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Modeling Immune Responses with Handling Time

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Simple predator–prey type models have brought much insight into the dynamics of both nonspecific and antigen-specific immune responses. However, until now most attention has been focused on examining how the dynamics of interactions between the parasite and the immune system depends on the nature of the function describing the rate of activation or proliferation of immune cells in response to the parasite. In this paper we focus on the term describing the killing of the parasite by cell-mediated immune responses. This term has previously been assumed to be a simple mass-action term dependent solely on the product of the densities of the parasite and the immune cells and does not take into account a handling time (which we define as the time of interaction between an immune cell and its target, during which the immune cell cannot interact with and/or destroy additional targets). We show how the handling time (i) can be incorporated into simple models of nonspecific and specific immunity and (ii) how it affects the dynamics of both nonspecific and antigen-specific immune responses, and in particular the ability of the immune response to control the infection.

1. INTRODUCTION

The immune response of hosts protects against a variety of invading parasites such as viruses, bacteria, and protozoa. The immune response of vertebrate hosts may be broadly divided into nonspecific and antigen-specific responses. In this paper we focus on the cellular components of both nonspecific and antigen-specific responses. We describe and analyse two models, one for macrophages and other phagocytic cells which constitute the nonspecific arm of cell-mediated immune

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responses and one for cytotoxic T lymphocytes (CTL) which constitute the antigen-specific arm of cell-mediated immune responses.

The cellular component of the nonspecific response is comprised predominantly of the uptake and destruction of parasites by activated macrophages, neutrophils and other phagocytic cells (Greenberg and Silverstein, 1993; Kuby, 1997). Such a response is rapidly induced by the parasite and consists of the rapid recruitment and activation of phagocytic cells from a limited population of these cells within the body. A simple mathematical model of nonspecific immunity (Antia and Koella, 1994) illustrated how the dynamics of nonspecific and antigen-specific responses might be expected to differ, nonspecific responses are clearing weak (slowly growing) infections without additional intervention of specific immunity, controlling somewhat stronger (faster growing) infections and permanently reducing the rate of growth of the strongest (fastest growing) infections.

In contrast to the nonspecific immune response, which can be very rapidly induced, the specific immune responses are generated somewhat more slowly by a process of clonal selection. In accord with clonal-selection theory (Burnett, 1959; Kuby, 1997), prior to the exposure to the parasite there is only a small number of cells which are capable of responding to the parasite. The parasite selects for expansion (proliferation) of parasite-specific cells into a population which is sufficiently large to control the parasite. There is extensive literature on models of antigen-specific responses [see, for example Bell (1970, 1973); Kevrikidis *et al*. (1988); Agur *et al*. (1989); McLean and Kirkwood (1990); Schweitzer *et al*. (1992); De Boer and Perelson (1995); Pilyugin *et al*. (1997)]. Most of these mathematical models feature predator–prey-like dynamics with the parasite as the replicating prey, and the immune response as the predator (Bell, 1973).

Until now the models of both nonspecific and specific immune responses have, for the most part, focused on the functional form of the term describing the generation of the immune response. The killing function was usually modeled by a mass-action term proportional to the product of the parasite and immune-cell densities, and thus to the probability rate of a single encounter between two randomly chosen individual cells of these populations. However, as has been described in the predator–prey literature [see for example Bell (1973); Bazykin (1975); Hsu (1978); Hofbauer and So (1990); Crawley (1992)] the form of the killing function is also important in determining the dynamics of the response. In this paper we describe a more realistic form for the killing of the parasite by the immune response. We do so by using a formalism similar to that employed earlier (De Boer and Perelson, 1995; Borghans *et al*., 1996; Burroughs and Rand, 1998). We first develop quasi-static steady-state approximations which allow us to examine how the *handling time* can be incorporated into the earlier models of parasite and immune response. Then we examine the consequences of this change for the dynamics of the infection and in particular the *vulnerability* of the hosts immune response. We say that a given type of immune response (specific or nonspecific) is *vulnerable* to a given parasite if the infection of a sufficiently large initial size is able to escape the control by this

type of immune response. If the immune response is able to control infections of arbitrarily large initial sizes, we say that such immune response is *invulnerable* to the parasite.

An outline of the paper is as follows. In Section 2, we briefly sketch the relevant biology, that is how cell-mediated responses kill the parasite. In Section 3, we propose a model for nonspecific immunity with handling time and show the effects of handling time on the killing term. In the same section, we show that introducing handling time always makes nonspecific immunity vulnerable, and estimate the largest infection size that can be controlled by nonspecific immunity alone. In Section 4, we derive a model for specific immunity with handling time, and present criteria for vulnerability of the specific immune response. Section 5 contains the discussion and concludes the paper. All mathematical derivations, steady-state and stability analysis are presented in Appendix A (nonspecific immunity) and Appendix B (specific immunity).

2. BIOLOGICAL BACKGROUND

We now give a brief background to the cellular components of the nonspecific and antigen-specific immune responses.

The cellular component of the nonspecific immune response includes macrophages and other phagocytic cells. The reader is directed to excellent reviews from Adams and Hamilton (1984), Bancroft *et al*. (1991), Greenberg and Silverstein (1993) and Aderem and Underhill (1999) and to the specific references provided at the end of this paper for additional details relevant to the model. There is a fixed total population of macrophages (they do not proliferate). In order for macrophages to kill parasites they must first be 'activated'. Prior to introduction of the parasite only a small fraction of macrophages are activated (Bancroft *et al*., 1986). Infection rapidly results in the activation of the 'resting' macrophages. This activation can occur upon contact with common parasite antigens or induced by cytokines such as interferon-γ, tumor necrosis factor (TNF), and interleukin-1 (IL-1) which are produced by a variety of cell types including natural-killer (NK) cells and T-cells. The initial activation is, however, predominantly T-cell independent: macrophage activation is much faster than the generation of a T-cell response; virtually all macrophages may be activated prior to the generation of the specific T-cell response; moreover, the dynamics of macrophage activation is similar in severe combined immuno deficiency disease (SCID) mice which lack specific immune cells (Bancroft *et al*., 1986). In the absence of stimulation, activated macrophages return to the inactive (resting) state. In order for nonspecific phagocytic cells to kill parasites, they must first encounter the parasite and then kill it either following phagocytosis or by secreting potent toxins such a reactive oxygen intermediates and nitroxide radicals.

CTL form the cellular component of the antigen-specific immune response. The reader is directed to excellent reviews from Weiss (1993), Berke (1994) and Kuby

(1997) and to the specific references provided at the end of this paper for additional details relevant to the model. In order for CTL to kill infected cells they must first encounter the parasite-infected cells and then deliver 'lethal hits' to these cells. Initial recognition between the T-cell receptor (TCR) complex on the T-cell and the MHC-peptide complex on the infected cell results in the redistribution of surface molecules and the upregulation of adhesion between these two cells (Grakoui *et al*., 1999) which results on the formation of a conjugate complex between the target cell and the T-cell which lasts for about 20–30 min (Zagury *et al*., 1975; Perelson and Bell, 1982; Yanelli *et al*., 1986). This interaction results in a variety of responses including specific cytotoxicity, cytokine secretion or proliferation in a manner which has been suggested to depend on the level of TCR occupancy (Valitutti *et al*., 1996). Delivery of the 'lethal hit' results predominantly in the exocytosis of the contents of granules which kill the target cell, as well as by FasL–Fas-induced apoptosis in the target cell. In order to elicit the expansion of CTL from naive precursors it requires specific stimulation by both an antigen-specific signal and cytokines such as IL-2 or, alternatively, presentation of an antigen on specialized presenting cells such as dendritic cells (Bennett *et al*., 1998; Ridge *et al*., 1998). However, subsequent stimulation with the antigen-specific signal alone is sufficient for further proliferation and will be expected to be the major factor in the overall expansion. The time scales for delivery of the killing and division are very different. Target-cell destruction is fairly rapid, being observed either during the period of contact or shortly thereafter (Zagury *et al*., 1975). In contrast, proliferation of T-cells takes a longer time-frame with the mean doubling time being at least 6 h (Murali-Krishna *et al*., 1998).

Both nonspecific and specific cellular immune responses require two processes, the first being the encounter between immune cells and the *target* (which corresponds to the parasite for macrophages and infected cells for CTL) and the second being the interaction between an immune cell and its target, during which the immune cell cannot interact with and/or destroy additional targets. When the numbers of both immune cells and targets are relatively low, the encounter rate will be the slower of these two processes and thus be a better approximation to the killing of targets by immunity. However, when either population becomes large, the rate at which a given immune cell can kill the target becomes the limiting factor. We address this situation by introducing the notion of *handling time* for immune responses. We define the handling time as the time for the interaction between an immune cell and its target, during which the immune cell cannot encounter and destroy additional targets. The handling time at the very least equals the time of the encounter between an immune cell and its target, but could be limited by other factors: for instance, for macrophages and other phagocytic cells the time required to ingest and then degrade phagocytosed bacteria, so as to be able to phagocytose additional bacteria, may be greater than the actual time for phagocytosis; and for cytotoxic T-cells, the time required to recycle between delivery of successive lethal hits to infected cells may be greater than the time of the encounter.

3. NONSPECIFIC IMMUNE RESPONSES WITH HANDLING TIME

In this section we introduce a model for nonspecific immune responses to infection which builds on an earlier model (Antia and Koella, 1994), and in addition incorporates a *handling* time.

The modified model features a parasite population and nonspecific phagocytic cells which are placed in one of three compartments: inactive or *resting* cells, *activated free* cells, and *activated engaged* cells. Inactive cells are unable to phagocytose parasites. Upon activation, but before they engage in clearing the parasite, immune cells become activated free. Activated engaged cells are those currently phagocytosing parasites and consequently unable to phagocytose additional parasites. The total population of nonspecific immune cells is set at the constant value J so that $(J - X - S)$, X, and S are the numbers of inactive, activated free, and activated engaged cells respectively. The modified model incorporates the following processes.

(1) Parasites grow at an exponential rate $r > 0$ and are removed following encounter and phagocytosis by activated nonspecific immune cells at a rate modeled by the mass-action term $h_1 X P$.

(2) Recruitment of cells from inactive to the activated free pool occurs at a background rate $a \geq 0$, and in a parasite-dependent manner which we model by a mass-action term $(a + sP)(J - X - S)$. We introduce the term a to take into account the low level of background activation observed prior to introduction of a parasite (Bancroft *et al.*, 1986). In the absence of parasite stimulation, activated free cells return to the inactive state at a background rate d as described by the term dX.

(3) We assume that activated free cells become engaged with the parasite at a rate proportional to the number of both parasite and activated free cells with a rate constant h_2. Adjusting the value of h_2 allows us to model the situation where a single free cell can encounter several parasites before becoming engaged. Upon completion of phagocytosis, engaged cells return to the free state at a rate g. Here, $1/g$ is an average time required for a single cell to complete phagocytosis, or simply the handling time.

The flow diagram of the model is presented in Fig. 1.
We describe the model by the following system of differential equations,

$$P'(t) = rP(t) - h_1 X(t) P(t), \tag{3.1}$$

$$X'(t) = (a + sP(t))(J - X(t) - S(t)) - h_2 X(t) P(t) + gS(t) - dX(t), \tag{3.2}$$

$$S'(t) = h_2 X(t) P(t) - gS(t), \tag{3.3}$$

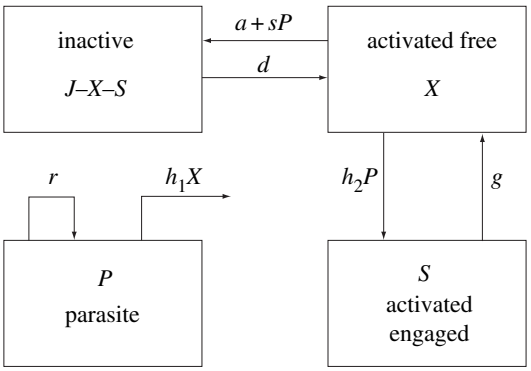

Figure 1. Flow diagram of the nonspecific immunity model.

with nonnegative initial conditions $P(0) \geq 0$, $X(0) \geq 0$, $S(0) \geq 0$ such that $X(0) + S(0) \leq J$.

Assuming that the average handling time $1/g$ is small, that is, the dynamics of (3.3) is fast compared with the dynamics of (3.1) and (3.2), we make the following quasi-steady-state assumption (QSSA-1):

$$0 = h_2 X(t)P(t) - gS(t) \qquad \text{or} \qquad S(t) = (h_2/g)X(t)P(t) = \alpha X(t)P(t),$$

where $\alpha = \frac{h_2}{g}$. We use QSSA-1 to reduce the model (3.1)–(3.3) to a system of only two equations:

$$P'(t) = rP(t) - h_1 X(t)P(t), \tag{3.4}$$

$$X'(t) = (a + sP(t))(J - X(t) - \alpha X(t)P(t)) - dX(t), \tag{3.5}$$

with $P(0) \geq 0$ and $0 \leq X(0) \leq J$.

We thus see that the incorporation of the handling time for the nonspecific response can be approximated by an appropriate reduction in the number of activated free immune cells (macrophages), that is, it removes macrophages at a rate of $\alpha X(t)P(t)$ from the population available for killing parasites.

In the remainder of this section we describe the steady states of (3.4) and (3.5) and their stability, show that nonspecific immunity is always vulnerable, and discuss asymptotic behavior of bounded solutions.

The system (3.4) and (3.5) can have anywhere from one to three steady states. There always exists a trivial steady state $E_0 = \left(0, \frac{aJ}{d+a}\right)$ which is stable if $\frac{aJ}{d+a} > r/h_1$ and unstable if $\frac{aJ}{d+a} < r/h_1$ (see Appendix A for details). The quantity $\frac{aJ}{d+a}$ represents the background level of activated free cells when P is absent. The quantity r/h_1 is the exact magnitude of nonspecific immunity to equilibrate the growth of P, thus stability of E_0 implies that the background level of activated free cells is already sufficiently high to control and clear the infection, while instability

of E_0 implies that infection will never be cleared. In the latter case, the parasite will either reach a nonzero plateau, or it will escape the control by nonspecific immunity. Whichever outcome occurs will depend on the number of nontrivial steady states (with $P > 0$), and the initial size of infection $P(0)$. In particular, if $J < r/h_1$ then nonspecific immunity will never be able to control the infection. For the remainder of this section, we will assume that $J > r/h_1$.

At any given steady state with $P > 0$, X must equal r/h_1. The P-coordinate of a nontrivial steady state must satisfy

$$F(P) = \frac{(a + sP)J}{d + (a + sP)(1 + \alpha P)} = \frac{r}{h_1}. \tag{3.6}$$

The function $F(P)$ increases for $P < \hat{P}$, has a maximum at $P = \hat{P}$, and declines to zero for $P > \hat{P}$ where $\hat{P} = \sqrt{\frac{d}{s\alpha}} - \frac{a}{s}$. $F(P)$ represents the number of activated free immune cells corresponding to a constant level of parasite P. For lower values of P, the response is low because of an insufficient activation rate, but when P becomes large, most of the activated free cells are engaged with the parasite, and nonspecific immunity loses control of the parasite. This property of our model is new compared with the previous model, which did not take into account a handling time (Antia and Koella, 1994).

If $\frac{aJ}{d+a} - \frac{r}{h_1} > 0$, then there exists a unique solution $P^* > \hat{P} > 0$ of (3.6), and the corresponding steady state $E_1 = (P^*, r/h_1)$ is unstable (a saddle). If $\frac{aJ}{d+a} - \frac{r}{h_1} < 0$ and $F(\hat{P}) < \frac{r}{h_1}$, then there are no steady states with $P > 0$. In this case, no infection can be controlled by nonspecific immunity. If $\frac{aJ}{d+a} - \frac{r}{h_1} < 0$ and $F(\hat{P}) > \frac{r}{h_1}$, then there exist two distinct steady states E_1 and E_2 with $0 < P_1^* < \hat{P} < P_2^*$; moreover, E_1 is stable, and E_2 is unstable (see Fig. 2 for details)

If there exists at least one steady state of (3.4) and (3.5) with positive $P = P^*$, then depending on the initial conditions, the infection will be either controlled, or it will escape the nonspecific control. Therefore, nonspecific immunity is always vulnerable (according to our definition of vulnerability). The steady state E of (3.4) and (3.5) with the largest value of P^* is always unstable (a saddle), whose stable manifold is a separatrix between the region of bounded solutions (immune control) which lies to the left of this separatrix, and the region of unbounded solutions (escape) which lies to the right of it. Due to nonlinearity of system (3.4) and (3.5), finding the shape of the separatrix is a tedious task. This separatrix is given by a graph of a strictly increasing function $X = X(P)$ passing through the saddle point E. Any solution of (3.4) and (3.5) with $X(0) < \frac{r}{h_1}$ and $P(0) > P^*$ lies to the right of the separatrix, and all solutions with such initial conditions are unbounded. The value of P^* gives a threshold for the largest infection that can be controlled by nonspecific immunity. P^* increases when either J or g increase, and decreases when $\frac{r}{h_1}$ increases. In the former case, increasing the total number of immune cells and/or reducing the handling time will certainly diminish chances of the parasite escaping control, while in the latter case, a parasite that requires a

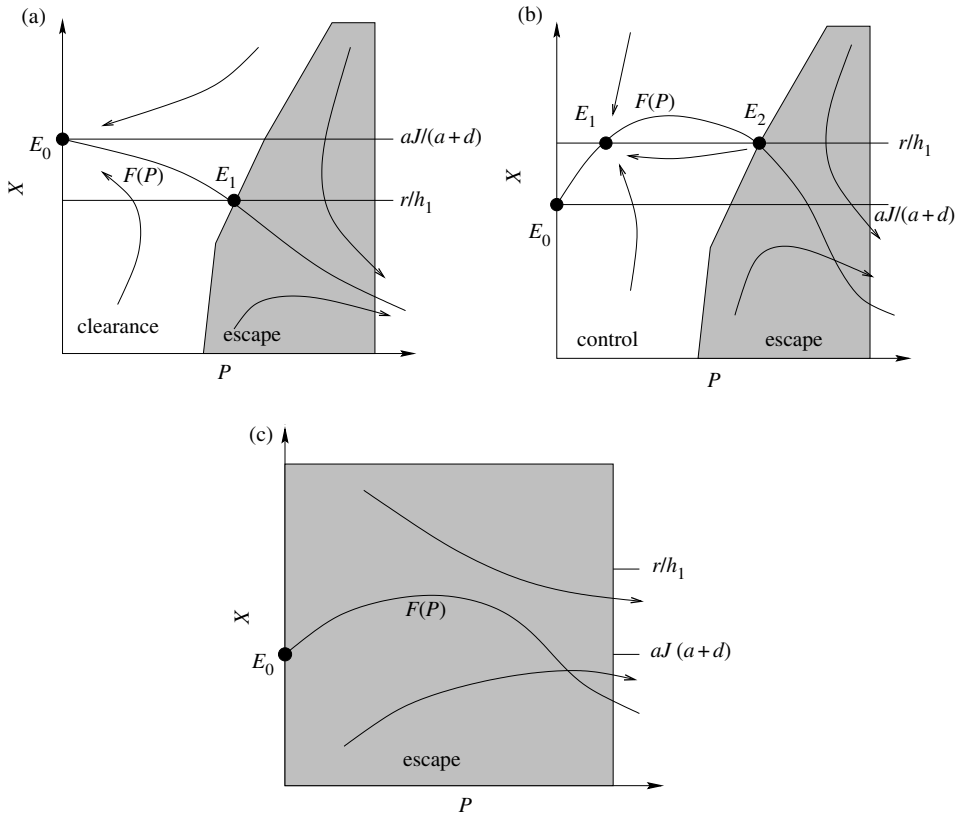

Figure 2. Vulnerability of nonspecific immunity. Steady states, regions of clearance, control and escape, and typical trajectories for three structurally distinct situations. (a) E_0 is stable, E_0 attracts all bounded solutions (region of clearance); E_1 is unstable, all solutions to the right of E_1 are unbounded (region of escape). (b) E_0 is unstable, E_1 attracts all bounded solutions (region of control); E_1 is unstable, all solutions to the right of E_1 are unbounded (region of escape). (c) E_0 is unstable, no positive steady state exists, all solutions are unbounded (escape).

greater magnitude of immune response naturally has higher chances to escape the control by nonspecific immunity.

If the infection cannot be controlled by the nonspecific immunity, then the parasite will replicate to large numbers and in particular, a saturation in the killing rate will be reached due to the handling time and QSSA-1 (for large P, $X \approx 0$). Consequently, the per capita replication rate of the parasite will not be affected by nonspecific immunity. This implies that nonspecific immunity is capable only of *transient* reduction in the replication rate of the parasite, thus it may be important for the outcome of the infection how fast the specific immunity can achieve levels sufficient to control the parasite. This feature is novel in comparison with the previous model of Antia and Koella (1994). In their model, the nonspecific immunity, although unable to completely control the parasite, was still capable of permanently reducing its per capita replication rate.

The asymptotic behavior of the bounded solutions of (3.4) and (3.5) is simple (we refer a more mathematically oriented reader to the analysis presented in Appendix A). Any bounded solution converges to a steady state. If only E_1 exists, then except for two critical solutions which correspond to the separatrix, all bounded solutions converge to E_0 (any controlled infection is cleared). If both E_1 and E_2 exist, then E_0 is unstable and all bounded solutions converge to E_1, and thus the model predicts that any controlled infection will persist at a positive level (see Fig. 2 for details).

4. SPECIFIC IMMUNE RESPONSES WITH HANDLING TIME

We model the specific immune response with the handling time in a similar manner to earlier approaches (De Boer and Perelson, 1995; Borghans *et al*., 1996; Burroughs and Rand, 1998), and use the resulting model to compare vulnerability of the specific response with that of the nonspecific response discussed in the previous section.

In accordance with earlier models, we let the pathogen density be represented simply by the infected (or target) cells and model the T-cell–target-cell interaction by the following reaction,

$$F + P \underset{k_c}{\overset{k_b}{\rightleftharpoons}} C \xrightarrow{k_d} (1+\gamma)F.$$

A free T-cell F forms a complex C with an infected cell P which presents the antigen. The rate at which these complexes are formed is proportional to the sizes of both populations F and P with the rate constant k_b. If the T-cell does not recognize the target the complex can dissociate with rate constant k_c, or if the target cell is recognized the complex is destroyed with rate constant k_d, in any case the T-cell is released from the complex. In this model, we only consider complexes including a single T-cell and a single infected cell. In general, this model must be modified to include the actual surface-to-surface interactions between cells when the number of infected cells approaches the carrying capacity of the infected tissue. Since our main goal is to study vulnerability of the immune response, we do not include such interactions here.

The flow diagram of this model is presented in Fig. 3.

We describe the model by the following system of differential equations,

$$P'(t) = r\,P(t) - k_d h_1 C(t), \tag{4.1}$$

$$F'(t) = a - dF(t) - k_b F(t)P(t) + k_c C(t) + k_d(1+\gamma)C(t), \tag{4.2}$$

$$C'(t) = k_b F(t)P(t) - (k_c + k_d)C(t) - dC(t), \tag{4.3}$$

where the following biological processes are included.

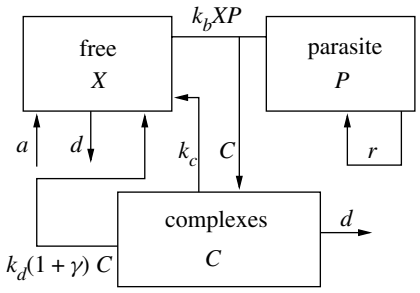

Figure 3. Flow diagram of the specific immunity model.

(1) *Viral growth/clearance.* In the absence of immune response, the number of infected cells grows exponentially at a rate r. We assume that a given hit delivered by a T-cell within the complex is successful with probability $0 < h_1 < 1$, so that the rate of destruction of infected cells equals that of the complex dissociation multiplied by h_1.
(2) *Formation/dissociation of complexes.* The rate at which complexes are formed is proportional to the sizes of both populations F and P with the rate constant k_b. The complex can dissociate without the T-cell getting a signal or delivering a lethal hit with rate constant k_c or following stimulation of the T-cell and destruction of the target cell with rate constant k_d.
(3) *T-cell proliferation/death.* We assume that T-cells divide upon receiving antigen-specific signals and dissociation from the complexes with infected cells with probability γ. A given T-cell destroys $1/\gamma$ targets on average before it divides. In this model, we also include an external source a of free T-cells, and a background death rate d of both free and bound T-cells $d \ll k_a, k_b, k_c$.

The processes featured in this model have different time scales. For CD8 cells, the lysis of the target can be performed on a time-scale of 20–30 min (Zagury *et al.*, 1975; Perelson and Bell, 1982; Yanelli *et al.*, 1986) which corresponds to k_d in the order of 50 d^{-1}. During an acute infection, one round of division for T-cells takes as long as 6–8 h ($\rho = k_d\gamma \approx 2$ d^{-1}). At present, there are no reliable estimates for the T-cell death rate, so we assume d to be in the order of 0.01–0.1 d^{-1}. A typical value of the viral growth rate r is between 0.1 and 0.5 d^{-1}. Therefore, it is reasonable to make the following QSSA (QSSA-2). Since the dynamics of complex dissociation (4.3) is faster than that of (4.1) and (4.2), we can approximate C by letting $C' = 0$ and solving for $C = \frac{k_b}{k_c+k_d+d}FP$, or equivalently,

$$C = \frac{P}{k+P}(F+C), \qquad k = \frac{k_c+k_d+d}{k_b}.$$

We let $X = F + C$ be the total number of T-cells, substitute the QSS value of C into (4.1), and add equations (4.2) and (4.3) to obtain the reduced model of the

form,

$$P'(t) = r\,P(t) - h\frac{P(t)}{k + P(t)}X(t), \tag{4.4}$$

$$X'(t) = a + \left(\rho\frac{P(t)}{k + P(t)} - d\right)X(t), \tag{4.5}$$

where $h = h_1 k_d$ and $\rho = k_d \gamma$.

In this model, both the proliferation rate of T-cells and the killing rate of infected cells saturate as the number of infected cells P becomes large (h is the maximal killing rate of the infected cells by specific immunity, and ρ is the maximal proliferation rate of the specific immune cells). In the reduced model, cytotoxic activity and proliferation of T-cells are directly correlated, which is supported by the experimental observation that effector and precursor T-cells are indistinguishable during the acute infection phase (Razvi *et al.*, 1995).

The reduced model (4.4)–(4.5) is easier to analyse because the system is two dimensional. We will show that the dynamic behavior of (4.4)–(4.5) closely resembles the behavior of standard predator–prey models (Bell, 1973; Bazykin, 1975; Schweitzer *et al.*, 1992), and in particular, the dynamics of the Gause model (Hsu, 1978; Hofbauer and So, 1990). We use this model to address the following questions. (i) For which values of the operating parameters is specific immunity vulnerable (invulnerable)? (ii) What does this model predict for the behavior of controlled infections in the long run?

Analysis presented in Appendix B (Theorem B1) shows that vulnerability of the specific immunity can be determined by comparing the quantities $\rho - d$ and r. If $\rho - d > r$ and $a > 0$, then all solutions with positive initial conditions are bounded which implies that infections of arbitrarily large initial sizes will be controlled by specific immunity. On the contrary, if $\rho - d < r$, then for a nonempty set of initial conditions, solutions of (4.4) and (4.5) become unbounded, so that the corresponding infections will escape the control by specific immunity (in this case, specific immunity is vulnerable). In Appendix B, we use an approximating system and show that if $\rho - d < r$, then all solutions starting on the right of the curve

$$P^*(X) = \frac{h(a + (\rho - d)X)[(r - (\rho - d))X + a]}{r(r - (\rho - d))} \tag{4.6}$$

are unbounded, and the ones starting on the left of this curve are bounded [see Appendix B, (B5)–(B7)].

If the infection cannot be controlled by the specific immunity, then the parasite will replicate to large numbers so that the killing rate will saturate at h, and the proliferation rate of immune cells will saturate at $\rho - d$, thus effectively making

$$X(t) \approx \tilde{X}e^{(\rho - d)t}, \qquad P(t) \approx \tilde{P}e^{rt}[1 - Ce^{(\rho - d - r)t}].$$

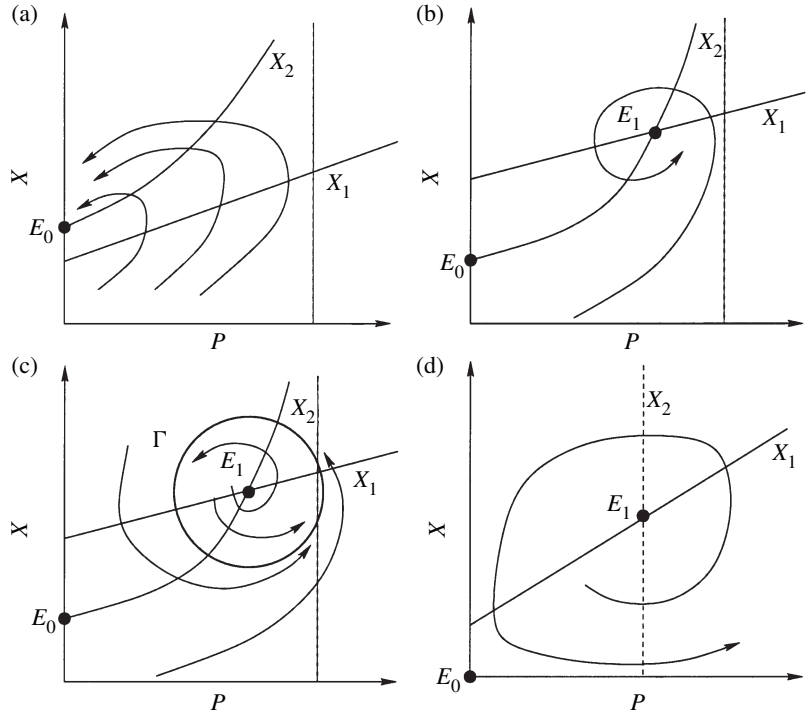

Figure 4. Bifurcations in the specific immunity model (see the detailed description in Appendix B). Steady states, limit cycles and typical trajectories for four structurally distinct situations. (a) $a > a^*$, E_0 is globally stable, E_1 does not exist. (b) $a_* < a < a^*$, E_0 is unstable, E_1 attracts all positive solutions; at $a = a_*$, E_1 undergoes Hopf bifurcation. (c) $0 < a < a_*$, both E_0 and E_1 are unstable, the limit cycle Γ attracts all positive solutions (except E_1). (d) $a = 0$, both E_0 and E_1 are unstable, all positive solutions are unbounded.

Since the term in square brackets approaches unity, the model predicts that specific immunity is capable of only transient reduction in the replication rate of the parasite.

It is also interesting that when $a = 0$, all nonconstant solutions are theoretically unbounded independently of the relation between $\rho - d$ and r (Appendix B, Theorem B1). Nevertheless, if $\rho - d > r$, every unbounded positive solution eventually passes through the region of arbitrarily small values of P, and thus any infection will be *practically* eliminated, see Fig. 4 ($a = 0$) for details and also Antia *et al.* (1996).

Finally, for bounded solutions of (4.4) and (4.5) which correspond to controlled infections, we describe their asymptotic behavior. The model (4.4) and (4.5) may have one or two steady states, E_0, a trivial steady-state $\left(P = 0,\ X = \frac{a}{d}\right)$, and E_1 with positive coordinates P^*, $X^* > 0$. In Appendix B, we choose a as a bifurcation parameter and show how the behavior of solutions of (4.4)–(4.5) changes as a goes from zero to infinity. In the biologically relevant case ($\rho - d > r$), behavior

of the model (4.4) and (4.5) belongs to one of the following four classes: when $a = 0$ (no input), then E_1 is unstable, and all positive solutions are unbounded; when $a < a_*$ is small but positive (low input), the positive steady state E_1 is still unstable, but there is a stable limit cycle which attracts positive solutions; when $a_* < a < a^*$ (intermediate input), E_1 is stable and attracts all positive solutions; finally, if $a > a^*$ (high input), E_1 does not exist, and all solutions converge to E_0. The bifurcation points $a = a_*$ and $a = a^*$ are discussed in detail in Appendix B.

5. DISCUSSION

Many of the conventional mathematical models of immunity were based on the assumption that the clearance of the parasite by cell-mediated immune responses obeys a simple mass action term dependent solely on the product of the densities of immune cells and the target (parasite or infected cells). This corresponds to the assumption that the time scale for an encounter between immune cells and the target is longer than the time scale for the subsequent handling of the target by immune cells. The relative magnitude of the encounter and handling time will be expected to change as the densities of pathogen and immune cells fluctuate during the course of the infection. At the onset of infection, the densities of immune cells and the targets are low, and thus reducing the model to the earlier approximation is reasonable; however, at a later stage when the density of the targets is high the length of the handling time may be the rate-limiting step. In this discussion we summarize our results by describing: (i) the biology of the handling time, (ii) how introduction of a handling time can be incorporated into simple predator–prey models of cellular immune responses, (iii) the consequences of doing this focusing on the vulnerability to infection, and (iv) critically discuss the limitations of the current models.

The handling time can arise for a variety of biological reasons. For phagocytic cells the handling time may arise due to the rate of phagocytosis or the rate of degradation of phagocytosed parasite. As more than one parasite can be phagocytosed at a time we might expect the latter to contribute more to the handling time. For cytotoxic cells the handling time may arise due to the killing of a given target by a CTL, or due to the exhaustion of lytic granules after multiple hits are delivered. More is known about the former than the latter. The ability of a CTL to kill only one target at a time has been documented, and even when CTL binds to more than one target cell the lethal hits are delivered sequentially (Zagury *et al*., 1975; Perelson and Bell, 1982).

We have added the handling time to the nonspecific and specific immunity by including an additional equation to describe the complexes of immune cells which are engaged in handling and killing their targets [see (3.1)–(3.3) and (4.1)–(4.3)]. Since changes in the numbers of complexes occur faster than changes in the population of immune cells or target cells we can set the rate of change in the density of complexes to zero allowing us to generate a simplified set of equations for

immunity and target cells. For the nonspecific immune response we find that incorporation of a handling time results in a decline in the number of activated free macrophages and can be approximated by a decline in the rate of activation of macrophages [see (3.4) and (3.5)]. For specific immunity the addition of a handling time results in the rate at which specific immune cells can kill the parasite and can be approximated by a saturation in the killing of infected cells by immunity [see (4.4) and (4.5)]. These modified equations may be useful in modeling the nonspecific and specific responses in more complex situations.

We have shown how the introduction of a handling time makes a qualitative change on the dynamics of both nonspecific and specific immune responses. The effect of introducing a handling time is to make the immune system vulnerable to high parasite inocula. We have shown that the nonspecific response is unable to clear a sufficiently large initial dose of even a slowly growing parasite, but the specific immunity is unable to clear only rapidly growing parasites. We have also shown that whenever a parasite is able to escape the control by immunity, the reduction in the parasites per capita rate of growth due to the immune response (specific or nonspecific) will only be transient. The qualitative change in dynamics has implications for our interpretation for measurements of the virulence of pathogens and provides us with ways in which the model can be experimentally tested.

A commonly employed measure of the virulence of a pathogen is the LD_{50}, the inoculum above which on average 50% of hosts die during the course of infection. The models proposed here suggest that the LD_{50} may arise for two reasons. The first is that the immune system would if given sufficient time be able to control the parasite, but when the inoculum size is greater than the LD_{50} the parasite transiently reaches levels which cause host mortality. The second possibility, suggested by the models in this paper, is that the LD_{50} corresponds to the inocula above which the immune system is not able to control the growth of the parasite.

The models we have described can be subject to experimental tests capable of discriminating between these models and earlier models. We illustrate one such test for the models of nonspecific immunity. Several experimental systems have followed nonspecific responses to pathogens by following viral or bacterial infection in SCID mice which are not able to generate specific immune responses. These models have typically shown that the nonspecific response alone is able to control the pathogen, but not to clear the pathogen (Bancroft *et al.*, 1986). The earlier models have 'dose-independent' steady states, while the models presented in this paper exhibit lack of control of pathogens when the inoculum density is high and feature dose-dependent behavior. We are currently conducting such tests of the models.

The models we have presented in this work are very simple and there are several possible directions for future improvements. First, we have modeled nonspecific and specific immunity acting in totally independent ways, yet there might be additional interactions between the cells involved in the immune responses, such as cytokine production and/or alternative signaling between different cells. Secondly, we have not included possible individual features of the parasite. These need to be

incorporated as appropriate for specific parasites. Thirdly, we have assumed that the host is a homogeneous environment. The consequences of spatial heterogeneity must be studied as well.

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APPENDIX A: DYNAMICS OF NONSPECIFIC IMMUNITY UNDER QSSA-1

This section contains the analysis of the dynamics of the model (3.4) and (3.5). First, we analyse steady states of the model and their stability. Then we study asymptotic behavior of bounded solutions.

The trivial steady state $E_0 = \left(0, \frac{aJ}{a+d}\right)$ always exists. The variational matrix of (3.4) and (3.5) at E_0 is given by

$$J(E_0) = \begin{pmatrix} r - h_1 \frac{aJ}{a+d} & 0 \\ \frac{J}{a+d}(sd - \alpha a^2) & -d \end{pmatrix},$$

so $J(E_0)$ has eigenvalues $r - h_1 \frac{aJ}{a+d}$ and $-d$. If $r - h_1 \frac{aJ}{a+d} < 0$ then E_0 is stable, and if $r - h_1 \frac{aJ}{a+d} > 0$ then E_0 is unstable.

Stability properties of a nontrivial steady state E can be determined from the variational matrix of (3.4) and (3.5) evaluated at this steady state. The variational matrix $J(E)$ is given by

$$J(E) = \begin{pmatrix} 0 & -h_1 P^* \\ s(J - X^* - \alpha X^* P^*) - \alpha X^*(a + sP^*) & -d - (a + sP^*)(1 + \alpha P^*) \end{pmatrix}, \tag{A1}$$

with $X^* = \frac{r}{h_1}$ and $F(P^*) = X^*$. The trace of $J(E)$ is always strictly negative, and the determinant of $J(E)$ is given by

$$\mathrm{Det}(J(E)) = h_1 P^*(s(J - X^* - \alpha X^* P^*) - \alpha X^*(a + sP^*)) = h_1 P \frac{\partial X'}{\partial P}.$$

Since $X' = (a+sP)(J - X - \alpha XP) - dX = (d + (a+sP)(1+\alpha P))(F(P) - X)$ from (3.5), it follows that

$$\frac{\partial X'}{\partial P}|_{X^*} = \nu(P^*)'(F(P^*) - X^*) + \nu(P^*)F'(P^*) = \nu(P^*)F'(P^*),$$

where $\nu(P) = (d + (a + sP)(1 + \alpha P)) > 0$ is a strictly positive function. Thus, the sign of $\mathrm{Det}(J)$ is the same as the sign of $F'(P^*)$ because $h_1 P^* > 0$. Now we apply Routh–Hurwitz criterion for matrices [see the appendix to Coppel (1965)] to determine the signs of the real parts of the eigenvalues of $J(E)$. If $F'(P^*) > 0$, then both eigenvalues of $J(E)$ have negative real parts and the corresponding steady state $E = (P^*, X^*)$ is linearly stable, and if $F'(P^*) < 0$, then $J(E)$ has one positive and one negative eigenvalue, and E is unstable (a saddle). We point out that in the case of a saddle, the negative eigenvalue exceeds the positive eigenvalue in the absolute value because their sum given by $\mathrm{Tr}(J(E))$ is negative. The case $F'(P^*) = 0$ is critical. This situation occurs when P^* coincides with the point of maxima of F, that is, $P^* = \hat{P}$ and $F(\hat{P}) = \frac{r}{h_1}$. A typical perturbation of the parameters in (3.4) and (3.5) will either destroy the steady state [if $F(\hat{P}) < \frac{r}{h_1}$], or produce two steady states: one sink (stable node) with smaller P^* and one saddle with greater P^* [if $F(\hat{P}) > \frac{r}{h_1}$]. This is a saddle-node bifurcation of a structurally unstable configuration of the system [for more details see Hale and Kocak (1991)]. In particular, at the point of bifurcation the resulting steady state is semi-stable degenerate with one zero and one negative eigenvalue.

In what follows we will make use of the Poincaré–Bendixson theorem for two dimensional vector fields (Coddington and Levinson, 1955). The theorem states that any bounded solution [of (3.4) and (3.5) in our case] must asymptotically approach a compact set of solutions of either of the following three types: a steady state, a periodic solution, or a saddle connection. We will eliminate the last two possibilities and thus show that every bounded solution of (3.4) and (3.5) approaches a steady state. In the proof, we use the fact that the only positive steady states of (3.4) and (3.5) are either saddles, or sinks.

LEMMA A1. *Any positive periodic solution of* (3.4) *and* (3.5) *is linearly stable.*

Proof. Let $\gamma(t) = (P(t), X(t))$ be a periodic solution of (3.4) and (3.5) of positive period $\omega > 0$. Let $J(P, X)$ be the variational matrix of (3.4) and (3.5). The trace $\mathrm{Tr}(J)$ is given by

$$\mathrm{Tr}[J(P(\rho), X(\rho))] = [r - h_1 X(\rho)] + [-d - (a + sP(\rho))(1 + \alpha P(\rho))].$$

The integral $\int_0^\omega \mathrm{Tr}(J(P(u), X(u))\, du$ is negative because the first term in $\mathrm{Tr}(J)$ integrates to zero [since $P(t)$ is ω-periodic by assumption], and the second term is strictly negative. Since $\int_0^\omega \mathrm{Tr}(J(P(u), X(u))\, du < 0$, stability of γ follows. □

The system (3.4) and (3.5) can have at most one saddle with positive P, therefore the only possible saddle connection is a homoclinic. Since the negative eigenvalue exceeds the positive eigenvalue in the absolute value at a saddle point, such a homoclinic must be linearly stable relative its interior. Consequently, if either a periodic solution or a homoclinic exists, it must contain at least one unstable steady state in its interior. The only unstable steady states are saddles, and by computing the corresponding indices, one concludes that neither a periodic solution nor a homoclinic

exists. Thus every bounded solution of (3.4) and (3.5) converges to a steady state by the Poincaré–Bendixson theorem.

APPENDIX B: DYNAMICS OF SPECIFIC IMMUNITY UNDER QSSA-2

This section contains the analysis of the dynamics of the model (4.4) and (4.5). First, we analyse steady states of the model and their stability. Then we present conditions for vulnerability of the specific immunity and obtain estimates for the critical threshold $P^*(0)$. Finally, we describe the asymptotic behavior of solutions of (4.4) and (4.5) for different values of the bifurcation parameter a.

The system (4.4) and (4.5) always has a trivial steady state E_0 with coordinates $P = 0$ and $X = \frac{a}{d}$. The variational matrix at E_0 is

$$J(E_0) = \begin{pmatrix} r - \frac{ha}{kd} & 0 \\ \frac{\rho a}{kd} & -d \end{pmatrix}, \tag{B1}$$

whose eigenvalues are $r - \frac{ha}{kd}$ and $-d$. If $r - \frac{ha}{kd} > 0$ then E_0 is unstable, and if $r - \frac{ha}{kd} < 0$ then E_0 is stable. We define $a^* = \frac{krd}{h} > 0$ so that E_0 is unstable for $a < a^*$. Positive steady states of (4.4) and (4.5) are the points of intersection of P- and X-isoclines given by the graphs of

$$X_1(P) = \frac{r}{h}(k + P), \qquad X_2(P) = \frac{a}{d - \frac{\rho P}{k+P}} = \frac{a(k+P)}{dk + (d-\rho)P}. \tag{B2}$$

Solving $X_1(P) = X_2(P)$ for $P > 0$ gives

$$P^* = \frac{ah - krd}{r(d - \rho)},$$

which is positive if and only if $ah - krd$ and $d - \rho$ have the same sign. In particular, E_1 exists if $\rho - d > 0$ and E_0 is unstable, or if $\rho - d < 0$ and E_0 is stable. The variational matrix at E_1 is

$$J(E_1) = \begin{pmatrix} \frac{rP^*}{k+P^*} & -h\frac{P^*}{k+P^*} \\ \frac{\rho r}{h(k+P^*)} & \rho\frac{P^*}{k+P^*} - d \end{pmatrix}. \tag{B3}$$

Direct calculation shows that $\mathrm{Det}(J(E_1))$ is positive for $\rho - d > 0$ and negative for $\rho - d < 0$. Thus, E_1 is always unstable (a saddle) for $\rho - d < 0$. The trace of $J(E_1)$ is given by

$$\mathrm{Tr}(J(E_1)) = (r + \rho)\frac{P^*}{k + P^*} - d. \tag{B4}$$

If $\rho - d > 0$, then $\mathrm{Tr}(J(E_1))$ is a continuous decreasing function of a which is positive for $a = 0$ $\left(P^* = \frac{dk}{\rho - d}\right)$ and negative for $a = a^*$ $(P^* = 0)$. We define

a_* ($0 < a_* < a^*$) as the unique value of a which solves $\mathrm{Tr}(J(E_1)) = 0$. It follows immediately that E_1 is stable for $a > a_*$ and unstable for $a < a_*$. As a crosses the value a_*, the Hopf bifurcation occurs producing a stable limit cycle in the neighborhood of E_1 for $a < a_*$.

The following result concerns the boundedness of positive solutions.

THEOREM B1. *If $a > 0$ and $\rho - d > r$, then every positive solution of* (4.4) *and* (4.5) *is bounded. If $a > 0$ and $\rho - d < r$, then there exists a nonempty region of initial conditions where positive solutions of* (4.4) *and* (4.5) *are unbounded. If $a = 0$, then all nonconstant positive solutions of* (4.4) *and* (4.5) *are unbounded.*

Proof. First, we consider the case when $a > 0$ and $\rho - d > r$. Let $\epsilon = \frac{1}{2}(\rho - d - r)$ and let $\hat{P} = \frac{k(\rho+d+r)}{\rho-d-r}$. Introduce two regions: $A = \{(P, X) | P > \hat{P}, 0 < X < X_1(P)\}$ where both X and P are increasing, and the neighboring region $B = \{(P, X) | P > \hat{P}, X > X_1(P)\}$ where X is increasing and P is decreasing. By construction, A and B have a common boundary along the graph of $X = X_1(P)$ which is transversal to the flow of (4.4) and (4.5). In A, the following inequalities hold,

$$P' \le rP, \quad X' \ge (r + \epsilon)X,$$

and therefore any trajectory starting at $(P_0, X_0) \in A$ satisfies

$$X(P) \ge X_0 \left(\frac{P}{P_0}\right)^{1+\frac{\epsilon}{r}},$$

and thus every solution leaves A in finite time and enters B. For any solution starting in B, $P'(t) \le P'(0) < 0$ [since P decreases and the distance from $X = X_1(P)$ increases along solutions in B] and thus $P(t)$ reaches the line $P = \hat{P}$ in finite time, so the solution leaves B in finite time. The direction field of (4.4) and (4.5) has a counterclockwise rotation, and it is geometrically obvious that all positive solutions remain bounded.

If $a > 0$ and $\rho - d < r$, we choose $\epsilon > 0$ so that $s - d + \epsilon < r$, and introduce the region $C = \left\{(P, X) | X > \frac{2a}{\epsilon}, P > \frac{2hX}{\epsilon}\right\}$. Any solution in C satisfies

$$X' < \left(r - \frac{\epsilon}{2}\right)X, \quad P' > rP - hX.$$

Introducing $\Phi = \frac{X}{P}$, we find that $\Phi' < -\frac{\epsilon}{2}\Phi + h\Phi^2 < 0$ in C. Therefore, C is a positively invariant region with no steady states. By the Poincaré–Bendixson theorem, any solution in C becomes unbounded.

If $a = 0$, both coordinate axes are invariant with the flow towards the origin on the X-axis, and towards infinity on the P-axis. E_1 exists and its unstable manifold is two dimensional. If a nonconstant solution were to be bounded, its ω-limit set

would be a limit cycle. Let Γ be such a limit cycle corresponding to an ω-periodic solution of period $\omega > 0$. The trace of the variational matrix J along Γ is given by

$$\mathrm{Tr}(J) = \left(r - h\frac{k}{(k+P)^2}X\right) + \left(\rho\frac{P}{(k+P)} - d\right),$$

where the periodicity in this case implies

$$\int_0^\omega \left(\rho\frac{P}{(k+P)} - d\right) = 0,$$

and

$$\int_0^\omega \left(r - h\frac{1}{(k+P)}X\right) = 0.$$

Since $\frac{k}{k+P} < 1$, the latter implies that

$$\int_0^\omega \left(r - h\frac{k}{(k+P)^2}X\right) > 0.$$

Therefore, $\int_0^\omega \mathrm{Tr}(J) > 0$, and Γ is unstable. This contradiction shows that all positive solutions must be unbounded. □

REMARK.

(1) Although mathematically in the case $a = 0$ all solutions become eventually unbounded, they come arbitrarily close to the X-axis. Biologically, that implies that after a certain number of oscillations the infection will be cleared.
(2) When a is small, the system (13) and (14) always possesses a limit cycle. As a increases, the behavior changes from unbounded ($a = 0$) to bounded oscillations ($0 < a < a_*$) and finally to convergence to a steady state ($a_* < a$). Thus, increasing the input has a stabilizing effect on the dynamics of specific immune responses.

To separate regions of bounded and unbounded solutions, we introduce the following approximating system,

$$P' = rP - hX, \qquad X' = a + (\rho - d)X = a + \lambda X,$$

which is obtained from (4.4) and (4.5) by replacing both the killing term and proliferation term by their maximal values and neglecting the input of T-cells. This approximation is expected to work well when P is large. Dividing P' by X', we obtain a single equation,

$$\frac{dP}{dX} = \frac{rP}{a + \lambda X} - \frac{hX}{a + \lambda X}. \tag{B5}$$

Equation (B5) allows an explicit solution

$$P(X) = \left(\frac{a+\lambda X}{a}\right)^{\mu-1}\left(P(0) - a^{\mu-1}h\int_0^X \frac{z\,dz}{(a+\lambda z)^{\mu}}\right), \tag{B6}$$

where $\mu = 1 + \frac{r}{\rho-d}$. If $\rho - d < r$, that is, if $\mu < 2$, then all solutions of (B5) with $P(0) > 0$ cross the P-isocline, and thus are bounded. If $\mu > 2$, then the integral in (B6) converges to $\frac{ha^2}{r(r-(\rho-d))}$, and solutions with $P(0) > P^* = \frac{ha^2}{r(r-(\rho-d))}$ become unbounded. Therefore, all solutions starting on the right of the curve

$$P^*(X) = \frac{h(a+(\rho-d)X)[(r-(\rho-d))X + a]}{r(r-(\rho-d))} \tag{B7}$$

are unbounded, and the ones starting on the left of this curve are bounded.

Finally, we describe the behavior of positive solutions in the case $\rho - d > r$. We choose a as a bifurcation parameter. If $a \geq a^*$, then E_0 is globally stable because E_1 does not exist. When a is decreased below a^*, E_0 becomes unstable and E_1 is born which is globally stable now. As a passes through $a = a_*$, its stability character changes and the Hopf bifurcation produces a stable limit cycle in the positive quadrant. The Hopf bifurcation is supercritical (we omit this calculation), so the limit cycle generated here is indeed unique and stable. As a decreases further to 0, subsequent bifurcations are possible (multiplication of limit cycles), but we have not observed such behavior numerically. The case $a = 0$ is studied in detail in the proof of Theorem B1.

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