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Modeling T-cell proliferation: an Investigation of the Consequences of the Hayflick Limit

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Somatic cells, including immune cells such as T-cells have a limited capacity for proliferation and can only replicate for a finite number of generations (known as the Hayflick limit) before dying. In this paper we use mathematical models to investigate the consequences of introducing a Hayflick limit on the dynamics of T-cells stimulated with specific antigen. We show that while the Hayflick limit does not alter the dynamics of T-cell response to antigen over the short term, it may have a profound effect on the long-term immune response. In particular we show that over the long term the Hayflick limit may be important in determining whether an immune response can be maintained to a persistent antigen (or parasite). The eventual outcome is determined by the magnitude of the Hayflick limit, the extent to which antigen reduces the input of T-cells from the thymus, and the rate of antigen-induced proliferation of T-cells. Counter to what might be expected we show that the persistence of an immune response (immune memory) requires the density of persistent antigen to be less than a defined threshold value. If the amount of persistent antigen (or parasite) is greater than this threshold value then immune memory will be relatively short lived. The consequences of this threshold for persistent mycobacterial and HIV infections and for the generation of vaccines are discussed.

Introduction

The classic studies of Hayflick and Moorhead demonstrated that normal human fibroblasts cultured *in vitro* display a finite proliferative capacity, with the mean total number of population doublings, known as the Hayflick limit ranging from 20 to 50 (Hayflick & Moorhead, 1961; Hayflick 1965). The generality of the Hayflick phenomenon has been confirmed for several types of somatic cells, including keratinocytes (Rheinwald & Green, 1975), vascular smooth muscle cells (Bierman, 1978), lens cells (Tassin *et al*., 1979), endothelial cells (Mueller *et al*., 1980), and T-cells (see below and also Discussion). While early limitations in T-cell culture techniques made it hard to determine whether T-cells were capable of a limited number of population doublings or were able to undergo an unrestricted growth (Effros *et al*. 1990; Effros & Walford, 1984), subsequent experiments (Perillo *et al*., 1988) resolved these culture problems and obtained estimates of the total number of population doublings of human T-cells obtained from the peripheral blood or neonatal chord samples of a total of 109 individuals. Of over 200 cultures only one generated an "immortal" cell line, and was subsequently shown to have signs of karyotypic abnormality. Their results demonstrated a Hayflick limit in all the samples but one, with the mean number of population doublings of 23 (Fig. 1). While this value of the Hayflick limit (for human T cells) is similar to that obtained for human fibroblasts, the Hayflick limit varies widely for cells obtained from different species.

In the case of fibroblasts and other somatic cells which are generally long-lived and have relatively slow replacement rates it has been proposed that the Hayflick limit will contribute to the process of aging and senescence of individuals [see Cristofalo &

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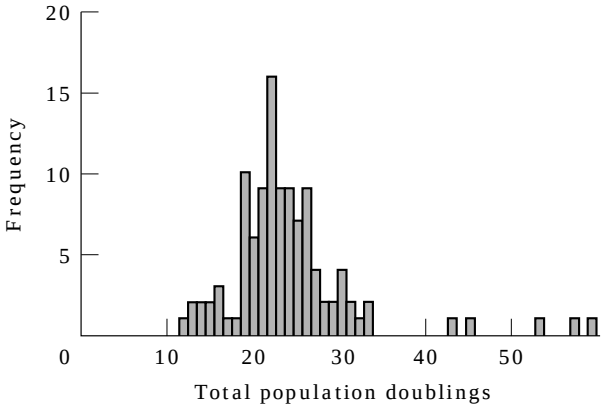


FIG. 1. The Hayflick limit for T-cells *in vitro*. Frequency distribution showing the maximum number of cell divisions of human peripheral blood or neonatal chord samples of a total of 109 individuals obtained by Perillo *et al*. (1988).

Pignolo (1993) for a review]. However, the Hayflick limit may have additional, shorter term consequences for antigen-specific immune cells which proliferate to large numbers following stimulation by specific-antigen. For example, immune cells undergoing rapid proliferation can have an average division time of about 1 day and will consequently attain a Hayflick limit of $n = 20$ to $n = 30$ within as many days. The principle factors which govern the effect of the Hayflick limit on the dynamics of antigen-specific cells are (i) the rate of recruitment of "naive" or new cells from the thymus, (ii) the rate at which they proliferate in response to antigen, and (iii) the rate at which they die, either as a consequence of a background death rate or as a consequence of reaching the Hayflick limit (Antia *et al*., 1996). In this paper we develop simple mathematical models to help us examine the impact of these processes on the dynamics of T-cells. The mathematical formalism which we develop can be applied to any process with birth, death and aging where the input of "young" or new cells immigrate from an external source, and where the age of both daughter cells equals that of the parent plus one, and where there is a Hayflick limit which governs aging.

Models and Results

In the ODE model described below we employ a system where we keep track of the subpopulations of cells in each age class (age being defined in terms of generation number ranging from zero for immigrant cells to the Hayflick limit). On division a cell leaves its age class and forms two daughter cells in the subsequent age class. In the Appendix we transform this model into a PDE model. This allows us to generate a system more amenable to analysis.

We begin by constructing a general model for the dynamics of T-cell-antigen interactions when there is a Hayflick limit. This model includes the input from the thymus and proliferation of T-cells as functions of antigen density (the term antigen will be used interchangeably with the term parasite). We use this model to determine the age distribution and the total magnitude of the T-cell response as functions of the Hayflick number, the rate of input from the thymus and the rate of proliferation of T-cells. We examine the consequences of these results for the magnitude of the immune response to persistent antigen (memory) as well as for the immune response to short duration (acute), and persistent infections.

In our model we let X (without a subscript) represent the population of T-cells specific for a given antigen P. By Q we denote a sequestered stage of antigen (parasite). We let X_i equal the number of immune cells in the i-th generation, where i goes from zero (for cells just recruited from the thymus) to n which equals the Hayflick limit. We let $A(P)$, $S(P)$ and d, respectively, represent the rate of recruitment of cells from the thymus, the rate of proliferation of cells and the death rate of these cells. We note that both the rate of recruitment of cells from the thymus and the rate of proliferation of cells will be functions of the antigen density P, and that the latter is independent of the "age" of the cells. The relative magnitudes of these variables (capitalized) and parameters (lower case) of the model are described in Table 1. With these definitions and assumptions we can write the following equations for the density of immune cells of the various generations.

$$\frac{dX_0}{dt} = A(P) - S(P)X_0 - dX_0,$$

$$\frac{dX_i}{dt} = 2S(P)X_{i-1} - S(P)X_i - dX_i,$$

$$\text{for} \quad i = 1, \ldots, n. \quad (1)$$

Adding up all equations in (1) we obtain

$$\frac{dX}{dt} = \frac{d}{dt}\sum_{i=0}^{n} X_i = A(P) + S(P)(X - 2X_n) - dX. \quad (2)$$

AGE DISTRIBUTION OF THE IMMUNE RESPONSE

We first determine the age distribution and total immune response which will be obtained at a

TABLE 1
Description of variables and parameters

Parameter or variable	Description	Range
P	density of antigen or parasite	we scale $P(0) = 1$
Q	density of antigen at sequestered stage	we set $Q(0) = 0$
X	total number of immune cells specific for antigen P	
X_i	number of immune cells in the i-th generation	
n	Hayflick limit	15–30, 20 in simulations
$A(P)$	rate of immigration from the thymus	0 to 1.0 depending on P
$S(P)$	replication rate of immune cells	0 to 1.0 depending on P
d	natural death rate of immune cells	0.1 unless otherwise specified
r	rate of growth of antigen/parasite	0.5 to 2.0, 1.0 in simulations
h	rate of killing of parasite by immunity	$h \ll X(0)$; 10^{-3} in simulations
k	parasite density for 1/2 max. proliferation rate	$k \gg P(0)$; 10^3 in simulations
s	maximum growth rate of immune cells	1.0 in simulations
a_1	maximum input from thymus	1.0 in simulations
a_2	parasite density for 1/2 max. input from the thymus	a_2 small
ϵ	cell division rate in absence of parasite	$\epsilon \ll d$, 0.01 in simulations
f, g	migration rates between stages P and Q	both 0.1 in simulations

steady state. The age distribution for the immune cells is obtained by setting the $dX_i/dt = 0$.

$$X_0 = \frac{A(P)}{S(P) + d},$$

$$X_1 = \frac{2S(P)X_0}{S(P) + d} = \left(\frac{2S(P)}{S(P) + d}\right)\frac{A(P)}{S(P) + d},$$

and in general

$$X_i = \left(\frac{2S(P)}{S(P) + d}\right)^i \frac{A(P)}{S(P) + d} \quad \text{for} \quad i = 0, \ldots, n. \quad (3)$$

As the density of antigen increases, T-cells rapidly proliferate, and leave the "naive" compartment thus reducing X_0. The number of T-cells in the i-th generation equals that in the preceding generation multiplied by a constant which increases with the rate of proliferation of immune cells and decreases with the death rate of these cells. This is illustrated in Fig. 2. In the Appendix we show that if the antigen density P is kept at a constant level, X approaches its asymptotic state at an exponential rate.

TOTAL MAGNITUDE OF THE IMMUNE RESPONSE

The total number of immune cells X at equilibrium equals the sum of the numbers of immune cells in all generations.

$$X = \sum_{i=0}^{n} X_i = \sum_{i=0}^{n} \left(\frac{2S(P)}{S(P) + d}\right)^i \frac{A(P)}{S(P) + d}$$

Using the formula for the sum of a geometric series we arrive at

$$X = \left(\frac{A(P)}{S(P) - d}\right)\left(\left(\frac{2S(P)}{S(P) + d}\right)^{n+1} - 1\right) \quad \text{if} \quad S(P) \neq d \quad (4)$$

$$X = n\left(\frac{A(P)}{S(P) + d}\right) \quad \text{if} \quad S(P) = d \quad (4')$$

We note that at equilibrium the total density of immune cells increases linearly with increasing input from the thymus, increases approximately exponentially with increasing Hayflick number, and decreases with increasing death rate [Fig. 3(a)].

In general, the total density of immune cells as a

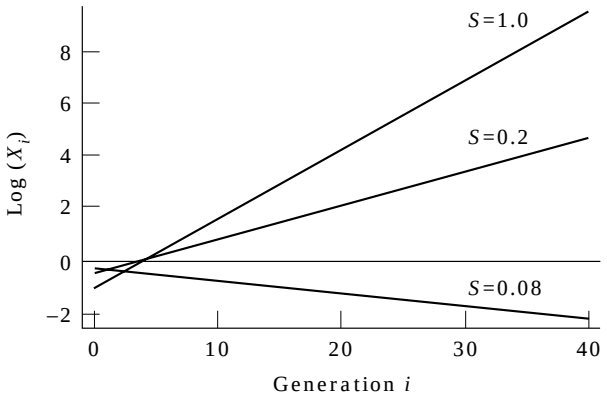


FIG. 2. If the level of T-cell proliferation S, and immigration, A are maintained constant then a unique stable age distribution is obtained. In the figure we plot the steady-state age distribution obtained from eqn (3) with $A = 0.1$, $d = 0.1$ and $S = 1$, 0.2, 0.08 respectively. We note that as the proliferation increases the number of immune cells in the naive class decreases due to more rapid migration of cells out from this class.

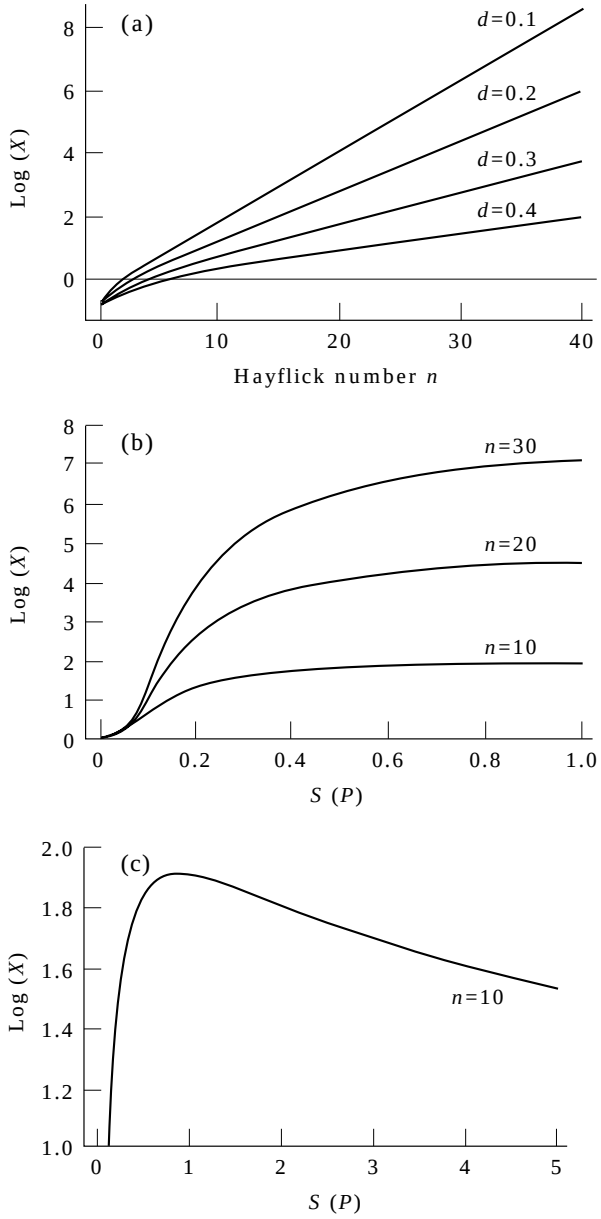


FIG. 3. The total immune response X is a function of the Hayflick number, n, the input from the thymus, A, the proliferation of the T-cells S, and the background death rate d. We plot the log of the total density X as a function of: (a) Hayflick number n with $A = 0.1$, $S = 0.5$ and $d = 0.1$, 0.2, 0.3, 0.4; (b) proliferation rate $S(P)$ with $A = 0.1$ and $d = 0.1$. In biologically reasonable region of parameters ($S < 1$ and $n \geqslant 20$), log(X) increases with $S(P)$, but for low n and high $S(P)$ as in (c), we observe a decline in log(X), reminiscent of "high-zone" tolerance.

function of proliferation rate has a maximum at intermediate values of $S(P)$ [Fig. 3(c)]. The initial increase in number of immune cells with increasing stimulation is intuitive, (stimulation is required for cell proliferation). The subsequent decline at very high rates of proliferation is due to the decreased transit time of these cells through generations from zero to the Hayflick limit n. However, we note that this drop is probably not observed in biologically reasonable parameter space ($S(P) < 1.0$; $n > 15$) and in a more reasonable parameter regime the total number of immune cells varies in a sigmoid way with their rate of proliferation [Fig. 3(b)].

SPECIFIC FUNCTIONS FOR PROLIFERATION AND INPUT FROM THE THYMUS

We now introduce specific functions to describe how the rate proliferation of cells and the rate of input of cells from the thymus varies with the density of antigen, P. As a first approximation we let $S(P)$ be a monotone increasing function of P saturating at the maximum rate of growth of immune cells. We use a modified Monod-type function

$$S(P) = \epsilon + \frac{sP}{k + P} \tag{5}$$

with a small additive term $\epsilon > 0$ which accounts for random proliferation of immune cells in the absence of parasite/antigen [Fig. 4(a)]. Note that $\epsilon < d$, and in

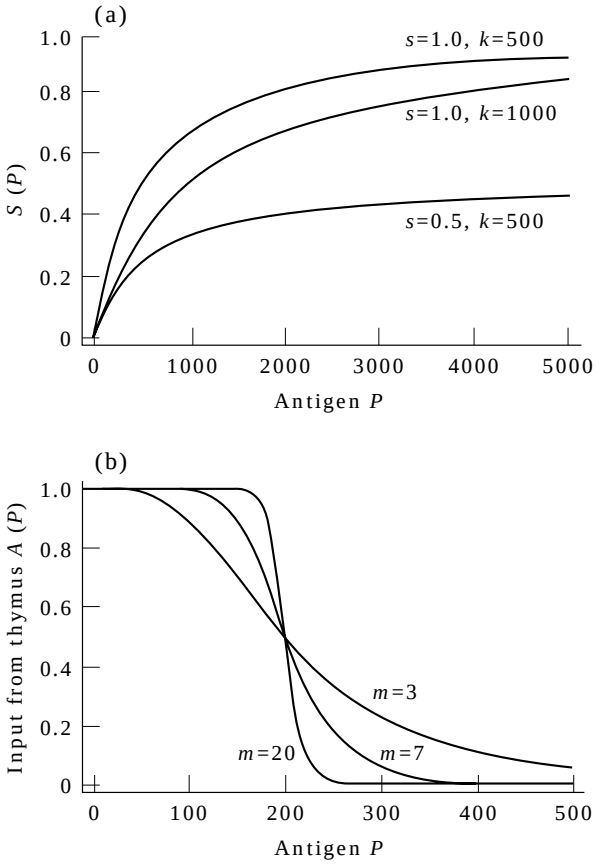


FIG. 4. We describe the dose response for proliferation of immune cells $S(P)$ and the input from the thymus $A(P)$ as functions of the density of antigen or parasite, P. (a) Plot of $S(P)$ with $\epsilon = 0.01$; (b) Plot of $A(P)$ with $a_1 = 1.0$, $a_2 = 200$, and $m = 3$, 7, 20.

the absence of antigen there will be $A/(\epsilon + d)$ cells in the "naive" compartment i.e. with $i = 0$. As the density of antigen increases, T-cells specific for this antigen will be deleted in the thymus (Kappler *et al*., 1987), and we would expect $A(P)$ to be a monotone decreasing function of the antigen density. At present, it is not known whether this function looks like a step function with a threshold parasite density above which input from the thymus falls to very low values or whether it is more gently sloping. In the absence of quantitative data we have as a first approximation used Hill functions of different orders m. By varying m we can approximate both these extremes. As illustrated in Fig. 4(b) when m increases the function $A(P)$ becomes closer to a step function.

$$A(P) = a_1 - \left(\frac{a_1 P^m}{a_2^m + P^m}\right) \tag{6}$$

In the above formula, a_1 is the maximum input from the thymus, and a_2 is the antigen density at which the input from the thymus is reduced in half. Qualitative description of thymic deletion (Miller, 1992) showing that even with barely detectable levels of antigen the input from the thymus is greatly reduced suggests that the constant a_2 is comparably small.

IMMUNE MEMORY TO PERSISTENT ANTIGEN

We now determine the immune response to different antigen densities. If, in fact, immune memory arises from the persistence of antigen, then this will tell us how the magnitude of immune memory changes with the concentration of antigen. In Fig. 5 we plot the magnitude of the immune response as a function of antigen density, obtained by substituting eqns (5) and (6) for $S(P)$ and $A(P)$ into (4). We find that the immune response attains its maximum at an intermediate density of antigen, and the immune response is low when the level of antigen increases beyond that density. The decline in the immune response when the density of antigen is high requires both the drop in immigration of cells from the thymus as well as the Hayflick limit for cells in the periphery. Either of these processes considered separately will not give rise to this result. If there is no Hayflick limit, then input from the thymus is relatively small compared to the density of cells in the periphery, and since the cells can divide indefinitely, lowering or even completely stopping the input from the thymus will only give rise to very slight changes in the total density of immune cells. On the other hand, if the input from the thymus is constant, then the total density of the immune cells will vary in a sigmoid way with the density of antigen.

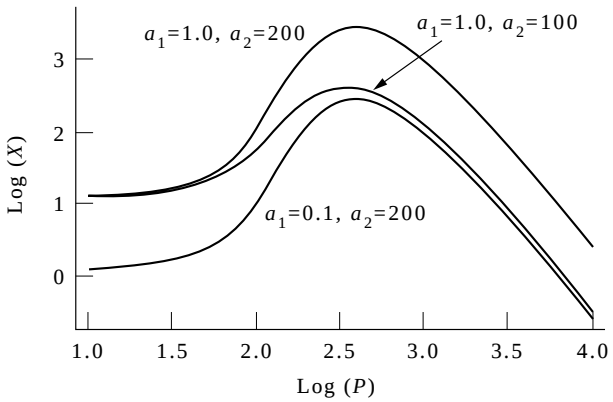


FIG. 5. Using the dose respnse functions for proliferation and input from the thymus described in Fig. 4 we plot the total immune response X as a function of antigen density with $m = 3$, $d = 0.1$, $\epsilon = 0.01$, $s = 1.0$ and $k = 10^3$.

IMMUNE RESPONSE TO A REPLICATING PARASITE (ANTIGEN)

We now consider immune response to a rapidly replicating micro-parasite with a dormant or sequestered stage. We do so by assuming that in the absence of the antigen-specific immune response the growth of the parasite is governed by the growth rate r, and that h equals the rate of the control of the parasite by the immune cells. We then have equations for the rate of change in the density of immune cells and parasite as

$$\frac{dP}{dt} = rP - hPX - fP + gQ \tag{7}$$

$$\frac{dQ}{dt} = fP - gQ \tag{8}$$

and from (2)

$$\frac{dX}{dt} = A(P) + S(P)(X - 2X_n) - dX.$$

Q here represents the level of the micro-parasite at a conceivable dormant stage. At a steady state we have

$$\frac{dQ}{dt} = 0, \quad Q = \frac{f}{g}P \tag{9}$$

$$\frac{dP}{dt} = 0, \quad X = \frac{r}{h} \tag{10}$$

$$\frac{dX}{dt} = 0, X = \left(\frac{A(P)}{S(P) - d}\right)\left(\left(\frac{2S(P)}{S(P) + d}\right)^{n+1} - 1\right) \tag{11}$$

what we already derived in (4).

In order for parasite and immune response to be at an equilibrium the two isoclines given by $dP/dt = 0$ and $dX/dt = 0$ must intersect. When they do intersect we see that there are at most two possible steady states corresponding to low and high levels of parasite. Points of intersection of these isoclines are given by

$$\frac{r}{h} = \left(\frac{A(P)}{S(P) - d}\right)\left(\left(\frac{2S(P)}{S(P) + d}\right)^{n+1} - 1\right) \qquad (12)$$

Here $A(P)$ and $S(P)$ are defined by eqns (5) and (6). The intersection of the isoclines for particular parameter values is shown in Fig. 6.

In the Appendix we introduce a PDE model of the Hayflick limit and provide similar analysis for the PDE model as for steady-states, stability and asymptotic dynamics.

Dynamics of Infection

In this section we investigate the effect of the Hayflick limit on the dynamics of immune cells following the introduction of a parasite (replicating antigen). We do so by examining the outcome at short times following infection (which would correspond to the "acute" phase of the infection) and at longer times during a persistent infection. Prior to the introduction of a parasite there are relatively few specific immune cells. During the initial acute phase of the infection we do not expect the Hayflick limit to prevent the control of the parasite. This can be seen if we note that the intensity of the immune response required to control the parasite is approximately r/h, which we would estimate to be in the region of 10^2 to 10^4. A single precursor cell can reach a density of 2^{23} or approximately 10^7 cells before the Hayflick limit is reached. For a parasite in general we might expect several clones specific for the different antigens on the parasite and thus increase in the number of cells available. The lack of an effect of introducing a Hayflick limit on an "acute" infection is seen in Fig. 7(a–d). We point out that for a short initial period of time, the dynamics of infection ("acute phase") closely follows the pattern of the model without the Hayflick limit. If the parasite is not driven to extinction after the acute phase of the infection, we might expect one of the following outcomes in the long term: (i) the eventual elimination of the parasite, (ii) a steady state between parasite and immunity, (iii) limit cycle oscillations of parasite and immune response, or (iv) escape of the parasite from immune control. Our numerical simulations of the model described above suggest that when the presence of a sequestered stage prevented parasite extinction following the initial acute phase of the infection the parasite does not go extinct in the longer term. However, the subsequent scenarios [i.e. outcomes (ii), (iii) and (iv) above] may be attained. Which outcome obtains depends on both the parameters and the initial conditions. We now illustrate these outcomes obtained by numerical simulations.

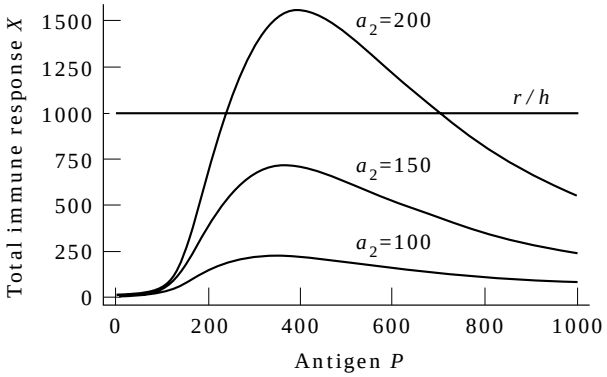


FIG. 6. Plot of the isoclines for $dX/dt = 0$ and $dP/dt = 0$ as a function of the antigen density for T-cell deletion in the thymus (a_2). Parameters, $r = 1.0$, $h = 10^{-3}$, $k = 10^3$, $s = 1.0$, $n = 20$, $d = 0.1$, $a_1 = 1.0$, $m = 3$, $a_2 = 100$, 150, 200. When $a_2 = 100$ and 150 the isoclines for $dX/dt = 0$ and $dP/dt = 0$ do not intersect and there is no steady state and the parasite cannot be controlled by the immune response. When $a_2 = 200$ there are two steady states corresponding to parasite densities of approximately 210 and 720 units. The former was numerically shown to be locally stable while the latter was shown to be unstable.

The numerical simulations reveal a possible bifurcation that the model undergoes when the parameter a_2 changes from its high values to low values. For high values of a_2 such as 200, the system has a stable steady state which loses its stability at intermediate values of a_2, and gives rise to an asymptotically stable limit cycle "around" now unstable steady state. When a_2 is lowered yet further, the unstable steady state is lost, but the limit cycle persists. If a_2 is lowered further, the limit cycle becomes unstable, and the parasite gains the ability to escape from the immune control. This bifurcation is illustrated in Fig. 7(a–c).

In order to get a better feel for the age distribution of T-cells during the dynamics observed in the simulations described in Fig. 7(a–c) we plot the age distributions of T-cells as a function of time in Fig. 8(a–c).

Discussion

The Hayflick limit equals the maximum number of cumulative doublings a cell population can undergo, and is a property of many somatic cells including

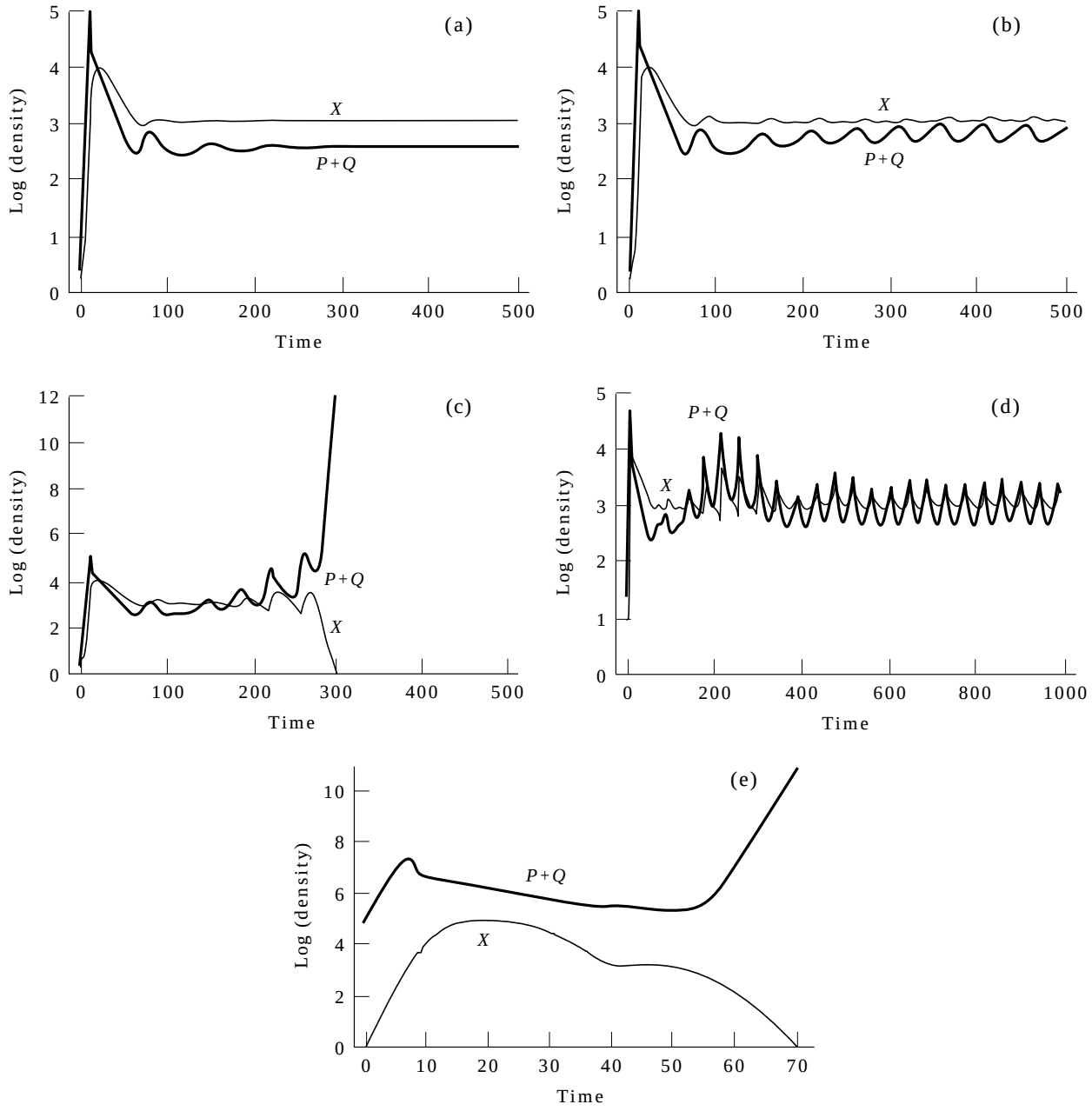
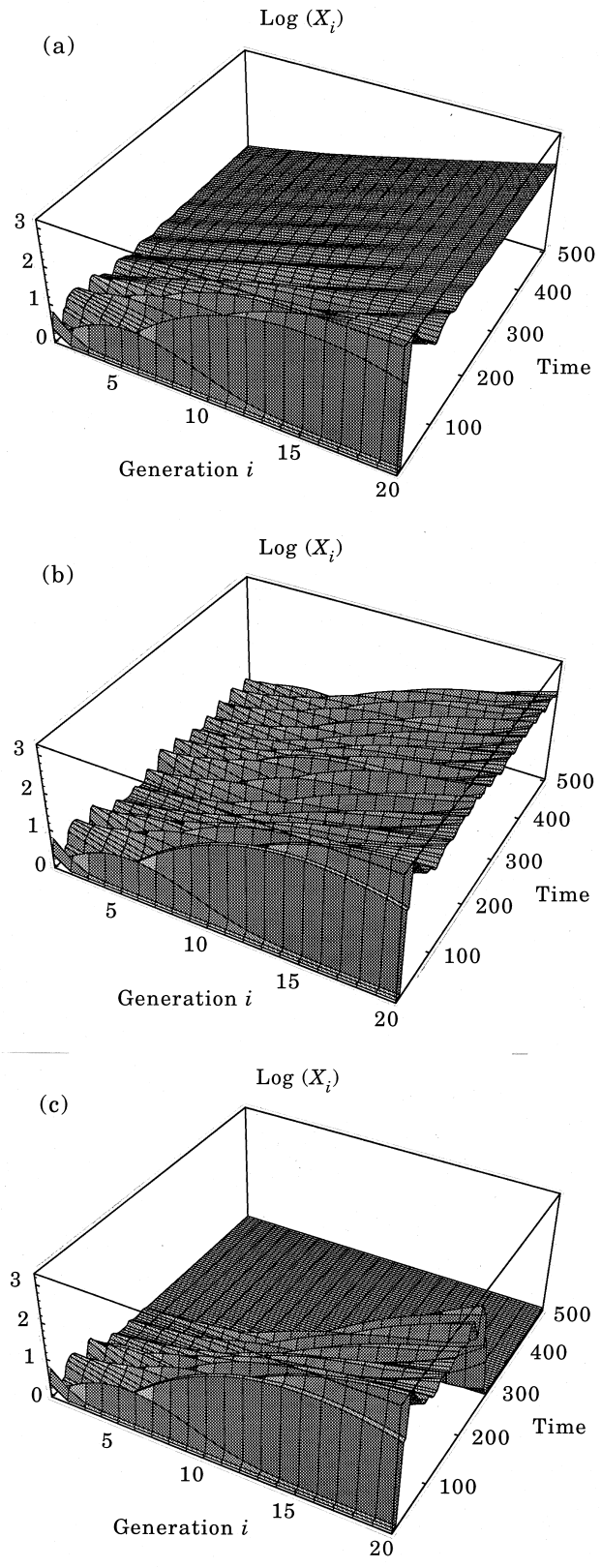


FIG. 7. Numerical simulations of the eqns (1–2) showing different outcomes of the infection course: (a) locally stable steady-state is reached, infection is controlled by the immune system, $a_2 = 200$, $X(0) = 1$, $P(0) = 1$, $Q(0) = 0$; (b) locally stable limit cycle is reached, infection is controlled, $a_2 = 100$, $X(0) = 1$, $P(0) = 1$, $Q(0) = 0$; (c) instability, parasite escape from the control, $a_2 = 50$, $X(0) = 1$, $P(0) = 1$, $Q(0) = 0$; (d) choosing different initial values in (c), it is possible to control the infection, $a_2 = 50$, $X(0) = 10$, $P(0) = 10$, $Q(0) = 0$; (e) situation when parasite escapes from the control, a counterpart to the outcome in (a), $a_2 = 200$, $X(0) = 1$, $P(0) = 5 \cdot 10^4$, $Q(0) = 0$. The rest of the parameters are described in Table 1.

lymphocytes. *In vitro* culture experiments suggest that the Hayflick limit of human T-cells is approximately 23 (see Introduction). On the molecular level the Hayflick limit arises from progressive loss of chromosome telomeres. The loss of telomeres is prevented in the germ line and in hematopoeitic stem cells by expression of the enzyme telomerase (Cristofalo & Pignolo, 1993; Hiyama *et al*., 1995). The level of telomerase is much lower in peripheral blood lymphocytes and is found to be upregulated in virally transformed lymphocytes such as those propagated in long-term cell culture which are



capable of indefinite proliferative capacity (Counter *et al*., 1994).

In this paper we examine the consequences of the Hayflick limit for the dynamics of expansion of antigen-specific T-lymphocytes. We do so by constructing a simple model which incorporates the following processes: input of "naive" cells from the thymus, antigen-induced proliferation, and death by two processes, namely at a fixed "background" rate and when the cells reach the Hayflick limit. We show that if the level of antigen is held constant the immune response will attain a steady-state "age" distribution with the total immune response obtaining a maximum at intermediate antigen densities and falling to lower levels at higher antigen densities. Two factors contribute to the decline in number of antigen-specific immune cells at high antigen densities; first, the input of T-cells from the thymus is reduced (due to clonal deletion), and second, immune cells progress more rapidly towards the Hayflick limit. When a replicating parasite is introduced, we find that although the early dynamics are not altered by the presence of the Hayflick limit, in the long term (i.e. in the case of a persistent infection) the dynamics of parasite and immune response can be relatively complex, allowing for stable fixed points, limit cycle behavior, and loss of control of the parasite. Furthermore, the final outcome is now dependent on both the parameters and the initial conditions which can determine whether the parasite densities cycle over time, or are controlled at a steady state, or the parasite escapes from control.

These results suggest that the inclusion of a Hayflick limit does not introduce a quantitative change for acute infections which are cleared by the immune response. However, the Hayflick limit may have a major impact on persistent infections, a situation which will obtain if the immune response does not drive the parasite to extinction, namely when there is a dormant stage, limitation on T-cell proliferation (De Boer & Perelson, 1994, 1995), or antigenic variation. We note that the Hayflick limit is an addition to the numerous mechanisms of immune suppression which include: an additional population of suppressor cells (Kaufman *et al*., 1985), idiotypic networks between immune cells (Jerne, 1974; De Boer & Hogeweg, 1989), adaptive cellular interactions

FIG. 8. Dynamics of age-distribution of T-cells in eqns (1–2) corresponding to the outcomes (a, b, c) in Fig. 7: (a) locally stable steady state is reached, infection is controlled by the immune system, $a_2 = 200$, $X(0) = 1$, $P(0) = 1$, $Q(0) = 0$; (b) locally stable limit cycle is reached, infection is controlled, $a_2 = 100$, $X(0) = 1$, $P(0) = 1$, $Q(0) = 0$; (c) instability, parasite escape from the control, $a_2 = 50$, $X(0) = 1$, $P(0) = 1$, $Q(0) = 0$.

(Grossman & Paul, 1992), the shape of the T-cell-antigen dose response (McLean & Kirkwood, 1990; Schweitzer & Anderson, 1992; Swinton *et al*., 1994), cross-regulation between Th1 and Th2 responses (Mosmann & Sad, 1996; Fishman & Perelson, 1994).

In the subsequent section we discuss the implications of this model for disease, and possible ways it can be experimentally tested. We also describe some of the caveats and limitations of the current models, outline some refinements, and suggest directions for future studies.

IMPLICATIONS FOR DISEASE

As mentioned above, the model has implications for persistent infections. We briefly consider the implications of a Hayflick limit for following three persistent infections: mycobacterial infections such as TB and leprosy, HIV, as well as for the immunity induced by live recombinant vaccines.

Mycobacteria causing tuberculosis and leprosy grow very slowly in the host and can remain dormant, sequestered in long-lived macrophages, in which they can also replicate. Both these mechanisms may contribute to the long-term persistence of mycobacteria within their hosts. Another feature of infection is that early control of the infection can be followed by later escape from control. This has led to the suggestion that the loss of control may be associated with the parasite specific immune cells reaching the Hayflick limit (Antia *et al*., 1996). An alternative (but not necessarily mutually exclusive) explanation is that an initially dominant Th1 response capable of controlling the parasite is lost at a later time due to the generation of a Th2 associated response which is characteristic of disease (Salgame *et al*., 1991; Bloom *et al*., 1992; Flynn *et al*., 1993). We are currently using mathematical models to investigate how these processes may interact to give rise to the dynamics observed during infection.

The Hayflick limit may also play an important role in AIDS pathogenesis. Infection with HIV is followed by a long and persistent ''asymptomatic period'' during which the infecting agent (''parasite'') persists at high densities. Studies of viral density changes following anti-viral drug treatment suggest that HIV replicates very rapidly in AIDS patients (Ho *et al*., 1995; Wei *et al*., 1995; Perelson *et al*., 1996). HIV may also reduce the rate at which naive T-cells immigrate into the body by destroying thymic tissues (Grody *et al*., 1985; Miller, 1992). These conditions may cause immune cells to reach their Hayflick limit more rapidly than in uninfected individuals. Support for this mechanism of HIV pathogenesis comes from a recent study by Effros *et al*. (1996) that reveals greatly shortened telomeres in CD28− CD8+ T-cells taken from HIV patients. CD28− CD8+ T-cells expand considerably during HIV infection (Saukkonen *et al*., 1993; Kammerer *et al*., 1996) and have been shown to display HIV-specific cytotoxic activity (Vingerhoets *et al*., 1995; Dalod *et al*., 1996; Fiorentino *et al*., 1996). At this point it is not clear whether or not telomere lengths also decrease in CD4+ T-cells over the course of HIV-infection (Cohen, 1996; Effros *et al*., 1996). Further experimental research should shed more light on the aging of both CD8+ and CD4+ populations during HIV infection.

Our conjecture that the intensity of the immune response to a persistent antigen is maximized at intermediate antigen levels (Fig. 6) has consequences for design and use of live recombinant vaccines. Recombinant vaccine could be constructed by inserting multiple antigens into a live vector such as the vaccinia virus or BCG (Perkus *et al*., 1985; Stover *et al*., 1991; Andino *et al*., 1994). In accord with our results it will be important to find the proper (intermediate) level of expression of these proteins since too high a level of expression could eventually push T-cell lineages beyond their Hayflick limit and, thus, either reduce the immune response to that protein, or even prevent future responsiveness to that protein.

TESTS OF THE MODEL

First we note that the central assumption of our model is based on experiments demonstrating the Hayflick limit for T-cells, which in turn is based on both direct measurements of the Hayflick limit for proliferating T-cells *in vitro*, and indirect observations of the length of telomeres *in vivo*. We have briefly reviewed this literature in the introduction, as well as in the beginning of this section. We now mention several predictions the model generates which make it amenable to experimental tests.

One way to test the model would be to determine the immune response to a fixed amount of antigen. In accord with this model the immune response would be maximum at intermediate antigen doses, and decline at both lower and higher antigen doses. Furthermore, analysis of the age distribution of antigen-specific immune (by looking at telomere lengths) should show age profiles similar to those in Fig. 6 as the density of antigen and thus immune proliferation is increased.

The relevance of the Hayflick limit during persistent infections is also amenable to empirical tests. If the Hayflick limit plays a dominant role in the

generation of disease following infection with mycobacteria such as *Mycobacterium leprae* and *M. tuberculosis*, then progression to disease might be expected to be accompanied by two phenomena: the aging of the dominant antigen-specific T-cell lineage with time, followed by the loss of this lineage.

CAVEATS, LIMITATIONS, EXCUSES, AND FUTURE DIRECTIONS

We have used simple deterministic models to describe the dynamics of antigen and the immune response. These models have the usual limitations in that they assume that the immune system is a "well-mixed vessel", and ignore both spatial and stochastic effects. The following assumptions common to many models are worthwhile to mention: (i) We have used a simple saturating function to describe the antigen-driven immune proliferation $\epsilon + sP/(k + P)$. While this is in accord with clonal expansion of immune cells in an antigen-dependent manner, it is only a rough approximation. However, by changing this to other commonly used terms such as sP, and sXP we still obtain similar qualitative behavior suggesting that our results are fairly robust. For simplicity we have considered a population of T-cells, and assumed that the efficacy of the immune response is proportional to this population. Further work also needs to be done to incorporate more complex immune response functions (Schweitzer & Anderson, 1992; McLean, 1994; De Boer & Perelson, 1995). (ii) We have used a simple product term to describe the control of parasite by immunity hXP. However, in some cases such as for cell mediated responses this term may be expected to saturate. Once again, provided the saturation is high many of the quantitative features of the models remain intact. (iii) While it is clear that the input from the thymus is a decreasing function of antigen concentration, the quantitative aspects of thymic deletion probably vary for different antigens dependent on how easily they access the thymus, and how well they are presented on the MHC. For this reason we used a function whose shape we could vary from steep to gently sloping. (iv) We have structured T-cells by the number of divisions they have undergone but assumed that all cells, prior to reaching the Hayflick, are identically "naive". More complex models will be required to take into account differences in the rate of aging of naive and activated cells (Weng *et al*., 1995). (vi) We have assumed the parasite is equivalent to a single antigen. In reality a parasite is composed of multiple antigens, and "competition" between the immune responses to these antigens may need to be considered (De Boer & Perelson, 1994; Nowak *et al*., 1995).

In conclusion we note that the value of this simple model is to explore the type of behavior which may be generated if T-cell proliferation is restricted by a Hayflick limit, and is not intended to generate precise numerical results. More complex interactions can be added stepwise. One particularly exciting addition which we are currently considering is competition between the immune responses to multiple parasite antigens. This may be relevant to the dynamics of immune responses to mycobacterial infections where competition between Th1 and Th2 responses may play an important role during infection. Another area of interest is to explore the effect of the Hayflick limit on the immune repertoire, and how it changes with aging, as well as during HIV infection.

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REFERENCES

Andino, R., Silvera, D., Suggett, S. D., Achacoso, P. L., Miller, C. J., Baltimore, D. & Feinberg, M. B. (1994). Engineering poliovirus as a vaccine vector for the expression of diverse antigens. *Science* **265,** 1448–1451.

Antia, R., Koella, J. C. & Perrot, V. (1996). Models of the within-host dynamics of persistent mycobacterial infections. *Proc. R. Soc. Lond. B*, **263,** 257–263.

Bierman, E. L. (1978). The effect of donor age on the in vitro life span of cultured human arterial smooth-muscle cells. *In vitro* **14,** 951–955.

Bloom, B., Modlin, R. L. & Salgame, P. (1992). Stigma variations: observations on suppressor T cell and leprosy. *Ann. Rev. Immunol*. **10,** 453–488.

Cohen, J. (1996). Selling the immune system short. *Science* **273**, 30–31.

Counter, C. M., Botelho, F. M., Wang, P., Harley, C. B. & Bacchetti, S. (1994). Stabilization of short telomeres and telomerase activity accompany immortalization of Epstein-Barr virus-transformed human B lymphocytes. *J. Virol*. **68,** 3410–3414.

Cristofalo, V. J. & Pignolo, R. J. (1993). Replicative senescence of human fibroblast-like cells in culture. *Physiol. Rev*. **73,** 617–638.

Dalod, M., Fiorentino, S., Delamare, C., Rouzioux, C., Sicard, D., Guillet, J. G. & Gomard, E. (1996). Delayed virus-specific CD8+ Cytotoxic T-lymphocyte activity in an HIV-infected individual with high CD4+ cell counts—correlations with various parameters of disease progression. *AIDS Res. Human Retroviruses* **12,** 497–506.

De Boer, R. J. & Hogeweg, P. (1989). Idiotypic networks incorporating T-B cell cooperation. The conditions for percolation. *J. theor. Biol*. **139,** 17–38.

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

De Boer, R. J. & Perelson, A. S. (1995). Towards a general function describing T cell proliferation. *J. theor. Biol*. **175,** 567–576.

Effros, R. B. & Walford, R. L. (1984). T cell cultures and the Hayflick limit. *Human Immunol*. **9,** 49–65.

Effros, R. B., Perillo, N. L., Bhuta, S. & Walford, R. L. (1990). *In vitro* studies of human T lymphocyte senescence. In *Molecular Biology of Aging* (Finch, C. E. & Johnson, T. E., eds) pp. 265–279. New York: Wiley-Liss.

Effros, R. B., Allsopp, R., Chiu, C. P., Hausner, M. A., Hirji, K.,

WANG, L., ET AL. (1996) Shortened telomeres in the expanded CD28−, CD8+ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. *AIDS* **10,** F17–F22.
FIORENTINO, S., DALOD, M., OLIVE, D., GUILLET, J. G. & GOMARD, E. (1996). Predominant involvement of CD8 + CD28 − lymphocytes in human immunodeficiency virus-specific cytotoxic activity. *J. Virol.* **70,** 2022–2026.
FISHMAN, M. A. & PERELSON, A. S. (1994). Th1/Th2 cross regulations. *J. theor. Biol.* **170,** 25–56.
FLYNN, J. L., CHAN, J., RIIEBOLD, K. J., SALTON, D. K., STEWART, T. A. & BLOOM, B. R. (1993). An essential role for interferon-γ in resistance to Mycobacterium tuberculosis infection. *J. Exp. Med.* **178,** 2249–2254.
GRODY, W. W., FLIGIEL, S. & NAEIM, F. (1985). Thymus involution in the acquired immunodeficiency syndrome. *Am. J. Clin. Pathol.* **84,** 85–95.
GROSSMAN, Z. & PAUL, W. E. (1992). Adaptive cellular interactions in the immune system: The tunable activation threshold and the significance of subthreshold responses. *Proc. Natl. Acad. Sci. U.S.A.* **89,** 10365–10369.
HAYFLICK, L. (1965). The serial cultivation of human diploid strains. *Exp. Cell Res.* **37,** 586–621.
HAYFLICK, L. & MOORHEAD, P. S. (1961). The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **25,** 585–621.
HIYAMA, K., HIRAI, Y., KYOIZUMI, S., AKIYAMA, M., HIYAMA, E., PIATYSZEK, M. A., *et al.* (1995). Activation of telomerase in human lymphocytes and hematopoietic progenitor. *J. Immunol.* **155,** 3711–3715.
HO, D. D., NEUMANN, A. U., PERELSON, A. S., CHEN, W., LEONARD, J. M. & MARKOWITZ, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373,** 123–126.
JERNE, N. K. (1974). Towards a network theory of the immune system. *Ann. Immunol.* (*Inst. Pasteur*) **125C,** 372–388.
KAMMERER, R., ITEN, A., FREI, P. C. & BURGISSER, P. (1996). Expansion of T-cells negative for CD28 expression in HIV-infection-relation to activation markers and cell-adhesion molecules, and correlation with prognostic markers. *Med. Micro. Immunol.* **185,** 19–25.
KAPPLER, J. W., ROEHM, N. & MARRACK, P. (1987). T cell tolerance by clonal elimination in the thymus. *Cell* **49,** 273–280.
MCLEAN, A. R. (1994). Modeling T cell memory. *J. theor. Biol.* **170,** 63–74.
MCLEAN, A. R. & KIRKWOOD, T. L. B. (1990). A model of human immunodeficiency virus (HIV) infection in T helper cell clones. *J. theor. Biol.* **147,** 177–203.
MILLER, J. A. F. P. (1992). The key role of the thymus in the body's defense strategies. *Phil. Trans. R. Soc. Lond. B* **337,** 105–124.
MOSMANN, T. R. & SAD, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* **17,** 138–146.
MUELLER, S. N., ROSEN, E. M. & LEVINE, E. M. (1980). Cellular senescence in a cloned strain of bovine fetal aortic endothelial cells. *Science* **207,** 889–891.
NOWAK, M. A., MAY, R. M. & SIGMUND, K. (1995). Immune responses against multiple epitopes. *J. theor. Biol.* **175,** 325–353.
PERELSON, A. S., NEUMANN, A. U., MARKOWITZ, M., LEONARD, J. M. & HO, D. D. (1996). HIV-1 dynamics in vivo: virion clearance rate, infected cell lifespan, and viral generation time. *Science* **271,** 1582–1586.
PERILLO, N. L., WALFORD, R. L., NEWMAN, M. A. & EFFROS, R. B. (1988). Human T lymphocytes possess a limited in vitro life span. *Exp. Gerontol.* **24,** 177–187.
PERKUS, M. E., PICCINI, A., LIPINSKAS, B. R. & PAOLETTI, E. (1985). Recombinant vaccinia virus: immunization against multiple pathogens. *Science* **229,** 981–984.
RHEINWALD, J. G. & GREEN, H. (1975). Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* **6,** 331–343.
SALGAME, P., CONVIT, J. & BLOOM, B. R. (1991). Immunological suppression by human CD8+ T cells is receptor dependent and HLA-DQ restricted. *Proc. Natl. Acad. Sci. U.S.A.* **88,** 2598–2602.
SAUKKONEN, J. J., KORNFELD, H. & BERMAN, J. S. (1993). Expansion of a CD8 + CD28 − cell population in the blood and lung of HIV-positive patients. *AIDS* **6,** 1194–1204.
SCHWEITZER, A. N., SWINTON, J. S. & ANDERSON, R. M. (1992). Complex outcomes in mouse leishmaniasis: A model for the dynamics of the Th1 response. In: *Theoretical and Experimental Insights into Immunology* (Perelson, A. S. & Weisbuch, G., eds), Berlin: Springer-Verlag.
STOVER, C. K., DE LA CRUZ, V. F., FUERST, T. R., BURLEIN, J. E., BENSON, L. A., BENNETT, L. T., *et al.* (1991). New use of BCG for recombinant vaccines. *Nature* **351,** 456–460.
TASSIN, J., MALAISE, E. & COURTOIS, Y. (1979). Human lens cells have an in vitro proliferative capacity inversely proportional to the donor age. *Exp. Cell Res.* **123,** 388–392.
VINGERHOETS, J. H., VANHAM, G. L., KESTENS, L. L., PENNE, G. G., COLEBUNDERS, R. L., VANDENBRUAENE, M. J., *et al.* (1995). Increased cytolytic T-lymphocyte activity and decreased B7 responsiveness are associated with CD28 down-regulation on CD8+ T-cells from HIV-infected subjects. *Clin. Exp. Immunol.* **100,** 425–433.
WEI, X., GHOSH, S. K., TAYLOR, M. E., JOHNSON, V. A., EMINI, E. A., DEUTSCH, P., *et al.* (1995). Virus dynamics in human immunodeficiency virus type 1 infection. *Nature* **373,** 117–122.
WENG, N. P., LEVINE, B. L., JUNE, C. H. & HODES, R. J. (1995). Human naive and memory T lymphocytes differ in telomeric length and replicative potential. *Proc. Natl. Acad. Sci. U.S.A.* **92,** 11091–11094.

APPENDIX

In order to simplify the analysis of the model we introduce an alternative partial differential equation (PDE). In so doing, we seek a PDE resembling the introduced system of ODE's.

For the PDE model we assume that the age of T-cells is a continuous variable that varies from 0 for naive cells to 1 for those cells having reached the Hayflick Limit. The discrete ages similar to successive generations can be incorporated in this model by dividing the interval [0, 1] into n subintervals each corresponding to a single generation. For instance, the generation X_i will correspond to the age distribution over the interval $[i/n, i + 1/n]$. Let now $U(t, a)$ be the age distribution of T-cells, that is the density of T-cells of age a at time t. Note, that we still keep the well-mixedness hypothesis for both T-cell and parasite populations. A continuous analogue of the total immune response X would be

$$X(t) = n \cdot \int_0^1 U(t, a)da. \tag{A.1}$$

So, we have the following integro-differential equation for the evolution of P and an ODE for Q:

$$\frac{\mathrm{d}P}{\mathrm{d}t} = P\left(r - hn \cdot \int_0^1 U(t, a)da\right) - fP + gQ, \tag{A.2}$$

$$\frac{dQ}{dt} = fP - gQ. \tag{A.3}$$

In order to set up the equation for $U(t, a)$ we rewrite eqn (1) as

$$\frac{dX_i}{dt} = 2S(P)(X_{i-1} - X_i) + (S(P) - d)X_i,$$

and replace the first term on the r.h.s. by

$$-2S(P)(U\left(t, a - \frac{1}{n}\right) - U(t, a)) = -2S(P)U_a(t, a)\frac{1}{n},$$

naturally assuming the distance between X_{i-1} and X_i being $1/n$, and n being large enough to give a good approximation. Thus, we arrive at the following PDE:

$$\frac{\partial}{\partial t}U(t, a) = \frac{-2S(P)}{n}\frac{\partial}{\partial a}U(t, a) + (S(P) - d)U(t, a) \quad \text{for} \quad 0 < a \leqslant 1, \quad 0 \leqslant t < \infty. \tag{A.4}$$

The differential equation for X_0 suggests the form of the boundary condition at the age $a = 0$. The rate at which newborn cells (naive cells) are being introduced to the system is $A(P)$, now these cells die at a rate d and are stimulated and move into the subsequent age class (thus, away from $a = 0$) at a rate $S(P)$. These three facts put together comprise the following equation at $a = 0$:

$$\frac{\partial}{\partial t}U + dU + S(P)U = A(P), \quad \text{for} \quad a = 0, \quad 0 \leqslant t < \infty. \tag{A.5}$$

Leaving out the standard question of existence and uniqueness for the solutions to the system (A.2–A.5) which couples a first order hyperbolic PDE with an integro-differential equation and an ODE, we look for the steady states of this system. Since at a steady state the T-cell distribution U is a function of a single variable a, we will denote it as $U(a)$. There are two different situations: $P = 0$ and $P \neq 0$.

If $P = 0$, eqns (A.2–A.3) are satisfied whenever $Q = 0$. For eqn (A.4) one has

$$0 = \frac{-2\epsilon}{n}\frac{\partial}{\partial a}U(a) + (\epsilon - d)U(a),$$

which can be solved directly using the boundary condition (A.5) which reads now $(\epsilon + d)U(0) = A(0)$. The solution is given by the following formula:

$$U(a) = \frac{A(0)}{\epsilon + d}e^{\frac{n(\epsilon - d)}{2\epsilon}a} \quad \text{for} \quad 0 \leqslant a \leqslant 1.$$

Again we point out that since $\epsilon \ll d$, the steady-state distribution is an exponentially decreasing function of the age a. Note that this steady state always exists for all values of parameters in $A(P)$ and $S(P)$.

If $P \neq 0$ we can always find the steady state distribution for $Q = f/gP$ and for U which is given by the formula

$$U(a) = \frac{A(P)}{S(P) + d}e^{\frac{n(S(P - d)}{2S(P)}a} \quad \text{for} \quad 0 \leqslant a \leqslant 1.$$

To satisfy eqn (A.2) we equate $r = h \cdot X$. X given by the eqn (A.1) can be calculated as:

$$X = n\int_0^1 \frac{A(P)}{S(P) + d}e^{\frac{n(S(P - d)}{2S(P)}a} \cdot da = \frac{2A(P)S(P)}{(S(P) + d)(S(P) - d)}\left(e^{\frac{(S(P) - d)}{2S(P)}} - 1\right) \tag{A.6}$$

if $S(P) \neq d$. However, if $S(P) = d$, then $U(a) \equiv U(0)$ and

$$X(P) = \frac{nA(P)}{S(P) + d} = \lim_{P \to S^{-1}(d)} X(P), \tag{A.6'}$$

so $X(P)$ is a continuous function of P, differentiable away from $P = S^{-1}(d)$. Now for a steady state to exist one must be able to find such a value of P that $X = X(P) = r/h$. We follow the steps similar to those in investigating the existence of nontrivial equilibria for the ODE model.

At this point we turn to the description of the dynamics generated by the eqns (A.2–A.5). The advantage we have is that the PDE was created in such a way that it yields the original system of ODE for Hayflick limit when solved by method of lines with the number of grid points equal exactly to n. Thus, we do not present the numerical results for the PDE model here. Rather we investigate what would happen if the parasite level were constant. The solution $U(t, a)$ would be presented by means of the explicit formula:

$$U(t, a) = e^{(S - d)t} \cdot U\left(0, a - \frac{2S}{n}t\right), \quad \text{for} \quad a - \frac{2S}{n}t \geqslant 0, \tag{A.7}$$

$$U(t, a) = e^{(S - d)\frac{an}{2S}}\left(e^{-\left(t - \frac{an}{2S}\right)} \cdot U(0, 0) + \frac{A}{S + d}\left(1 - e^{-\left(t - \frac{an}{2S}\right)}\right)\right) \quad \text{for} \quad a - \frac{2S}{n}t \leqslant 0, \tag{A.7'}$$

where $S = S(P)$ and $A = A(P)$ are evaluated at the fixed level of parasite density P. This solution (A.7–A.7′) is found by integration along characteristics of (A.2–A.5), that is along straight lines $a = 2S/nt + \text{Const}$.

We would like to point out several useful properties of the above equations. First of all, as time goes past $n/2\epsilon$ where ϵ is the minimal possible value of $S(P)$, the solution depends exclusively upon its value at $(0, 0)$, that is $U(0, 0)$. Second, for t sufficiently large ($t \to +\infty$) the solution is given by the formula (A.7′), and therefore its limit is calculated as

$$U(a) = \frac{A(P)}{S(P) + d} e^{\frac{n(S(P) - d)}{2S(P)} a}.$$

Thus, we expect the T-cell distribution to reach a certain asymptotic distribution, different for each value of P. Third, given a parasite density P, the time required for T-cells to reach the Hayflick limit can be estimated as $t = n/2S(P)$.