J. theor. Biol. (1995) **175,** 567–576

Towards a General Function Describing T Cell Proliferation

ROB J. DE BOER† AND A. S. PERELSON‡

†*Theoretical Biology, Utrecht University, Padualaan* 8, 3584 *CH Utrecht, The Netherlands and* ‡*Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM* 87545, *U.S.A.*

(*Received on* 3 *October* 1994, *Accepted in revised form on* 3 *February* 1995)

A new function is proposed for describing the rate of T cell proliferation in response to peptides on antigen-presenting cells. The model improves an earlier model of ours by allowing for a true maximum proliferation rate of the T cells. This is achieved by a simple change of variables that markedly relaxes the conditions for a conventional quasi-steady-state assumption. The new model has the same "ecological" properties as the previous one. Thus the natural competition in the model allows for regulation of T cell population size in the presence of continuous stimulation by antigen. An important feature is the competitive exclusion of T cell clones recognizing the same peptide with different affinities allowing for "affinity selection". Models for the population dynamics of experienced, naïve and activated T cells are also developed. These T cell subpopulations compete with one another for antigen. In models with lymphokine production a "proliferation threshold" is obtained that allows for tolerance.

Introduction

During a cellular immune reaction T cells proliferate in response to antigen (typically peptides) presented on antigen-presenting cells (APCs). Models for T cell proliferation are most reasonable when they are based upon a saturation function such that individual T cells have a well-defined maximal rate of proliferation. This is achieved when the proliferation rate saturates as a function of the antigen concentration. An attempted "rigorous" derivation of the T cell proliferation rate, based upon a conventional quasi-steady-state assumption, however yielded a proliferation rate that saturated as a function of the concentration of all T cells recognizing the antigen, but which did not saturate as a function of the antigen concentration (De Boer & Perelson, 1994). Thus this function failed to define a maximal proliferation rate.

Problems arise with this function when the antigen concentration is larger than that of the T cells. Since this is typical during the initial phase of an immune response we think it is important to improve upon our earlier work (De Boer & Perelson, 1994). Borghans *et al*. (1995) show that the problem is due to a violation of the conditions of the quasi-steady-state assumption, and that a solution of the problem can be obtained by changing variables to "total T cells". This yields a simple "competitive saturation" function with a well-defined maximum proliferation rate. Here we generalize the results in Borghans *et al*. (1995) to account for case in which several T cell clones interact with one antigen. We also show that this new model can be applied to a system in which T cell clones are decomposed into naïve, experienced and activated T cell sub-populations.

Our results lead to an ecological view of the immune system. T lymphocyte populations compete for resources, i.e., for antigens presented as peptides on the surface of APCs. Thus the ecological principle of competitive exclusion applies and indeed is a consequence of the basic requirement of T cells interacting with APCs in order to become activated (De Boer & Perelson, 1994; Fishman & Perelson, 1995). In the immunological literature similar ecological views are now being expressed. Cyster *et al*. (1994) interpret their recent data on selective exclusion

Emails: rdb@alive.biol.ruu.nl and asp@receptor.lanl.gov.

of self-reactive B cells from the recirculating B cell repertoire by cellular competition for niches in lymphoid follicles. Freitas & Rocha (1993) discuss how competition may regulate total lymphocyte populations.

Previous Model

De Boer & Perelson (1994) modeled T cell growth on the basis of schemes similar to

$$T_i + S \underset{k_i^d}{\overset{k_i^b}{\rightleftarrows}} C_i \xrightarrow{k_i^p} 2T_i + S, \tag{1}$$

where S is the concentration of sites on APCs, such as dendritic cells, presenting a specific peptide from an antigen, and T_i, $i=1,\ldots,n$, is a set of T cell populations all of which recognize the peptide but with different affinities. The concept of a site on an APC is discussed in De Boer & Perelson (1994) and how the number of sites depends on the antigen concentration is dealt with later in this paper [see eqns (29)–(31)]. Here k_i^b, k_i^d, and k_i^p are rate constants for binding, dissociation and activation/proliferation, respectively. These constants may all be a function of the affinity of T cell receptor i for the peptide presented on the MHC of the APC. The differential equations describing the model are

$$\frac{dT_i}{dt} = -k_i^b T_i S + (k_i^d + 2k_i^p) C_i, \tag{2}$$

$$\frac{dC_i}{dt} = k_i^b T_i S - (k_i^d + k_i^p) C_i, \tag{3}$$

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

$$S = \bar{S} - \sum_{i=1}^{n} C_i, \tag{4}$$

and where $\bar{S}$ is the total concentration of available sites. Making a quasi-steady-state assumption for C_i gives

$$C_i = K_i T_i S, \quad \text{where} \quad K_i \equiv \frac{k_i^b}{k_i^d + k_i^p}. \tag{5a,b}$$

Note that K_i is some function of the affinity between T_i and the presented peptide (cf De Boer & Perelson, 1994). Substituting eqn (5a) into eqn (4) gives

$$S = \frac{\bar{S}}{1 + \sum_{j=1}^{n} K_j T_j}. \tag{6}$$

Since at quasi-steady state, $dC_i/dt = 0$, we can add the right-hand side of eqn (3) to (2) to obtain

$$\frac{dT_i}{dt} = k_i^p C_i = \frac{k_i^p K_i T_i \bar{S}}{1 + \sum_{j=1}^{n} K_j T_j}. \tag{7}$$

Thus we "naturally" obtain a term indicating that there is competition between T cells for antigen-presenting sites.

Adding a source and decay term, we thus proposed the following model of T cell growth

$$\frac{dT_i}{dt} = \sigma + T_i \left(\frac{k_i^p K_i \bar{S}}{1 + \sum_{j=1}^{n} K_j T_j} - \delta \right), \tag{8}$$

where σ is the source of naive T cells from the thymus, and δ defines the T cell death rate. For a single T cell population this gives

$$\frac{dT}{dt} = \sigma + T \left(\frac{\rho \bar{S}}{1/K + T} - \delta \right), \tag{9}$$

where $\rho \equiv k_i^p$ is a proliferation parameter. The properties of eqn (8) and (9) are discussed fully by De Boer & Perelson (1994). The model is similar to ecological models for populations exploiting a limited resource. Thus, we demonstrated that eqn (8) accounts for competitive exclusion, and that eqn (9) accounts for a carrying capacity when $\bar{S}$ has some fixed value. Both results remain valid for our new model below.

The main problem with this model is that it has an unbounded per capita rate of T cell growth, i.e. as $\bar{S} \to \infty$, cells grow at an infinite per capita rate.

New Model

Following Borghans *et al*. (1995) we resolve these difficulties by changing variables to total T cells

$$\bar{T} \equiv T_i + C_i. \tag{10}$$

The new equations are

$$\frac{d\bar{T}_i}{dt} = \frac{dT_i}{dt} + \frac{dC_i}{dt} = k_i^p C_i, \tag{11}$$

$$\frac{dC_i}{dt} = k_i^b \left[\left(\bar{S} - \sum_{j=1}^{n} C_j \right) (\bar{T}_i - C_i) - C_i / K_i \right] = 0. \tag{12}$$

Assuming $k_i^b \neq 0$ for all i, and making the quasi-steady state assumption on all C_i yields a system of the n dependent quadratic equations defined by eqn (12).

Since it is not feasible to solve these equations exactly, we approximate the solutions by assuming that the concentrations of the complexes are sufficiently small so that we can ignore all C_iC_j terms. The same result can also be obtained by making more sophisticated approximations (Cha & Cha, 1965; Borghans *et al.*, 1995). In the Appendix we show that these approximations give

$$C_i \simeq \frac{K_i \bar{T}_i \bar{S}}{1+K_i\bar{S}+\sum_{j=1}^{n} K_j \bar{T}_j \dfrac{1+K_i\bar{S}}{1+K_j\bar{S}}}. \tag{13}$$

Thus, we propose as a general T cell population dynamic equation

$$\frac{\mathrm{d}\bar{T}_i}{\mathrm{d}t} = \sigma + \bar{T}_i\left(\frac{k_i^p K_i \bar{S}}{1+K_i\bar{S}+\sum_{j=1}^{n} K_j \bar{T}_j \dfrac{1+K_i\bar{S}}{1+K_j\bar{S}}} - \delta\right), \tag{14}$$

where σ and δ are defined as above.

For a single T cell population eqn (14) reduces to

$$\frac{\mathrm{d}\bar{T}}{\mathrm{d}t} = \sigma + \bar{T}\left(\frac{\rho\bar{S}}{1/K+\bar{S}+\bar{T}} - \delta\right). \tag{15}$$

Note that the proliferation rate is now defined by a "competitive saturation" term, and ρ defines a true maximum proliferation rate per T cell. This is the main result of this paper.

Ignoring the source term by setting $\sigma = 0$, we find the carrying capacity of eqn (15) to be

$$\bar{T} = \bar{S}(\rho/\delta - 1) - \frac{1}{K}. \tag{16}$$

Thus, as in the previous model, in response to continuous stimulation by a fixed antigen concentration a T cell population attains an equilibrium density wherein proliferation balances death. However, if $\bar{S} < [K(\rho/\delta - 1)]^{-1}$, there is too little "resource" to sustain the T cell population: the only equilibrium solution is $\bar{T} = 0$.

The new model also accounts for competitive exclusion. To see this, assume $\sigma = 0$, and consider an antigen giving rise to a fixed concentration $\bar{S}$. Then we may rewrite the non-trivial equilibrium of eqn (14) for population i as

$$\frac{k_i^p \alpha_i}{(1+\alpha_i)(1+\beta)} = \delta, \tag{17}$$

where

$$\alpha_i \equiv K_i\bar{S}, \quad \text{and} \quad \beta \equiv \sum_{j=1}^{n} K_j \bar{T}_j/(1+K_j\bar{S}).$$

Note that β is the same for all populations i; however, α_i and k_i^p need not be. Assuming that k_i^p and K_i are monotonically increasing functions of the true affinity of T_i for this antigen, and assuming that all clones have a different affinity, we can order all clones with respect to their affinity. Thus if clone 1 has the largest affinity, and forms a non-trivial equilibrium by satisfying eqn (17), all other clones fail to attain a non-zero equilibrium. Since $k_i^p\alpha_i/(1+\alpha_i)(1+\beta)$ is maximal for clone number 1 the other clones will have a rate of proliferation that is smaller than δ, as a consequence they can only decrease. This argument implies that a stable equilibrium will be reached with only the highest affinity clone present. The model thus exhibits "affinity selection", i.e. the clone with the highest affinity is expected to outcompete all others during an immune response (De Boer & Perelson, 1994; Fishman & Perelson, 1995).

A major difference between the two models is that the T cell variable in the new model is total T cells, whereas in the previous model the variable represented only the T cells not bound to APCs.

T cell Subpopulations

A T cell population interacting with antigen may be viewed as being composed of subpopulations of naïve (T_N), experienced (T_E), and activated (T_A) T cells. Here we consider a simplified version of a scheme proposed by De Boer & Perelson (1994)

$$T_N + S \underset{k_N^d}{\overset{k_N^b}{\rightleftharpoons}} C_N \xrightarrow{k_N^a} T_A + S, \tag{18a}$$

$$T_E + S \underset{k_E^d}{\overset{k_E^b}{\rightleftharpoons}} C_E \xrightarrow{k_E^a} T_A + S, \tag{18b}$$

$$T_A \xrightarrow{p} 2T_E. \tag{18c}$$

With respect to the complexes this scheme is identical to scheme (1). Thus if we define

$$\bar{T}_N \equiv T_N + C_N, \quad \text{and} \quad \bar{T}_E \equiv T_E + C_E, \tag{19}$$

we obtain the equivalent of eqn (13), i.e.

$$C_N \simeq \frac{K_N \bar{T}_N \bar{S}}{1+K_N\bar{S}+K_N\bar{T}_N+K_E\bar{T}_E \dfrac{1+K_N\bar{S}}{1+K_E\bar{S}}}, \tag{20a}$$

$$C_E \simeq \frac{K_E \bar{T}_E \bar{S}}{1+K_E\bar{S}+K_E\bar{T}_E+K_N\bar{T}_N \dfrac{1+K_E\bar{S}}{1+K_N\bar{S}}}, \tag{20b}$$

where

$$K_N \equiv \frac{k_N^b}{k_N^d + k_N^a} \quad \text{and} \quad K_E \equiv \frac{k_E^b}{k_E^d + k_E^a}. \tag{21}$$

The proliferation of T cells is generally controlled by lymphokines, e.g. IL-2, which are produced by activated T cells. If we assume that lymphokines L are produced at rate k_S per activated T cell and destroyed as a first process with rate constant d_L, then

$$\frac{dL}{dt} = k_S T_A - d_L L. \tag{22}$$

At quasi-steady-state

$$L = \frac{k_S}{d_L} T_A. \tag{23}$$

We assume that the proliferation rate is $pf(L)$, where $f(L)$ is a simple saturation function of the lymphokine concentration, i.e.

$$f(L) = \frac{L}{\hat{\theta} + L}, \tag{24}$$

and $\hat{\theta}$ is the lymphokine concentration at which proliferation is half maximal. Using eqn (23),

$$f(L) = f(T_A) = \frac{T_A}{\theta + T_A}, \tag{25}$$

where $\theta = d_L \hat{\theta} / k_S$.

Thus, after making quasi-steady-state assumptions the differential equations become

$$\frac{d\bar{T}_N}{dt} = \sigma - d_N \bar{T}_N - k_N^a C_N, \tag{26}$$

$$\frac{d\bar{T}_E}{dt} = 2pT_A f(L) - d_E \bar{T}_E - k_E^a C_E, \tag{27}$$

$$\frac{dT_A}{dt} = k_N^a C_N + k_E^a C_E - d_A(1 - f(L))T_A - pf(L)T_A, \tag{28}$$

where we assume that activated cells die or become anergic at a rate d_A if they do not see a sufficient concentration of lymphokine. If they do see enough lymphokine then they proliferate into two experienced daughter cells. These assumptions are translated into a model by assuming that a fraction of activated cells, $f(L)$, proliferate and the remaining fraction, $1 - f(L)$ become anergic or die.

Antigen

One can derive a simple expression for the concentration of sites presenting a certain peptide, $\bar{S}$, as a function of the antigen concentration, say A, by considering

$$A + S_F \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \bar{S}, \tag{29}$$

where $S_F \equiv S_T - \bar{S}$ is the concentration of sites not presenting this antigen and S_T is the total concentration of sites available in the local environment. Writing

$$\frac{d\bar{S}}{dt} = k_1 A(S_T - \bar{S}) - k_{-1}\bar{S} = 0, \tag{30}$$

we obtain

$$\bar{S} = S_T \frac{A}{\kappa + A}, \tag{31}$$

where $\kappa \equiv k_{-1}/k_1$. This function can be substituted in any of the above proliferation functions.

A simple differential equation for a growing antigen is

$$\frac{dA}{dt} = rA(1 - cA) - A \sum_{i=1}^{n} k_i^e T_i, \tag{32}$$

where r is the growth rate of the antigen, and k_i^e is the rate of antigen elimination by T cells. Here $1/c$ is the maximum antigen concentration. In the subpopulation model, T_i in eqn (12) is replaced by T_A since we assume only activated cells act as effectors or produce the lymphokines needed by effectors. The equilibria are

$$A = 0, \quad \text{and} \quad A = \frac{1}{cr}\left[r - \sum_{i=1}^{n} k_i^e T_i \right]. \tag{33}$$

For growing antigens, i.e. $r > 0$, we can scale A such that $c = 1$ without loss of generality. We model non-growing, i.e., decaying, antigens by setting $r > 0$ and $c = 0$. Note that when $r > 0$ the only equilibrium is $A = 0$.

Parameter Values

We consider a local immune reaction in, for instance, a T cell area of a lymph node. Our population sizes are measured as number of cells in the local region. Thus we assume that there are say 10^3 APCs available for antigen presentation. If each APC can interact with ten lymphocytes at a time, i.e. if each APC has ten sites, we obtain $S_T = 10^4$. Since the antigen concentration is scaled between $0 \leqslant A \leqslant 1$, we choose the saturation constant of the presentation process to be $\kappa = 0.1$. This means that maximally 91% of the locally available APCs can present this antigen [see eqn (31)], $0 \leqslant \bar{S} < 0.91 \times 10^4$. The rate at which antigen grows or decays might vary between say $-0.01\ \text{day}^{-1} \leqslant r \leqslant 1\ \text{day}^{-1}$. Choosing

$k^e = 0.01$ day^{-1}, eqn (33) implies that a population of about a hundred activated T cells is required for the elimination of a growing antigen in the local region (for $r = 1$ day^{-1}).

When all $S_T = 10^4$ sites on the APCs are presenting the same peptide, we assume that a T cell with high affinity for this peptide will bind to one of these sites on a time scale of ten minutes, i.e., $k_b = 144 \times 10^{-4}$ day^{-1} site^{-1}. Note that we have made a mistake in our previous paper (De Boer & Perelson, 1994), where k_b had units day^{-1}. Since the corrected value of k_b remains large, the model behavior is not sensitive to this error.

We also assume that the time scale at which a highly specific T-cell–MHC complex breaks apart by activation or dissociation is of the order of 1 hour. We estimate $k_d = 4$ day^{-1} and $k_a = 20$ day^{-1}, i.e. a T cell with high affinity is most likely to become activated before dissociating. Thus we consider $K_i = 6 \times 10^{-4}$ site^{-1} to be a high affinity interaction. A typical rate of T cell proliferation is on the order of one doubling per day. We thus assume that activated cells either become anergic, die or proliferate at a time scale of one day, i.e. $d_A = 1$ day^{-1} and $p = 1$ day^{-1}. We assume naive cells to have a intermediate life, i.e. $d_N = 0.1$ day^{-1} and assume experienced cells to be long-lived, i.e. $d_E = 0.01$ day^{-1}. Choosing $\theta = 10$ we assume that the lymphokine production of ten activated cells suffices for half maximal proliferation. Assuming that all T cells specific for the antigen localize in the compartment under consideration, the source term σ is the rate at which each specificity is produced in the thymus. Hence, a typical value is $\sigma = 1$ cell day^{-1}.

In the simple model of eqn (14) in which we have not considered T cell subpopulations we have lumped proliferation and activation into one step, and k_a is replaced by k_p, which is a maximum proliferation rate. We here choose $k_p = \rho = 2$ day^{-1} and $\delta = 1$ day^{-1}, i.e. a doubling time and a lifetime of 1 day. The immune response generated by the two models can be made equivalent by again choosing $K_i = 6 \times 10^{-4}$ site^{-1} as a high affinity interaction.

Numerical Results

Our models have been derived by making a Michaelis–Menten type quasi-steady-state assumptions for the biochemical processes. Thus we assume that the time scale of T cell activation by binding APCs is much faster than that of proliferation and death. The standard Michaelis–Menten quasi-steady-state assumption is however invalid when the reaction rate is limited by the substrate (here T) rather than the enzyme (here S) (Segel, 1988; Segel & Slemrod, 1989). In our old model, which used the standard Michaelis–Menten quasi-steady-state analysis, this gave rise to unrealistically high rates of T cell division when $S \gg T$. By making the change of variables to total substrate (here $\bar{T}$) this problem is avoided (Borghans *et al*., 1995). Indeed, we see that after our change of variables the proliferation function has a true maximum.

We illustrate these results numerically by comparing the immune response of the old quasi-steady-state model [eqn (8)], the exact model [eqns (2–3) or (11–12)], and the new quasi-steady-state model [eqn (14)], all coupled to antigen dynamics given by eqns (31) and (32). For low and high antigen concentrations, the new model and the exact model behave similarly. The old model deviates by exhibiting oscillations for growing antigens, and by showing a huge peak of proliferation during the initial phase of the response to a large dose of antigen (see Fig. 1).

Memory

All of the immume responses in Fig. 1 have a time scale of about a week, which is biologically realistic. The immune responses to the growing antigen all attain an equilibrium in which the antigen and the T cells persist at $\bar{T} \simeq 91$ and $A \simeq 0.09$. Thus a small amount of antigen persists maintaining the stimulation of the T cell population. This may account for immune memory (Gray & Matzinger, 1991). Since the concentration of the persisting antigen is low, the antigen-specific T cell clone will remain small, despite its continuous activation. This is unlike some previous models for T cell memory, which 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 (De Boer & Perelson, 1994).

The size of the T cell population in this equilibrium is determined by our parameter choice $k^e = 0.01$: about a hundred T cells are required to constrain the growth of the antigen. We further investigate this equilibrium in the phase plane of A and $\bar{T}$ depicted in Fig. 2. The nullclines of A and $\bar{T}$ intersect in a stable equilibrium in which the (growing) antigen is controlled by an increased T cell population. The stability of the equilibrium can be checked graphically by the direction of the vector field in the local neighborhood of the equilibrium. Thus, the qualitative Jacobian is

$$J = \begin{pmatrix} \dfrac{\partial(\mathrm{d}A/\mathrm{d}t)}{\partial A} & \dfrac{\partial(\mathrm{d}A/\mathrm{d}t)}{\partial \bar{T}} \\ \dfrac{\partial(\mathrm{d}\bar{T}/\mathrm{d}t)}{\partial A} & \dfrac{\partial(\mathrm{d}\bar{T}/\mathrm{d}t)}{\partial \bar{T}} \end{pmatrix} = \begin{pmatrix} - & - \\ + & - \end{pmatrix}. \qquad (34)$$

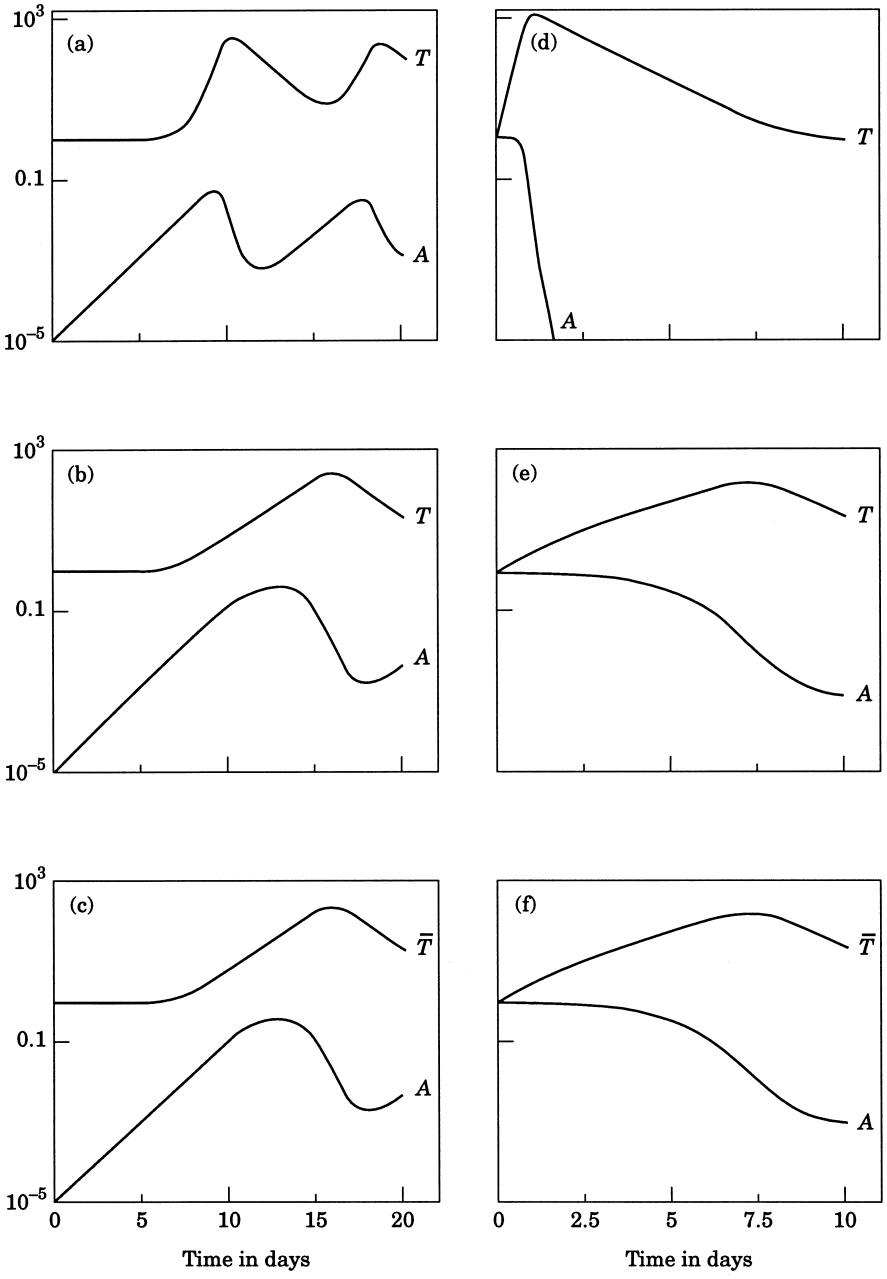

FIG. 1. Immune responses to small and large doses of antigen. From top to bottom we depict the old quasi-steady-state, the exact, and the new quasi-steady-state model, respectively. See the text for further explanation. Parameters: $\delta = 1$ day^{-1}, $k^e = 0.01$ day^{-1}, $K = 6 \times 10^{-4}$ site^{-1}, $k^p = 2$ day^{-1}, $\kappa = 0.1$, $\sigma = 1$ day^{-1}, and $S_T = 10^4$. In panels (a)–(c) a small dose of a growing antigen (i.e., $A_0 = 10^{-3}$, $r = 1$ day^{-1}, $c = 1$) evokes a similar immune response in all three models. In panels (d)–(f) a large dose of a decaying antigen (i.e., $A_0 = 1$, $r = -0.01$ day^{-1}, $c = 0$) evokes an unrealistic immune response in the old quasi-steady-state model, and a similar immune response in the exact and new quasi-steady-state model.

The equilibrium is always stable because trace $J < 0$ and determinant $J > 0$. The other equilibrium $\bar{T} = \sigma/\delta = 1$, $A = 0$ is the naïve/virgin state of the system. It is unstable against the introduction of antigen.

Note that the memory results only apply to growing antigens. Decaying antigens cannot persist in a stable equilibrium. They may transiently however stimulate the T cells and thus maintain a short-term memory.

T CELL SUBPOPULATIONS

In Fig. 3 we depict the immune response of the subpopulation model to a small growing and a large decaying antigen (for $\sigma = 2$ and $\sigma = 1$, respectively).

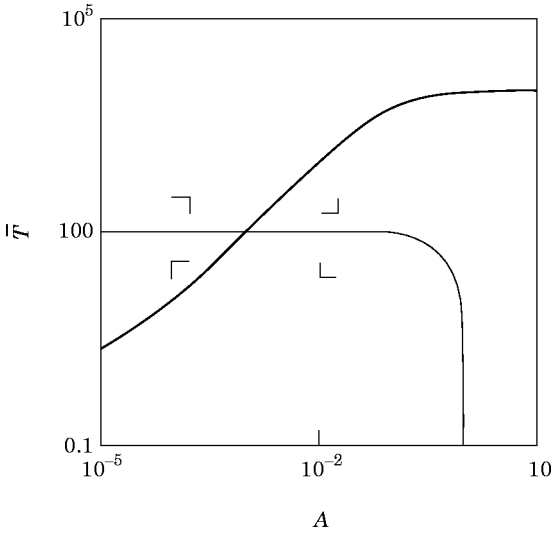

FIG. 2. Nullclines of the simple model. Parameters as in Fig. 1 with $r=1$ day^{-1}, $c=1$, i.e. a growing antigen with a carrying capacity of 1. The heavy line is the A-nullcline, the light line is the $\bar{T}$-nullcline. The horizontal part of the A-nullcline shows that it takes about a hundred T cells to control the antigen. The vertical part corresponds to the carrying capacity.

The typical time scale of the immune response in panels (b) and (c) is again a week, which seems realistic. We observe that once the antigen concentration drops most of the T cells revert to the long-lived stage of experienced T cells. When antigen concentrations are high the naïve T cells disappear due to depletion by activation. The response to the growing antigen again attains a "memory equilibrium" with $T_A \simeq 100$. However, because most of the T cells are in the experienced stage, the total T cell population is much larger. In the case of the non-growing antigen the activated cells disappear as the antigen is eliminated.

The quadratic dependence of proliferation on the number of activated cells, which is due to the lymphokine production [see eqn (27)], may lead to a critical density of total T cells below which proliferation fails to get started (De Boer & Hogeweg, 1987; Kevrekidis & Perelson, 1988). We have called this the "proliferation threshold". This is shown in Fig. 3(a) in which $\sigma=1$. Stimulating the system with a growing antigen in the naïve state $\bar{T}_N=10$, $\bar{T}_E=T_A=0$ we attain a stable "non-responsive" equilibrium $A\simeq 1$, $T_A\simeq 1$ and $\bar{T}_N\simeq\bar{T}_E\simeq 0$. During the immune response the naïve T cells are slowly activated and die, or become anergic, without much proliferation. Introducing a similar antigen at high concentration [Fig. 3(c)] we obtain a strong immune response. Panels (a) and (c) together show low zone tolerance (see also De Boer & Hogeweg, 1987).

The proliferation threshold is illustrated by the bifurcation analysis depicted in Fig. 4. Changing σ or θ as a bifurcation parameter we continue both the memory equilibrium and the non-responsive equilibrium. The latter is involved in a saddle-node equilibrium at $\sigma\simeq 1.7$ [$\theta=10$, Fig. 4(a)] and $\theta\simeq 5.8$ [$\sigma=1$, Fig. 4(b)]. Thus immune responses are only possible when $\sigma>1.8$ [Fig. 4(a)] or $\theta<5.8$ [Fig. 4(b)], otherwise the system gets trapped in the non-responsive equilibrium due to a lack of lymphokine production. The total T cell density in the saddle equilibrium that is born at the bifurcation point in fact defines the proliferation threshold: it is this density that has to be exceeded for proliferation to initiate.

Following σ and θ as bifurcation parameters we studied a two parameter continuation of the saddle-node bifurcation (not shown). The continuation formed a straight line. Thus decreasing σ has the same effect on the proliferation threshold as increasing θ.

Discussion

Using quasi-steady-state analysis we have derived a new saturation function to describe the per capita rate of T cell proliferation [see eqns (14) and (15)]. For a single T cell clone this leads to the following T cell population dynamic equation:

$$\frac{\mathrm{d}\bar{T}}{\mathrm{d}t}=\sigma+\bar{T}\left(\frac{\rho\bar{S}}{1/K+\bar{S}+\bar{T}}-\delta\right), \tag{15}$$

where $\bar{T}$ is the total T cell population, σ is the rate of creation of new T cells, ρ is the maximum proliferation rate, δ is the per capita T cell death rate, $\bar{S}$ is an "antigen concentration" here measured as the concentration of "antigen presenting sites" on APCs, and K is an affinity like parameter measuring the tendency of the T cell to bind and become activated by the antigen presenting site. Note that the per capita rate of proliferation is given by

$$f(\bar{T},\bar{S})=\frac{\rho\bar{S}}{1/K+\bar{S}+\bar{T}}. \tag{35}$$

The function has two important properties: (i) at high antigen concentration the function saturates, so that the rate of T cell division remains bounded, and (ii) the function decreases with increasing numbers of T cells owing to competition among T cells for antigen. Further, if we examine the *net* per capita rate of T cell proliferation by subtracting the per capita rate of cell death, then we observe a third important property; (iii) net expansion only occurs if the antigen concentration is above a threshold level (here given by $K(\rho/\delta-1)^{-1}$).

The function (35) is monotonically increasing in antigen presenting sites, $\bar{S}$. Thus we expect that as the antigen concentration or the number of APCs

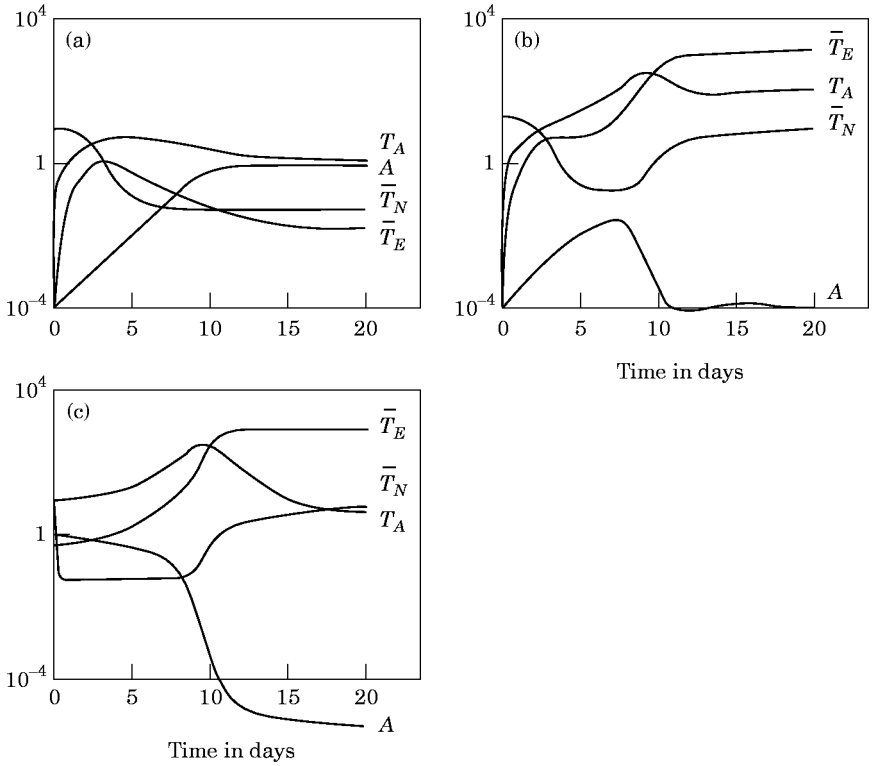

FIG. 3. Immune responses of the subpopulation model to small and large doses of antigen. Parameters $d_A = 1\ \text{day}^{-1}$, $d_E = 0.01\ \text{day}^{-1}$, $d_N = 0.1\ \text{day}^{-1}$, $k^e = 0.01\ \text{day}^{-1}$, $K_N = K_E = 6 \times 10^{-4}\ \text{site}^{-1}$, $k_N^a = k_E^a = 20\ \text{day}^{-1}$, $\theta = 10$, $\kappa = 0.1$, $\sigma = 1\ \text{day}^{-1}$, and $S_T = 10^4$. In panel (a) we set $\sigma = 1\ \text{day}^{-1}$ and introduce a small dose of a growing antigen (i.e., $A_0 = 10^{-3}$, $r = 1\ \text{day}^{-1}$, c = 1). This leads to tolerance. In panel (b) we set $\sigma = 2\ \text{day}^{-1}$ and introduce a small dose of a growing antigen (i.e. $A_0 = 10^{-3}$, $r = 1\ \text{day}^{-1}$, c = 1). This leads to immunity by persistence of antigen. In panel (c) we set $\sigma = 1\ \text{day}^{-1}$ and introduce a large dose of a decaying antigen (i.e., $A_0 = 1$, $r = -0.01\ \text{day}^{-1}$, $c = 0$). Antigen is removed in about 10 days. In all cases antigen is introduced into the naïve state of the system, i.e. $\bar{T}_N = \sigma/d_N$, $\bar{T}_E = T_A = 0$.

increase, the T cell proliferation rate should increase. While some experimental data shows this pattern (Taylor *et al*., 1987; Go & Miller, 1992; Croft *et al*., 1992), other data suggest that the rate of T cell proliferation may decrease when antigen concentrations become very high. This high dose inhibition gives rise to bell or "hump-shaped" dose-response functions when response is plotted against the logarithm of the antigen concentration (cf. Matis, 1983; Knight, 1987; Suzuki *et al*., 1988; Sebzda *et al*., 1994). This decrease in proliferation may be an effect of excess cytokine or may involve other processes of anergy or tolerance induction that we have not yet considered in our model. Thus, while our model has a number of biologically reasonable properties, e.g. (i)–(iii) above, further work may be needed to describe T cell behavior at high antigen concentrations.

Recent findings on cytotoxic T cells cast doubt on the hypothesis of T cell memory by antigen persistence (Lau *et al*., 1994; Hou *et al*., 1994). These investigations indicate life-long memory of cytotoxic T cell clones to virus infection in the absence of retained antigen. In these experiments memory appears to be determined by the "clonal burst size" attained during the immune response. In our model this would correspond to parameter conditions in which either the memory equilibrium has a negligible amount of antigen, or in which the antigen is removed during the initial transient of the immune response. In our subpopulation model the long-lived experienced T cells would then still account for a long-lived (non-equilibrium) memory.

Due to algebraic complexity we have been unable to derive via quasi-steady-state analysis a simple model for the case in which many T cell clones interact with many antigens. This is the situation considered in our earlier paper (De Boer & Perelson, 1994). Thus, with the more realistic competitive saturation proliferation function derived here one can only study the response of many T cell clones to one antigen, or the response of one T cell clone to many antigens. Thus, it seems best to use the present model whenever possible, and to use the earlier model when needed.

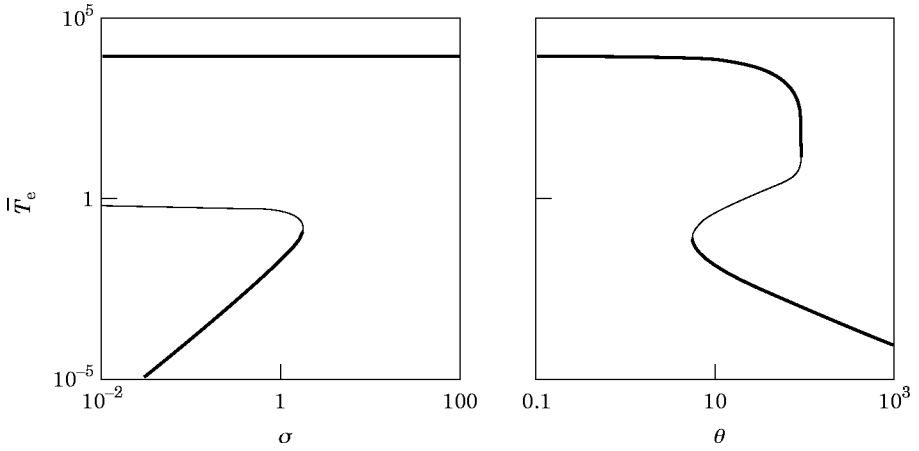

FIG. 4. Bifurcation diagrams of the subpopulation model. Parameters as in Fig. 3 with $r=1$ day^{-1}, $c=1$, i.e. a growing antigen with a carrying capacity of one. Following either (a) σ (with θ fixed at 10), or (b) θ (with σ fixed at 1) as bifurcation parameters, we observe that the virgin state is involved in a saddle-node bifurcation around $\sigma \simeq 1.7$ ($\theta=10$) and $\theta \simeq 5.5$ ($\sigma=1$), respectively. The saddle point defines the proliferation threshold of the model. When it exists the memory equilibrium may be unattainable. The diagram in panel (b) is more complicated because large values of θ require large T cell clones. Due to the competition for antigens such large clones cannot be maintained. We observe the memory equilibrium is involved in a Hopf bifurcation and a saddle-node bifurcation around $\theta=101$. This not investigated further because it is not realistic.

We have borrowed a number of concepts from ecology. Our T cell clones competing for antigen can indeed be viewed as an "ecosystem" of populations competing for some resource. In ecology the interaction between a predator and its prey is described by a functional response. Our competitive saturation function defines the functional response of a predator Y feeding on a prey X as:

$$f(X, Y)=\frac{aXY}{k+X+iY} \tag{36}$$

where a is the maximum number of X that Y can eat per time unit, $i=1$ is an interference parameter, and k is a saturation constant. Functions like this, i.e. eqn (36) with $i\neq 1$, have been proposed and derived before (Beddington, 1975; DeAngelis *et al*., 1975; Ruxton *et al*., 1992). Because of the interference term, these functions have been called "predator-dependent" functional responses (Abrams, 1994). We have shown that eqn (36) can formally be derived if one adopts the conventional scheme in which the predator and prey form a transient complex that leads to death of the prey and the growth of the predator. Additionally, our work suggests a generalization of eqn (36) for the case in which the prey is eaten by several predators.

The simple equation that we derive for antigen presentation, i.e. eqn (31), says that the concentration of sites presenting some antigen (i.e. $\bar{S}$) is proportional to the concentration of APCs, and saturates as a function of antigen. If the antigen concentration is low, i.e. if $A \ll \kappa$, this means that $\bar{S}$ is proportional to the product of the concentration of antigen and APCs. This in agreement with the experimental data of Matis *et al*. (1983).

The proliferation threshold defined here and before (De Boer & Hogeweg, 1987; Kevrekidis & Perelson, 1988) provides a formal model for a recent hypothesis put forward by Brunner *et al*. (1994). They argue that the number of T cells clustered around an APC has to exceed some critical value to initiate proliferation. They suggest that if self-reactive cells are rare that this may be relevant for self tolerance. This is a "recessive" form of tolerance by which populations become anergic. Indeed, we have shown before that such a form of self tolerance can be accounted for by a proliferation threshold and "precursor depletion" (De Boer & Hogeweg, 1987). If during early development naïve cells appear in the presence of a self antigen that they recognize, they become activated, short-lived cells and hence fail to accumulate. Because their numbers are small the population remains below the proliferation threshold and they remain unresponsive. Conversely, clones not recognizing any self antigen will accumulate a population of naïve cells, and become responsive as soon as this population exceeds the proliferation threshold.

This work was partially performed under the auspices of the U.S. Department of Energy. It was supported by NIH Grant AI28433 (A.S.P.) and the Santa Fe Institute through a grant from the Joseph P. and Jeanne M. Sullivan Foundation.

REFERENCES

Abrams, P. (1994). The fallacies of "ratio-dependent" predation. *Ecology* **75,** 1842–1850.

Beddington, J. R. (1975). Mutual interference between parasites or predators and its effects on searching efficiency. *J. Anim. Ecol.* **51,** 597–624.

Borghans, J. A. M., De Boer, R. J. & Segel, L. A. (1995). Improving the Michaelis-Menten assumption by changing variables. *Bull. math. Biol.*, in press.

Brunner, M. C., Mitchison, N. A. & Schneider, S. C. (1994). Immunoregulation mediated by T-cell clusters. *Folia Biol.* (Prague) **40,** 359.

Cha, S. & Cha, C.-J. M. (1965). Kinetics of cyclic enzyme systems. *Molec. Pharmacol.* **1,** 178–189.

Croft, M., Duncan, D. D. & Swain, S. L. (1992). The response of naive antigen-specific CD4+ T cells *in vitro*: Characteristics and antigen-presenting cell requirements. *J. expl. Med.* **176,** 1431–1437.

Cyster, J. G., Hartley, S. B. & Goodnow, C. C. (1994). Competition for follicular niches excludes self-reactive cells from the recirculating repertoire. *Nature* **371,** 389–395.

DeAngelis, D. L., Goldstein, R. A. & O'Neill, R. V. (1975). A model for trophic interaction. *Ecology* **56,** 881–892.

De Boer, R. J. & Hogeweg, P. (1987). Immunological discrimination between self and nonself by precursor depletion and memory accumulation. *J. theor. Biol.* **124,** 343–369.

De Boer, R, J. & Perelson, A. S. (1994). T cell repertoires and competitive exclusion. *J. theor. Biol.* **169,** 375–390.

Fishman, M. A. & Perelson, A. S. (1995). Lymphocyte memory and affinity selection. *J. theor. Biol.* **173,** 241–262.

Freitas, A. A. & Rocha, B. B. (1993). Lymphocyte lifespans, homeostasis, selection and competition. *Immunol. Today* **14,** 25–29.

Go, C. & Miller, J. (1992). Differential induction of transcription factors that regulate the interleukin 2 gene during energy induction and restimulation. *J. Expl. Med.* **175,** 1327–1336.

Hou, S., Hyland, L., Ryan, K. W., Portner, A. & Doherty, P. C. (1994). Virus-specific $CD8^+$ T-cell memory determined by clonal burst size. *Nature* **369,** 652–654.

Kevrekidis, I. G., A. D. Zecha & A. S. Perelson (1988). Modeling dynamical aspects of the immune response; I. T cell proliferation and the effect of IL-2. In: *Theoretical Immunology* (Perelson, A. S., ed.) Part One, pp. 167–197. *SFI Studies in the Science of Complexity*, Vol. II. Reading, MA: Addison-Wesley.

Knight, S. C. (1987). Lymphocyte proliferation assays. In: *Lymphocytes: A Practical Approach* (Klaus, G. G. B., ed.) pp. 189–207. Oxford: IRL Press.

Lau, L. L., Jamieson, B. D., Somasundaram, T. & Ahmed, R. (1994) Cytotoxic T-cell memory without antigen. *Nature* **369,** 648–652.

Matis, L. A., Glimcher, L. H., Paul, W. E. & Schwartz, R. H. (1983). Magnitude of response of histocompatibility-restricted T-cell clones is a function of the product of the concentrations of antigen and Ia molecules. *Proc. natn. Acad. Sci. U.S.A.* **80,** 6019–6023.

Ruxton, G. D., Gurney, W. S. C. & De Roos, A. M. (1992). Interference and generation cycles. *Theor. Popul. Biol.* **42,** 235–253.

Sebzda, E., Wallace, V. A., Mayer, J., Yeung, R. S. M., Mak, T. W. & Ohashi, P. S. (1994). Positive and negative thymocyte selection induced by different concentrations of a single peptide. *Science* **263,** 1615–1618.

Segel, L. A. (1988). On the validity of the steady state assumption of enzyme kinetics. *Bull. math. Biol.* **6,** 579–593.

Segel, L. A. & Slemrod, M. (1989). The quasi-steady-state assumption: a case study in perturbation. *SIAM Rev.* **31,** 446–477.

Suzuki, G., Kawase, Y., Koyasu, S., Yahara, I., Kobayashi, Y. & Schwartz, R. H. (1988) Antigen-induced suppression of the proliferative response of T cell clones. *J. Immunol.* **140,** 1359–1365.

Taylor, P. M., Thomas, D. B. & Mills, K. H. G. (1987). In vitro culture of T cell lines and clones. In: *Lymphocytes: A Practical Approach* (Klaus, G. G. B., ed.) pp. 133–147. IRL Press, Oxford.

APPENDIX

Approximating eqn (12), in the main text, by ignoring all C_iC_j terms we obtain a system of n linear equations of the form

$$C_i=\left(\bar{S}-\sum_j C_j\right)\frac{\bar{T}_i}{\bar{S}+k_i}, \quad \text{for } i=1, 2,\ldots n \tag{A.1}$$

where $k_i \equiv 1/K_i$. The sum over all C_i is

$$\sum_i C_i=\left(\bar{S}-\sum_j C_j\right)\sum_i \frac{\bar{T}}{\bar{S}+k_i}, \tag{A.2}$$

where

$$\sum_i \frac{\bar{T}_i}{\bar{S}+k_i}=\frac{\bar{T}_1}{\bar{S}+k_1}+\frac{\bar{T}_2}{\bar{S}+k_2}+\cdots+\frac{\bar{T}_n}{\bar{S}+k_n}$$

$$=\frac{\sum_j \bar{T}_j \prod_{l\neq j}(\bar{S}+k_l)}{\prod_l (\bar{S}+k_l)}. \tag{A3}$$

Hence (A.2) becomes

$$\sum_i C_i=\bar{S}\frac{\sum_j \bar{T}_j \prod_{l\neq j}(\bar{S}+k_l)}{\prod_l (\bar{S}+k_l)+\sum_j \bar{T}_j \prod_{l\neq j}(\bar{S}+k_l)}. \tag{A.4}$$

Substituting (A.4) into (A.1) we write

$$C_i=\frac{\bar{S}\bar{T}_i}{\bar{S}+k_i}\frac{\prod_l (\bar{S}+k_l)}{\prod_l (\bar{S}+k_l)+\sum_j \bar{T}_j \prod_{l\neq j}(\bar{S}+k_l)}, \tag{A.5}$$

or

$$C_i=\frac{\bar{S}\bar{T}_i}{\bar{S}+k_i}\frac{1}{1+\sum_j \bar{T}_j \frac{1}{\bar{S}+k_j}}, \tag{A.6}$$

or

$$C_i=\frac{\bar{S}\bar{T}_i}{k_i+\bar{S}+\sum_j \bar{T}_j \frac{\bar{S}+k_i}{\bar{S}+k_j}}. \tag{A.7}$$

Substituting $K_i \equiv 1/k_i$ we obtain eqn (13) in the text.