Models of the within-host dynamics of persistent mycobacterial infections

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SUMMARY

We use mathematical models to investigate the within-host dynamics of mycobacterial infections. In particular, we investigate the mechanisms by which bacteria such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* persist at low densities for extended periods, and attain high densities much later. We suggest that the persistence of bacteria in face of immune pressure may result from the bacteria having a very slow growth rate, or having a dormant stage. We show that whereas these mechanisms may lead to long-term persistence, this will be obtained at relatively low densities. We then suggest that the long-term persistence of bacteria may result in the loss of immunity because of the deletion of specific T-cells arriving from the thymus, and the exhaustion of the specific T-cells as these cells reach the Hayflick limit and die. This loss of immunity will allow the bacteria to attain a high density. We propose experiments capable of testing our models and discuss the implications of the models for the treatment of infected hosts.

1. INTRODUCTION

While recent advances have greatly enhanced to our understanding of the molecular and cellular biology of *Mycobacterium tuberculosis* and *Mycobacterium leprae* and the immune responses they elicit (Gaylord & Brennan 1987; Ellner & Wallis 1989; Bloom 1990; Jacobs *et al.* 1991; Kaufmann 1993; Britton *et al.* 1994), much less attention has been directed towards understanding how this biology leads to the observed dynamics of these bacteria within their hosts.

The dynamics of *M. tuberculosis* and *M. leprae* are different from those of bacteria and viruses causing acute infections of short duration, as well as from those of antigenically varying microparasites such as *Trypanosoma vivax* which maintain a high parasitemia for extended periods of time. Following infection with *M. tuberculosis* and *M. leprae*, progression to disease is relatively slow (Comstock & Cauthem 1993), suggesting that during the early phases of infection the immune response is able to control the bacterial density at a low level, and it is only later that the density of bacteria may increase to a high level. Several aspects of the biology of *M. tuberculosis* and *M. leprae* and the immune responses they elicit are worth noting. These bacteria replicate at a lower rate than their free living relatives and bacteria causing acute infections. *M. tuberculosis* has a doubling time of approximately one day, whereas that of *M. leprae* is an order of magnitude slower. In comparison, the free living *M. smegmatis* has a doubling time of two hours and *E. coli* has a doubling time of 0.4 hour. Following infection, *M. tuberculosis* and *M. leprae* enter mononuclear cells such as macrophages. After entering a macrophage, the bacteria may either proliferate or remain dormant (Small *et al.* 1994). During this 'dormant stage' the bacteria are hidden from the immune response and may be resistant to many drugs (Grange 1992; Britton *et al.* 1994). Mycobacteria antigens elicit strong cell-mediated Th1 responses that are capable of controlling these bacteria. It is thought that shortly after infection, it is this response that maintains the bacterial density at low levels, and if this response is absent, as is the case in mice unable to produce interferon-γ, the bacteria can attain an extremely high density (Cooper *et al.* 1993; Flynn *et al.* 1993). The observation of elevated Th2 associated cytokines in leprosy patients with high bacterial densities (Salgame *et al.* 1991; Yamamura *et al.* 1991; Bloom *et al.* 1992) suggests that Th1/Th2 cross-regulation may be responsible for disease[1]. However, a simple Th1/Th2 model does not explain the relatively slow progression from infection to disease: if the initial response is of a Th1 type then it might be expected that the infection is controlled (the Th1 response preventing a Th2-like response from developing); and if the initial response is of a Th2 type then a rapid progression to disease might be expected (the Th2 response preventing a Th1 response from developing).

We first construct simple models for the within-host dynamics of microparasites and use these models to examine how the biology of mycobacteria and their

[1] In this paper we use Th1 and Th2 responses to represent type 1 and type 2 T-cell responses on the basis of the cytokines which these cells secrete. These cells may be of either the CD4+ or the CD8+ phenotype (Seder *et al.* 1994) and in leprosy it may be that the type 1 responses are generated by CD4+ cells while the type 2 responses are generated by CD8+ cells (Bloom *et al.* 1992).

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interaction with the immune response gives rise to the observed pattern of infection. In particular, we examine the mechanisms which allow parasites to avoid clearance by the immune response, and how the long-term persistence can lead to the down-regulation of the immune response. In what follows the term parasite is used interchangeably with bacteria.

2. MODELS

(*a*) *Short-term dynamics*

Our basic model for the growth of microparasites shortly after infection resembles in many ways previous models (Reibnegger *et al.* 1989; Antia *et al.* 1994). We let P and X represent the parasite density and the intensity of the immune response to the parasite. In the absence of immunity we assume that the parasite grows exponentially at rate r. The intensity of the immune response is assumed to be proportional to the density of the T-cells specific to the parasite. These cells immigrate from the thymus at rate a, and die at rate d. The parasite stimulates the proliferation of T-cells at a rate that is proportional to the density of parasites at low parasite densities and that saturates at high parasite densities. The rates of growth of parasite and immunity are thus given by:

$$\frac{\mathrm{d}P}{\mathrm{d}t} = rP - hPX, \tag{1}$$

$$\frac{\mathrm{d}X}{\mathrm{d}t} = a + sX\left(\frac{P}{k+P}\right) - \mathrm{d}X, \tag{2}$$

These equations are scaled so that in the absence of parasite the density of immune cells equals unity (i.e. $a = d$) and the initial density of parasite is set at unity. The relative magnitudes of the various parameters for biologically reasonable cases are (Antia *et al.* 1994):

$$h < a = d < r,\ s \approx 1 \ll k. \tag{3}$$

Equations (1) and (2) have a trivial steady state, where parasite density is equal to zero and immunity is equal to a/d, and another steady state, where the parasite density P^* is balanced by immunity X^*

$$P^* = \frac{k\mathrm{d}r - ahk}{(s-\mathrm{d})r + ah} \approx \frac{k\mathrm{d}}{s-\mathrm{d}} \quad \text{and} \quad X^* = \frac{r}{h}. \tag{4}$$

Using formal stability analysis it can be shown that this steady state is stable when the input from the thymus, a is greater than zero. As we expect the input from the thymus to be small, we find that the parasite and immune response oscillate and only gradually converge on the steady-state. This can also be noted by observing that the isoclines corresponding to $\mathrm{d}P/\mathrm{d}t = 0$ and $\mathrm{d}X/\mathrm{d}t = 0$ are almost perpendicular (see inset to figure 1*a*). Figure 1*a* shows that rapidly growing parasites stimulate a strong immune response, which results in the parasite density (and immune response) exhibiting lightly damped oscillations of large amplitude. During these oscillations, the parasite density passes through several minima, where the density of parasites is so low, that we would expect the parasite to be driven to extinction by the immune response (because a fraction of a parasite cannot exist). In contrast, the oscillations in the density of a slowly growing parasites have a smaller amplitude, and the parasite does not reach as high a maximum or as low a minimum density before attaining the steady state. The relation between the growth rate and the minimum parasitemia is shown in figure 1*b*. These results suggest that by growing slowly, parasites may avoid being driven to extinction by the immune response and persist within the host.

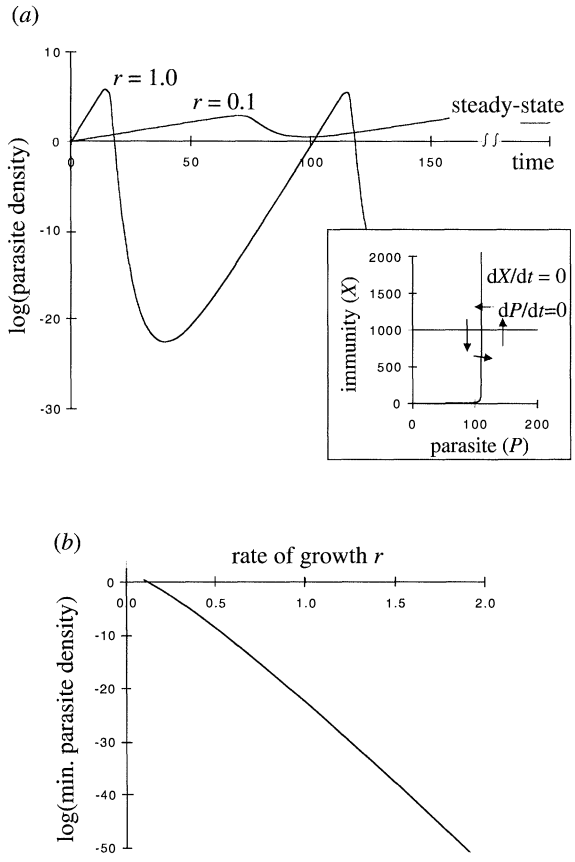

Figure 1. (*a*) Dynamics of a slowly ($r = 0.1$) and rapidly ($r = 1$) growing parasite: slow growth can prevent extinction of the parasite. The inset shows the isoclines for $\mathrm{d}X/\mathrm{d}t = 0$ and $\mathrm{d}P/\mathrm{d}t = 0$. (*b*) Minimum parasite density as a function of the growth rate of the parasite. At high growth rates the minimum density is so low that the parasite will go extinct. The density of parasite (P) within the host was calculated by numerical solution of equations (1) and (2) with parameters $h = 10^{-3}$, $d = 0.1$, $s = 1$, $k = 10^3$, $a = 0.1$, and initial conditions $P(0)$ and $X(0) = 1.0$.

To incorporate a dormant stage (or refuge) into the model of the parasite dynamics, we assume that the parasite exists in two forms: a growth stage P; and a dormant stage Q which is sequestered from the immune response. The rate constants f and g describe the conversions between the growth and dormant stages. The dormant stage neither stimulates immunity nor is it susceptible to the immune response. The equations

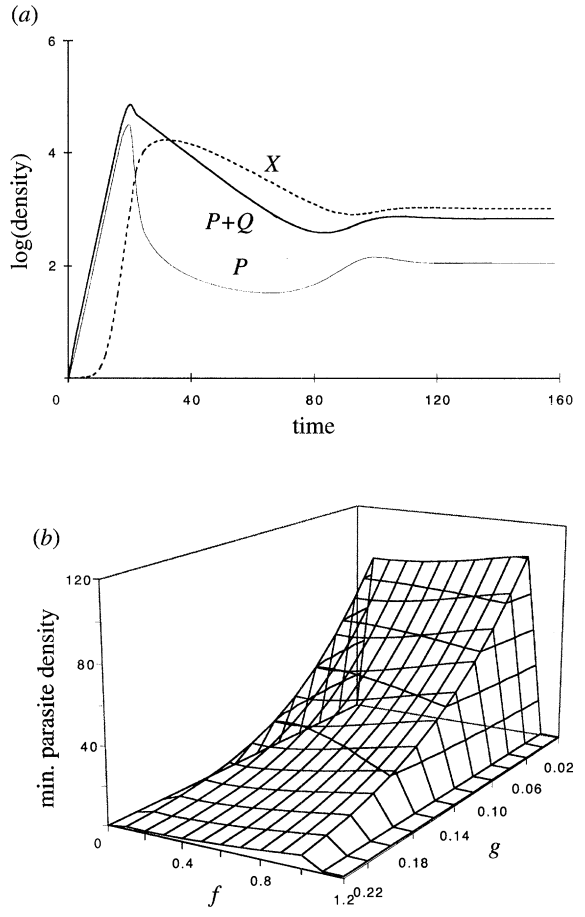

Figure 2. Dynamics of parasites having a dormant stage: the dormant stage provides a refuge and prevents extinction. (*a*) The density of the immune response (X) and both the growth (P) and dormant stage (Q) of the parasite as a function of time. (*b*) The minimum density of the growth stage (Pmin) as a function of f and g. The density of parasite within the host was calculated by numerical solution of equations (3)–(5) with parameters as in figure 1 and $r = 1.0$, the initial density of the dormant stage $Q(0) = 0$, and in (*a*) $f = 0.5$, $g = 0.1$.

describing the dynamics of parasite and immunity are now:

$$\frac{dP}{dt} = rP - hPX - fP + gQ, \tag{5}$$

$$\frac{dQ}{dt} = fP - gQ, \tag{6}$$

$$\frac{dX}{dt} = a + sX\left(\frac{P}{k+P}\right) - dX. \tag{7}$$

These equations have a trivial steady state where parasite density is zero, immunity equals a/d, and a steady state with the densities of the various populations given by:

$$P^* \approx \frac{kd}{s-d}; \quad Q^* = \frac{f}{g}P^* \quad \text{and} \quad X^* = \frac{r}{h}. \tag{8}$$

Note that the density of the growth stage and the level of immunity reach the same values as in the previous model (i.e. as in the absence of a dormant stage). However, the total parasite density, $P+Q$, is elevated by a factor of $(1+f/g)$.

While the steady-state density of the growth stage remains identical to its value in the absence of a dormant stage, the stability of this equilibrium can be altered dramatically. As shown in figure 2*a* a dormant stage very effectively damps the oscillations in parasite density, increasing the minimum parasite density, and preventing extinction of the parasite. In figure 2*b* we show that dormancy prevents parasite extinction for a broad regime of parameters, including: (i) when the lifespan of the dormant stage is sufficiently long to allow the immune response to return from its peak level to its steady state density (i.e. $g < d$); and (ii) when the parasite's replication rate exceeds the rate at which it is converted to the dormant stage (i.e. $r > f$), allowing the parasite to proliferate during the initial stages of infection.

(*b*) *Long-term dynamics*

The previous models consider the dynamics shortly after infection with a parasite, and need to be modified to consider the dynamics of persistent infections. In the following discussion we modify the equations for the dynamics of T-cells, keeping the equations for the dynamics of parasite the same as in the previous model (i.e. equations (5) and (6)). In the model described below, we investigate whether the combination of two known phenomenon (the antigen-dependent deletion of T-cells in the thymus, and the presence of a Hayflick limit to the proliferation of T-cells) can generate immune suppression whose dynamics is similar to that observed in mycobacterial infections. The relation of this model with previous models is described in §3.

First, we note that if the parasite persists, the presence of parasite antigens could lead to clonal deletion of parasite-specific immune cells in the thymus, resulting in a decrease in the immigration of T-cells from the thymus (parameter a) in a manner dependent on the density of parasite, as follows:

$$a(P) = a_1 - \frac{a_1 P^m}{(a_2)^m + P^m}. \tag{9}$$

The parameter m regulates the shape of this function (from gently sloping to step function) and a_2 is the density of parasite (or antigen) at which the input from the thymus is reduced to half its maximum value. In accord with experiments by Miller (1992), we expect a_2 to be small and we set it to be somewhat greater than the initial parasite inoculum, but much less than the parasite density (k) at which T-cells in the periphery proliferate at half the maximum rate of proliferation.

Second, we note that – analogous to the limited proliferative capacity of epithelial cells observed by Hayflick (Hayflick & Moorhead 1961) – immune cells are only capable of a limited number of cell divisions (Perillo *et al.* 1988). The limited proliferation of immune cells is incorporated by introducing a 'Hayflick limit' of n generations, after which the T-cells die. In accord with the experimental literature we set $n = 20$–25 (Perillo *et al.* 1988). If we let x_i equal the

number of T-cells of the i^{th} generation, and $X = \Sigma x_i$ represents the total number of T-cells, then the equations for the proliferation of T-cells are given by:

$$\frac{dx_1}{dt} = a(P) - s\left(\frac{P}{k+P}\right)x_1 - dx_1$$

$$\frac{dx_i}{dt} = 2\,s\left(\frac{P}{k+P}\right)x_{i-1} - s\left(\frac{P}{k+P}\right)x_i - dx_i \qquad (10)$$

$$\frac{dx_n}{dt} = 2\,s\left(\frac{P}{k+P}\right)x_{n-1} - s\left(\frac{P}{k+P}\right)x_n - dx_n.$$

The dynamics shortly after infection is not affected by the addition of the Hayflick limit. This is because if the Hayflick limit is in a biologically reasonable range (i.e. between 20 and 25 (Perillo *et al.* 1988)), very few immune cells reach the Hayflick limit during this time, and the densities of parasite and immunity reach approximately those given in equation (8). In the long term however, the outcome depends on whether the parasite restricts input from the thymus sufficiently so as to prevent the immune response (which as before is assumed to be proportional to the total density of T-cells) from controlling its density. The steady state solution to these equations can be obtained by collapsing the $n+2$ equations (corresponding to n compartments for the T-cells and two for the parasite) into two, one for the growth stage of the parasite and one for the total density of T-cells. It can be shown that the total parasite density P and immunity X at equilibrium must satisfy the following conditions (Pilyugin *et al.* 1996):

$$\frac{dP}{dt} = 0 \Rightarrow X = \frac{r}{h}, \qquad (11)$$

and

$$\frac{dX}{dt} = 0 \Rightarrow X = \left(a_1 - \frac{a_1 P^m}{(a_2)^m + P^m}\right)\left(\frac{k+P}{(s-d)P - kd}\right)\left(\left(\frac{2sP}{(s+d)P+kd}\right)^n - 1\right). \qquad (12)$$

In figure 3*a* we see that only if a_2 is sufficiently large will the two lines intersect, allowing the parasite to be controlled at steady-state. We use numerical simulations of the complete $(n+2)$ dimensional system to examine the dynamics of parasite and immunity and evaluate the stability of the steady-states inferred from figure 3*a*. When a_2 is sufficiently small, (figure 3*b*), in the long term the immune response declines and the parasite escapes control. When a_2 is sufficiently large (figure 3*c*) the immune system is able to control the parasite, and we find that the densities of parasite and immune response oscillating around the values obtained from the solution of equations (11) and (12) shown in figure 3*a*. As the parameter a_2 is increased yet further the limit cycle collapses to a fixed point. A more detailed description of this behaviour can be found in Pilyugin *et al.* (1996), however, the general form of the behavior of the system as the parameter a_2 increases follows the pattern described here, i.e. escape of the parasite from immune control when a_2 is small and control of the parasite when a_2 is sufficiently large.

3. DISCUSSION

The models suggest two elements of a mechanism for the pattern of infection shown by *M. tuberculosis* and *M. leprae*. First, either slow growth or the production of a dormant stage can prevent the bacteria from being driven to extinction by the immune response. Second, if the density of bacteria is sufficiently high, then the reduction in input of naive cells from the thymus and the loss of cells as they approach the Hayflick limit may, in the long-term, result in the loss of the specific immune response to the bacteria and their proliferation will no longer be controlled. We now consider whether the models are consistent with the experimental observations of *M. tuberculosis* and *M. leprae*, and suggest how the models can be experimentally tested.

Both *M. tuberculosis* and *M. leprae* have a much slower growth rate than the free-living mycobacterium *M. smegmatis*, suggesting that slow growth is not a phylogenetic constraint on all mycobacteria but is associated with persistent infection. Evidence suggesting mycobacteria have a dormant stage includes reports that these bacteria can be separated into different populations (Khomenko 1987; Grange 1992). This dormant stage has been suggested to be resistant to many drugs and has sometimes been referred to as a mycobacterial persistor (Grange 1992). The dormant stage could be generated in several ways. Following invasion of a macrophage, the bacteria could remain dormant within the cell (Britton *et al.* 1994). The antigens from the dormant bacteria would not appear on the surface of the cell, thus rendering more difficult the recognition and destruction of the infected cell. Alternatively, the bacteria may persist in the tuberculous lesions characteristically observed following infection with *M. tuberculosis*. The model presented here formally demonstrates how the presence of a dormant stage or refuge for the bacteria could lead to the long-term coexistence of the parasite and the immune response. In this respect, the model with the dormant stage is the application of the principle of the 'refuge' in ecology to the within-host dynamics of an infections disease.

The two mechanisms for persistence (i.e. slow growth versus a dormant stage) may be experimentally distinguished by adding a large parasite inoculum to a host in the early stages of infection. If slow growth is responsible for persistence then a large inoculum will result in the development of a sufficiently large immune response to clear the parasite. In contrast, if there is a dormant stage then the infection need not be cleared following the additional inoculation. These two outcomes are illustrated in figure 4.

As there are multiple pathways by which the immune response can be regulated, it is not surprising that a variety of models are needed to describe the different processes. The down-regulation of the immune response may arise from: (i) an additional population of suppressor cells (Kaufman *et al.* 1985); (ii) idiotypic networks between immune cells (De Boer & Hogeweg 1989); (iii) the shape of the T-cell–antigen dose response (McLean & Kirkwood 1990; Schweitzer & Anderson 1992; Swinton *et al.* 1994);

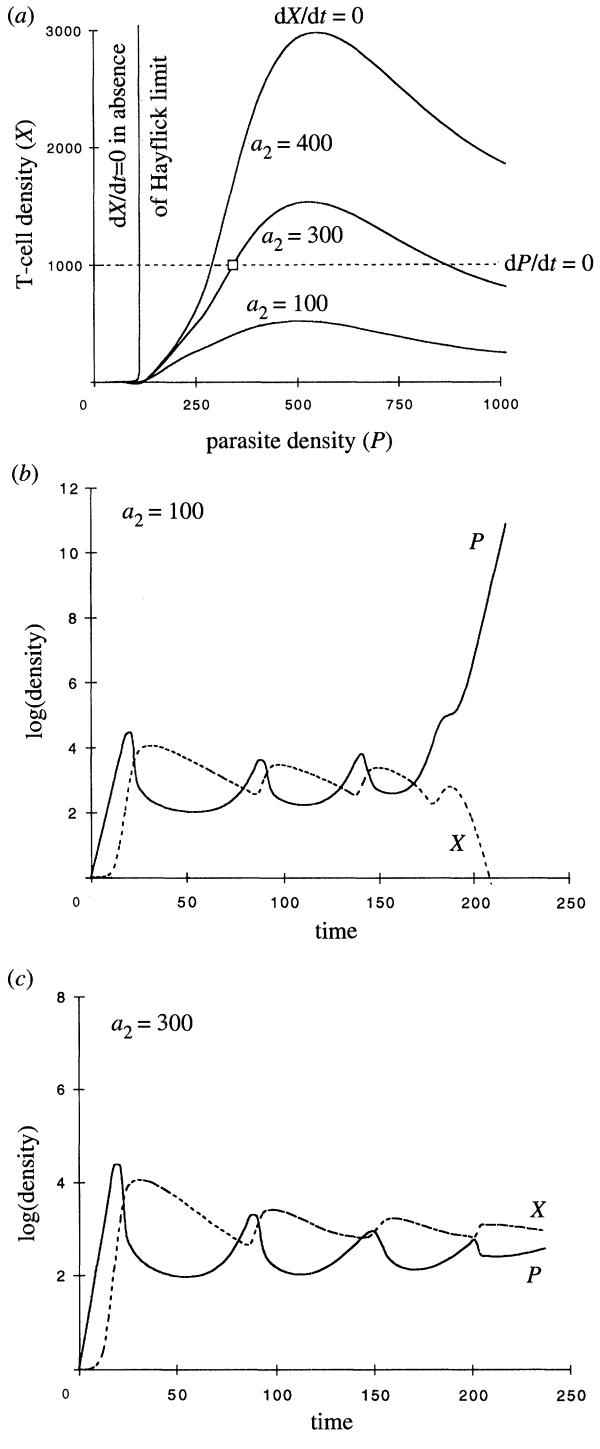

Figure 3. Dynamics when the immune system is regulated by the Hayflick limit. (*a*) Isoclines of $dP/dt = 0$ (---), and $dX/dt = 0$ (——) for the reduced system of equations given by equations (11) and (12) and different values of a_2, the parasite density that reduces input from the thymus by half. If a_2 is low, the isoclines for $dP/dt = 0$ and $dX/dt = 0$ do not intersect, and in the long-term the immune response is not able to control the parasite. When the isoclines intersect the immune response may be able to control the parasite. In (*b*) and (*c*) we examine the dynamics with the help of numerical solutions of the complete model (equations (5), (6), (9) and (10)). The dynamics for a small ($a_2 = 100$) and a large ($a_2 = 300$) value of a_2 is shown in (*b*) and (*c*) respectively. For the parameters and initial conditions of the simulation in (*b*) these two isoclines in (*a*) do not intersect, and for the simulations in (*c*) the parasite exhibits limit cycles around the intersection between the isoclines which is marked by the box. The parameter values are as in figure 2*a* with $n = 23$, $a_1 = 0.1$, and $m = 3$.

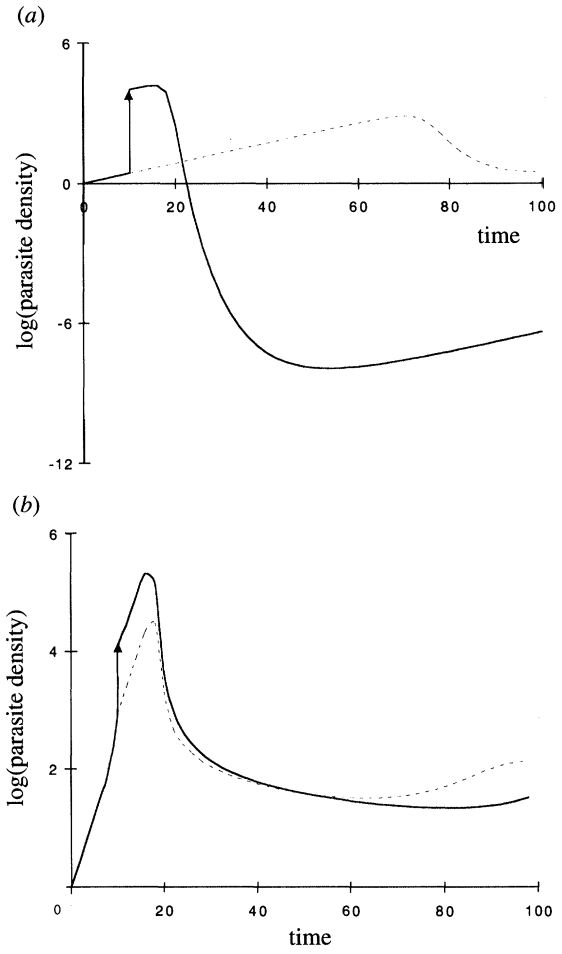

Figure 4. Treatment of infected hosts: a large inoculum of parasite delivered shortly following the initial infection is able to clear a slowly growing parasite (*a*), but is not able to clear a parasite with a dormant stage (*b*). Parameters as in figure 1 with $r = 0.1$ for (*a*), and as in figure 2*a* for (*b*). In both cases a second inoculum of 10^4 parasites is given at $t = 10$ days as indicated by the arrow, and the dotted lines represent the parasitemia in the absence of treatment.

(iv) cross-regulation between Th1 and Th2 responses (Fishman & Perelson 1994); and (v) the Hayflick model presented here. The Hayflick model accounts for both the initial control of the bacteria and their later escape from immune control. While, at this stage, we cannot definitively rule out the alternative models we note that in many cases the outcome (control–clearance or loss of control of the bacteria) is obtained relatively rapidly, rather than the pattern of initial control of the bacteria followed by subsequent loss of control of the bacteria which may characterize disease. Because Th1/Th2 cross regulation has been suggested to be responsible for the down-regulation of the Th1 immune response (Bloom *et al.* 1992), we now compare the outcome of these models with the Hayflick model. As mentioned in the introduction, it is not clear how the shift from an initial Th1 response to a Th2 type of response would occur: the initial Th1 response, which

controls the parasite, should down-regulate the Th2 type response and thus prevent its emergence (however, since the intensity of the Th1 response ($X = r/h$) required to control the bacteria is relatively low, it will exert a relatively weak downregulatory force on the Th2 response). In the Hayflick model the protective Th1 response capable of clearing the parasite is lost as the Hayflick limit for these cells is reached. While the Hayflick model presented does not explicitly consider the Th2 response, we note that the loss of the Th1 response might allow a competing Th2 response to take over. In this (Hayflick limit) scenario the elevated Th2 response would arise a consequence of the loss of the Th1 response, and not cause of the loss of the Th1 response as is the case in the Th1/Th2 scenario. At present we do not believe there is sufficient experimental data to discriminate between Th1/Th2 cross regulation model or the Hayflick model for mycobacterial infections. However it is, at least in principle, possible to test and reject the Hayflick model which predicts that the loss of Th1 cell clones specific for these mycobacteria during the course of infection.

What implications do our models have for the treatment of patients? First, both the slow growth model and the dormant stage model are consistent with observations that prolonged treatment with many antimicrobial agents is required to control the infection. This could arise as a consequence of the antimicrobial agents killing only dividing bacteria or because the bacteria in the dormant stage are physically inaccessible to the antimicrobial agent. Second, as mentioned earlier, if the slow-growth model is correct, inoculation shortly after infection with large amounts of either live or dead bacilli will result in the clearance of the infection. If however the initial persistence of mycobacteria is the result of a dormant stage, the inoculation of bacilli need not clear the infection.

The models presented here form the basis for adding further complexity and suggest directions for future studies. First, in the models described here the parasite is reduced to an equation describing a single replicating antigen. We need to consider the effect of introducing several antigens and competition between the immune responses to these antigens (De Boer & Perelson 1994). Second we need to consider the effect of incorporating Th1 and Th2 immune responses and cross-regulation between these responses in our models. We expect that further theoretical and experimental work will help to address these questions.

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