incorporation, as a function of the antigen concentration. These measurements result in sigmoidal/saturating or in convex/humped dose–response functions (Matis *et al.*, 1983; Knight, 1987; Suzuki *et al.*, 1988). In our model thymidine incorporation corresponds to the "total proliferation" term $T_i k_{ij}^a K_{ij} S_j$ in eqn (28). In Figs 3(b) and 3(c) we show that in our model total proliferation is a saturating function of P_j and of T_i. According to the data of Matis *et al.* (1983), T-cell proliferation depends on the product of the concentrations of antigens and MHC molecules.

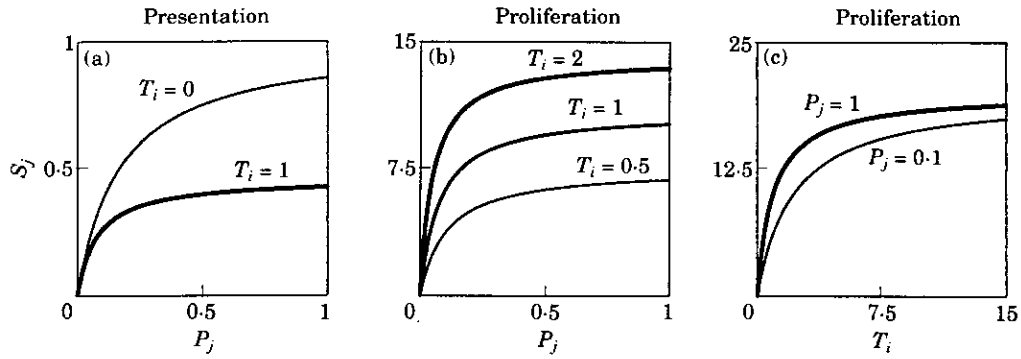

FIG. 3. Presentation and proliferation as a function of the concentrations of peptide and T cells. Parameters: $n = 1$, $m = 1$, $k_a = 20$, $k_b = 24$, $k_d = 4$, $K^u = 6$, $S_T = 1$, $\kappa_{ij} = 1$. (a) The number of sites presenting peptide, i.e. S_j or eqn (A.8), as a function of the peptide concentration P_j for $T_i = 0$ and $T_i = 1$. (b) T-cell proliferation, i.e. $T_i k^a_{ij} K_{ij} S_j$, as a function of the peptide concentration P_j for $T_i = 0{\cdot}5, 1, 2$. (c) T-cell proliferation, i.e. $T_i k^a_{ij} K_{ij} S_j$, as a function of the T-cell concentration T_i for $P_j = 0{\cdot}1$ and $P_j = 1$.

In our model the number of MHC molecules is proportional to S_T. Thus our model is in agreement with these data since S_T determines the height of all saturation functions in Fig. 3 [see eqn (29)]. Our model however can not account for convex dose–response functions, in which the response falls at high doses. This fall may be due to the effects of excess lymphokine production, a phenomenon outside the scope of our model.

In the interests of realism we have also developed a second APC model in which we distinguish various subpopulations of T cells. For each specificity i we distinguish (i) "virgin" cells, T_i^V, which have never seen antigen before; (ii) "memory" cells, T_i^M, which have seen antigen but have reverted to a resting stage, (iii) "activated" cells, T_i^*, which appear by activation of virgin and of memory cells, and (iv) "proliferated" cells, T_i^P, which appear by cell division of activated T cells. The corresponding kinetic scheme is

$$T_i^V + S_j \underset{k^d_{ij}}{\overset{k^b_{ij}}{\rightleftharpoons}} C_{ij}^V \xrightarrow{k^a_{ij}} T_i^* + S_j,$$

$$T_i^M + S_j \underset{k^d_{ij}}{\overset{k^b_{ij}}{\rightleftharpoons}} C_{ij}^M \xrightarrow{k^a_{ij}} T_i^* + S_j,$$

$$T_i^* \xrightarrow{k_p} 2T_i^P \quad \text{and} \quad T_i^P \xrightarrow{k_r} T_i^M, \qquad (30)$$

where k_r is the rate at which proliferated cells revert to the memory stage. The processes involving antigen presentation remain the same as in the first model.

In Appendix B we write a conservation equation for the total number of sites, and make quasi-steady-state assumptions for the complexes C_{ij}, the sites S_j, the virgin T cells T_i^V, the activated T cells T_i^*, the proliferated T cells T_i^P. After some algebra we obtain for the memory T cells

$$\dot{T}_i^M = T_i^M\left(\frac{k_r - d_P}{k_r + d_P}\sum_j^m k^a_{ij} K_{ij} S_j - d_M\right), \qquad (31)$$

where we have set $s = 0$. This is of the same form as eqn (28).

APC results

Since eqns (28) and (31) are of the same form as eqn (1), the principle of competitive exclusion also holds for the two APC models (also see Appendices A and B). This shows the generality of our main result. The form of the competition between the T cells resembles that of the ecological model [compare eqns (9b) and (29)]. In response to fixed peptide concentrations T-cell clones again attain an equilibrium level. In conclusion all results obtained with the ecological model hold true for the two APC models.

PARAMETER VALUES

In the next section we provide some numerical experiments illustrating the principle of competitive exclusion. Before studying our model numerically we need to choose parameter values. We have chosen the parameter $c = 0{\cdot}1$ for scaling the antigen concentration. At this scale the immune response to the antigen introduced at maximum concentration takes a few days to develop. The antigen has a doubling rate of one day, i.e. $r = 1\ \text{day}^{-1}$. To obtain a timescale at which antigen is removed of the order of one week we have chosen $k_e = 0{\cdot}1\ \text{day}^{-1}$. The lifespan of T cells is taken to be 10 days, i.e. $d_T = 0{\cdot}1\ \text{day}^{-1}$. Taking a longer lifetimes does not qualitatively affect the results, but does slow down the competitive exclusion.

The uptake of antigen by APCs, its degradation, and presentation in the form of peptides differs

between the different types of APC. For B cells it takes about 60 min for processed antigen to appear on the cell surface (Lanzavecchia, 1987). However, processed antigen continues to accumulate on the cell surface, and the cell becomes more efficient at stimulating T cells. For example, in experiments using splenic B cells and pigeon cytochrome c (Pc) as the antigen, Lakey *et al.* (1988) found half-maximal T-cell stimulation is achieved after 4 to 5 hr of incubation of B cells with antigen. Once processed, Pc is lost from the surface in 8 to 12 hr (Lakey *et al.*, 1988). If we use the 8 hr lifetime then $k^u \simeq 1/4\ \mathrm{hr}^{-1}$ and $d_s \simeq 1/8\ \mathrm{hr}^{-1}$. In our model we only deal with K^u, the ratio of these estimates. For B cells, $K^u \simeq 2$. For peritoneal exudate cells, which are largely macrophages, Harding & Unanue (1989) found after 15–20 min the cells were capable of presenting antigen, with maximal T cell stimulation occurring after 1 hr. Antigen–MHC complexes were lost from the cell surface with a half-life of 5·5 hr (Harding & Unanue, 1989). Using, say, 0·5 hr for half-maximal stimulation (cf. Harding *et al.*, 1989), then $K^u = 11$. Dendritic cells pick up antigen in the periphery and carry it to a lymph node or to the spleen, where they present it to T cells. It takes of the order of 1 day for a dendritic cell to appear in the T-dependent area of the spleen (Austyn *et al.*, 1988). Dendritic cells exposed to antigen *in vitro* and then injected in an animal stimulate T cells. This responsiveness peaks at 5 days, wanes by day 9–12, and can be rapidly reinduced by rechallenge with antigen-pulsed dendritic cells. From this we infer that dendritic cells lose antigen with a half-life of, say, 5 days. Hence for dendritic cells $K^u \simeq 5$. Given the variability in values, we choose the average value $K^u = 6$.

We assume that a T cell with high affinity will bind to a site on an APC on a timescale of 1 hr, i.e. $k_b = 24\ \mathrm{day}^{-1}$. We also assume that the timescale at which a highly specific T-cell–MHC complex breaks apart by activation or dissociation is on the order of 1 hr. We estimate $k_d = 4\ \mathrm{day}^{-1}$ and $k_a = 20\ \mathrm{day}^{-1}$, i.e. a T cell with high affinity is most likely to become activated before dissociating. Although the parameters involved in antigen presentation do not directly apply to the ecological model we have taken the same values for k_b, k_a, and k_d.

NUMERICAL EXAMPLES

Here we study our ecological model, eqns (13) and (17), by computer analysis. First consider the interaction between one clone of T cells, T_1, responding with maximum affinity, i.e. $\kappa_{11} = 1$, to one antigen, A_1. This yields the two-dimensional system that was studied in eqns (19–23) (see Fig. 4). In Fig. 4(a) the heavy line depicts the solutions of the equation $\dot{A}_1 = 0$ and the light line those of the equation $\dot{T}_1 = 0$. The two nullclines intersect in the stable equilibrium of the system [see eqn (22)] in which the antigen persists maintaining T-cell proliferation. In Fig. 4(b) we show a trajectory representing the response of clone T_1, initially in the virgin state, i.e. $T_1 = s/d_T$, to an antigen dose $A_1 = 0{\cdot}1$. In the initial phase antigen grows. This evokes T-cell proliferation until the antigen concentration drops by immune elimination. The trajectory attains the stable equilibrium. In the stable equilibrium the population level of the T cells is regulated by competition for antigen. As we have argued above this provides the regulation for having T-cell memory on the basis of antigen persistence (Gray & Matzinger, 1991).

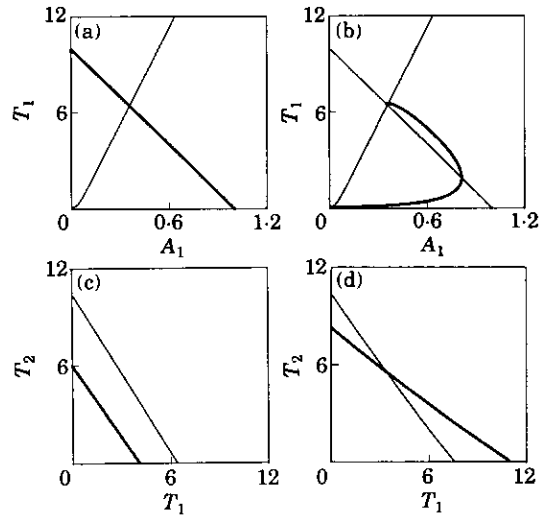

FIG. 4. State space analysis. Parameters: $c = 0{\cdot}1$, $d_T = 0{\cdot}1$, $k_a = 20$, $k_b = 24$, $k_d = 4$, $k_e = 0{\cdot}1$, $K_A = 1$, $r = 1$. (a) Parameters: $n = 1$, $m = 1$, $s = 0{\cdot}01$, $\kappa_{11} = 1$. The light and the heavy lines depict the T_1 and A_1 nullclines, respectively. (b) For the same parameters the heavy line depicts a trajectory from $A_1(0) = 0{\cdot}1$ and $T_1(0) = s/d_T = 0{\cdot}1$. The light lines are the same nullclines. In panels (c) and (d) we make a quasi-steady-state assumption for the antigens, i.e. the systems obeys eqn (18b), in order to study the competition between two T-cell clones. The light and heavy lines depict the T_1 and T_2 nullclines, respectively. (c) Parameters: $n = 2$, $m = 1$, $s = 0$, $\kappa_{11} = 1$, $\kappa_{21} = 0{\cdot}5$. The two parallel nullclines resemble those of competitive exclusion situations in ecosystem models. (d) Parameters: $n = 2$, $m = 2$, $s = 0$, $\kappa_{11} = \kappa_{22} = 1$, $\kappa_{21} = 0{\cdot}5$, $\kappa_{12} = 0{\cdot}25$. The two intersection nullclines allow for stable co-existence of the two clones.

We can study the competition between two T-cell clones by phase plane analysis if we make a quasi-steady-state assumption for the antigens [eqn (18b)]. In Fig. 4(c) and (d) we depict the two qualitatively different nullcline situations corresponding to exclusion and co-existence, respectively. They resemble the two well-known cases in ecological competition models. If the two T-cell clones compete for the same antigen (e.g. $\kappa_{11} = 1$, $\kappa_{12} = 0{\cdot}5$) then their nullclines are

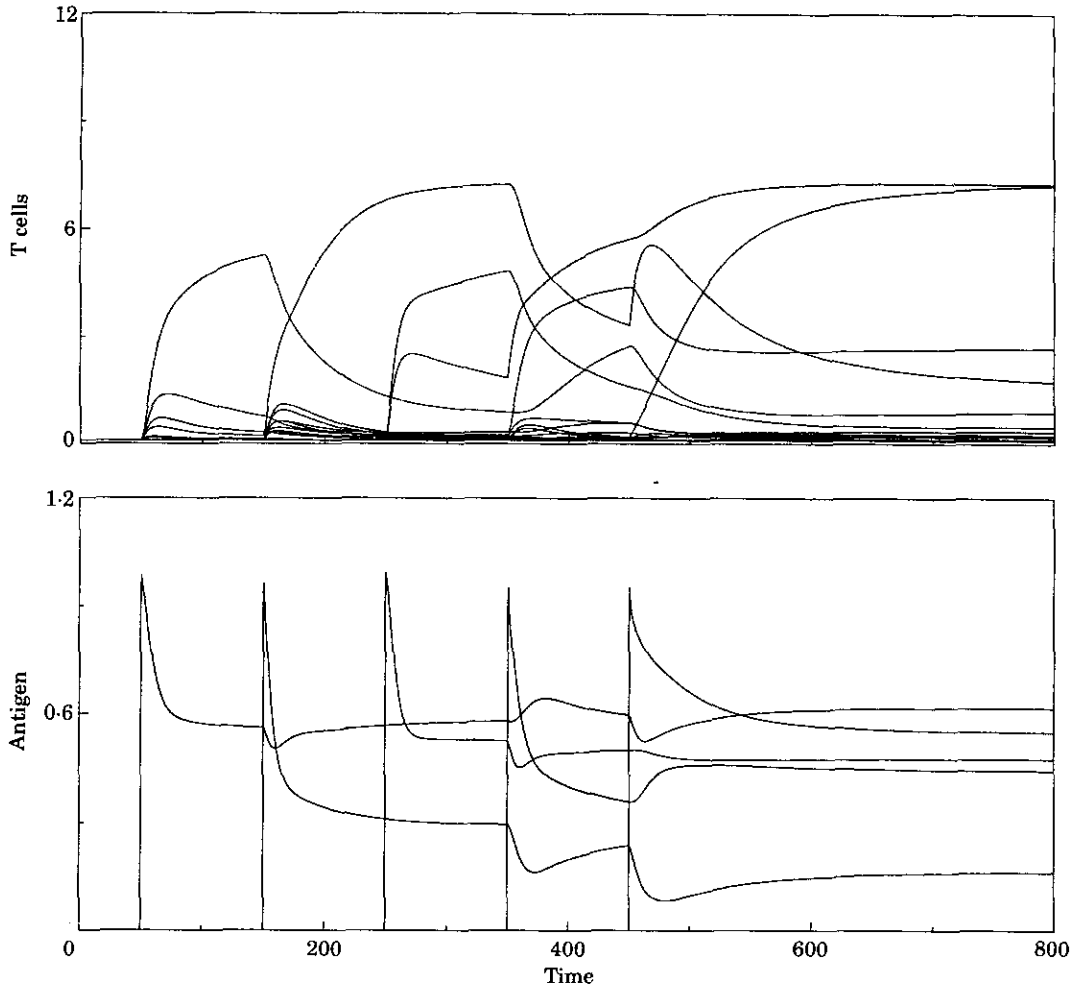

FIG. 5. Competitive exclusion and memory to five foreign antigens. Parameters: $n = 25$, $m = 5$, $c = 0{\cdot}1$, $d_T = 0{\cdot}1$, $k_a = 20$, $k_h = 24$, $k_d = 4$, $k_e = 0{\cdot}1$, $K_A = 1$, $r = 1$, $0 < \kappa_{ij} < 1$, $s = 0{\cdot}01$. The connectivity of the κ_{ij} was 20%. Thus, on average, each antigen was seen by five T-cell clones.

parallel and they exclude each other [Fig. 4(c)]. On the other hand, if the two T-cell clones compete for two antigens (e.g. $\kappa_{11} = 1$, $\kappa_{12} = 0{\cdot}25$, $\kappa_{21} = 0{\cdot}5$, $\kappa_{22} = 1$), they may co-exist because the nullclines intersect in a stable equilibrium [Fig. 4(d)].

High-dimensional systems can no longer be studied by phase plane analysis. By numerical simulation we study a system of 25 T-cell clones responding to five different foreign antigens (see Fig. 5). The affinities of the T cells for these antigens were chosen randomly (see the figure caption for details). Antigens are introduced at day 50, 150, 250, 350 and 450. They all expand initially, they all evoke an immune response involving several T-cell clones, and they all persist afterwards. For each antigen we see several clones responding but ultimately one clone becomes much larger than the others. The others return to a level close to the virgin state and may only increase again in response to another antigen. This illustrates our result on affinity selection. Following the exposure to five antigens four clones persist at a high level in the repertoire. [If the source of virgin cells s were to decrease in time (see Fig. 6), the 21 small clones would disappear entirely].

Diversity

We now come to the most speculative implication of our results. It is generally assumed that the adult T-cell repertoire is largely maintained by peripheral expansion and not by a constant source of virgin cells from the thymus (Rocha *et al.*, 1989; Beverly, 1990). This assumption is based on the observation that thymectomized adults maintain normal T-cell numbers (cf. Helbert *et al.*, 1993). It is not known what form of antigenic stimulation is responsible for activating peripheral T cells to maintain the repertoire. However, owing to the competitive exclusion, the system would have to maintain more than 10^6 different

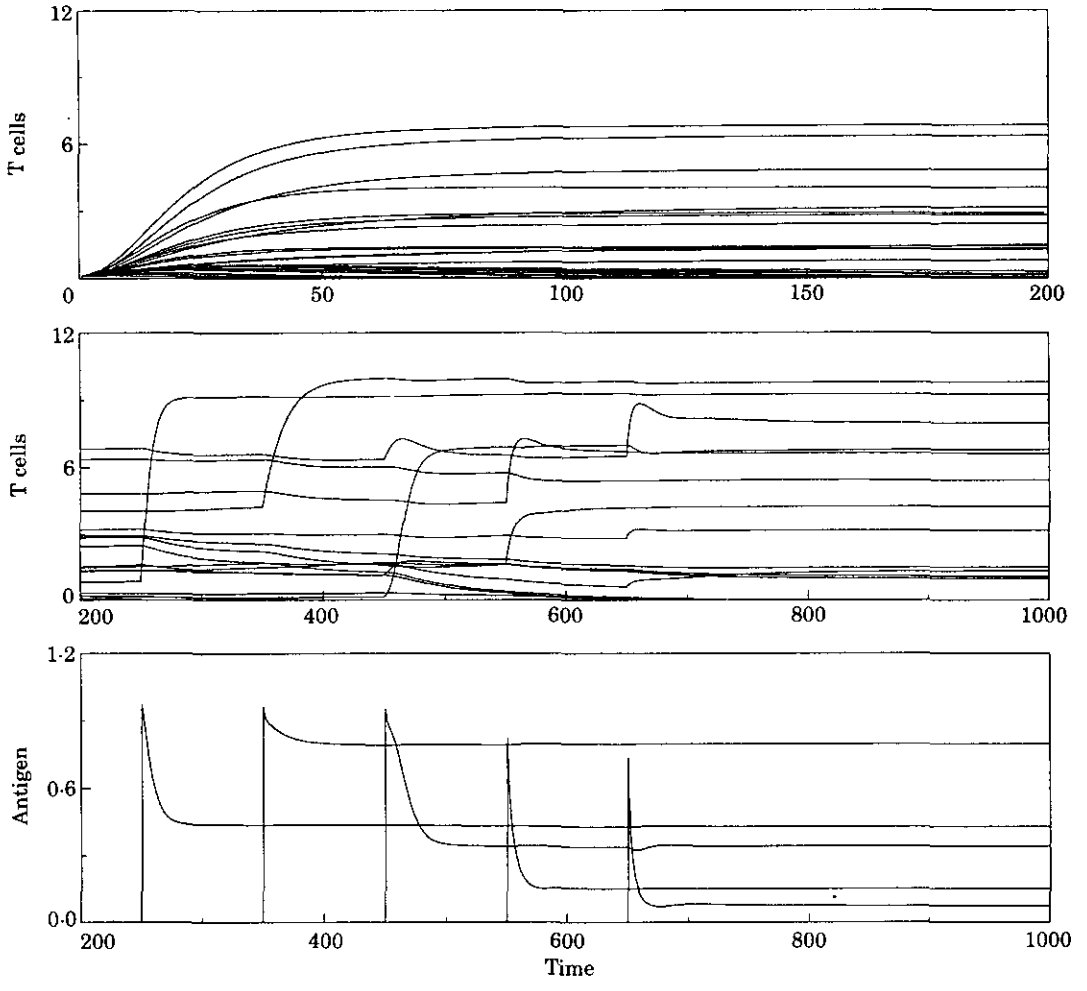

FIG. 6. Repertoires maintained by cross-reactivities with self antigens, and memory to foreign antigens. Parameters: $n = 25$, $m = 105$, $c = 0{\cdot}1$, $d_T = 0{\cdot}1$, $k_a = 20$, $k_b = 24$, $k_d = 4$, $k_e = 0{\cdot}1$, $K_A = 1$, and $r = 1$. In panel (a) $t \leqslant 200$, $s = 0{\cdot}01/(1 + t)$. In panels (b) and (c) $t > 200$, $s = 0$. The connectivity of the κ_{ij} was 20%. The first 100 antigens are considered to be self antigens. They remain present at a fixed concentration and the affinities are distributed between $0 < \kappa_{ij} < 0{\cdot}1$, for $j = 1, \ldots, 100$. The last five antigens are considered to be foreign. They obey eqn (17) and the affinities are distributed between $0 < \kappa_{ij} < 1$, for $j = 101, \ldots, 105$.

antigenic peptides to allow for a T-cell repertoire of 10^6 specificities. It is not known whether the foreign antigens can account for such a diversity. Another hypothesis is that the peripheral expansion is driven by cross-reactivities with self-antigens (Rock & Benacerraf, 1984; Stutman, 1986; Rocha *et al.*, 1989; Beverly, 1990). For such a system our results suggest that the diversity of the T-cell repertoire would be of the order of the number of self antigens in the system. This suggestion is in agreement with an earlier model that was based upon an entirely different approach (De Boer & Perelson, 1993). Note that our results on competitive exclusion only say that the number of T-cell clones, in the absence of a source, must be less than or equal to the number of effectively presented peptides. These peptides could all be foreign, all be self, or a combination of the two. Also, our results on competitive exclusion are equilibrium results. It could take a substantial length of time before clones were eliminated in a competition for peptide. Thus, in the period before equilibrium was reached more clones could be present than peptides. Our numerical results show this effect.

Cross-reactivities with self-antigens are analyzed numerically in Fig. 6. In the first 200 days of the simulation we let 25 T-cell clones respond to 100 self antigens in the absence of foreign antigens. The T cells have low random affinities for the self antigens. (We assume that T cells with high affinities for presented self-peptides would have been eliminated in the thymus.) During this period we allow the source from the thymus to gradually decrease according to $s_t = s/(1 + t)$. At day 200 we set $s = 0$ and we set all T-cell clones that are smaller than s/d_T to zero.

(This method allows us to preclude immune reactions from clones that are not maintained by self renewal.) We find that the repertoire at day 200 is composed of 17 specificities. We then introduce five self-reproducing foreign antigens, one at a time, at days 250, 350, ..., 650. The T cells have random affinities that can be up to ten-fold larger for the foreign antigens than for self antigen. All foreign antigens evoke an immune response and persist afterwards. The presence of the persisting antigens accounts for shifts in the repertoire causing some clones to attain a larger equilibrium and other clones to decrease and/or disappear. This again accounts for memory by persistence of the foreign antigen (Gray & Matzinger, 1991).

Discussion

The data that we have discussed in the Introduction suggested regulation of T-cell numbers at the level of total T-cell numbers (Freitas & Rocha, 1993). It is straightforward to account for such a "global" regulation of T-cell numbers by letting the rates of T-cell proliferation and/or turnover depend on total T-cell numbers. It would be more challenging though if the global regulation were to be an emergent property of the local processes. This has been achieved for the control of total antibody levels by means of idiotypic networks (De Boer & Perelson, 1991). As yet we have no insights on how the same result might be achieved in T-cell repertoires regulated by clonal competition.

In earlier work (Merrill *et al.*, 1994) we have developed a model in which we made a distinction between "local" effects, i.e. clonal competition for antigen, and "global" effects, i.e. regulation at the level of total T-cell numbers. This model allowed us to study the T-cell repertoire as a frequency distribution of clone sizes. We showed that local competition was essential to avoid competitive exclusion. However, the local competition in that model was defined at the level of single clones. Within clones there was competition for binding antigens, but between clones there was only regulation at the level of total T-cell numbers. The competition terms that we derived for the models developed in this paper are more realistic because they involve competition between clones and such competition appears to be intrinsic to the process of T-cell activation.

The two APC models incorporate a second level of competition, namely the competition amongst peptides for antigen presentation. This becomes a problem when a foreign peptide has to compete with a large diversity of self peptides, that may be present at high concentrations (Adorini *et al.*, 1989; Lorenz *et al.*, 1990; Singer & Linderman 1990, 1992). It has been estimated that T-cell activation requires roughly 100–1000 peptide–MHC molecules per B cell or macrophage (Demotz *et al.*, 1990; Harding & Unanue, 1990). These cells have about 10^5 MHC molecules on their surface. Considering the case where all peptide concentrations are equal this implies that the system can only present of the order of 100–1000 different peptides. If some peptides are present at much higher concentrations than others, then even fewer peptides can be effectively presented per APC. Similar problems arise in both our ecological and APC models—the peptide concentration needs to be above some critical level in order to maintain a T-cell clone. One possible solution to this problem is that there may be a large number (10^3–10^4) of distinct microenvironments in the immune system between which peptide concentrations may differ markedly. To study this requires a model with a large number of compartments. This is too complicated as a first model, and thus as an illustration of our main point we have only simulated the simple ecological model.

Our most speculative conclusion, namely, that the diversity of the T-cell repertoire can be determined by the diversity of the environment of self antigens, corresponds very closely to earlier results (De Boer & Perelson, 1993). These earlier results were based upon a very different approach. In that model we studied the repertoire size as a function of the specificity of the T-cell receptor. We required that the functional repertoire respond to an unpredictable set of pathogens. For a given receptor specificity, we calculated the fraction of the repertoire that would be rendered non-functional by self tolerance. Then we searched for the receptor specificity that would fulfill the requirement of being able to reliably recognize foreign antigens with a minimal repertoire. This ideal specificity turned out to be inversely related to the number of self antigens. The corresponding repertoire, after tolerance induction, was then shown to be linearly related to the number of self antigens. Thus, we argued that the immune system is diverse because the self environment is diverse (De Boer & Perelson, 1993).

One difficulty with envisioning the repertoire being maintained by self peptides is that T cells exposed to high concentrations of self peptides in the thymus are deleted from the repertoire. However, even if this is the case, such deletion mechanisms will not eliminate all self reactive T cells. Self peptides that contribute to maintaining the repertoire may be derived from proteins that are not effectively presented in the

thymus. Alternatively, the peptides may be what Sercarz has called "cryptic": peptides that are not normally processed and presented at high enough concentrations to stimulate T cells or lead to their deletion in the thymus, but which may at certain times be upregulated and displayed in an effective or "dominant" form (Sercarz *et al.*, 1993).

The results presented in this paper have all been equilibrium results. Hence, our analysis only shows that clones will *ultimately* be eliminated by competitive exclusion. However, if the time scale of this elimination process is sufficiently long the immune repertoire stays more diverse. Indeed, the immune system need not be operating at equilibrium and the diversity of the immune repertoire may be due to transients. Important parameters determining the behavior of these models, and the validity of our equilibrium analysis, are the rates of T-cell turnover. The T-cell lifetime determines the rate at which clones are eliminated by the competitive exclusion process. Unfortunately, the empirical estimates of T-cell lifetimes (Freitas & Rocha, 1993; Michie *et al.*, 1992) differ by more than three orders of magnitude.

Interestingly, if we do not make the quasi-steady-state assumptions that we have used here to prove competitive exclusion, our models have very complex dynamical behavior. Examples of this have been analyzed before (De Boer & Hogeweg, 1987; Kevrekidis *et al.*, 1988). Thus, we also have to consider co-existence of clones in periodic or chaotic attractors. Because in our models, i.e. eqns (13), (A.15) and (B.8), the maximum growth rate of the clones (i.e. populations) is a linear function of the peptide or site densities (i.e. resources) competitive exclusion is expected to hold for such attractors (Levin, 1970; Kaplan & Yorke, 1977; Armstrong & McGehee, 1980).

However the growth of T-cell clones can also be limited by other factors and need not be limited by antigen (i.e. resources) only. For instance, regulator T cells that are activated by proliferating T cell clones and that inhibit their proliferation may exist (cf. Lohse *et al.*, 1993). Such regulatory T cells would play the role of a predator regulating its prey. Levin (1970) has shown that such additional processes have to be included in the set of "limiting factors" and may thus increase the system diversity. Unfortunately, this remains inconclusive because it is a matter of debate whether or not immune reactions are regulated largely by antigen or by "regulator cells".

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APPENDIX A

Here we show the analysis of our first APC model [scheme (27) in the main text]. For the complexes C_{ij} we write the quasi-steady-state equation

$$\dot{C}_{ij} = k_{ij}^b T_i S_j - C_{ij}(k_{ij}^d + k_{ij}^a) = 0, \qquad \text{(A.1)}$$

where $i = 1, 2, \ldots, n$. This gives

$$C_{ij} = K_{ij} T_i S_j, \qquad \text{(A.2a)}$$

where

$$K_{ij} \equiv \frac{k_{ij}^b}{k_{ij}^d + k_{ij}^a}. \qquad \text{(A.2b)}$$

Owing to conservation of sites we have

$$S_T = S_F + \sum_i^n \sum_j^m C_{ij} + \sum_j^m S_j. \qquad \text{(A.3)}$$

For the sites presenting peptide j we write

$$\dot{S}_j = k_j^u P_j S_F - d_S S_j, \qquad \text{(A.4)}$$

where $j = 1, 2, \ldots, m$. Making quasi-steady state assumptions we obtain

$$S_j = K_j^u P_j S_F, \tag{A.5a}$$

where

$$K_j^u \equiv k_j^u / d_S. \tag{A.5b}$$

Substituting eqn (A.2a) into eqn (A.3) gives

$$S_T = S_F + \sum_i^n \sum_j^m K_{ij} T_i S_j + \sum_j^m S_j. \tag{A.6}$$

Substituting eqn (A.5), and re-ordering, gives

$$S_F = \frac{S_T}{1 + \sum_i^n \sum_j^m K_j^u K_{ij} T_i P_j + \sum_j^m K_j^u P_j}, \tag{A.7}$$

which upon substitution into eqn (A.5) gives

$$S_j = \frac{S_T K_j^u P_j}{1 + \sum_i^n \sum_j^m K_j^u K_{ij} T_i P_j + \sum_j^m K_j^u P_j}. \tag{A.8}$$

This says that the number of sites presenting peptide j is a saturating function of the free peptide concentration P_j. The number of available sites is a decreasing function of the number of specific T cells in the system. These two effects were illustrated in Fig. 3.

For the activated T cells, T_i^*, we write another quasi-steady-state equation

$$\dot{T}_i^* = \sum_j^m k_{ij}^a C_{ij} - k_p T_i^* = 0, \tag{A.9a}$$

hence

$$T_i^* = \frac{1}{k_p} \sum_j^m k_{ij}^a C_{ij}. \tag{A.9b}$$

For the T cells we write

$$\dot{T}_i = s - \sum_j^m k_{ij}^b T_i S_j + \sum_j^m k_{ij}^d C_{ij} + 2k_p T_i^* - d_T T_i, \tag{A.10}$$

where s is the source of T cells from the thymus and d_T is the rate of T-cell turnover. Since $\dot{C}_{ij} = 0$ we may add eqn (A.1) to $\dot{T}_i$. Further, we substitute eqn (A.9b) to obtain

$$\dot{T}_i = s + T_i \left(\sum_j^m k_{ij}^a K_{ij} S_j - d_T \right), \tag{A.11}$$

which says that T-cell proliferation is proportional to S_j, the number of sites presenting peptides.

Equations (A.8) and (A.11) may be combined to obtain the full model

$$\dot{T}_i = s + T_i \left(\frac{S_T}{1 + \sum_l^n \sum_j^m K_{lj} K_j^u T_l P_j + \sum_j^m K_j^u P_j} \times \sum_j^m k_{ij}^a K_{ij} K_j^u P_j - d_T \right), \tag{A.12}$$

which shows that proliferation decreases as a function of the total T-cell concentration and of the total peptide concentration.

We prove competitive exclusion by observing that solving the equilibrium of eqn (A.11), while setting $s = 0$, gives

$$\sum_j^m k_{ij}^a K_{ij} S_j = d_T; \quad i = 1, \ldots, n, \tag{A.13}$$

which has the same linear form in S_j as eqn (2) in the main text has in α_j. Thus, solving eqn (A.13) for S_j can maximally give rise to m solutions. Whenever $n > m$ the system is overdetermined and maximally m T-cell clones can have a non-zero concentration. Thus, our results on competitive exclusion are equally valid for this more realistic model.

Note that if we were to allow for turnover of the activated T cells, i.e. if we were to replace eqn (A.9) by

$$\dot{T}_i^* = \sum_j^m k_{ij}^a C_{ij} - T_i^* (k_p + d_T) = 0, \tag{A.14a}$$

so that at quasi-steady state

$$T_i^* = \frac{1}{k_p + d_T} \sum_j^m k_{ij}^a C_{ij}, \tag{A.14b}$$

we would end up with

$$\dot{T}_i = s + T_i \left(\frac{k_p - d_T}{k_p + d_T} \sum_j^m k_{ij}^a K_{ij} S_j - d_T \right), \tag{A.15}$$

which is similar to eqn (A.11) and similar to the model derived in Appendix B. Thus, allowing for a turnover of activated T cells does not affect our results.

APPENDIX B

Here we present the analysis of our second model in which we distinguish "virgin" cells, T_i^V, "memory" cells, T_i^M, "activated" cells, T_i^*, and "proliferated" cells, T_i^P [see scheme (30) in the text]. By analogy to the analysis of the first model we write

$$\dot{C}_{ij}^V = k_{ij}^b T_i^V S_j - C_{ij}^V (k_{ij}^d + k_{ij}^a) = 0, \tag{B.1a}$$

and

$$\dot{C}_{ij}^M = k_{ij}^b T_i^M S_j - C_{ij}^M (k_{ij}^d + k_{ij}^a) = 0, \quad \text{(B.1b)}$$

or

$$C_{ij}^V = K_{ij} T_i^V S_j, \quad \text{(B.2a)}$$

and

$$C_{ij}^M = K_{ij} T_i^M S_j, \quad \text{(B.2b)}$$

where K_{ij} is defined by eqn (A.2). Owing to conservation of sites we have

$$S_T = S_F + \sum_i^n \sum_j^m (C_{ij}^V + C_{ij}^M) + \sum_j^m S_j. \quad \text{(B.3a)}$$

For the sites presenting peptide j we adopt eqns (A.4) and (A.5). By a similar analysis we arrive at

$$S_j = \frac{S_T P_j}{1/K^u + \sum_i^n \sum_l^m K_{il}(T_i^V + T_i^M) P_l + \sum_l^m P_l}. \quad \text{(B.3b)}$$

For the virgin cells we write

$$\dot{T}_i^V = s - d_V T_i^V - \sum_j^m k_{ij}^b T_i^V S_j + \sum_j^m k_{ij}^d C_{ij}^V,$$

where s is the source for virgin T cells from the thymus and d_V is the turnover rate of virgin cells. Upon addition of eqn (B.1a) and substitution of eqn (B.2a) this gives

$$\dot{T}_i^V = s - T_i^V \left(\sum_j^m k_{ij}^a K_{ij} S_j + d_V \right). \quad \text{(B.4a)}$$

For the memory cells we write

$$\dot{T}_i^M = k_r T_i^P - d_M T_i^M - \sum_j^m k_{ij}^b T_i^M S_j + \sum_j^m k_{ij}^d C_{ij}^M,$$

where d_M is the turnover rate of memory cells. Upon addition of eqn (B.1b) and substitution of eqn (B.2b) this gives

$$\dot{T}_i^M = k_r T_i^P - T_i^M \left(\sum_j^m k_{ij}^a K_{ij} S_j + d_M \right). \quad \text{(B.4b)}$$

For the activated cells we write the quasi-steady-state equation

$$\dot{T}_i^* = \sum_j^m k_{ij}^a (C_{ij}^V + C_{ij}^M) - k_p T_i^* = 0,$$

or

$$T_i^* = \frac{1}{k_p} \sum_j^m k_{ij}^a (C_{ij}^V + C_{ij}^M). \quad \text{(B.4c)}$$

For the proliferated cells we write

$$\dot{T}_i^P = 2k_p T_i^* - T_i^P (k_r + d_P),$$

where d_P is the turnover rate of the proliferated cells. Upon substitution of eqns (B.2) and (B.4c) this gives

$$\dot{T}_i^P = 2(T_i^V + T_i^M) \sum_j^m k_{ij}^a K_{ij} S_j - T_i^P (k_r + d_P). \quad \text{(B.4d)}$$

Making quasi-steady-state assumptions for T_i^V and T_i^P, we obtain

$$T_i^V = \frac{s}{d_V + \sum_j^m k_{ij}^a K_{ij} S_j} \leqslant \frac{s}{d_V}, \quad \text{(B.5a)}$$

where S_j is still a function of T_i^V, and

$$T_i^P = \frac{2}{k_r + d_P} (T_i^V + T_i^M) \sum_j^m k_{ij}^a K_{ij} S_j, \quad \text{(B.5b)}$$

which upon substitution in eqn (B.4b) gives

$$\dot{T}_i^M = \frac{2k_r}{k_r + d_P} (T_i^V + T_i^M) \sum_j^m k_{ij}^a K_{ij} S_j - T_i^M \left(\sum_j^m k_{ij}^a K_{ij} S_j - d_M \right). \quad \text{(B.6)}$$

When $d_P \ll k_r$ we see that this equation:

$$\dot{T}_i^M = 2T_i^V \sum_j^m k_{ij}^a K_{ij} S_j + T_i^M \left(\sum_j^m k_{ij}^a K_{ij} S_j - d_M \right), \quad \text{(B.7)}$$

is of the same form as eqn (A.8) if we consider the first term as a small source (remember $T_i^V \leqslant s/d_V$).

We prove competitive exclusion by rewriting eqn (B.6) while setting $s = T_i^V = 0$:

$$\dot{T}_i^M = T_i^M \left(\frac{k_r - d_P}{k_r + d_P} \sum_j^m k_{ij}^a K_{ij} S_j - d_M \right), \quad \text{(B.8)}$$

where we require $d_P > k_r$. In equilibrium all clones i have to satisfy

$$\sum_j^m k_{ij}^a S_j = d_M \frac{k_r + d_P}{k_r - d_P}, \quad \text{(B.9)}$$

which has the same linear form in S_j as eqn (2) in the text has in α_j. Thus, solving eqn (B.9) for S_j can maximally give rise to m solutions, i.e. maximally m different clones can have a non-zero concentration. Thus, our results on competitive exclusion are equally valid for this more realistic model.