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Immune Responses Against Conserved and Variable Viral Epitopes

B. BITTNER[a,*] and L.M. WAHL[b]

[a]*Institute of Algebra and Computational Mathematics, Vienna University of Technology, Vienna, Austria and* [b]*Theoretical Biology, Institute for Advanced Study, Princeton, New Jersey*

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We extend well-known mathematical models of viral infection to examine the response of cytotoxic T lymphocytes (CTL) to both conserved and variable viral epitopes. Because most viruses are subject to error-prone reproduction, CTL recognition may be faced with highly variable epitopes, while other CTL epitopes may remain conserved across viral strains. In this paper we examine the steady state conditions for a simple model of viral-immune system dynamics in which the viral strain can be limited by either a specific immune response, a cross-reactive immune response, or host cell availability. We find that the most important factors determining the type of immune response elicited and viral diversity are the relative proliferation rates of the two types of immune response. If the immune response to variable epitopes is strong compared with the response to conserved epitopes, diversity will be *negatively* correlated with the total burden of infected cells. In this situation high diversity may be indicative of a strong immune response and slower disease progression. In contrast, for patients whose immune response is directed predominantly towards conserved viral epitopes, our model predicts that diversity and viral load will be *positively* correlated.

Keywords: HIV, mathematical model, cytotoxic T lymphocytes, virology, immunology, mutation

1 INTRODUCTION

Cytotoxic T lymphocytes (CTLs) constitute an important immune defense to viral infection. These lymphocytes recognize and respond to small sections of viral proteins called epitopes. Because most viruses are subject to highly error-prone reproduction, CTL recognition can be confronted with viral mutations (Eigen and Schuster 1977). Within some epitopes, mutations may allow the virus to escape immune recognition ("variable" epitopes); conversely, epitopes may exist in conserved regions of the viral genome where mutations are not possible, or may not lead to immune escape ("conserved" epitopes). Clearly the interactions between the immune system and these conserved or variable epitopes will differ, and may impact disease progression.

We hope to shed light on possible interactions between viral mutations and the immune system by translating the key features of this complex system into

* Corresponding Author, present address: Department of Medical Statistics, University of Vienna, Schwarzspanierstraße 17, 1090 Wien, Austria, e-mail: barbara.bittner@univie.ac.at, phone: + 43 1 4277 63203, fax: + 43 1 4277 9632

a mathematical framework; this process makes our assumptions about the underlying dynamics explicit (Kirschner 1996; Blower and Ganem 1998). In particular, we classify CTL responses into two categories: the CTL response to conserved epitopes ("cross-reactive" CTL response); and the CTL response to variable epitopes ("specific" CTL response). In this way we are able to investigate how small changes in the environment or the appearance of a new mutant might affect both the type of immune response elicited and the number of mutants present (diversity).

Our analysis builds on earlier work investigating the effects of immune responses against multiple epitopes (Nowak et al. 1995a; Nowak et al. 1995b); similar models have been studied recently in Ribeiro and Bonhoeffer 1999; Regoes et al. 1998, and Ribeiro et al. 1998. In this paper we extend earlier work by explicitly examining the inherent competition between cross-reactive and specific CTL responses (Antia and Koella 1994), that is, we consider the situation when some epitopes are variable and others are conserved across all viral strains. This work forms the underpinning for a more complete understanding of the immunological changes which may occur in response to the appearance of new mutations during viral infections (Wahl et al. 2000; Borrow et al. 1997).

2 THE MODEL

A number of possible models for virus-immune system dynamics have been suggested in the literature (for review see Marchuk 1997; DeBoer and Perelson 1998); to capture the key effects of conserved and variable viral epitopes, however, we propose the following: the number of healthy cells is given by x, and these are produced (activated) at a constant rate λ and die at rate dx. The number of cells which are infected is given by $x \Sigma \beta_i y_i$ where y_i stands for the number of cells which are infected with virus type i, while β_i is the infectivity parameter. Without loss of generality the virus types shall be numbered so that $\beta_1 > \beta_2 > \ldots > \beta_n$ (the higher the infectivity parameter the smaller the index). Virus types may differ at one or more epitopes, but we assume that at least one epitope is conserved across all viral types. Note that we do not explicitly include circulating virus in the model, assuming that the circulating virus and infected cells are at effective equilibrium at the time scale of interest.

Infected cells die at rate ay_i (here we allow a to be smaller than, equal to or larger than d). Cytotoxic T lymphocytes z_i which are specific for mutants of type i kill infected cells at rate py_iz_i, while the cross-reactive immune response (w) is responsible for the death of qwy_i infected cells. A clear limitation of this model, for antigenically heterogeneous viral populations, is that we do not include the effects of CTL which may recognize some, but not all, of the existing viral strains. We have instead made the simplifying asumption that any epitope that is conserved between some viral strains is conserved between all viral strains.

Although the lymphatic system produces highly diverse CTL, the abundance of a specific response type might not be large. When the virus is detected by a suitable CTL, i.e., the epitope fits the immune cell's paratope (the corresponding surface proteins of the immune cell), this immune response will proliferate. In our model the proliferation rate is $c_iy_iz_i$, implying that the specific immune response will be elicited more quickly if more infected cells exist. The natural death rates of the immune cells – whether they are specific or cross-reactive – are by_i and bw respectively. The response to the conserved epitope is evoked at rate $w \Sigma k_iy_i$ since all mutants carry this surface structure.

Further we assume that there is a maximum number of different mutant strains, n. This number can be large. Given certain conditions (parameter values, immunological influences) only some of the possible virus mutants will exist at equilibrium.

Thus it is necessary to differentiate between the number of surviving mutants, m, and the maximum possible number of mutants, n.

This yields the following system of differential equations:

$$\dot{x} = \lambda - dx - x \sum_{i=1}^{n} \beta_i y_i \qquad (1)$$

$$\dot{y}_i = y_i(\beta_i x - a - pz_i - qw) \quad i = 1, \ldots, n \qquad (2)$$

$$\dot{z}_i = z_i(c_i y_i - b) \quad i = 1, \ldots, n \tag{3}$$

$$\dot{w} = w\left(\sum_{i=1}^{n} k_i y_i - b\right) \tag{4}$$

3 THE CASE OF TWO VIRUS STRAINS

To gain some insight into the dynamics of this system, we first consider the simple case of two viral strains, and determine the steady states of this system and the conditions under which they exist and are stable. Once again we note that these viral sub-types may differ at one or more epitopes, but have in common a conserved epitope which is theoretically capable of eliciting an immune response. In this case the system of differential equations can be re-written:

$$\dot{x} = \lambda - dx - x\beta_1 y_1 - x\beta_2 y_2 \tag{5}$$

$$\dot{y}_1 = y_1(\beta_1 x - a - pz_1 - qw) \tag{6}$$

$$\dot{y}_2 = y_2(\beta_2 x - a - pz_2 - qw) \tag{7}$$

$$\dot{z}_1 = z_1(c_1 y_1 - b) \tag{8}$$

$$\dot{z}_2 = z_2(c_2 y_2 - b) \tag{9}$$

$$\dot{w} = w(k_1 y_1 + k_2 y_2 - b) \tag{10}$$

We find seven possible equilibrium states for this system, as described in Table I. These states correspond to the uninfected state (E_x), three states in which only a single virus type exists, kept in check by (*i*) target cell availability ($E_v^{(1)}$), (*ii*) a specific immune response ($E_z^{(1)}$), or (*iii*) a cross-reactive immune response ($E_w^{(1)}$), and three states in which both virus types are present and there is (*i*) a specific immune response against only one type ($E_v^{(2)}$), (*ii*) a specific immune response against both types ($E_z^{(2)}$), or (*iii*) a cross-reactive immune response and a specific immune response against one type ($E_w^{(2)}$).

TABLE I Equilibrium loads and conditions for existence and stability of steady states of the system with two virus types. Note that the basic reproductive ratio, $R_i = \frac{\beta_i \lambda}{ad}$. The notation $E_*^{(m)}$ is used for consistency with the general model, and is discussed in greater detail in Section 4.

E_x	$\hat{x} = \frac{\lambda}{d}$	$R_1 < 1$
$E_v^{(1)}$	$\hat{x} = \frac{a}{\beta_1}$, $\hat{y}_1 = \frac{\lambda}{a} - \frac{d}{\beta_1}$	$1 < R_1 < \min\left\{1 + \frac{\beta_1 b}{c_1 d}; 1 + \frac{\beta_1 b}{k_1 d}\right\}$
$E_v^{(2)}$	$\hat{x} = \frac{a}{\beta_2}$, $\hat{y}_1 = \frac{b}{c_1}$, $\hat{y}_2 = \frac{\lambda}{a} - \frac{d}{\beta_2} - \frac{b\beta_1}{\beta_2 c_1}$, $\hat{z}_1 = \frac{a}{p}\left(\frac{\beta_1}{\beta_2} - 1\right)$	$1 + \frac{\beta_1 b}{c_1 d} < R_2 < \min\left\{1 + \frac{\beta_1 b}{c_1 d} + \frac{\beta_2 b}{c_2 d}; 1 + \frac{\beta_1 b}{c_1 d} + \frac{\beta_2 b}{k_2 d}\left(1 - \frac{k_1}{c_1}\right)\right\}$, $\frac{k_1}{c_1} < 1$
$E_z^{(1)}$	$\hat{x} = \lambda : \left(d + b\frac{\beta_1}{c_1}\right)$, $\hat{y}_1 = \frac{b}{c_1}$, $\hat{z}_1 = \frac{1}{p}(\beta_1 \hat{x} - a)$	$R_2 < 1 + \frac{\beta_1 b}{c_1 d} < R_1$, $\frac{k_1}{c_1} < 1$

$E_z^{(2)}$	$\hat{x} = \lambda : \left(d + b\left(\frac{\beta_1}{c_1} + \frac{\beta_2}{c_2}\right)\right),$	$1 + \frac{\beta_1 b}{c_1 d} + \frac{\beta_2 b}{c_2 d} < R_2,$
	$\hat{y}_1 = \frac{b}{c_1},$	$\frac{k_1}{c_1} + \frac{k_2}{c_2} < 1$
	$\hat{y}_2 = \frac{b}{c_2},$	
	$\hat{z}_1 = \frac{1}{p}(\beta_1 \hat{x} - a),$	
	$\hat{z}_2 = \frac{1}{p}(\beta_2 \hat{x} - a)$	
$E_w^{(1)}$	$\hat{x} = \lambda : \left(d + \frac{\beta_1 b}{k_1}\right),$	$1 + \frac{\beta_1 b}{k_1 d} < R_1,$
	$\hat{y}_1 = \frac{b}{k_1},$	$1 < \frac{k_1}{c_1}$
	$\hat{w} = \frac{1}{q}(\beta_1 \hat{x} - a)$	
$E_w^{(2)}$	$\hat{x} = \lambda : \left(d + \frac{\beta_1 b}{c_1} + \frac{\beta_2 b}{k_2}\left(1 - \frac{k_1}{c_1}\right)\right),$	$1 + \frac{\beta_1 b}{c_1 d} + \frac{\beta_2 b}{k_2 d}\left(1 - \frac{k_1}{c_1}\right) < R_2,$
	$\hat{y}_1 = \frac{b}{c_1},$	$\frac{k_1}{c_1} < 1 < \frac{k_1}{c_1} + \frac{k_2}{c_2}$
	$\hat{y}_2 = \frac{b}{k_2}\left(1 - \frac{k_1}{c_1}\right),$	
	$\hat{z}_1 = \frac{1}{p}(\beta_1 - \beta_2)\hat{x},$	
	$\hat{w} = \frac{1}{a}(\beta_2 \hat{x} - a)$	

From Table I it is clear that the existence of the various equilibrium states depends on two factors: the relative proliferation rates (c_i and k_i) of the specific and cross-reactive immune responses; and the basic reproductive ratios (R_i) of the two virus types. The basic reproductive ratio gives the average number of cells newly infected by one infected cell during its lifetime. We note first that if R_1 is sufficiently small, no immune response is necessary to keep the system in equilibrium, and state E_x or $E_v^{(1)}$ results.

Neither of the virus subtypes can survive in the body as long as R_1 and R_2, are both smaller than one, where $R_i = \frac{\beta_i \lambda}{ad}$. Since we assume that the transmission rates are ordered so that $\beta_1 > \beta_2$, this implies simply that R_1 is smaller than 1. When this condition is met, the uninfected state, E_x, is the only stable equilibrium.

When R_1 is large enough that an immune response is evoked, several possible equilibrium states exist. If the cross-reactive immune response is elicited more readily by y_1 than the specific immune response $\left(\frac{k_1}{c_1} > 1\right)$, we find that *only one* equilibrium state $(E_w^{(1)})$ is possible, and it is clear that a second virus type with a lower infectivity can never invade. If, on the other hand, $\frac{k_1}{c_1} < 1$, the equilibrium state will depend on R_2: if R_2 is small, state $E_z^{(1)}$ will be stable (the second virus type is not present at equilibrium); if R_2 is slightly larger, state $E_v^{(2)}$ will result (the second virus type is present but regulated by host cell availability); if R_2 is larger yet, state $E_z^{(2)}$ or $E_w^{(2)}$ will be

stable (either the specific or cross-reactive immune response will be evoked).

In conclusion we note that no stable equilibrium exists in which both virus types are present and *only* the cross-reactive immune response is elicited, neither is there an equilibrium state in which the cross-reactive and specific immune responses to *both* types are present.

4 THE GENERAL CASE OF N VIRAL STRAINS

For the more general system, we likewise determine the steady states and the conditions under which they exist and are saturated (i.e. no other mutants would survive), and then discuss the effects of environmental (immunological) changes.

In theory there exist six different categories of possible equilibria, but two can be excluded, because they either contradict our assumption that all β_i are different or are subject to a condition which is very unlikely.

As for the $n = 2$ case, we find that the uninfected state exists only under the condition that the basic reproductive ratio of all virus types is less than one.

In general within our model infection cannot be controlled by target cell availability alone. Only one strain of the virus can be controlled by target cell availability; this is a special case of the situations discussed below. An equilibrium with m different types of virus and no immune response at all would mean $\beta_1 = \beta_2 = \ldots = \beta_m$, which we exclude. Hence, if more than one viral sub-type is present, the immune response must be active in some way. In fact, we discover three possible constellations.

TABLE II Steady states, description and conditions

E_x	$\hat{x} \neq 0$	$R_1 < 1$
	$\hat{x} \neq 0$, $\hat{y}_i \neq 0\ \forall i = 1, \ldots, m$	$\beta_1 = \beta_2 = \ldots = \beta_m$ $\Rightarrow$ contradicts $\beta_1 > \ldots > \beta_n$
$E_v^{(m)}$	$\hat{x} \neq 0$, $\hat{y}_i \neq 0\ \forall i = 1, \ldots, m$, $\hat{z}_i \neq 0\ \forall i = 1, \ldots, m-1$	$L_1^{(m-1)} < R_m < \min\{L_1^{(m)}; L_1^{(m-1)} + L_2^{(m-1)}\}$ $\Rightarrow \sum_{i=1}^{m-1} \frac{k_i}{c_i} < 1$
$E_z^{(m)}$	$\hat{x} \neq 0$, $\hat{y}_i \neq 0\ \forall i = 1, \ldots, m$, $\hat{z}_i \neq 0\ \forall i = 1, \ldots, m$	$R_m > L_1^{(m)}, R_{m+1} < L_1^{(m)}$, $\sum_{i=1}^{m} \frac{k_i}{c_i} < 1$
$E_w^{(m)}$	$\hat{x} \neq 0$, $\hat{y}_i \neq 0\ \forall i = 1, \ldots, m$, $\hat{z}_i \neq 0\ \forall i = 1, \ldots, m-1$, $\hat{w} \neq 0$	$R_m > L_1^{(m-1)} + L_2^{(m-1)}$, $\sum_{i=1}^{m-1} \frac{k_i}{c_i} < 1 < \sum_{i=1}^{m} \frac{k_i}{c_i}$
	$\hat{x} \neq 0$, $\hat{y}_i \neq 0\ \forall i = 1, \ldots, m$, $\hat{z}_i \neq 0\ \forall i = 1, \ldots, m$, $\hat{w} \neq 0$	$\sum_{i=1}^{m} \frac{\kappa_i}{c_i} = 1$ $\Rightarrow$ unlikely

$$L_1^{(m)} := 1 + \sum_{i=1}^{m} \frac{\beta_i b}{c_i d},$$

$$L_2^{(m)} := \frac{\beta_{m+1} b}{d k_{m+1}} \left(1 - \sum_{i=1}^{m} \frac{k_i}{c_i}\right)$$

Note that m is the largest number i which meets the conditions given above.

First, m virus mutants and m-1 types of specific immune response are present and establish a steady state. We call this kind of equilibrium $E_v^{(m)}$. While the m-1 most infectious virus subtypes are controlled by the specific immune responses, the least infectious subtype is held in check by the limited availability of healthy cells. (Note that m-1 might equal zero.)

Second, m virus mutants and the same number of specific immune responses exist in equilibrium. This fixed point is referred to as $E_z^{(m)}$. If more different mutants are present at equilibrium, we see that the specific immune response must proliferate more quickly (c_i large) or else the cross-reactive immune response will be evoked. In other words, the more mutants, the higher the probability that a cross-reactive response spreads.

Thirdly, a cross-reactive immune response is present with m different types of infected cells and m-1 types of specific immune response. We use $E_w^{(m)}$ to denote these equilibrium states. In this case the cross-reactive response plus diminished target cell availability is enough to keep y_m under control. (Again, m-1 might equal zero.)

The steady state in which all the specific immune responses and the cross-reactive response are present is excluded because this is only possible under very specific (and therefore unlikely) conditions.

All these equilibria are shown in Table II with the conditions which must be fulfilled to make their existence possible.

5 IMMUNE RESPONSE PARAMETERS AND THEIR EFFECTS

Note that neither p nor q appears in the conditions in Table II. This means that the size of the immune response parameters, i.e. the rate of killing by the specific and cross-reactive T cells, has no effect on the qualitative behavior of the system. The more efficiently the specific immune response works (p large), however, the fewer immune cells will be employed ($\hat{z}_{i\ \mathrm{i}}$ small). The same is true for the cross-reactive immune response ("q large" corresponds to "$\hat{w}$ small" and vice versa). So, immune reaction parameters have only a scaling effect on the equilibrium loads, as can be seen in Table III.

TABLE III Equilibrium loads

E_x	$\hat{x} = \frac{\lambda}{d}$	
$E_v^{(m)}$	$\hat{x} = \frac{a}{\beta_m}$,	
	$\hat{y}_i = \frac{b}{c_i}$	$\forall$ i = 1,..., m-1,
	$\hat{y}_m = \frac{\lambda}{a} - \frac{d}{\beta_m} - \frac{b}{\beta_m} \sum_{i=1}^{m-1} \frac{\beta_i}{c_i}$	
	$\hat{z}_i = \frac{a}{p}\left(\frac{\beta_i}{\beta_m} - 1\right)$	$\forall$ i = 1,..., m-1,
$E_z^{(m)}$	$\hat{x} = \lambda : \left(d + b\sum_{i=1}^{m} \frac{\beta_i}{c_i}\right)$	
	$\hat{y}_i = \frac{b}{c_i}$	$\forall$ i = 1,..., m,
	$\hat{z}_i = \frac{1}{p}(\beta_i \hat{x} - a)$	$\forall$ i = 1,..., m,

$E_w^{(m)}$

$$\hat{x} = \lambda : \left(d + b \sum_{i=1}^{m-1} \frac{\beta_i}{c_i} + \frac{\beta_m b}{k_m} \left(1 - \sum_{i=1}^{m-1} \frac{k_i}{c_i} \right) \right)$$

$$\hat{y}_i = \frac{b}{c_i} \qquad \forall \text{ i} = 1,\ldots, m\text{-}1,$$

$$\hat{y}_m = \frac{b}{k_m} \left(1 - \sum_{i=1}^{m-1} \frac{k_i}{c_i} \right)$$

$$\hat{z}_i = \frac{1}{p}(\beta_i - \beta_m)\hat{x} \qquad \forall \text{ i} = 1,\ldots, m\text{-}1,$$

$$\hat{w} = \frac{1}{q}(\beta_m \hat{x} - a)$$

To isolate the effect of immunogenicity, we assume that the immunogenicity factors do not differ greatly for all viral mutants, and therefore we can simplify the mathematical analysis by setting $c_i = c$ and $k_i = k$ for all i. If the specific and cross-reactive immune responses are elicited more or less equally, both response types will be present at equilibrium. The dynamics will approach $E_w^{(m)}$ with m small, i.e., the abundance of virus types will be small. If the specific immune response reacts more quickly to the infection than the cross-reactive response, then the variety of mutants will increase. With increasing immunogenicity c, the cross-reactive response decreases and may disappear entirely. In contrast, if the cross-reactive immune response reacts more quickly than the specific response, the variety of mutants will decrease, and the specific immune response may disappear.

These effects are illustrated in Figures 1 and 2, where the parameters c_i and k_i are varied, respectively. In Figure 1, the c_i are assumed to be equal for all viral strains and vary from $c = 0$ to $c = 3$. In the top panel diversity (the number of surviving viral strains) is plotted against c; the second panel shows the equilibrium load of uninfected cells (dashed line) and the total burden of infected cells (solid line); the third panel gives the equilibrium loads for the total specific immune response (dashed line) and the cross-reactive immune response (solid line). In each panel vertical dotted lines show the transitions when an additional viral strain survives at equilibrium.

For small c we observe a single virus type with a cross-reactive immune response and no specific response $(E_w^{(1)})$. For slightly larger c, a second mutant can survive and a specific response to the first mutant emerges. This is followed by states $E_w^{(3)}$ and then $E_z^{(3)}$ as c increases further (the cross-reactive response disappears and a specific response to the least infectious strain appears). For even larger values of c another virus type can survive, but a specific immune response to this type does not appear without a further increase in c.

Examining in particular the equilibrium load of uninfected cells (dashed line), we see that the number of healthy cells increases with higher immunogenicity, and therefore with higher diversity. Likewise, we see that increases in specific immunogenicity correlate with decreases in the equilibrium load of infected cells, $\hat{y}$ (solid line).

We also note that the equilibrium loads for both types of immune response are discontinuous, exhibiting 'all or nothing' behavior. This indicates that the survival of a new type of specific immune response at equilibrium results in a significant difference to the total frequency of specific immune cells. As the frequency of the specific immune response increases, the cross-reactive response falls to lower levels. At $c = 1.2$ the immunogenicity of the cross-reactive response is so small relative to that of the specific response that the cross-reactive response disappears altogether.

Figure 2 shows similar results, but in this case c has been held constant while the cross-reactive immunogenicity, k, is varied between 0.02 and 1. Once again, as the efficiency of the immune response increases, the equilibrium load of uninfected cells increases and the total burden of infected cells decreases. In this

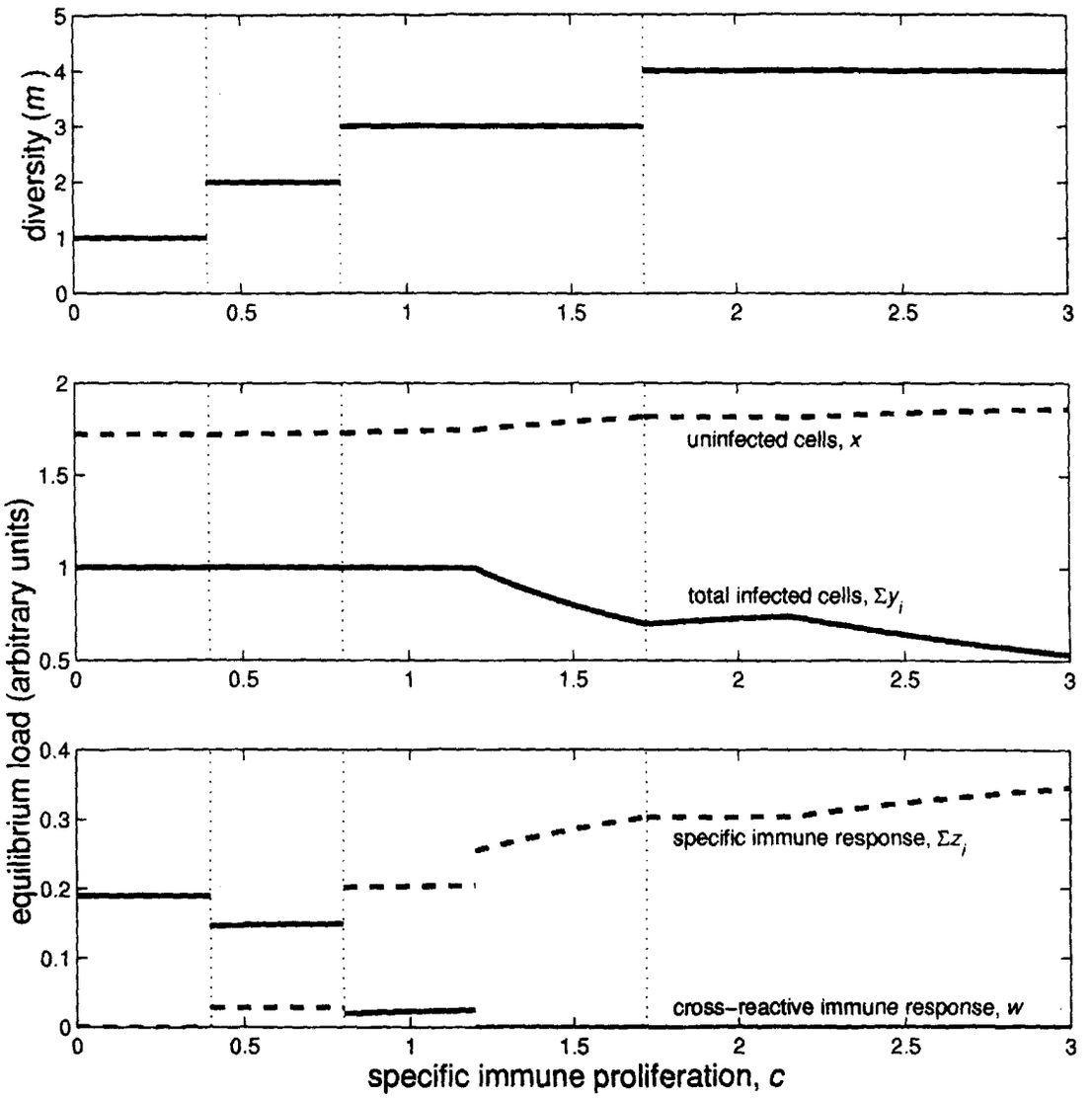

FIGURE 1 Effects of increasing specific immunogenicity. In each panel the x-axis represents the proliferation of specific CTLs in response to infection, parameter c in the model. Thus towards the right the specific immune response proliferates more effectively than the cross-reactive immune response; the immune response to conserved epitopes is weaker. The first panel illustrates the number of viral strains surviving at equilibrium, which increases with specific immunogenicity. The vertical dotted lines show transitions where an additional viral strain survives; these lines are repeated in the second and third panels. The second panel shows the equilibrium load of uninfected cells (dashed line) and the total burden of infected cells (solid line). The third panel shows corresponding changes to the equilibrium load of the specific (dashed line) and cross-reactive (solid line) immune response. For $c \in (0; 0.4)$ one mutant plus the cross-reactive response can be observed. At 0.4 and 0.8 another virus type can survive, together with the corresponding specific immune response to the more infectious type. With even stronger specific immunogenicity ($c \geq 1.2$) the cross-reactive response disappears while a specific response even to the least infectious strain emerges. For $c \in (1.72; 2.16)$ a fourth mutant is able to survive and is regulated by target cell availability alone. When ($c \geq 2.16$), the specific response to this mutant also survives. We note two counter-intuitive results: the number of healthy cells increases with the number of surviving mutants; and likewise the total burden of infected cells *decreases* with increasing diversity. As explained in the text, we have chosen $c_i = c$ for all mutants, to isolate the effect of varying immunogenicity for the specific immune response. Note that the corresponding immunogenicity parameters k_i are likewise assumed to be the same for all mutants, hence $k_i = k = 0.4$. Other parameter values are: $a = 0.1$, $b = 0.4$, $\beta_1 = 0.08$, $\beta_2 = 0.075$, $\beta_3 = 0.06$, $\beta_4 = 0.055$, $\beta_5 = 0.05$, $d = 0.5$, $k_i = k = 0.4$, $\lambda = 1$, $p = 0.3$, $q = 0.2$

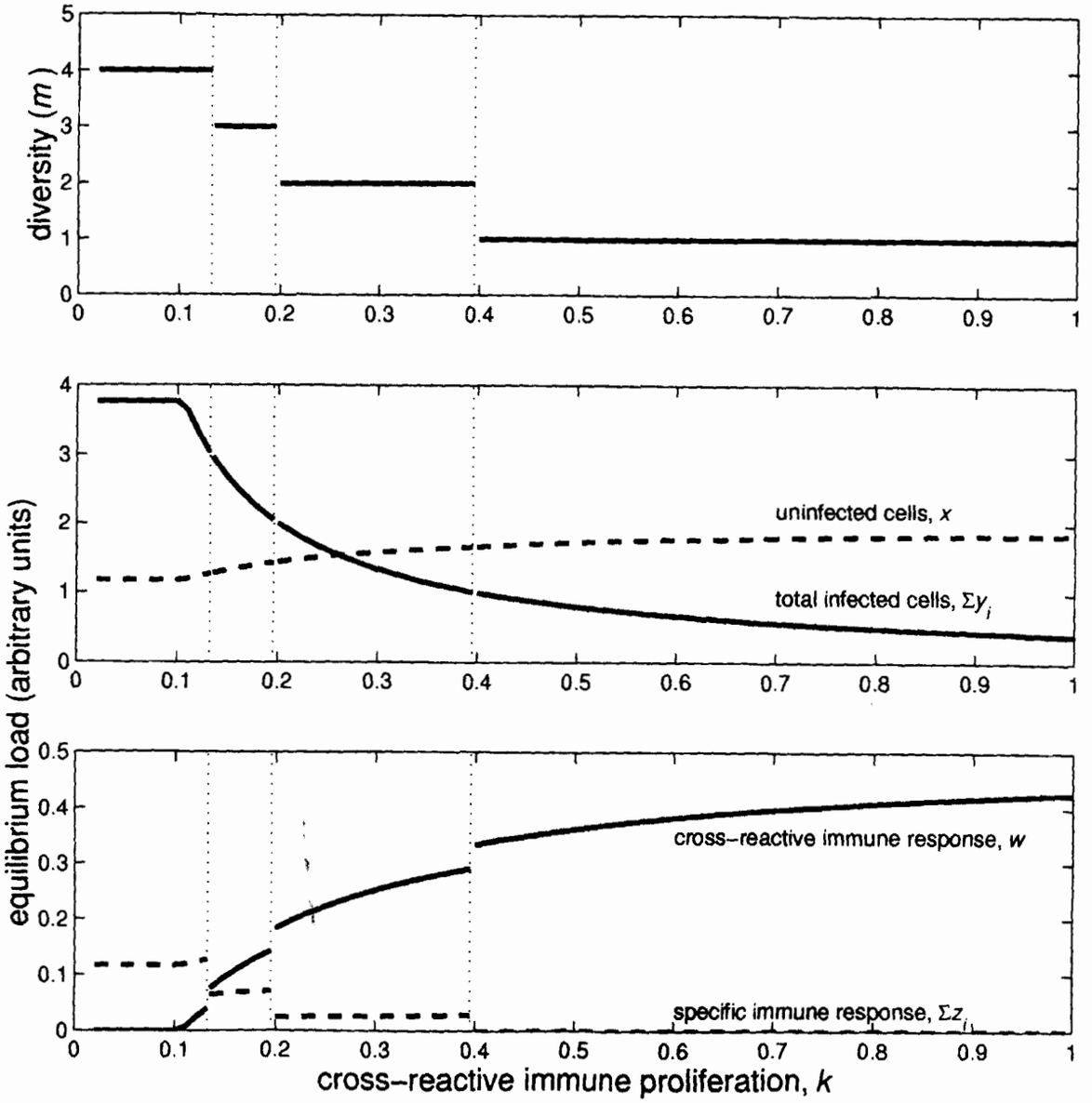

FIGURE 2 Effects of increasing cross-reactive immunogenicity. In each panel the x-axis represents the proliferation of cross-reactive CTLs in response to infection, parameter k in the model. Thus towards the right the cross-reactive immune response proliferates more effectively than the specific immune response; the immune response to conserved epitopes is stronger. The first panel illustrates the number of viral strains surviving at equilibrium, which decreases with cross-reactive immunogenicity. The vertical dotted lines show transitions where an additional viral strain disappears; these lines are repeated in the second and third panels. The second panel shows the equilibrium load of uninfected cells (dashed line) and the total burden of infected cells (solid line). The third panel shows corresponding changes to the equilibrium load of the specific (dashed line) and cross-reactive (solid line) immune responses. We note that in this case the number of healthy cells increases with decreasing diversity, and likewise the total burden of infected cells decreases with decreasing diversity. As explained in the text, we have chosen $k_i = k$ for all mutants, where k varies between 0.02 and 1; likewise $c_i = c = 0.4$. Other parameter values are: $a = 0.1$, $b = 0.4$, $\beta_1 = 0.1$, $\beta_2 = 0.995$, $\beta_3 = 0.990$, $\beta_4 = 0.985$, $\beta_5 = 0.980$, $d = 0.5$, $k_i = k = 0.4$, $\lambda = 1$, $p = 0.3$, $q = 0.2$

case, however, diversity *decreases* as the cross-reactive immune response becomes more effective. This interesting constrast between Figures 1 and 2 will be taken up again in the discussion.

6 CHANGES DUE TO A NEW MUTANT

The analysis above sets the stage for a detailed investigation of changes in the immune response which may be elicited by the emergence of a novel mutation in this system. With the emergence of a new viral strain, it seems clear that a shift in the steady state might occur, diversity might change and virus load (mirrored by the number of infected cells at equilibrium) may be adjusted. Most importantly, we expect that shifts in the type of immune response elicited may be possible. Although a thorough analysis of this behavior is beyond the scope of this paper (see Wahl et al. 2000), we offer an example of one such change to illustrate the interesting effects this model allows us to investigate.

As an example, let us consider an immunological system at an equilibrium with both specific and cross-reactive immune responses, and with m different virus mutants, i.e., at steady state $E_w^{(m)}$. Now a new virus type (k) emerges (observed in the appearance of infected cells y_k). Since there will be hardly any specific immune response to the new mutant, it will be able to invade the body iff $\beta_k \hat{x} - a > q\hat{w}$, which is equivalent to $\beta_k - \beta_m > 0$. From this condition it seems clear that only virus strains which infect cells more efficiently than the least infectious strain m can invade in this case. We expect that the vast majority of mutants will *not* be more infectious than the wild-type virus, nonetheless some mutants may be more infectious than one of the viral sub-types present at equilibrium. It is also likely that certain mutations could confer an infective advantage if the virus is facing strong selective pressures, such as during drug therapy.

In the simplest case only one strain of the virus (y_j) is present, and the abundance of this strain is regulated by an immune response directed against it. Suppose a second type of infected cell (y_k) invades the equilibrium, and that the new viral strain does not escape from the immune response directed at y_j, i.e, the relevant epitope is conserved. It seems likely in this case that an immune response specific to one of the viral strains must also emerge, i.e, an immune response directed against the epitope that differs between the two viral strains will proliferate. If this were not the case, the new equilibrium would be specified by $\hat{x} \neq 0$, $\hat{w} \neq 0$, $\hat{y}_j \neq 0$ and $\hat{y}_k \neq 0$ which is only possible under the condition that $\beta_j = \beta_k$, contradicting our previous assumption that $\beta_1 > \ldots > \beta_n$.

The question is, which specific immune response will emerge, $z_j$or z_k? Assume $\hat{z}_k \neq 0$ and $\hat{z}_j = 0$. Then $q\hat{w}$ will equal $\beta_j \hat{x} - a$ and

$$\hat{z}_k = \frac{1}{p}\hat{x}(\beta_k - \beta_j) > 0. \tag{11}$$

In contrast, if we assume $\hat{z}_k = 0$ and $\hat{z}_j \neq 0$, then $\hat{z}_j$ would be negative. Thus we conclude in this simple case that the new equilibrium will include a specific immune response to the invading viral strain.

A diagram illustrating this type of transition is shown in the top panel of Figure 3.

Initially (on the left) the system is in equilibrium $E_w^{(3)}$; three viral strains are controlled by the cross-reactive immune response and specific immune responses against all but the least infectious strain. When a mutant appears which is more infectious, however, the system moves to equilibrium $E_z^{(3)}$. A specific immune response to the invading mutant appears. In this example, however, the appearance of the new mutant also causes the cross-reactive immune response and the least infectious viral strain to disappear. The lower two panels show a simulation of this transition. At the beginning of the simulation the system is seeded with viral strains y_2, y_3 and y_4, as well as small amounts of both the specific and cross-reactive immune responses. We see that the cross-reactive response proliferates, as well as specific responses to y_2 and y_3. At (arbitrary) time 1000 a small amount of a new mutant, y_1 (dotted line), is injected. Shortly afterwards y_4 (dashed line) disappears. The immune response to this transition is shown in the lowest panel; after an interlude when the cross-reactive

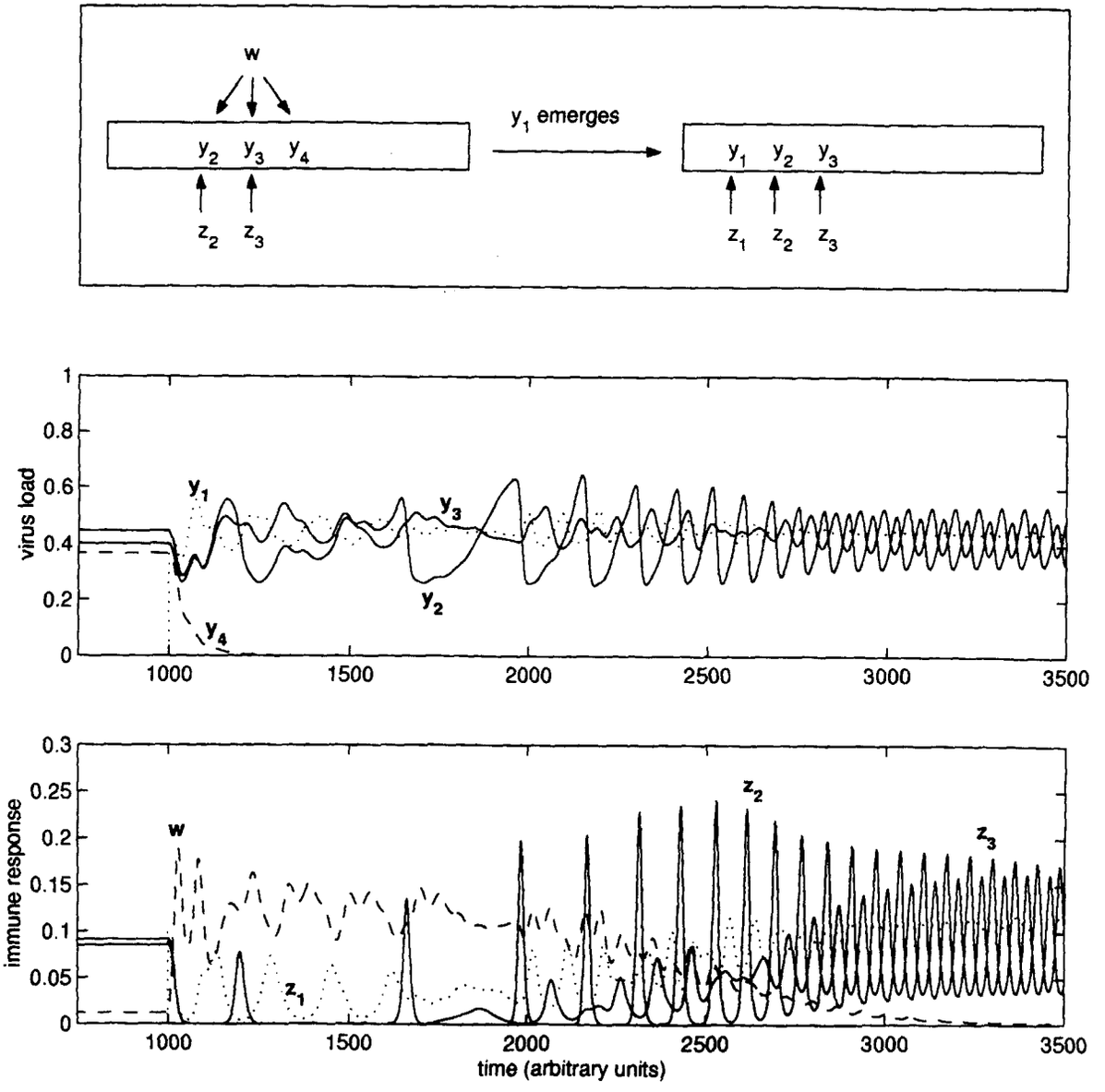

FIGURE 3 Fixpoint transition from E_w to E_z. In the top row we illustrate a population of virus which consists of three viral strains, y_2, y_3 and y_4. Each is controlled by the cross-reactive immune response w; specific immune responses z_2 and z_3 also control y_2 and y_3 respectively. The system is in equilibrium state $E_w^{(3)}$. After a new mutant arises (y_1), the system moves to equilibrium state $E_z^{(3)}$. Here the new mutant causes the emergence of its own specific immune response, z_1, as well as the *disappearance* of the cross-reactive immune response; the viral strain y_4 also disappears. The second panel shows a simulation of this transition; the population is seeded with viral strains y_2, y_3 and y_4 initially, as well as all specific and cross-reactive immune responses. The system quickly reaches equilibrium $E_w^{(3)}$. At an arbitrary time (1000), a small amount of viral strain y_1 is injected (dotted line), as well as (again) small amounts of every possible immune response. Only the three most infective viral strains survive the transition; y_4 (dashed line) is rapidly out-competed. The third panel illustrates that the cross-reactive immune response (w, dashed line) disappears, while the specific immune response z_1 (dotted line) emerges. Parameter values are: $\lambda = 1$; $d = 0.5$; $a = 0.1$; $p = 0.3$; $q = 0.2$; $b = 0.4$; $\beta_1 = .08$; $\beta_2 = .076$; $\beta_3 = .075$; $\beta_4 = .06$; c_1, c_3, and $c_4 = 0.9$; $c_2 = 1$; k_1 and $k_3 = 0.4$; $k_2 = .1$; $k_4 = 0.5$

immune response w dominates (dashed line), w also is eliminated from the equilibrium and a specific response to the new mutant (z_1, dotted line) appears. Thus the emergence of a new mutant causes, in this example, the disappearance of a different, less infective viral strain, and the appearance of a specific immune response directed against the new-comer. Most importantly perhaps, the new mutant causes the cross-reactive immune response (which affects and is elicited by every other viral species in the system), to disappear. (For a discussion of the oscillatory dynamics of this system, refer to Nowak et al. 1995b).

7 DISCUSSION

In this paper we compare the immune responses to conserved and variable epitopes in viral infection. We examine the dynamics of this system by determining all possible steady states (equilibria) of the model and the conditions under which they exist and are stable. Our model predicts the existence of four different types of steady state: first, there is no infection; second, infection is controlled by immune responses to variable epitopes and the least infectious mutant is contained by limited target cell availability; third, all mutants are held in check by specific CTL responses to variable epitopes; fourth, responses to both conserved and variable epitopes control infection. In the latter three equilibria, the diversity of CTL is limited by viral diversity; this effect is analogous to the principle of competitive exclusion in theoretical ecology, and has been described previously for T-cell dynamics (DeBoer and Perelson 1994; DeBoer and Perelson 1995).

We also note that when a cross-reactive immune response is present, the immune response to the variable epitope does not recognize the least infectious viral strain, which is held in check by the cross-reactive immune response alone. This implies that there is no stable equilibrium in which the immune system recognizes a conserved epitope of the virus *and* each variant at a variable epitope. If a mix of cross-reactive and specific immune responses are present, there will always appear to be one viral strain that "escapes" detection by the specific immune response.

We find that the most important factors in determining the type of immune response and the number of mutants at steady state are the relative proliferation rates of the two types of immune response. These proliferation rates (k_i and c_i in the model) reflect the magnitude of the immune response generated per infected cell by conserved and variable epitopes, respectively. The ratio of cross-reactive to specific proliferation is decisive for the number of surviving mutants – the smaller this ratio is, the more mutants can survive. In other words, the more effectively CTLs respond to viral epitopes which are conserved across all viral strains, the fewer mutations will survive at the steady state. Conversely, we find that if the immune response is directed predominantly against variable viral epitopes, the immune system itself exerts selective pressure on the virus and thereby favors mutation. For further discussion on the correlation between diversity and infection see Lukashov and Goudsmit 1998; Bittner et al. 1997; Nowak and Bangham 1996; Nowak et al. 1996; Wolinsky et al. 1996a, and Wolinsky et al. 1996b.

Although total viral diversity may increase with increasing CTL response to variable epitopes, the correlation between diversity and viral load is not straightforward. If the immune response to variable epitopes is strong compared with the response to conserved epitopes, diversity will be *negatively* correlated with the total burden of infected cells. In this situation high diversity may be indicative of a strong immune response and slower disease progression. These results agree with empirical results in Ogg et al. 1998, where an inverse correlation was found between the levels of HIV-specific CTLs and viremia. In contrast, for patients whose immune response is directed predominantly towards conserved viral epitopes, diversity and viral load will be *positively* correlated. Since the immunogenicities of the conserved and variable epitopes may differ between patients, trends which may be true for some patients will not generalize to all infected individuals. These phenomena are discussed further in Bittner et al. 1997, and Nowak and Bangham 1996, and have led to

minor differences of opinion in the scientific community, see Nowak et al. 1996, Wolinsky et al. 1996a, and Wolinsky et al. 1996b.

Our model naturally lends itself to further analysis of possible transitions between the steady states we have determined (Wahl et al. 2000). We illustrate one example of such a transition and find a surprising range of changes in response to the emergence of a single, novel mutation; this rich behaviour compares well with the results of Nowak et al. 1995b, for a multiple epitope model with no cross-reactive immune response. Although the emergence of a specific immune response to a new viral strain may be expected, we also find that the emergence of a new mutant may cause the disappearance of other viral sub-species and, in fact, the disappearance of the cross-reactive immune response. As experimental characterization of the short-term kinetics of viral and CTL diversity becomes increasingly feasible (see for example Borrow et al. 1997), understanding of immune transitions during disease progression will likewise become increasingly necessary.

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