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The race between initial T-helper expansion and virus growth upon HIV infection influences polyclonality of the response and viral set-point

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Infection with HIV is characterized by very diverse disease-progression patterns across patients, associated with a wide variation in viral set-points. Progression is a multifactorial process, but an important role has been attributed to the HIV-specific T-cell response. To explore the conditions under which different set-points may be explained by differences in initial CD4 and CD8 T-cell responses and virus inoculum, we have formulated a model assuming that HIV-specific CD4 cells are both targets for infection and mediators of a monoclonal or polyclonal immune response. Clones differ in functional avidity for HIV epitopes. Importantly, in contrast to previous models, in this model we obtained coexistence of multiple clones at steady-state viral set-point, as seen in HIV infection. We found that, for certain parameter conditions, multiple steady states are possible: with few initial CD4 helper cells and high virus inoculum, no immune response is established and target-cell-limited infection follows, with associated high viral load; when CD4 clones are initially large and virus inoculum is low, infection can be controlled by several clones. The conditions for the dependence of viral set-point on initial inoculum and CD4 T-helper clone availability are investigated in terms of the effector mechanism of the clones involved.

Keywords: HIV; viral set-point; mathematical model; polyclonality; CD4 T help

1. INTRODUCTION

Infection with the human immunodeficiency virus type 1 (HIV-1) is characterized by an asymptomatic phase of variable length, during which the viral load fluctuates around a stable level, the viral set-point. This set-point is a good predictor of disease progression (Mellors *et al.* 1996; Pedersen *et al.* 1997). The extent of early viral replication, in turn, is a determinant of viral set-point (Lifson *et al.* 1997; Staprans *et al.* 1999). Another important factor influencing the set-point is the breadth of the HIV-specific immune response. Indeed, an inverse correlation has been observed between early CD8 T-cell repertoire diversity and viral set-point (Pantaleo *et al.* 1997). Similarly, there is some evidence to suggest that individuals with broadly directed CD8 T-cell responses have a lower viral set-point than those with narrow responses (Dalod *et al.* 1999; Hay *et al.* 1999; Edwards *et al.* 2002).

Here, we investigate whether variation in viral set-point may be explained by the polyclonality of the immune response, using a mathematical model assuming that clones differ in their avidity for antigen. So far, formal mathematical descriptions of HIV infection have shown that, depending on the initial conditions, an individual may or may not mount an immune response (Wodarz & Nowak 1999; Korthals Altes *et al.* 2002). However, these models cannot address the variation in viral set-point among individuals who mount an immune response but differ in the breadth of that response. Prior mathematical models did not allow for the coexistence of multiple clones. Instead, systems predicted competitive exclusion between clones; it was assumed that the biological reality of multiple T-cell clones responding in a typical infection corresponded to a transient state before the steady state in which there would be only one clone (Wodarz & Nowak 2000). However, the coexistence of multiple clones is important in the control of HIV infection (Dalod *et al.* 1999; Hay *et al.* 1999; Edwards *et al.* 2002). Here, we study a model of HIV infection that allows for clonal coexistence, rather than competitive exclusion, and we show for the first time, to our knowledge, that differences in set-point, associated with different levels of polyclonality of the response, can be explained by different initial abundances of each HIV-specific CD4 T-cell clone. This observation is crucial in the context of HIV vaccination, where the aim is to increase the initial abundances of different HIV-specific clones. The influence on viral set-point of the mechanism, lytic or non-lytic, through which T-cell clones act on the virus is also discussed.

2. MODEL

We developed a deterministic model of HIV infection describing the dynamics of the cell populations of importance. We made three main biological assumptions. First, we simplified an earlier model (Korthals Altes *et al.* 2002) by assuming that the immune response is directly proportional to the number of HIV-specific CD4 cells (Callaway *et al.* 1999; Kalams *et al.* 1999). These cells have experimentally been shown to be required for an effective response: they are involved in the priming of the cytotoxic T-lymphocyte (CTL) response (Ridge *et al.*

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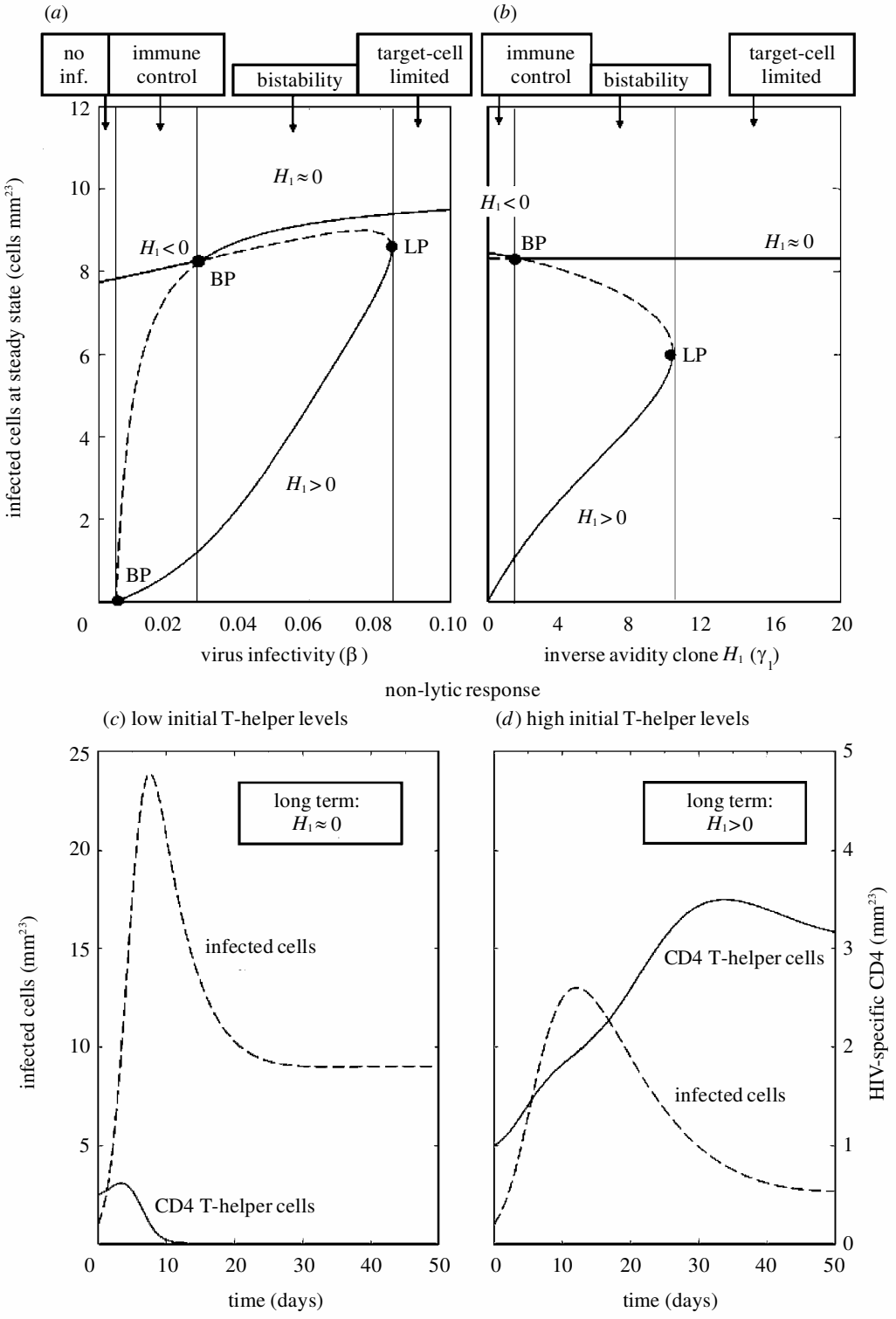


Figure 1. (*Caption overleaf.*)

1998; Schoenberger *et al.* 1998; Livingstone & Kuhn 1999) and in the generation (Borrow *et al.* 1996, 1998; Ostrowski *et al.* 2000) and re-expansion (Janssen *et al.* 2003) of the memory CD8 response. This assumption simplifies the model, but we do not expect the results to be affected qualitatively. Second, we modelled the lytic and non-lytic components of the immune response assuming that the latter protects a cell against infection, for instance through the production of cytokines such as RANTES and interferon-γ (Price *et al.* 1998), and the former is responsible for the direct killing of infected cells, for instance through the action of cytokines such as perforin (Russell & Ley 2002; Wodarz *et al.* 2002). Also, each clone may have both a lytic and a non-lytic effect, albeit with different strengths. Third, T-helper clone abundance is controlled through intraspecific competition, reflected in the density-dependent death rate (quadratic rate of cell loss) (Fraser *et al.* 2002). A biological explanation for intraclone competition would be that competition between HIV-specific T-helper cells occurs during the

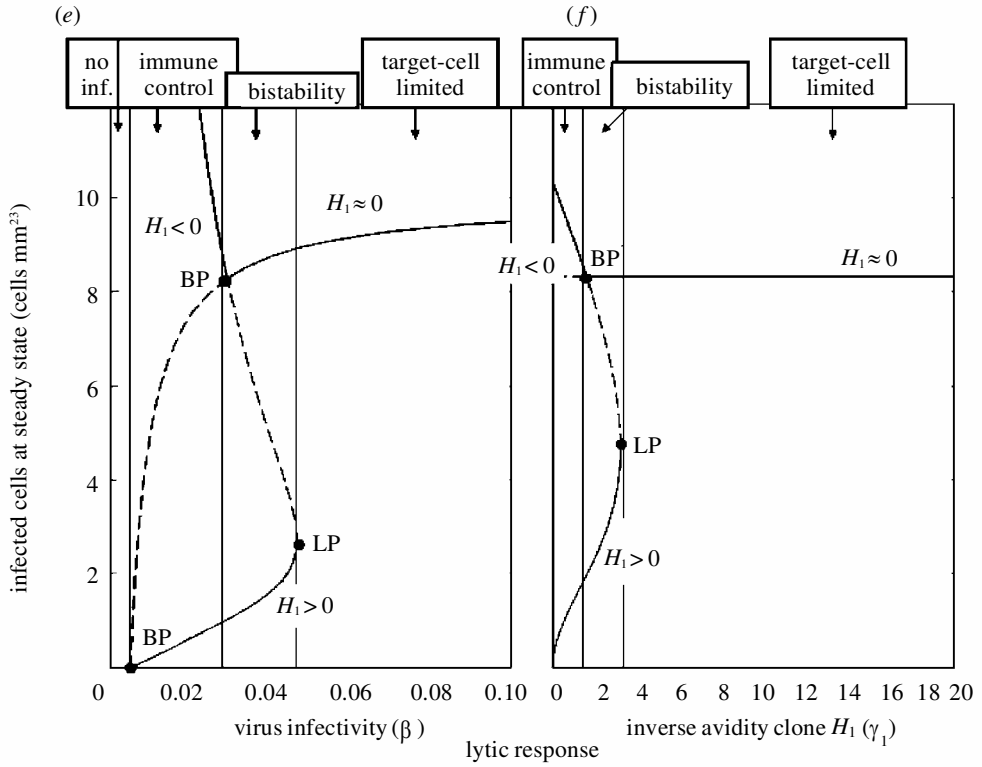


Figure 1. Monoclonal model. (*a*,*b*) Bifurcation diagrams representing steady-state infected-cell numbers $\hat{I}$ as a function of (*a*) virus infectivity β and (*b*) avidity of the T-helper clone, for the monoclonal model. Stability of a steady state (indicating convergence to that state) is indicated by a continuous line; instability (no convergence) is indicated by a broken line. BP, branching point; LP, limit point; no inf., no infection. $H_1 > 0$, immune-controlled infection with one clone; $H_1 \approx 0$, target-cell-limited infection, in which helper cells are at naive levels, very close to zero; $H_1 < 0$, the number of T-helper cells is negative, so this is a biologically irrelevant steady state. Bistability occurs when two steady states are stable for a given value of the parameter on the *x*-axis: for intermediate infectivity or avidity, either immune-controlled or target-cell-limited infection can occur. See § 3a for an explanation. (*a*) Non-lytic immune response with varying infectivity β ($\gamma_1 = 2$, $n_1 = 2$, $k_1 = 0$). (*b*) Non-lytic immune response with varying HIV-specific CD4 avidity γ_1 ($\beta = 0.03$, $n_1 = 2$, $k_1 = 0$). (*c*) Infected-cell and HIV-specific CD4 T-helper cell numbers over the course of early infection, for low initial T-helper cell levels ($T_0 = \sigma/\delta_T = 40$, which corresponds to the number of activated CD4 cells and other possible target cells; $I_0 = 1$, $H_{1(0)} = 0.5$, $\gamma_1 = 2$, $n_1 = 2$, $k_1 = 0$, $\beta = 0.05$). (*d*) Infected-cell and HIV-specific CD4 T-helper cell numbers over the course of early infection, for high initial T-helper cell levels (as in (*c*), but with $H_{1(0)} = 1$). (*e*) As in (*a*), but with lytic immune response and varying infectivity β ($\gamma_1 = 2$, $n_1 = 0$, $k_1 = 0.4$). (*f*) As in (*b*), but with lytic immune response and varying HIV-specific CD4 avidity γ_1 ($\beta = 0.03$, $n_1 = 0$, $k_1 = 0.2$). Other parameter values are $\sigma = 2$, $\delta_T = 0.05$, $\sigma_H = 10^{-7}$, $\alpha = 0.3$, $\beta_H = \beta$, $\varepsilon = 0.05$, $\delta_T = 0.2$, $g \rightarrow \infty$. In (*e*) and (*f*) k_1 was chosen so that equivalent steady-state values for infected cells were found in the non-lytic and lytic models.

expansion phase, leading mainly to within-clone competition, as clones expand locally in the lymphoid tissue. In addition, time-series of responses to different epitopes do not provide evidence for competition between clones. In fact, it has been observed that if the dominant response is removed, the subdominant response stays at the same level as before (Van der Most *et al.* 1996; Vijh *et al.* 1999), which would argue against interclone competition. Moreover, in our model, the absence of a linear death rate could account for some form of immunological memory. Indeed, when T-helper cells are present in minimal concentrations (as is the case when there is no active infection), their loss rate, being quadratic, is minimal too, leading to their maintenance at low levels.

There are four populations of cells: target cells (T) that do not participate in the immune response, mainly non-HIV-specific T cells; HIV-specific CD4 T-cell clones (H_1 and H_2) that play a role in priming the immune response but can also be infected; and, finally, infected cells (I), which we assume do not have an immune function:

$$\frac{dT}{dt} = \sigma - \delta_T T - \beta I T N(H_1, H_2),$$

$$\frac{dH_1}{dt} = \sigma_H + \frac{\alpha I H_1}{\gamma_1 + I} - \varepsilon H_1^2 - \beta_H I H_1 N(H_1, H_2),$$

$$\frac{dH_2}{dt} = \sigma_H + \frac{\alpha I H_2}{\gamma_2 + I} - \varepsilon H_2^2 - \beta_H I H_2 N(H_1, H_2),$$

$$\frac{dI}{dt} = I[\beta T + \beta_H(H_1 + H_2)]N(H_1, H_2) - \delta_I I - K(H_1, H_2)I.$$

With the non-lytic response:

$$N(H_1, H_2) = \frac{1}{1 + n_1 H_1 + n_2 H_2},$$

and the lytic response:

$$K(H_1, H_2) = k_1 H_1 + k_2 H_2.$$

Also,

$$\gamma_2 = g\gamma_1 \text{ and } g > 1.$$

Target cells other than HIV-specific CD4 T cells, T, are produced at rate σ cells per day, die at rate δ_T and are

infected by virus at rate $\beta IN(H_1, H_2)$. The non-lytic component of the immune response is reflected in the protection of target cells, through the term $N(H_1, H_2)$ limiting the infection, where n_1 and n_2 refer to the strengths of the non-lytic responses generated through T-helper clones H_1 and H_2, respectively. The lytic component, however, acts by direct killing of infected cells, as seen by the term $K(H_1, H_2)I$ in the equation describing infected cells, I (Wodarz *et al.* 2002). One or two clones may be mobilized during an infection. Helper CD4 T cells, H_i, are produced at very low levels, σ_H cells mm^{-3} day^{-1}, and proliferate in response to antigen presented by infected cells I. Their proliferation saturates as a function of antigen ($\alpha I H_i/(\gamma_i + I)$): indeed, over a certain threshold of antigen, there is no increase in proliferation as more antigen is presented (Ferguson *et al.* 1999; Fraser *et al.* 2002). CD4 T-helper clones differ in avidity, described by the parameter γ_i: the lower γ_i, the lower the antigen concentration needed to elicit an immune response. The avidity is defined here as functional avidity (Derby *et al.* 2001; O'Connor *et al.* 2002). In fact, γ_i is the amount of antigen needed for half-maximum proliferation of H_i, so γ_i is inversely related to functional avidity. The avidity of clone H_2 is scaled to that of clone H_1 ($\gamma_2 = g\gamma_1$); because we demand that $g > 1$, clone H_1 is dominant and clone H_2 is subdominant. T-helper cell numbers are controlled through density-dependent death (εH_i^2) (Ferguson *et al.* 1999; Fraser *et al.* 2002). They become infected at rate $\beta_H IN(H_1, H_2)$, and it is assumed that HIV-specific CD4 T cells lose their helper function upon infection.

Three scenarios are studied. We shall start with a monoclonal system, by setting g at a very high value (when $g \rightarrow \infty$, the second clone cannot expand because of its poor avidity). In the second scenario, both T-helper clones elicit either a fully lytic or a fully non-lytic response ($n_1 = n_2 = 0$, $k_1 = k_2 \neq 0$ or vice versa). In the last scenario, the two clones induce different types of response ($n_2 = 0$ and $k_1 = 0$ or $n_1 = 0$ and $k_2 = 0$). We chose a set of parameters yielding approximately realistic cell numbers. As we are more interested in the qualitative features of the model, we have not sought to match all aspects of the model with the course of infection.

Previous models describing the within-host dynamics of multiple clones do not allow for the steady-state coexistence of different clones (Wodarz & Nowak 2000). An important feature of the model presented here is that multiple clones may stably coexist in a host at steady state. This is possible because there is competition within clones (see Appendix A). Competition between clones also occurs, but indirectly: different clones compete for the antigen by which they are activated. Indeed, if clone 1 expands massively, it will remove the infected cells, so there will be less scope for activation of clone 2.

3. RESULTS

(a) *Monoclonal model*

To get a better insight into the long-term behaviour of the model, we analyse in detail the scenario in which one clone induces a non-lytic response ($g \rightarrow \infty$, $k_1 = 0$). We focus on the outcome of infection ('steady state') for varying virus infectivity β. A virus with very low infectivity cannot infect an individual, because its reproductive number is too low (figure 1*a*, 'no inf.') (Anderson & May 1991). As infectivity increases, the steady state with an immune response (so-called immune-controlled equilibrium, '$H_1 > 0$') becomes stable: there is a balance between T-helper cells as targets for viral infection and as mediators of the immune response. When virus infectivity β is too high, no immune response can be maintained, regardless of the initial conditions ('target-cell limited', '$H_1 \approx 0$' in figure 1*a*).

For intermediate infectivity β, an important characteristic of the model becomes apparent: there are two possible outcomes, depending on the initial conditions. For low initial T-helper cell numbers and high virus inoculum, an immune response will not be established, and target-cell-limited infection follows ('$H_1 \approx 0$', figure 1*a*). However, with the inverse initial conditions, the infection is immune-controlled ('$H_1 > 0$', figure 1*a*). This behaviour is termed bistability, as two steady states are simultaneously stable. This is illustrated in figure 1*c*,*d*, where infected-cell numbers and CD4 T-helper numbers are shown over time. When there are initially few HIV-specific CD4 cells, they are infected before they can mediate an immune response, leading to the absence of an immune response and an associated high viral load in the long term (figure 1*c*). Conversely, high initial CD4 T-helper levels control the infection before too many cells are infected, leading to an immune-controlled situation with a low viral load (figure 1*d*).

If an individual mounts an immune response involving a clone that needs little antigen to be activated (low γ_1, i.e. high avidity), T-helper cells are very effectively stimulated by antigen. Control of infection is therefore efficient and viral load is low (figure 1*b*). For intermediate avidity, there are again two possible outcomes depending on the initial conditions: immune-controlled or target-cell-limited infection ('bistability' in figure 1*b*). If T-helper cells have low avidity (high γ_1), no immune response can be established: too much antigen is needed to activate the immune response; the number of infected cells (antigen) is limited by the number of target cells, and a T helper response cannot be generated. Bistability disappears when HIV-specific T-helper cells are not infected (i.e. $\beta_H = 0$, results not shown). In other words, the bistability we have described is a direct result of the fact that T-helper cells have a dual role in HIV infection, both as targets for infection and as mediators of the immune response.

We then perform the same analyses in a model with a lytic immune response only ($g \rightarrow \infty$, $n_1 = 0$); k_1 is chosen such that similar steady-state numbers of infected and HIV-specific CD4 cells are reached, with all other parameters remaining the same. The results are qualitatively similar to those in which the immune response acts only through a non-lytic mechanism (compare figure 1*e* with figure 1*a* and figure 1*f* with figure 1*b*). However, the scope for bistability between target-cell-limited and immune-controlled infection is reduced in a system with only a lytic response, because now both infection and immune response contribute to the removal of HIV-specific T-helper cells. Thus, the infection easily becomes target-cell limited (figure 1*e*,*f*). Consequently, a non-lytic response can be maintained in the face of a virus with a higher infectivity, compared with a lytic response (Wodarz *et al.* 2002).

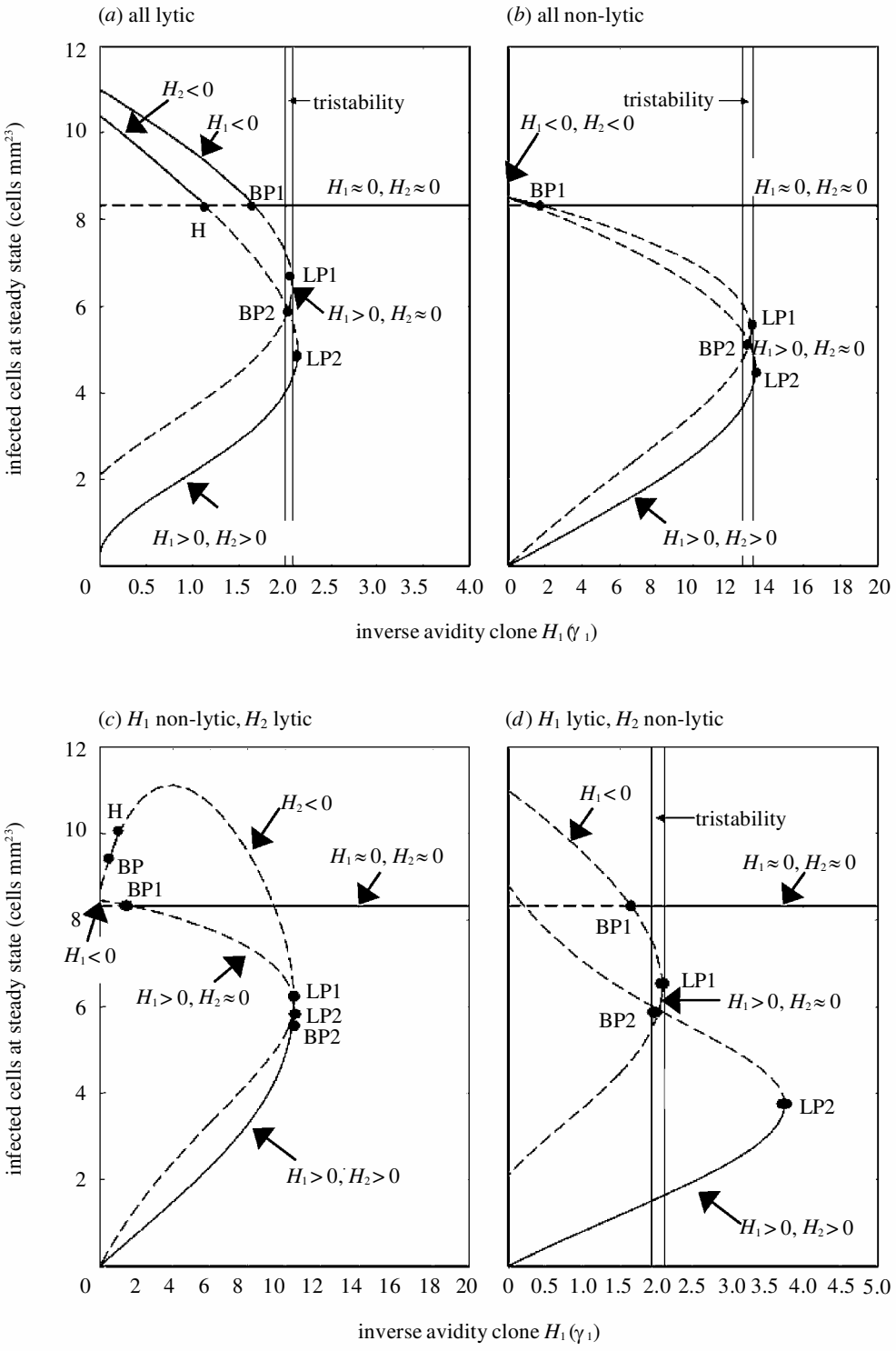


Figure 2. Polyclonal model. Bifurcation diagrams representing steady-state infected-cell numbers $\hat{I}$ as a function of the avidity of the T-helper clone H_1. The horizontal line ('$H_1 \approx 0$, $H_2 \approx 0$') refers to the target-cell-limited steady state. The other steady state is immune-controlled infection, with two T-helper clones ('$H_1 > 0$, $H_2 > 0$'); $H_1 < 0$ or $H_2 < 0$ refers to a biologically irrelevant steady state. Parameters are as in figure 1*a* except where indicated (with $\beta = \beta_H = 0.03$ and $g = 2$). BP1 is the transition from an unstable to a stable steady state without T-helper cells. BP2 is the point where H_2 can invade in the steady state with only H_1. At LP1, H_1 can invade in the steady state without T-helper cells. At LP2, both H_1 and H_2 can invade in the steady state without T-helper cells. H refers to a Hopf bifurcation. For simplicity, branches where variables have only negative values are not indicated, as they are biologically irrelevant. (*a*) Lytic response ($n_1 = n_2 = 0$; $k_1 = k_2 = 0.1$). (*b*) Non-lytic response ($n_1 = n_2 = 3$; $k_1 = k_2 = 0$). (*c*) H_1 non-lytic, H_2 lytic ($n_1 = 2$, $k_1 = 0$, $n_2 = 0$, $k_2 = 0.1$). BP is a branching point that is not further characterized because it occurs for $H_2 < 0$ in a biologically irrelevant parameter regime. (*d*) H_1 lytic, H_2 non-lytic ($n_1 = 0$, $k_1 = 0.1$, $n_2 = 2$, $k_2 = 0$). As in figure 1*b*, high-avidity clones lead to an immune response involving all clones, and low-avidity clones are associated with target-cell-limited infection. Also, for intermediate avidities, either no immune response is mounted or a full immune response is established (bistability). A new element is that, within this range, when clones have the lowest avidity (highest γ_1), a third steady state is stable in which only the clone with higher avidity, H_1, becomes established (region of 'tristability').

(b) *Polyclonal model*

Having looked at the case with only one clone, we turn to the scenario with two clones. It is assumed, first, that the clones have the same mechanism of action: they are either both lytic ($n_1 = n_2 = 0$) or both non-lytic ($k_1 = k_2 = 0$). The steady state with only one clone, analogous to that in figure 1*b*,*f* ('$H_1 > 0$'), is represented by the curve denoted '$H_1 > 0$, $H_2 \approx 0$' (figure 2*a*,*b*). If there was no other HIV-specific clone present, this steady state would be stable for low values of γ_1 (as in figure 1*b*, *f*). Adding a second clone yields multiclonal immune control, the so-called 'coexistence' of multiple clones ('$H_1 > 0$, $H_2 > 0$'). Clone H_2 can always invade the steady state with only clone H_1 for γ_1 below BP2: the steady state with two clones coexisting is stable, whereas the steady state with only one clone is unstable. This results from the assumption of intraclone competition: expansion of a single clone is limited, so it does not control the viral load as well as it would if there was no such competition. As clones do not compete interclonally, a second clone, even with lower avidity, can expand and lead to a lower viral load. This would not occur if there was no intraclone competition, because the clone with the higher avidity would not be limited in numbers and would be able to control the viral load by itself; a second clone with lower avidity would receive too little antigenic stimulation to expand. Thus, interclone competition would simply lead to competitive exclusion between clones.

When avidity is intermediate (LP2 $> \gamma_1 >$ BP2), high virus levels and the associated infection of helper cells require a minimum initial number of lower avidity cells (H_2) for the second clone to be maintained. Essentially, clone H_2 must have a minimum initial abundance to be able to invade in the steady state with only H_1. Finally, when T-helper cells have poor avidity ($\gamma_1 >$ LP2), no immune response can be established and target-cell-limited infection results (figure 2*a*,*b*).

For intermediate avidity (when BP2 $< \gamma_1 <$ LP1), target-cell-limited infection results if T-helper clones are very rare in the naive state. However, if an HIV-specific T-helper clone is abundant enough, the viral load can be reduced compared with the target-cell-limited state to such an extent that a new balance is obtained, in which one T-helper clone maintains the viral load at a lower level than would occur without an immune response. Interestingly, in this avidity range, if two T-helper clones were stimulated at high enough levels, the viral load could be controlled to even lower levels, reaching yet another steady state ('$H_1 > 0$, $H_2 > 0$' in figure 2*a*,*b*). Essentially, there are three simultaneous steady states in the narrow range of γ_1 between BP2 and LP1 (target-cell limited, '$H_1 \approx 0$, $H_2 \approx 0$'; immune control with one clone, '$H_1 > 0$, $H_2 \approx 0$'; and immune control with two clones, '$H_1 > 0$, $H_2 > 0$'), a feature we call tristability. This property is important from the point of view of therapeutic interventions aiming at boosting T-helper cell clones: if new clones can be stimulated, a new steady state with a lower viral load could be reached as additional clones become involved in the immune response.

When the two clones are non-lytic, the range of avidity for which two clones can control infection ('$H_1 > 0$, $H_2 > 0$') is relatively broad compared with the lytic scenario (compare figure 2*b* with figure 2*a*; note the different *x*-axis scales). Tristability can also be observed in the non-lytic scenario, but the clones stimulated must be very effective at blocking viral transmission (e.g. $n_1 = n_2 = 3$). Owing to within-clone competition, a clone with poor effector function (low n_1) cannot contain the infection by itself, and the steady state with only one clone is always unstable (results not shown).

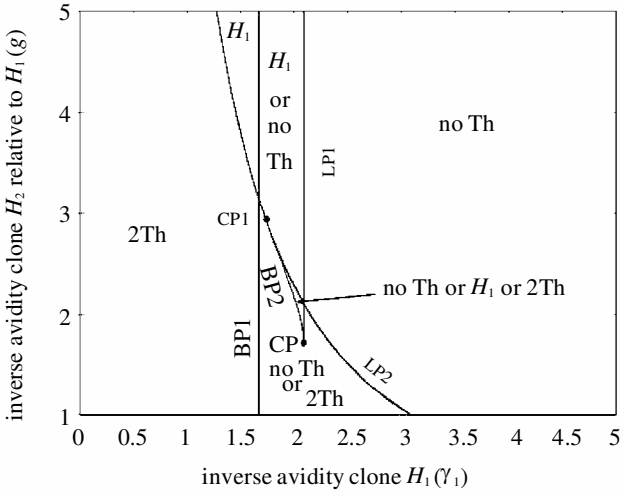
(c) *Mixed responses*

Finally, we are interested in the scenario with mixed responses, in which clones have specialized effector mechanisms, one being lytic and the other non-lytic. When the clone with higher avidity, H_1, is non-lytic and the other, H_2, is lytic, the steady state with only one clone is always unstable ('$H_1 > 0$, $H_2 \approx 0$' in figure 2*c*). Essentially, either there is no immune response, or two clones control the infection. There is a range of avidities (between BP1 and LP2) for which there is bistability between a target-cell-limited steady state ('$H_1 \approx 0$, $H_2 \approx 0$') and immune control by two clones ('$H_1 > 0$, $H_2 > 0$' in figure 2*c*). When the clone with higher avidity, H_1, is lytic (figure 2*d*), the curve labelled '$H_1 > 0$, $H_2 \approx 0$' is identical to that in figure 2*a*. However, the steady state with two clones ('$H_1 > 0$, $H_2 > 0$') is stable over a much broader range of avidities than in the case with two lytic clones. When the second, subdominant, clone is non-lytic, it allows for immune control at lower ranges of avidities (figure 2*d*: steady state with two clones stable for γ_1 up to ± 3.7) than when the second clone is lytic (figure 2*a*). There is tristability when clonal avidity is intermediate (BP2 $< \gamma_1 <$ LP1), as in the case of a fully lytic or fully non-lytic response.

(d) *Different combinations of clones*

Finally, we analyse the outcome of infection for different combinations of avidities of clones H_1 and H_2 (figure 3), first in a system with two lytic clones, and then with the higher avidity clone being lytic (H_1) and the other non-lytic (H_2). In either scenario, when both clones have high avidity (lower left-hand corner of figure 3*a*,*b*), the two coexist and control infection ('2Th'). We first describe figure 3*a*. When clone H_2 has low avidity (high *g*), only H_1 can control infection ('H_1'). For intermediate avidity of H_1, when H_2 has very poor avidity, either no response is mounted or only H_1 controls the infection ('H_1 or no Th'). When H_2 has high avidity, either no response is established or two clones control infection ('no Th or 2Th'). In the intermediate range of H_2 avidity, three outcomes are possible ('no Th, H_1 or 2Th'). Finally, clones with very poor avidity (upper right-hand corner) are associated with target-cell-limited infection ('no Th'). In a system where H_1 is lytic and H_2 is non-lytic (figure 3*b*), there are two main differences compared with the case of two lytic clones. First, there is an additional possibility when H_1 has high avidity and H_2 comparatively poor avidity: either only H_1 is stimulated, or both clones may become established ('H_1 or 2Th' in figure 3*b*). This is because H_2 is non-lytic and can as such be maintained at lower avidities than in the scenario with two lytic clones. Second, and importantly, when H_1 is lytic and H_2 is non-lytic, the region of tristability ('no Th, H_1 or 2Th') is larger than when both clones are lytic (compare figure 3*a* with figure 3*b*).

(*a*) all lytic



(*b*) H_1 lytic, H_2 non-lytic

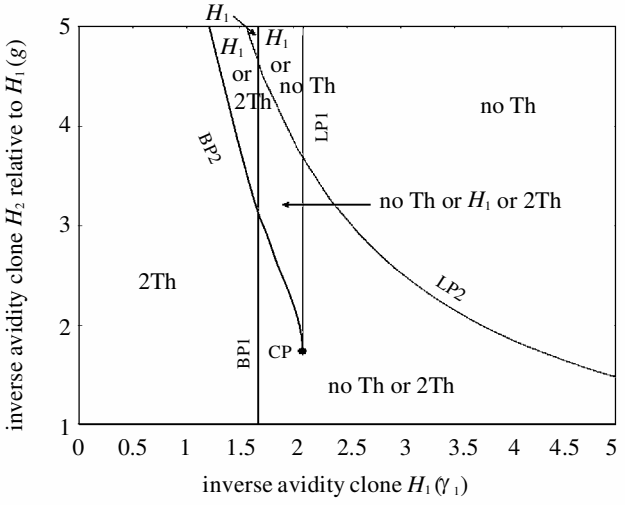


Figure 3. Two-parameter bifurcation plots. For varying avidities of the clones involved in the response, the different outcomes are indicated: infection with two clones ('2Th'), with one clone (H_1) or without immune response ('no Th'). LP1, LP2, BP1 and BP2 refer to the bifurcation points as indicated in figure 2; in addition there are two cusp points CP and CP1. (*a*) Two lytic clones, parameters as in figure 2*a*. (*b*) H_1 is lytic and H_2 is non-lytic, parameters as in figure 2*d*.

To summarize, immune correlates of the viral set-point are the avidity of the clones involved in the immune response, the strength of the immune response (a similar bifurcation pattern is seen for varying k_1, k_2, n_1 or n_2) and the level of pre-existing immunity, i.e. the initial abundance of the HIV-specific T-helper clones. The last is more likely to influence the outcome of infection in the context of two non-lytic responses or when the higher avidity clone is lytic and the other non-lytic.

4. DISCUSSION

Infection with HIV is characterized by very diverse disease-progression patterns across patients. Several factors are thought to contribute to this process (Kinter *et al.* 2000; Rowland-Jones *et al.* 2001). In this work, we focus on the possible role of the breadth of the immune response in determining the set-point. Patients with a high set-point have narrow responses, i.e. a limited number of clones responding to HIV epitopes, whereas patients with a low set-point control infection through broadly directed responses. We have formulated a theoretical framework that reflects the essential features of HIV infection, in order to explore the conditions under which different viral set-points, associated with immune responses of varying breadth, may be explained by differences in the initial CD4 and CD8 T-cell responses and virus inoculum. We have been concerned with the qualitative features of the long-term dynamics, because we are interested in the possible occurrence of multiple stable steady states. We have therefore not looked for parameter values that would exactly match infections observed in patients, but rather that would result in approximately realistic cell numbers.

In very early models of HIV infection, polyclonal responses resulted from the stochastic appearance of new antigenic variants (Nowak *et al.* 1991). A major caveat of later models describing responses of multiple lymphocyte clones to a pathogen not (necessarily) generating new antigenic variants (Nowak & May 2000; Wodarz & Nowak 2000) is the so-called competitive-exclusion principle, which results in dominance of a single clone. Although it is true that the anti-HIV response during the asymptomatic phase can be monoclonal in some individuals, this is certainly not the rule (Harrer *et al.* 1996; Ogg *et al.* 1999; Betts *et al.* 2000). This caveat has been addressed by arguing that the asymptomatic phase of HIV infection might be a transient rather than a steady state (Wodarz & Nowak 2000). We believe that our approach, which assumes a linear relationship between the numbers of CD4 T-helper clones and CD8 T-cell clones, offers a more realistic picture, because it allows for multiple coexisting clones, through within-clone competition.

The outcome of infection as described through our model can be dependent on the initial conditions. Indeed, for a virus with intermediate infectivity (or for HIV-specific CD4 cells with intermediate functional avidity), an immune response is established only if initial HIV-specific T-helper cells are present in sufficiently high numbers and viral inoculum is low. With the reverse initial conditions, the patient will not mount an immune response to control the infection, and has a high viral set-point. This is not the first time this has been documented (Wodarz & Nowak 1999). However, here we explicitly attribute it to the dual role of HIV-specific CD4 T cells, as targets for infection and as mediators of the immune response. Also, a novel result is that multiple steady states occur in a small parameter range, corresponding to different possible courses of infection, characterized by different viral set-points controlled by various numbers of T-helper cell clones. This means that, depending on the abundance of each clone and the size of virus inoculum at the moment of infection, a different viral set-point, controlled by different numbers of clones, is reached. As expected, the broadest response is associated with the lowest viral load (Edwards *et al.* 2002). Our results can be extended to a model with n clones ($n > 2$): for varying T-helper clone avidities, we would see a range of stable steady states characterized by different numbers of clones. In a certain parameter range, we speculate that '$n + 1$-stability' could

occur, just as we saw tristability in our model with two clones. It is also relevant to note that the coexistence of several T-cell clones leads, in our model, to lower viral loads. This means that vaccination strategies directed at stimulating a broad immune response may have a beneficial effect on the outcome of infection.

We observed that tristability is most likely to occur (i.e. is possible for more combinations of avidities of the clones involved in the response) when the clone with higher avidity is lytic and the other is non-lytic. It is slightly artificial to speak of T-helper clones involved in strictly lytic or non-lytic responses, as we do not know whether such specialized responses exist. However, we can imagine that some CTL clones may constitutively express more perforin, for example, than RANTES. Also, this distinction may help us to envisage under what conditions a therapeutic intervention manipulating CTL responses might influence the clonality of the response and the associated viral load. Adding a clone when one clone is already involved in the immune response does not seem to reduce viral load considerably. In the parameter range with multiple stable solutions, we found that the second clone can reduce the number of infected cells to one-sixth of the number controlled by only one clone (see figure 2*d*). A bigger effect could be obtained if the second, lower avidity, clone had a stronger non-lytic effect: for $n_2 = 10$, there is a 20-fold reduction in viral load (results not shown). Our results can help interpret the possible effects of therapeutic interventions in the context of HIV infection. Altfeld *et al.* (2002) observed that only previously existing clones could be expanded by structured therapy interruptions. We show here that it may in theory be possible to stimulate other clones, albeit for clones with a limited, intermediate range of avidities.

Our results suggest that differences in viral set-point across patients who might otherwise have similar viral and immune-response parameters (e.g. virus infectivity and patient human leukocyte antigen (HLA)-type) could be explained by the number of HIV-specific T-cell clones controlling the infection. This in turn would depend on virus inoculum and the abundance of each clone at the moment of infection. Across patients with different HLA-types, variation in viral set-point can also be attributed to the nature of the response elicited by the virus: a patient mounting a response with low-avidity clones will have a higher viral set-point than a patient mounting a high-avidity response. The viral set-point shows up to a 1000-fold variation across patients (Piatak *et al.* 1993). This has been attributed to variation in immune responsiveness (Nowak & Bangham 1996); in a similar vein, we could explain this 1000-fold variation by variation in immune responses involving clones with different avidities; such large variations cannot be accounted for solely by the switching behaviour we observed between steady states with immune responses involving different numbers of clones. However, immune responsiveness is not the only factor likely to be involved, as small variations in other parameters may account for large variations in the viral set-point (Muller *et al.* 2001). We have indeed shown that viral set-point could also be determined by variation in killing efficiency across CTL clones.

No measurements of HIV-specific CD4 responses have to our knowledge been carried out early in untreated infections and correlated with disease progression, which we could compare with the above results. Fidler *et al.* (2002) have observed the maintenance of the CD4 response with early therapy, but they have not been able to follow up on the long-term outcome of infection. There is only indirect evidence for the importance of HIV-specific CD4 responses. First, a correlation between levels of HIV-specific T-helper cells and delayed progression has been documented (Rosenberg *et al.* 1997; Pitcher *et al.* 1999; Oxenius *et al.* 2000). Second, there is evidence that CD4 T-helper responses elicited by vaccination confer a protective advantage (Heeney 2002); and third, strong HIV-specific Th1 responses have been measured in exposed uninfected individuals compared with unexposed seronegative individuals (Shearer & Clerici 1996). Interestingly, transient early antiretroviral therapy in rhesus macaques has been associated with stronger SIV-specific proliferative responses and lower viral set-points (Lifson *et al.* 2000).

Thus, we believe that our model can help us to understand the variation in viral set-points across patients with HIV infection. Differences in viral set-point, associated with different levels of polyclonality of the response, can be explained by different initial abundances of each HIV-specific CD4 T-cell clone. Also, our model can serve as a framework to study the effect of vaccination and structured treatment interruptions on the establishment of polyclonal immune responses against HIV. Our work would suggest that broadening immune responses through structured therapy interruptions (amounting to a shift between steady states in the parameter region where there is *n*-stability) is possible only in a very narrow parameter range—except when the patient starts without any HIV-specific immunity (i.e. in target-cell-limited infection). Even if a shift could be achieved, this would not lead to a great reduction in viral load. More promising would seem to be to stimulate previously unsolicited higher-avidity clones through (therapeutic) vaccination, thereby shifting to a steady state with a much lower viral set-point.

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APPENDIX A

Here, we show that clones cannot coexist when there is no competition within clones. For an activated clone, the production of cells by proliferation ($\alpha I H_i/(\gamma_i + I)$) should be much larger than the production by the thymus. Thus, letting $\sigma_H \to 0$, we solve the equations of the model referring to the T-helper cell populations at steady state:

$$\begin{cases} \dfrac{\mathrm{d}H_1}{\mathrm{d}t} = \dfrac{\alpha \bar{I} \bar{H}_1}{\gamma_1 + \bar{I}} - \dfrac{\beta_H \bar{I} \bar{H}_1}{1 + n_1 \bar{H}_1 + n_2 \bar{H}_2} - \varepsilon \bar{H}_1^m = 0 \\ \dfrac{\mathrm{d}H_2}{\mathrm{d}t} = \dfrac{\alpha \bar{I} \bar{H}_2}{\gamma_2 + \bar{I}} - \dfrac{\beta_H \bar{I} \bar{H}_2}{1 + n_1 \bar{H}_1 + n_2 \bar{H}_2} - \varepsilon \bar{H}_2^m = 0 \end{cases}.$$

When $m = 1$, death of T-helper cells is linear:

$$\Leftrightarrow \begin{cases} \dfrac{f\beta}{1+n_1\bar{H}_1+n_2\bar{H}_2}\bar{H}_1 = \left(\dfrac{\alpha}{\gamma_1+\bar{I}}-\varepsilon\right)\bar{H}_1 \\ \dfrac{f\beta}{1+n_1\bar{H}_1+n_2\bar{H}_2}\bar{H}_2 = \left(\dfrac{\alpha}{\gamma_2+\bar{I}}-\varepsilon\right)\bar{H}_2 \end{cases},$$

$$\Leftrightarrow \begin{cases} \bar{H}_1 = 0 \\ \bar{H}_2 = \dfrac{f\beta(\bar{I}+\gamma_2)}{[\alpha-\varepsilon(\bar{I}+\gamma_2)]n_2} - \dfrac{1}{n_2} \end{cases} \vee \begin{cases} \bar{H}_1 = \dfrac{f\beta(\bar{I}+\gamma_1)}{[\alpha-\varepsilon(\bar{I}+\gamma_1)]n_1} - \dfrac{1}{n_1} \\ \bar{H}_2 = 0 \end{cases} \begin{cases} \bar{H}_1 = 0 \\ \bar{H}_2 = 0 \end{cases}.$$

In other words, when the constant source term is very small, coexistence is not possible in the absence of competition within clones. However, when $m > 1$, we can show that analogous steady-state equations lead to a solution with both H_1 and H_2 positive, i.e. coexistence rather than competitive exclusion (see our simulations in § 3).

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