J. theor. Biol. (1995) **175,** 325–353

Immune Responses against Multiple Epitopes

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(*Received on* 31 *August* 1994, *Accepted on* 19 *December* 1994)

The current understanding of antigenic escape dynamics is based on models with single epitopes. The usual idea is that a mutation which enables a pathogen (virus, bacteria, etc) to escape from a given immune response confers a selective advantage. The "escape mutant" may then increase in abundance until it induces a new specific response against itself. In this paper a new picture is developed, based on mathematical models of immune responses against several epitopes; the simplest such models can have very complicated dynamics, with some surprising features. The emergence of an escape mutant can shift the immunodominant response to another epitope. Even in the absence of mutations, antigenic oscillation is found, with distinct peaks of different virus variants and fluctuations in the size and specificity of the immune responses. The model also provides a general theory for immunodominance in the presence of antigenic variation. Immunodominance is determined by the immunogenicity and by the antigenic diversity of the competing epitopes. Antigenic oscillations and fluctuations in the cytotoxic T-lymphocyte response have been observed in infections with the human immunodeficiency virus (HIV). Shifting the immune responses to weaker epitopes can represent a mechanism for disease progression based on evolutionary dynamics and antigenic diversity of the virus.

1. Introduction

An important part of the human immune response against viral infections are cytotoxic T lymphocytes (CTL). These recognize and kill cells which are infected by virus. The CTL receptor binds to viral epitopes in association with major histocompatibility complex (MHC) class I molecules. (An epitope is a part of a (viral) protein which is recognized by immune responses; CTL epitopes are roughly ten amino acids long; a viral protein can have several epitopes.) CTL responses have been demonstrated in many viral infections, and they are generally believed to be important factors for controlling viral infections (for a recent review, see McMichael, 1993). However, in the biological outline of this introduction we shall concentrate on observations from patients infected with the human immunodeficiency virus (HIV).

CTL responses arise early in HIV infections and suppress viral loads after the primary phase of the infection (Pantaleo *et al*., 1994). Most asymptomatic HIV-infected individuals have a high antiviral CTL activity, which disappears as AIDS develops. This could suggest that the CTL response is a relevant factor in controlling HIV infection. Phillips *et al*. (1991) performed a longitudinal study of CTL responses against epitopes in the HIV *gag* protein in six HIV infected patients. Three of these patients recognized *gag* through HLA B27 (HLA stands for human leucocyte antigen and is the human MHC). They recognized only a single epitope, which remained conserved throughout the study. The three other patients recognized three different epitopes in *gag* restricted by HLA B8. The HIV quasispecies of these patients showed genetic variation in these epitopes, and some virus mutants were not recognized by the patients' CTL response. Furthermore, Phillips *et al*. (1991) observed unexpected fluctuations in the specificity of the CTL responses. At different time points responses against different epitopes were predominant. In July 1989, patient 020 recognized

peptide sequence p24-13 (residues 255–269) in the *gag* p24 protein. In December 1989 the same patient had CTL activity against peptide p24-20 (residues 325–339), but no longer against p24-13. In February 1990, there was hardly any response against p24-13 or p24-20—instead the CTL response recognised the peptide sequence p17-3 (residues 21–35) from p17 *gag*. During 1990 there were fluctuating responses against these three epitopes. Throughout 1989 and 1990 there was extensive genetic variation in the epitopes p17-3 and p24-13, although p24-20 was rather conserved. Interestingly, the three patients with oscillating responses seemed to develop AIDS faster than the three patients with constant responses.

Phillips *et al*. (1991) did not propose a mechanism for these oscillations, but conjectured that "in these longitudinal studies, dominant HLA B8-restricted responses apparently shifted from one epitope to another raising the possibility that antigenic variation in the virus may underlie these unexpected changes."

Here we propose a mechanism. We shall first show how antigenic diversity can indeed drive oscillations in immune responses against several different epitopes. These oscillations arise as an intrinsic feature of the population dynamics of different virus variants and immune responses against different epitopes. For these oscillations it is not necessary that new virus variants arise over time; the oscillations occur for a fixed number of mutants that may be present right from the beginning. There is an important distinction between this phenomenon and the previously-discussed "antigenic drift", where it is assumed that peaks in viral concentrations are caused by the emergence of new variants. Antigenic drift has been described for several systems, for example, Equine infectious anemia virus (Salinovich *et al*., 1986; Carpenter *et al*., 1990), or HIV (Nara *et al*., 1990, Wahlberg *et al*., 1991, Holmes *et al*., 1992). (For a recent review, including evidence for spontaneously arising antigenic variants, see Domingo *et al*., 1993. For mathematical models of antigenic drift, see Nowak *et al*., 1990, 1991; Nowak & May, 1992, 1993; Anderson, 1994; Sasaki, 1994; R. Antia *et al*., unpublished data.) We propose the term "antigenic oscillation" for the new and different biological phenomenon studied in the present paper. We shall develop a rigorous mathematical framework for it, which enables us to calculate the damping times and other dynamical properties of these oscillations for various assumptions about the underlying dynamics of the immune response.

There are several important biological consequences of these new ideas about antigenic oscillations (Nowak *et al*., 1995). First, the peaks in viral abundance need not reflect new antigenic material. Mutants that have been around for some time, and that were clearly recognized by earlier immune responses, may cause such peaks, because at some specific time the intrinsic dynamics of the system may have caused the response against them to fluctuate to very low levels. This causes the oscillations. Second, predominant viral variants need not be extinguished by a strong immune response against them, but may persist and come back at a later time. Third, persistence of some predominant variants is not an argument against the importance of the selection pressure exerted by the immune response. Clearly, virus variants do not have to escape in all epitopes simultaneously to cause antigenic oscillations.

After discussing these basic aspects, we proceed to combine "antigenic oscillations" with the emergence of new mutants. Given the fast mutation rate of HIV, it seems likely that new mutants will emerge within the damping time of antigenic oscillations. This can keep the system oscillating. We will also show how new mutants can account for a long-term (effectively permanent) loss of the response against a particular epitope. As an intuitive example, imagine the following situation: a virus population is down-regulated by CTL responses against two different epitopes, which we denote A and B (we consider two epitopes just for the sake of the argument; in a real situations there may be more). Suppose epitope A is immunodominant; i.e. most of the patients' CTLs are directed against this epitope. B is only weakly recognized. Now assume that mutation generates a new variant in epitope A that can escape recognition (from the response against epitope A). Our mathematical models will show that, following the emergence of such a mutant, there are four dynamical possibilities, which depend on the relative immunogenicities and replication rates of the new and old mutant:

(i) the new mutant may induce a new specific CTL response against itself, but not affect the (weak) response against epitope B (this is simply a diversification in epitope A);

(ii) the new mutant may not induce a new response in epitope A, but lead to an increase of the response against epitope B (this means that the old response against epitope A, with specificity for the original variant, coexists with a response against epitope B; this represents a partial shift in immunodominance);

(iii) the new mutant may induce a new response in epitope A which outcompetes the original response in A (this occurs simultaneously with the stimulation of a response in epitope B; hence again a partial shift of immunodominance to epitope B);

(iv) finally, the new mutant may induce a complete shift in immunodominance to epitope B (the response against A vanishes).

We shall develop a variety of mathematical models for the dynamics of the immune responses, but all such models will have the generic feature that there is some intrinsic competition between the responses directed against A versus B. This is similar to simple ecological situations in which two different predators (the CTL responses) feed on a single prey species (the virus). The result is, in effect, competition between the two CTL responses/predators.

The mathematical model suggests that the competition between the responses against A and B is governed by two different factors: the immunogenicity of the epitope and the antigenic variablility. The more immunogenicity the better; the less antigenic variation the better. Thus increasing antigenic variation can shift the response from A to B. But then the immunological pressure against A is removed, and we may observe neutral genetic drift among the various A sequences. In this sense, antigenic variation in A may not result in the takeover of the virus population by an escape mutant in A, but may shift the immunodominance to epitope B. In the beginning, we assumed that B is generally less immunogenic than A, so that the virus population may now be less well controlled. The response against A may or may not come back, depending on the (random) events of the antigenic drift among the different variants in A. This example shows how antigenic variation can lead to "shifting immunodominance", and hence to disease progression.

In Section 2 we develop the simplest mathematical model, in which all virus mutants have the same overall replication rates and all variants of a given epitope have the same immunogenicity (different epitopes can, however, have different immunogenicities). CTL responses proliferate proportional to the product of their own abundance and (specific) viral abundance.

In Section 3 we allow different replication rates and immunogenicities for the different virus mutants. The equations of Sections 2 and 3 are specific Lotka–Volterra systems (see May, 1974, or Hofbauer & Sigmund, 1988, for comprehensive treatments).

In Section 4 we consider more complicated immune response dynamics, where CTLs proliferate as before, but may also be activated from a (constant) pool of precursor cells. This assumption seems biologically necessary. It will introduce some complications and new aspects.

Throughout the paper we analyse models with two epitopes. There may be n_1 variants in epitope A and n_2 variants in epitope B. This leads to a total of $n_1 \times n_2$ potential virus mutants. Together with n_1 specific CTL clones against epitope A and n_2 specific CTL clones against epitope B, we have a system with $n_1 \times n_2 + n_1 + n_2$ dimensions. We do not consider models with more than two epitopes, but the extension to such models seems straightforward.

In general we cannot give a complete analysis of the dynamical possibilities for situations where different virus mutants have different replication rates. Therefore we give complete classifications of some low-dimensional cases. In Section 5 we discuss the 2×1 case (i.e. $n_1 = 2$, $n_2 = 1$), which provides a complete description of the dynamical events after the emergence of an escape mutant in a homogeneous virus population. We show how antigenic variation can shift immunodominance.

In Section 6 we examine the effect of cross-reactive immune responses within an epitope. In Section 7 we include intra-cellular competition for MHC presentation. In Section 8 we discuss some consequences for immunotherapy. Section 9 lists open questions and future direction. Section 10 gives a summary of the biological implications of this paper.

The five Appendices are mostly for the mathematically interested reader and may be skipped by a reader needing only the general character of our models, rather than the details.

2. The Simplest Model

First we will develop a simple model that describes the dynamics of immune responses against two different epitopes:

$$\begin{aligned} \dot{v}_{ij} &= v_{ij}(r - px_i - qy_j) \\ \dot{x}_i &= x_i(cv_{i*} - b) \quad \text{with} \quad i = 1, \ldots, n_1 \\ \dot{y}_j &= y_j(kv_{*j} - b) \quad \text{with} \quad j = 1, \ldots, n_2. \end{aligned} \tag{1}$$

Here v_{ij} denotes the abundance of virus variants with sequence i in epitope A and sequence j in epitope B. There are n_1 different sequences for epitope A and n_2 for epitope B. Thus in total we consider $n_1 \times n_2$ different virus variants. The variables x_i and y_j denote CTLs directed at sequence i of epitope A and sequence j of epitope B, respectively. There are n_1 CTL clones directed at the various A variants and n_2 against the B variants. In this simplest model, all the virus variants reproduce at rate r. They are killed by CTL responses at the rates $-pv_{ij}x_i$ and $-qv_{ij}y_j$. CTLs are stimulated by their specific epitope sequence (in association with

HLA presentation) and replicate at the rates cx_iv_{i*} and ky_jv_{*j}, with the notation

$$v_{i*}=\sum_{j=1}^{n_2} v_{ij} \quad \text{and} \quad v_{*j}=\sum_{i=1}^{n_1} v_{ij}. \tag{2}$$

Thus a particular CTL clone recognizes all viruses that have the specific sequence in the right epitope, i.e. x_i is directed at (and is stimulated by) $v_{i1}+v_{i2}+\ldots+v_{in_2}=v_{i*}$, whereas y_j recognizes $v_{1j}+v_{2j}+\ldots+v_{n1j}=v_{*j}$. The constants c and k describe the immunogenicities of the two epitopes. If $c>k$ then epitope A is more immunogenic and will provide a stronger stimulus for replication of the relevant CTL clones. Finally we assume that in the absence of antigenic stimuli the activated CTLs decline at the rates $-bx_i$ and $-by_j$. In total the model has seven parameters (r, p, b, c, k, n_1 and n_2) and $n_1n_2+n_1+n_2$ variables (dimensions).

The above model assumes a very simple dynamics for the immune responses. We will first analyse this simple model and then study how alterations in the dynamics of the immune response will affect the outcome.

We start by looking for equilibrium solutions. Setting $\dot{x}_i=0$ we obtain the non-trivial solution $v_{i*}=b/c$ (alternatively, $x_i=0$). For the total virus abundance this yields the equilibrium value $v=bn_1/c$. From $\dot{y}_i=0$ we similarly get $v_{*j}=b/k$ and hence $v=bn_2/k$. Both relations cannot be fullfilled (as long as $n_1/c\neq n_2/k$, which is the generic assumption). Hence there is no interior equilibrium of system (1). The competition between the responses against the two different epitopes is decided by the relative magnitudes of the ratios c/n_1 and k/n_2. If, for example, $c/n_1>k/n_2$ then all y_j converge to zero and the system (1) reduces to

$$\begin{aligned}\dot{v}_{i*}&=v_{i*}(r-px_i)\\ \dot{x}_i&=x_i(cv_{i*}-b)\,.\end{aligned} \tag{3}$$

This is a simple Lotka–Volterra system with neutral oscillations around $v_{i*}=b/c$ and $x_i=r/p$. The eigenvalues of the Jacobian matrix are given by $\pm i\sqrt{rb}$, and hence the period of the oscillations is roughtly $T\approx 2\pi/\sqrt{rb}$ (lengthening in the usual way for oscillations of larger amplitudes). The total amount of virus and of immune cells fluctuate indefinitely around their long-term averages $v=bn_1/c$ and $x=n_1r/p$. Note that both quantities increase linearly with the number of variants n_1. Such neutral oscillations are structurally unstable (see May, 1974), and therefore we consider structural modifications of the above model in the subsequent sections.

The model also has an interesting kind of degeneracy: the long-term averages of v_{i*} are exactly specified, but the individual v_{ij} remain undefined (within the limits set by v_{i*}). For our entirely deterministic model this means the variants in epitope B are fixed in some arbitrary population structure (satisfying $v_{i*}=b/c$), once the immune responses against B have vanished. In a real situation genetic drift will follow.

Figure 1 illustrates the dynamics of eqn (1) for $n_1=n_2=2$. Thus there are two variant sequences in each epitope, which induce $2x$- and $2y$-responses. All mutants are present in the beginning ($t=0$); there is no production of new mutants over the time of the simulation. Nevertheless, the system displays antigenic oscillations. The y-responses will slowly converge to zero, leaving the x_i-v_{i*}-system in neutral oscillations. Antigenic oscillations can occur without the emergence of new mutants.

3. Different Parameters of Different Mutants

In the above model we assumed that all virus mutants have the same replication rates, are killed by CTL responses at equal rates, and induce CTL responses at equal rates. We shall now generalize these assumptions.

Let us first consider the system, where the virus mutants differ in their immunological parameters, but still have the same replication rates

$$\begin{aligned}\dot{v}_{ij}&=v_{ij}(r-p_ix_i-q_jy_j)\\ \dot{x}_i&=x_i(c_iv_{i*}-b) \quad \text{with} \quad i=1,\ldots,n_1\\ \dot{y}_j&=y_j(k_jv_{*j}-b) \quad \text{with} \quad j=1,\ldots,n_2.\end{aligned} \tag{4}$$

The immunogenicity of sequence i in epitope A is now given by c_i, and similarily k_j for j in epitope B. We still maintain that the natural death/decay rate of CTLs, b, is the same for all different specificities. Biologically this seems plausible, but we stress that it is not an essential assumption for the mathematical analysis.

A simple rescaling, $x_i'=p_ix_i$ and $y_j'=q_jy_j$, shows that we can neglect the parameters p_i and q_j. Thus without loss of generality we can write (after dropping the primes)

$$\begin{aligned}\dot{v}_{ij}&=v_{ij}(r-x_i-y_j)\\ \dot{x}_i&=x_i(c_iv_{i*}-b) \quad \text{with} \quad i=1,\ldots,n_1\\ \dot{y}_j&=y_j(k_jv_{*j}-b) \quad \text{with} \quad j=1,\ldots,n_2.\end{aligned} \tag{5}$$

Generically, there is again no interior equilibrium, but competitive exclusion between the x- and y-response. In fact there are only two possible (non-trivial) equilibria: either all x_i converge to zero or all y_j converge to zero. The x-responses will eventually win if

$$\sum_{i=1}^{n_1} \frac{1}{c_i} < \sum_{j=1}^{n_2} \frac{1}{k_j} . \tag{6}$$

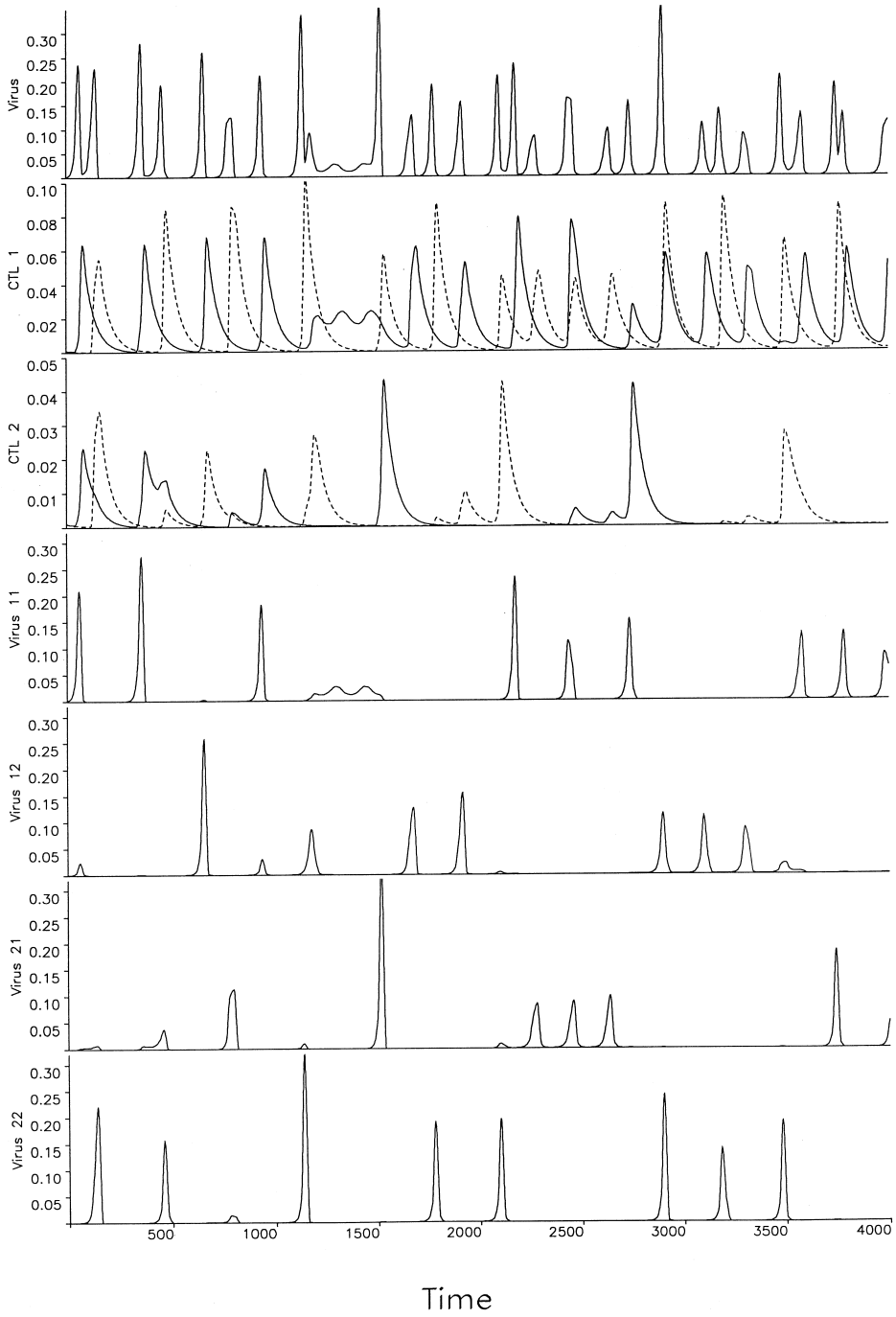


FIG. 1. Computer simulation of the basic model given by eqn (1). There are two different epitopes with two sequence variants in each epitope (thus altogether four different virus species). All virus mutants replicate at the same rate and are present at the beginning ($t=0$) in different abundances. There is no subsequent production of new antigenic material. Nevertheless, we observe sequential peaks in viral abundance that correspond to antigenically different variants. Thus antigenic oscillations can occur without antigenic drift. As discussed in the text, we have a clear understanding of the long term behaviour of the system: the y_i (CTL 2) will converge to zero, and there are undamped neutral oscillations with the x_i and v_{i*}. The parameters are: $n_1=n_2=2$, $r=0.1$, $p=5$, $c=1.1$, $k=1$, $b=0.02$. The time axes is in arbitrary units (but the biological observations suggest these oscillations to occur on a time scale of weeks or months).

This can be seen by considering the products

$$P=\prod_{i=1}^{n_1} x_i^{1/c_i} \quad \text{and} \quad Q=\prod_{j=1}^{n_2} y_j^{1/k_j}. \tag{7}$$

We obtain

$$\mathrm{d}\left(\frac{P}{Q}\right)\Big/\mathrm{d}t=\frac{P}{Q}\left(\sum_{j=1}^{n_2}\frac{1}{k_j}-\sum_{i=1}^{n_1}\frac{1}{c_i}\right)b. \tag{8}$$

Thus if inequality (6) holds, the ratio P/Q will be an exponentially increasing function of time, suggesting that the x-response outperforms the y-response. P/Q is a Lyapunov function. There is asymptotic convergence to the boundary where at least one y_j is zero. For specificity, let us assume that y_1 converges to zero. What happens next?

We can show that it is not possible to have an equilibrium in the face $\{y_1=0\}$ with some other y_j and v_{*1} being positive. This can be seen by considering the ratios v_{ih}/v_{i1} for different i and h. (Since $v_{*1}>0$ we can always find at least one $v_{i1}>0$). With $y_1=0$ we get

$$\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{v_{ih}}{v_{i1}}\right)=-\frac{v_{ih}}{v_{i1}}y_h. \tag{9}$$

Thus for $y_h>0$ we have that v_{i1} always grows faster than v_{ih} except if $v_{ih}=0$. But at least some v_{ih} have to be strictly positive. If $v_{*h}=0$ then y_h converges to 0, and hence there is indeed no equilibrium with some y_j being positive.

If all y_j have vanished, we are left with the system

$$\begin{aligned}\dot{v}_{i*}&=v_{i*}(r-x_i)\\ \dot{x}_i&=x_i(c_iv_{i*}-b) \quad \text{with} \quad i=1,\ldots,n_1\end{aligned} \tag{10}$$

This represents, essentially, n_1 uncoupled oscillators. Note that the ratios v_{ij}/v_{ik} remain constant. Then x_i has neutral oscillations around the equilibrium (time average) r/p_i and v_{i*} oscillates around b/c_i. The period of the oscillation close to the equilibrium is approximately $T\approx 2\pi/\sqrt{rb}$.

The general system with different replication rates for different mutants is more difficult to understand. Let us assume that the virus mutant v_{ij} replicates with rate r_{ij}. We have

$$\begin{aligned}\dot{v}_{ij}&=v_{ij}(r_{ij}-x_i-y_j)\\ \dot{x}_i&=x_i(c_iv_{i*}-b) \quad \text{with} \quad i=1,\ldots,n_1\\ \dot{y}_j&=y_j(k_jv_{*j}-b) \quad \text{with} \quad j=1,\ldots,n_2.\end{aligned} \tag{11}$$

Equation (8) remains true. Let us again assume that inequality (6) holds. Then the ratio P/Q is exponentially increasing over time, implying convergence to a boundary where at least one y_j is zero. Again, let us assume that $y_1=0$. Now it is indeed possible to have an equilibrium in the face $\{y_1=0\}$ with all other x_i and y_j being positive (see Section 6). From $\dot{x}_i=0$ for all $i=1,\ldots,n_1$ we get $v=b\ \Sigma_{i=1}^{n_1}1/c_i$. From $\dot{y}_j=0$ for all $j=2,\ldots,n_2$ we get $v=v_{*1}+b\ \Sigma_{j=2}^{n_2}1/k_j$. Thus an equilibrium in the interior of the face $\{y_1=0\}$ requires

$$v_{*1}=b\left(\sum_{i=1}^{n_2}1/c_i-\sum_{j=2}^{n_2}1/k_j\right)>0. \tag{12}$$

Hence, combining this inequality with the earlier (6), we have

$$\sum_{j=1}^{n_2}1/k_j>\sum_{i=1}^{n_1}1/c_i>\sum_{j=2}^{n_2}1/k_j \tag{13}$$

as a necessary condition for the existence of such an equilibrium. We do not know any sufficient condition, but for the ratios v_{ih}/v_{i1} we get

$$\frac{d}{dt}\left(\frac{v_{ih}}{v_{i1}}\right)=\frac{v_{ih}}{v_{i1}}\left(r_{ih}-r_{i1}-y_h\right). \tag{14}$$

Thus an equilibrium is only possibly if $r_{ih}>r_{i1}$ for those indices i and h where $v_{ih}>0$.

We lack a complete understanding of system (11), but conjecture the following dynamics. Suppose inequality (6) holds. Then one y_j will converge to zero (say y_1). If $\Sigma_{i=1}^{n_1}1/c_i<\Sigma_{j=2}^{n_2}1/k_j$, then another y_j will converge to zero. Several y_j will become extinct until the inequality reverses, e.g. $\Sigma_{i=1}^{n_1}1/c_i>\Sigma_{j=l}^{n_2}1/k_j$. (Thus we have assumed that all y_j with $j=1,\ldots,l-1$ have converged to zero.) Now a coexistence between the remaining y_j and the x_i is possible, but depends on the r_{ij} (with $j=1,\ldots,l-1$) being small compared to the replication rates of other mutants that are present at this equilibrium—as specified by eqn (14). (The right-hand side of eqn (14) can only be zero if $r_{ih}>r_{i1}$.)

Other complications are possible, too. Suppose, by chance, one of the x_i dies out first. Then the y-responses may become immunodominant. Thus clonal exhaustion can indeed lead to switching immunodominance. And which epitope is immunodominant in the long run is partly determined by the random events of extinction of CTL clones at very low frequencies.

Appendix A contains a general result. Any fixed point of (11) is neutrally stable within its face, and a generalization of Volterra's function represents an invariant of motion.

Another observation is that *either* some x_i or some y_j become extinct, but never both. Consider a situation

where a specific x_i and a specific y_j have become extinct. Then $\dot{v}_{ij} = r_{ij} v_{ij} > 0$, and v_{ij} is able to invade this fixed point. Hence such a fixed point cannot be saturated (i.e. stable against invasion by those variables which are close to zero).

3.1. WHAT DETERMINES IMMUNODOMINANCE?

Even for system (11), where the virus mutants have different replication rates, it can happen that all responses against one epitope will vanish, and the whole immune response is entirely directed against the other epitope. We call this state "complete immunodominance". What are the conditions for complete immunodominance?

We have seen that the inequality (6),

$$\sum_{i=1}^{n_1} \frac{1}{c_i} < \sum_{j=1}^{n_2} \frac{1}{k_j},$$

implies that at least one y_j will tend towards zero. Furthermore, we also know that for a saturated equilibrium *either* some x_i or y_j responses can vanish, but never both. Thus inequality (6) is a necessary condition for complete immunodominance of the x-response.

If all r_{ij} are the same then inequality (6) is sufficient for complete immunodominance. The epitope which minimizes the sum of the reciprocals of the immunogenicities of all variants is immunodominant. The responses against all other epitopes will vanish.

If the r_{ij} are different then complete immunodominance would imply that we are left with the subsystem

$$\begin{aligned} \dot{v}_{ij} &= v_{ij}(r_{ij} - x_i) \\ \dot{x}_i &= x_i(c_i v_{i*} - b). \end{aligned} \tag{15}$$

For each i, only one v_{ij} will persist. This will be the v_{ij} with the largest r_{ij} in this row of the r_{ij}-matrix. More precisely, let us define the index m_i as $r_{im_i} = \max_j \{r_{ij}\}$. The equilibrium of (15) is given by

$$x_i = r_{im_i} \quad \text{and} \quad v_{i*} = v_{im_i} = b/c_i. \tag{16}$$

This equilibrium is saturated with respect to the y_j-responses if $\partial \dot{y}_j / \partial y_j = k_j v_{*j} - b < 0$. We have at equilibrium

$$v_{*j} = \sum_{i=1}^{n_1} \delta(m_i, j) v_{im_i} = b \sum_{i=1}^{n_1} \delta(m_i, j)/c_i, \tag{17}$$

where δ is the Kronecker symbol, i.e. $\delta(m_i, j) = 1$ if $m_i = j$ and $\delta(m_i, j) = 0$ otherwise. Thus saturation requires

$$\sum_{i=1}^{n_1} \delta(m_i, j) \frac{1}{c_i} < \frac{1}{k_j}, \quad \forall j. \tag{18}$$

This is a necessary and sufficient condition for complete immunodominance of the x-responses.

4. Activated CTLs Arise from Inactivated Precursors

In this Section we include the biologically essential assumption that the CTLs are not only produced by replication of already activated cells, but are also generated by activation of specific precursor cells at rates proportional to the specific antigen abundance. This leads to

$$\begin{aligned} \dot{v}_{ij} &= v_{ij}(r_{ij} - p_i x_i - q_j y_j) \\ \dot{x}_i &= \eta c_i v_{i*} + x_i(c_i v_{i*} - b) \\ &\quad \text{with} \quad i = 1, \ldots, n_1 \\ \dot{y}_j &= \eta k_j v_{*j} + y_j(k_j v_{*j} - b) \\ &\quad \text{with} \quad j = 1, \ldots, n_2. \end{aligned} \tag{19}$$

A sensible assumption is that the activation signals for precursor cells and already activated cells depend on the immunogenicity of the epitope sequence and are proportional to each other, with η being the proportionality constant. Again this is just one way of writing things more neatly; the assumption of a common η-value is not essential for the following analysis.

Since η is positive it is clear that the x- and y-responses can coexist. An interesting problem immediately arises: What are the possible equilibria of system (19)? Can some v_{ij} converge to zero? Are there interior equilibria?

Let us first perform the helpful transformation $x_i' = p_i x_i$ and $y_j' = q_j y_j$. This produces (where we have at once dropped the primes):

$$\begin{aligned} \dot{v}_{ij} &= v_{ij}(r_{ij} - x_i - y_j) \\ \dot{x}_i &= \eta c_i p_i v_{i*} + x_i(c_i v_{i*} - b) \\ &\quad \text{with} \quad i = 1, \ldots, n_1 \\ \dot{y}_j &= \eta k_j q_j v_{*j} + y_j(k_j v_{*j} - b) \\ &\quad \text{with} \quad j = 1, \ldots, n_2. \end{aligned} \tag{20}$$

4.1. THE NEUTRAL CASE: ALL MUTANTS HAVE THE SAME REPLICATION RATES

An important special case arises when all virus mutants replicate at the same rate, i.e. $r_{ij} = r$. Without

loss of generality we then set $r=1$. Different epitope sequences may have different immunogenicities and different rates at which they are recognized by the CTL response, but all mutations in these epitopes are essentially neutral with respect to the replication rate.

For the interior equilibria of this neutral system (with $r_{ij}=1$) we obtain the relations

$$1=x_i+y_j, \quad \forall i, j. \tag{21}$$

This immediately implies that all x_i (and all y_j) have to be the same, i.e.

$$x_i=\xi \quad \text{and} \quad y_j=1-\xi, \quad \forall i, j. \tag{22}$$

For the virus population, we obtain, at equilibrium,

$$v_{i*}=\frac{b\xi}{c_i(\eta p_i+\xi)}, \quad v_{*j}=\frac{b(1-\xi)}{k_j(\eta q_j+1-\xi)}. \tag{23}$$

This specifies the set of all interior equilibria. The constant ξ is obtained from $\Sigma_i v_{i*}=\Sigma_j v_{*j}$, which can be a high-order polynomial. If all p_i are the same and all q_i are the same, then it reduces to a quadratic equation in ξ. Equation (23) specifies the equilibrium values for v_{i*} and v_{*j}. The individual v_{ij} can take arbitrary values within this envelope. Essentially there are n_1+n_2 constraining relations for $n_1 \times n_2$ variables. Appendix B shows local stability of this set of equilibria (at least for some special cases).

There are also boundary equilibria (with some $v_{ij}=0$), but we will show that none of these can be saturated (i.e. stable to invasion by the v_{ij} in question). First note that in each row (or column) at least one v_{ij} has to be positive at a saturated equilibrium point. Otherwise the corresponding x_i or y_j would vanish and at least one v_{ij} of this row (or column) can invade. In general, this invasion is specified by the transversal eigenvalue $\lambda_{ij}=\partial \dot{v}_{ij}/\partial v_{ij}=1-x_i-y_j$. Consider an equilibrium where the x_i and y_j take some arbitrary values. But note that for each x_i there is at least one y_j such that $x_i+y_j=1$. This follows from the fact that in each row (or column) at least one v_{ij} has to be positive. Similarily for each y_j there is at least one x_i such that $y_j+x_i=1$. Denote the smallest of all x_i, by x_1 and denote the smallest of all y_j by y_1. Clearly $x_1+y_1 \leqslant 1$. We have to distinguish two cases:

(i) If $x_1+y_1=1$ then it follows that all x_i are equal to some constant ξ and y_j are equal to $1-\xi$. But in this case for any $v_{ij}=0$ the eigenvalue λ_{ij} has to be zero, hence no saturation (which requires $\lambda_{ij}<0$).

(ii) If $x_1+y_1<1$ then $v_{11}=0$ otherwise we are not at an equilibrium. But $x_1+y_1<1$ implies $\lambda_{11}>0$ and hence no saturation.

Thus there is no saturated equilibrium at the boundary. Putting this fact together with the evidence of local stability (Appendix B) and the numerical simulations, we conjecture that all trajectories converge to the manifold of interior equilibria specified by eqns (22) and (23).

Figure 2 gives a numerical example of such a slow convergence towards an interior equilibrium. We chose the same system as for Fig. 1, but included the immigration term of eqn (19) with $\eta=0.001$. Again there are antigenic oscillations over a long period, without the emergence of new mutants.

4.2. THE MUTANTS HAVE DIFFERENT REPLICATION RATES

We do not have a complete understanding for the case where the r_{ij} can take arbitrary (positive) values. The numerical simulations suggest that all trajectories converge to fixed points, which are generically on the boundary. But we cannot rule out the existence of cyclic solutions or chaotic attractors.

There is an interesting *exclusion principle*. For simplicity, consider a case with two epitopes and two sequence variants in each epitope. The conditions for an interior equilibrium are

$$\begin{aligned} r_{11}&=x_1+y_1 \quad r_{12}=x_1+y_2 \\ r_{21}&=x_2+y_1 \quad r_{22}=x_2+y_2. \end{aligned} \tag{24}$$

This can be fulfilled if and only if $r_{11}-r_{12}=r_{21}-r_{22}$, as is the case if mutations in one epitope have no effect on replication rates or if the contributions of the mutations in different epitopes are additive. But this relation will not hold for an arbitrary choice of parameters r_{ij} (except for a set with measure zero). Hence, in general, it is not possible to have an interior equilibrium, and at least one of the v_{ij} has to be zero. This means that for a virus population expressing simultaneously two different variants at two different epitopes it is not possible to obtain a stable *selection* equilibrium with all four virus variants present. The generalization of this result to m epitopes and n sequences in each epitope is obvious: for a generic choice of the r_{ij}, all but $(m+n-1)$ of the v_{ij} must vanish. Of course, for a fast mutating virus like HIV, such variants can be maintained at finite values in a mutation equilibrium (facilitated by the additional effect of recombination).

Figure 3 shows a computer simulation of eqn (19) for $n_1=n_2=3$. The replication rates and immunogenicities of the individual virus variants are randomly assigned. We observe damped antigenic oscillations to a boundary equilibrium with only three virus species surviving. These are v_{12}, v_{21}, and v_{33}. Note that there is no cross-reactivity between these three mutants. It seems that the virus population often adopts such a

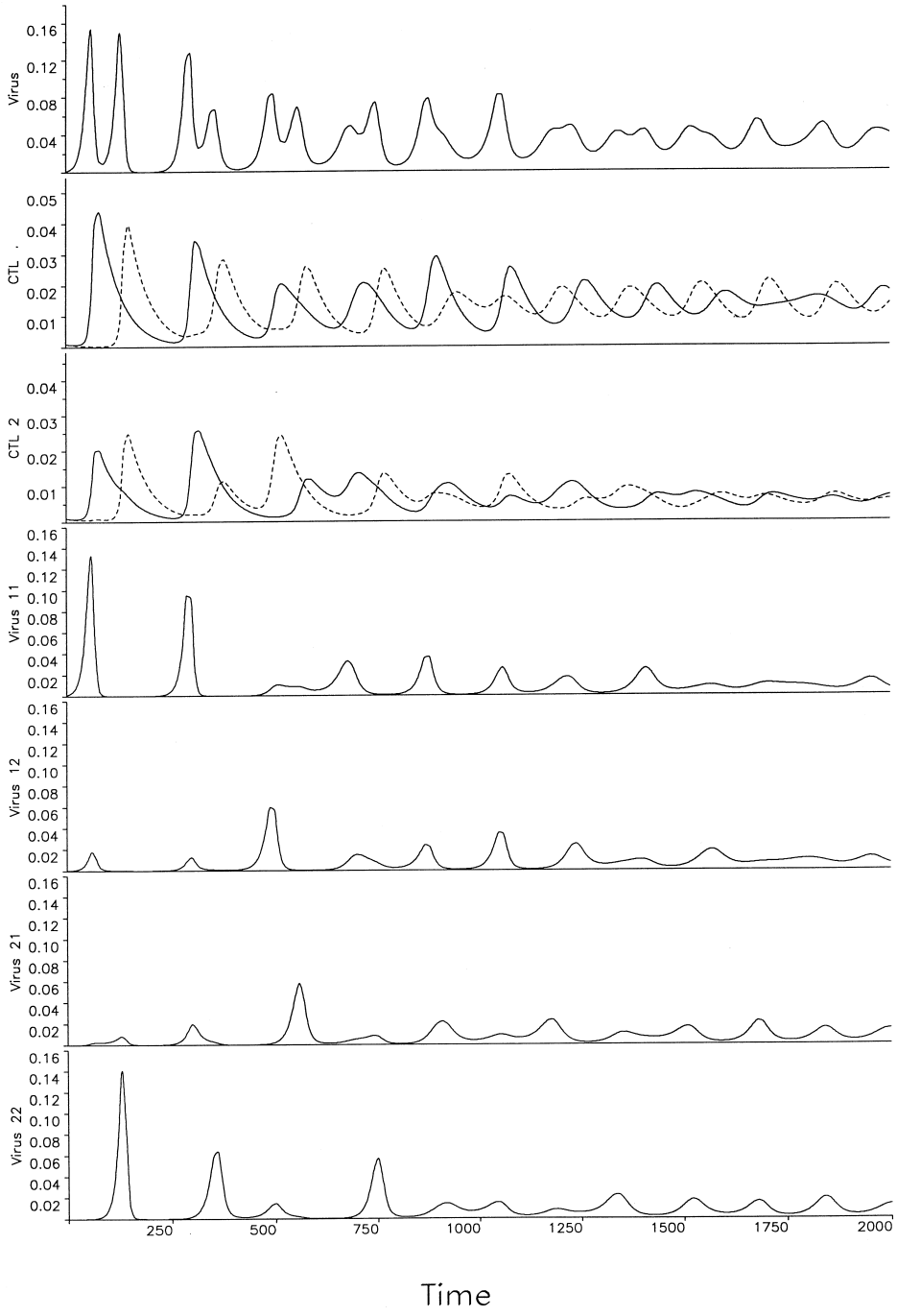


FIG. 2. The model with immigration from a pool of precursor cells as given by eqn (19). All virus mutants have the same replication rates, $r_{ij}=r$. As shown in Section 4.1 there is convergence to a degenerate set of interior equilibria, given by eqns (22) and (23). The oscillations are damped on a very slow time scale. The parameters are the same as in Fig. 1: $n_1=n_2=2$, $r=0.1$, $p=5$, $c=1.1$, $k=1$, $b=0.02$; except $\eta=0.001$.

structure. In this example all x_i- and y_j- responses coexist.

4.3. THE LIMIT OF LARGE η

In the limit of large η we obtain the system

$$\begin{aligned}
\dot{v}_{ij} &= v_{ij}(r_{ij}-x_i-y_j)\\
\dot{x}_i &= \eta c_i p_i v_{i*} - b x_i \quad \text{with} \quad i=1,\ldots,n_1\\
\dot{y}_j &= \eta k_j q_j v_{*j} - b y_j \quad \text{with} \quad j=1,\ldots,n_2.
\end{aligned} \tag{25}$$

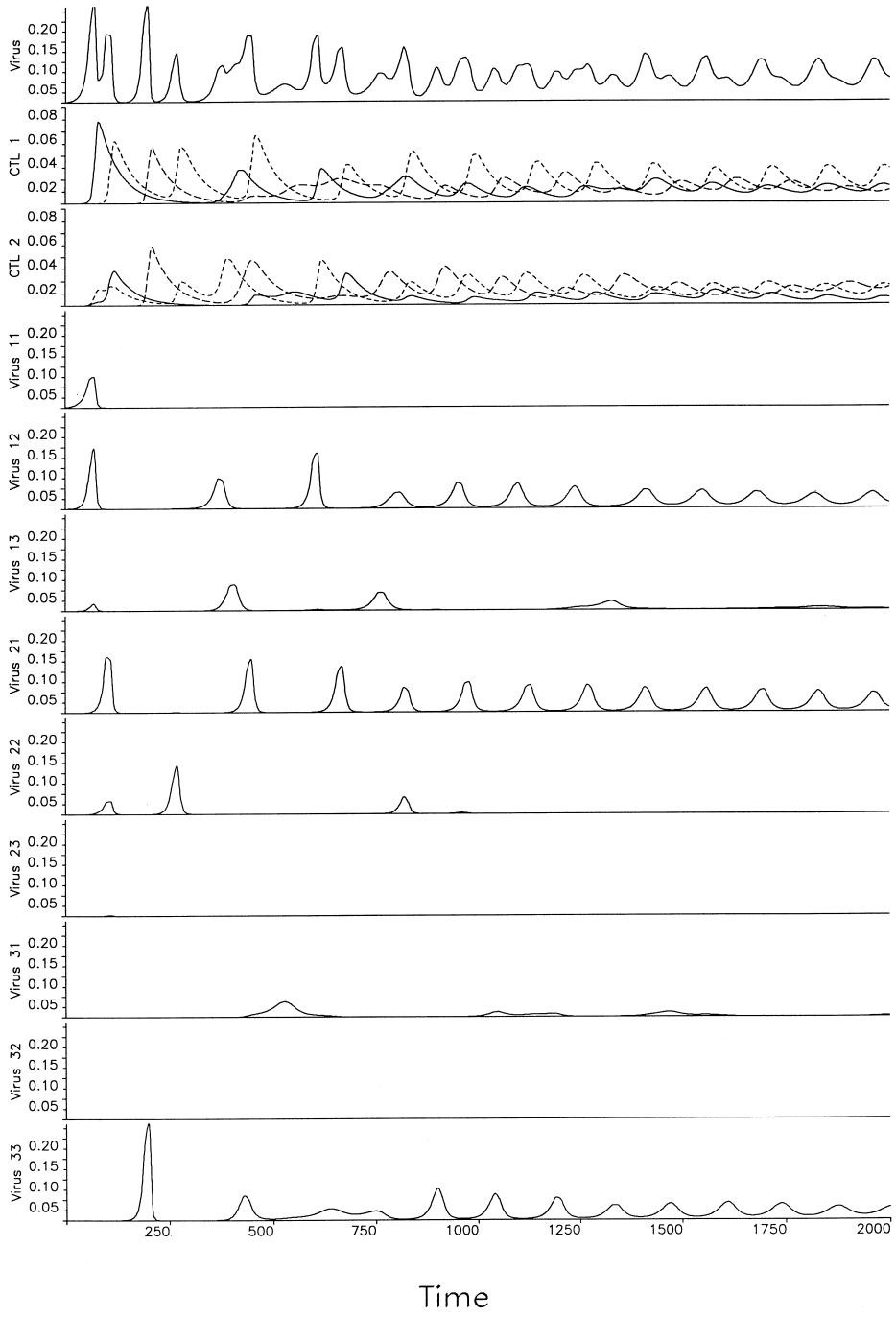


FIG. 3. An example of the dynamics of a higher-dimensional system with randomly assigned parameters. The figure shows a computer simulation of eqn (19) with $n_1 = n_2 = 3$. The randomly assigned parameters are: $r_{11} = 0.0739$, $r_{12} = 0.1175$, $r_{13} = 0.1170$, $r_{21} = 0.1261$, $r_{22} = 0.1478$, $r_{23} = 0.1217$, $r_{31} = 0.0925$, $r_{32} = 0.0738 = 0.1256$, $p_1 = 5.1361$, $p_2 = 5.2294$, $p_3 = 5.3526$, $q_1 = 4.6811$, $q_2 = 4.9450$, $q_3 = 4.6972$, $c_1 = 0.8719$, $c_2 = 1.0150$, $c_3 = 0.8753$, $k_1 = 0.7797$, $k_2 = 1.0080$, $k_3 = 0.8708$. In addition we chose $b = 0.02$ and $\eta = 0.001$. There are slowly damped oscillations to a boundary fixed point with all virus mutants zero, except v_{12}, v_{21}, v_{33}. Note that these three virus mutants do not share a single epitope. We often observe that the virus population converges to such a state with minimal (zero) cross-reactivity.

TABLE 1
Saturated fixed points for the 2×1 *system with* $\eta=0$ *as specified by eqn* (28)

Fixed point	(v_1, v_2)	(x_1, x_2)	y	Conditions of existence and stability
P_1	$(+, +)$	$(+, +)$	0	$1/c_1+1/c_2<1/k$
P_2	$(+, +)$	$(0, +)$	+	$1/c_2<1/k<1/c_1+1/c_2 \quad r_1<r_2$
P_3	$(0, +)$	$(0, 0)$	+	$1/k<1/c_2 \quad r_1<r_2$
P_4	$(+, +)$	$(+, 0)$	+	$1/c_1<1/k<1/c_1+1/c_2 \quad r_1>r_2$
P_5	$(+, 0)$	$(0, 0)$	+	$1/k<1/c_1 \quad r_1>r_2$

The table shows the conditions of existence and stability of the five different stable equilibria, which occur for five distinct parameter regions. The sign of the coordinates of the fixed points are shown; the actual values can easily be calculated from eqn (28). The equilibria P_1, P_3, and P_5 specify complete immunodominance, whereas P_2 and P_4 imply stable coexistence between one of the x-responses and the y-response.

This is derived from eqn (20), neglecting x_i compared to ηp_i in $\dot{x}_i=c_i v_{i*}(\eta p_i+x_i)-bx_i$. In the same way we derive the equation for the y_j.

If all virus mutants have the same replication rates, i.e. $r_{ij}=r$, then system (25) converges to a set of interior equilibria, which is given by

$$x_{i*}=\xi; \quad y_j=r-\xi; \quad v_{i*}=\frac{b}{\eta c_i p_i}\,\xi;$$

$$v_{*j}=\frac{b}{\eta k_j q_j}\,(r-\xi). \tag{26}$$

From $\Sigma_{i=1}^{n_1} v_{i*}=\Sigma_{j=1}^{n_2} v_{*j}$ we obtain

$$\xi=r\sum_{j=1}^{n_2}\frac{1}{k_j q_j}\Bigg/\left(\sum_{i=1}^{n_1}\frac{1}{c_i p_i}+\sum_{j=1}^{n_2}\frac{1}{k_j q_j}\right). \tag{27}$$

There are no saturated equilibria on the boundary. The proof for this is equivalent to the one in Section 4.2.

For the system with arbitrary replication rates, r_{ij}, we cannot give a complete analysis. The same exclusion principle as in Section 4.2 applies. Thus in general there are no interior equilibria. In Appendix C we give a complete classification of all dynamical possibilities for the system $n_1=n_2=2$.

5. The 2×1 case

5.1. $\eta=0$

Since we cannot derive a general analysis for the case with different replication rates r_{ij}, we shall now describe some low-dimensional cases. Let us first consider a system with two mutants in epitope A and only a single variant in epitope B, i.e. $n_1=2$ and $n_2=1$. For $\eta=0$ we have, from eqn (11),

$$\begin{aligned}
\dot{v}_1&=v_1(r_1-x_1-y)\\
\dot{v}_2&=v_2(r_2-x_2-y)\\
\dot{x}_1&=x_1(c_1v_1-b)\\
\dot{x}_2&=x_2(c_2v_2-b)\\
\dot{y}&=y[k(v_1+v_2)-b].
\end{aligned} \tag{28}$$

We have avoided unneccessary indices by setting $v_1=v_{11}$, $v_2=v_{21}$, $r_1=r_{11}$, $r_2=r_{21}$, and $y=y_1$. For system (28) we can distinguish five parameter regions, which are mutually exclusive and cover the whole parameter space. For each parameter region there is exactly one saturated equilibrium. All trajectories from the interior of the phase-space converge to the face that contains the saturated equilibrium, and within this face there are neutral oscillations around the equilibrium. The five equilibria, P_1 to P_5, and their conditions of existence and stability are listed in Table 1.

We can now understand the dynamics following the emergence of a new mutant in a homogeneous virus population. Consider a virus population with only one type of virus; i.e. $v_2=0$. There is no coexistence between the two immune responses. Assume that $c_1>k$ (i.e. $1/c_1<1/k$). Thus $x_1=r_1$ and $y=0$. A mutation in epitope A, i.e. the emergence of mutant v_2, can lead to four different possibilities:

(i) It can simply lead to a diversification in epitope A without stimulating an immune response against epitope B. This happens if $1/c_1+1/c_2<1/k$. The system converges to the face containing equilibrium p_1 (see Table 1).

(ii) The new mutant, v_2, may not elicit an immune response against itself, but may induce a partial shift in immunodominance. This happens if $1/c_1+1/c_2>1/k$ and $r_1>r_2$. The system converges to the face containing equilibrium P_4.

(iii) The new mutant may induce a response against itself, which outcompetes the response against the original virus, and induces a partial shift in immunodominance. The conditions for this behaviour are $1/c_1+1/c_2>1/k>1/c_2$ and $r_1<r_2$. We end up in the face containing equilibrium P_2.

(iv) Finally, the new mutant can induce a complete shift in immuno dominance to epitope B. This happens for $1/c_2>1/k$ and $r_1<r_2$, which brings us to the face containing equilibrium P_3.

Note that equilibrium P_5 is excluded by our original assumption that $c_1>k$. In other words, a mutant in the immunodominant epitope can always invade.

In all four cases there will be undamped oscillations around the relevant equilibrium. Only the time averages will converge towards the equilibrium. In general these oscillations will be very complex. Figure 4 shows a computer simulation of eqn (28). The parameters are chosen such that there is convergence towards the face $x_1=0$. The system is specified by the quasi-periodic oscillations. It is interesting to note that such complex, and unpredictable dynamics can occur for a system with only two virus variants and two immune responses against different epitopes.

5.2. $\eta>0$

Next we consider the 2×1 system with positive v. For small η it is clear that there can be only one fixed point in the interior, because for $\eta>0$ only the saturated fixed points of the system with $\eta=0$ can migrate into the interior. Since the $\eta=0$ system

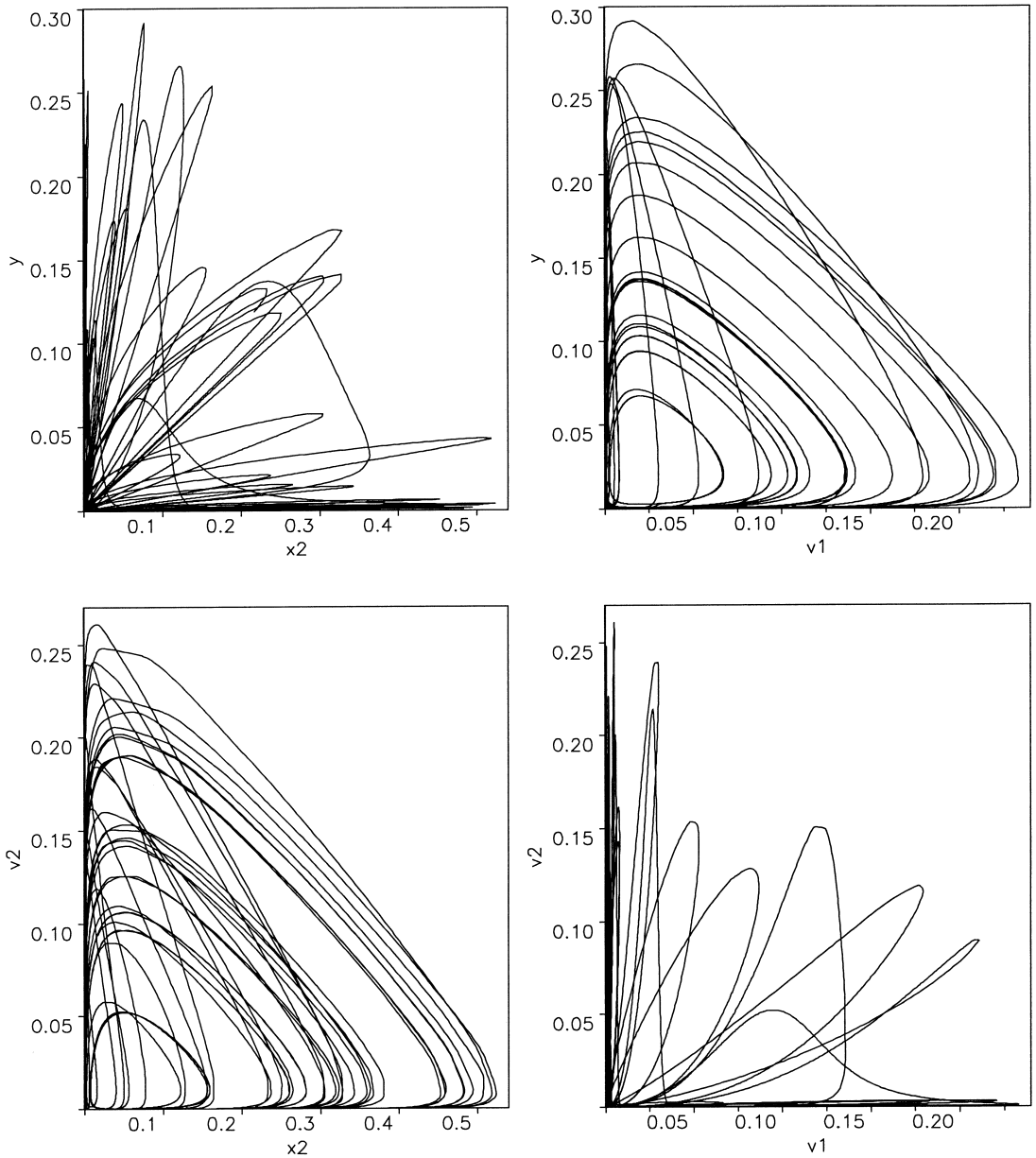


FIG. 4. The quasi-periodic behaviour of eqn (28), i.e. the 2×1 model wtih $\eta=0$. The parameters are: $r_1=0.02$, $r_2=0.05$, $c_2=2$, $k=1$ and $b=0.02$. The parameter c_1 is chosen such that $1/k<1/c_1+1/c_2$. This implies convergence to the face with $x_1=0$, which corresponds to case P_2 in Table 1. We are left with a subsystem containing the variables v_1, v_2, x_2 and y. This is the actual system we consider for the computer simulation. There is an invariant of motion (see Appendix A) which reduces the dimension to three. There we find quasi-periodic behaviour. The figure shows the time trajectories of y vs. x_2, y vs. v_1, v_2 vs. x_2, and v_2 vs. v_1.

has only one saturated fixed point for any one choice of parameters, we can conclude that there can be only one saturated (and hence at most one interior) fixed point for the system with positive (but small) η. We have

$$\begin{aligned}\dot{v}_1 &= v_1(r_1 - p_1x_1 - qy)\\ \dot{v}_2 &= v_2(r_1 - p_2x_2 - qy)\\ \dot{x}_1 &= x_1(c_1v_1 - b) + \eta c_1v_1\\ \dot{x}_2 &= x_2(c_2v_2 - b) + \eta c_2v_2\\ \dot{y} &= y[k(v_1+v_2) - b] + \eta k(v_1+v_2).\end{aligned} \tag{29}$$

If v_1 and v_2 are both strictly positive, there is no equilibrium without x_1, x_2 and y all being present. An interior equilibrium satisfies

$$\begin{aligned}x_i &= \frac{\eta c_i v_i}{b - c_i v_i}\\ y &= \frac{\eta k(v_1+v_2)}{b - k(v_1 - v_2)}\end{aligned} \tag{30}$$

and

$$r_i = p_i x_i + qy. \tag{31}$$

For this, we must of course have $v_i < b/c_i$ and $v_1 + v_2 < b/k$.

There are two equilibria on the boundary of the state space. Let us denote by P_1 the equilibrium with $v_2 = x_2 = 0$ and by P_2 the equilibrium with $v_1 = x_1 = 0$. For P_1, the equilibrium must satisfy

$$r_1 = p_1x_1 + qy, \quad x_1 = \frac{\eta c_1 v_1}{b - c_1 v_1}, \quad y = \frac{\eta k v_1}{b - k v_1}, \tag{32}$$

with $v_1 < b/c_1$ and $v_1 < b/k$. This implies

$$\begin{aligned}r_1(b - c_1v_1)(b - kv_1) &= \eta p_1 c_1 v_1 (b - kv_1)\\ &\quad + \eta q k v_1 (c_1 v_1),\end{aligned} \tag{33}$$

i.e. after setting $v_1 = bw$ and dividing by $b^2 k c_1$,

$$\begin{aligned}f(w) \equiv r_1\left(w - \frac{1}{k}\right)\left(w - \frac{1}{c_1}\right) + \eta w\left[p\left(w - \frac{1}{k}\right)\right.\\ \left. + q\left(w - \frac{1}{c_1}\right)\right] = 0.\end{aligned} \tag{34}$$

Since f is negative at the smaller of the two values $1/c_1$ and $1/k$, and positive at the larger of the two values as well as at the origin, we have exactly one root between 0 and $\min\{1/c_1, 1/k\}$. This yields the desired equilibrium P_1 on the boundary face $x_2 = v_2 = 0$. A straightforward application of the Routh–Hurwitz criterion shows that P_1 is stable *within* the corresponding boundary face. It is saturated iff v_2 cannot invade, i.e. iff

$$r_2 < q\hat{y}_1. \tag{35}$$

We note that it is impossible to have both P_1 and P_2 saturated since $q\hat{y}_1 < r_1$ and hence $r_2 < r_1$ whenever P_1 is saturated. Therefore we cannot have a bistable situation with both boundary fixed points being saturated.

The condition (35) for saturation of the boundary fixed point P_1 can explicitly be written as:

(i) $r_1 > r_2$ and
(ii) if $c_1 > k$ then

$$\begin{aligned}\eta k q r_1/r_2 > (r_1 - r_2)(c_1 - k) + \eta(kq + c_1p_1)\\ > r_2(c_1 - k),\end{aligned} \tag{36}$$

if $c_1 < k$ then

$$(r_1 - r_2)\left(k - c_1 + \frac{\eta k q}{r_2}\right) > \eta c_1 p_1. \tag{37}$$

A similar condition determines saturation of P_2.

But even for the simple 2×1 system the general model with arbitrary η leaves some open questions. For example, we do not know if a saturated boundary equilibrium is compatible with the existence of an inner equilibrium (for the same choice of parameters). We also do not know if it is possible to have several interior equilibria. The answers to these questions seem to require explicit solutions of third and fourth order polynomials. However, we conjecture that there is always only one stable equilibrium. This is certainly true in the limit of small η, and in Section 5.3 we will show that it also holds in the limit of large η.

Computer simulations of system (29) for $\eta = 0.01$ are shown in Fig. 5. All simulations are originally started with a homogeneous virus population (variant v_1). It is assumed that $c_1 > k$ such that the x_1-response is immunodominant. Since $\eta > 0$ the y-response also survives, but at much lower levels. After some time, mutant v_2 is introduced. The figure shows four dynamical possibilities, which correspond to the four cases we have described analytically for $\eta = 0$. In Fig. 5(a) the new virus mutant induces a significant x_2-response, but does not affect the y-response. In Fig. 5(b) the new mutant induces only a very weak x_2-response (maybe below the limit of detection), but greatly enhances the y-response, thereby shifting immunodominance. In Fig. 5(c) the new mutant induces a strong x_2-response. The x_1 response is significantly weakened, but the y-response is somewhat enhanced. In Fig. 5(d) the new mutant outcompetes the original v_1 variant, induces only a

weak x_2-response, but a strong y-response. This corresponds to a more or less complete shift in immunodominance.

5.3. THE LIMIT OF LARGE η

In the limit of large η we can write (see Section 4.3)

$$\begin{aligned}\dot{v}_1 &= v_1(r_1 - p_1 x_1 - qy)\\ \dot{v}_2 &= v_2(r_2 - p_2 x_2 - qy)\\ \dot{x}_1 &= \eta c_1 v_1 - b x_1\\ \dot{x}_2 &= \eta c_2 v_2 - b x_2\\ \dot{y} &= \eta k(v_1 + v_2) - by. \end{aligned} \tag{38}$$

This system has three non-trivial equilibria: one fixed point in the interior and two boundary fixed points. There are three parameter regions, which are mutually exclusive. For each parameter region exactly one of these fixed points is stable.

If $r_1/r_2 > (c_1 p_1 + kq)/kq$ then v_2 and x_2 converge to zero.

If $kq/(c_2 p_2 + kq) > r_1/r_2$ then v_1 and x_1 converge to zero.

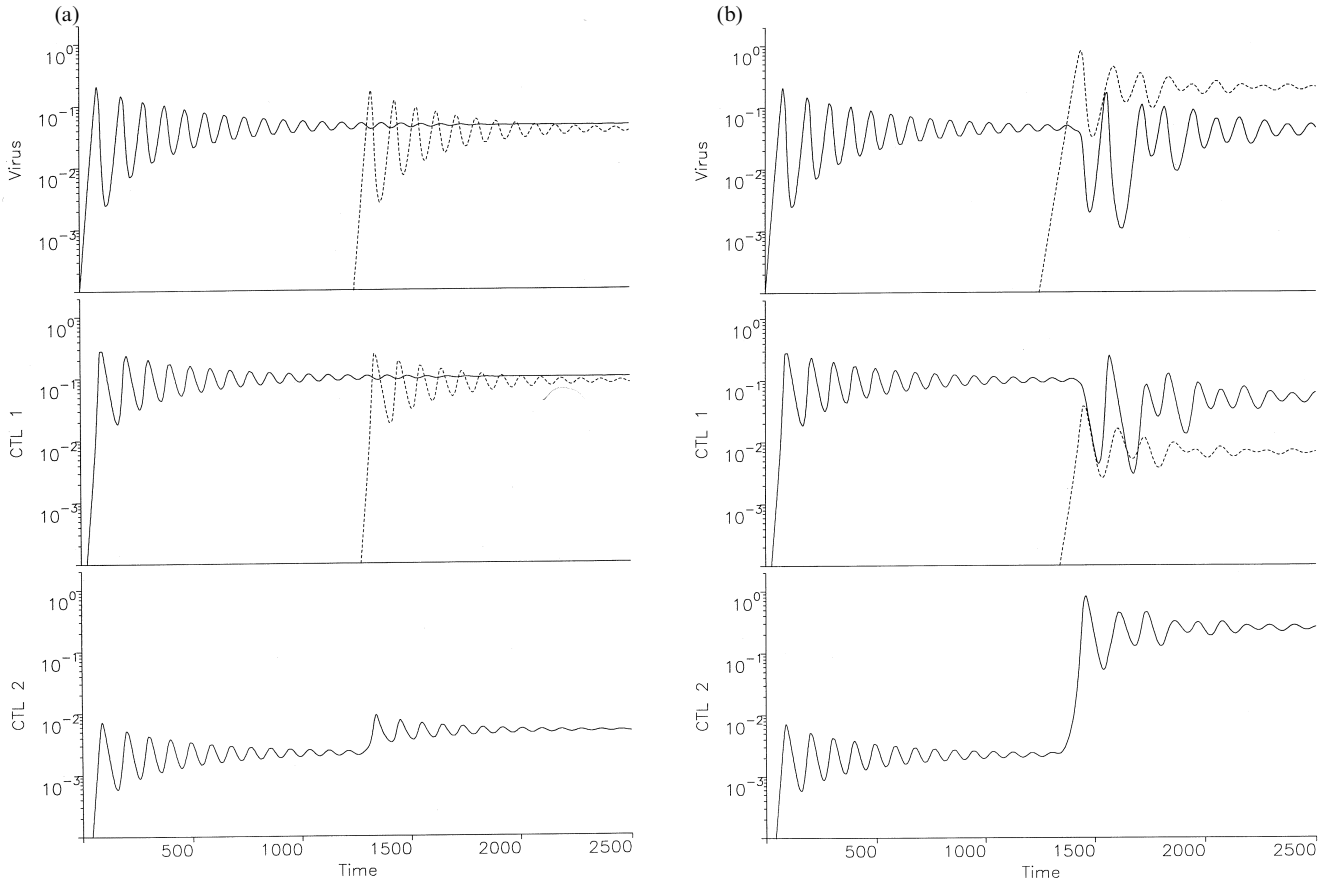
If $(c_1 p_1 + kq)/kq > r_1/r_2 > kq/(c_2 p_2 + kq)$ then the interior fixed point is stable.

Again for any choice of parameters there is exactly one stable equilibrium.

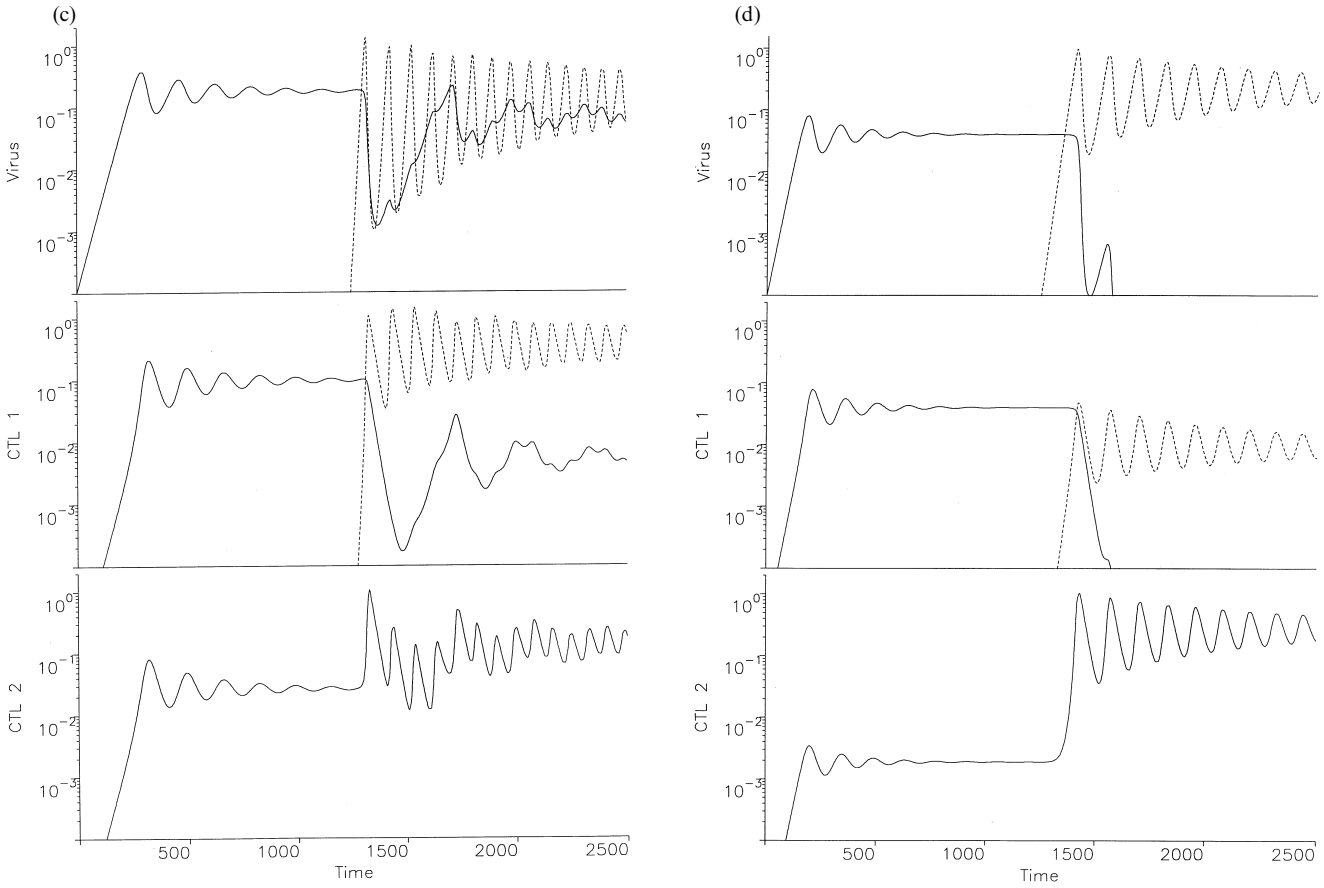
In Appendix C we discuss the 2×2 system and give a complete classification for the two limiting cases $\eta = 0$ and η very large.

6. Cross-reactivity within the Variants of a Given Epitope

In this section we analyse the effect of cross-reactivity within the sequences of a given epitope. We assume that sequence i of epitope A can cross-stimulate the response against sequence j of epitope A, at a rate c_{ij}. Similarily we define k_{ij} for all the variants of epitope B. We include this cross-reactivity in both the stimulation term and in the term for the CTL response



FIGS 5(a–b)—caption on p. 339.



FIGS 5(c–d)

FIG. 5. The figure shows the four different dynamical possibilities following the emergence of a new mutant. Equation (29) with $\eta = 0.01$ is used for the computer simulations. Originally only one virus variant (v_1) is present. The x_1-response is immunodominant, because we chose $c_1 > k$. After some time mutant v_2 is introduced. There are four different possibilities which correspond to the four cases which were discussed for the $\eta = 0$ model in Section 5. (a) The mutant induces an x_2-response but does not significantly affect the y-response. This is simply a diversification in epitope A, without changing immunodominance. (b) The mutant only induces a very weak x_2-response (maybe below an experimental detection limit), but stimulates a very strong y-response. The x_1-response is more or less unaffected. This corresponds to a partial shift in immunodominance. (c) The mutant induces a strong x_2-response, reduces the x_1-response, and increases the y-response. (d) The mutant outcompetes the original v_1 variant (together with the x_1-response), induces only a very weak x_2-response, but a very strong y-response. This represents an almost complete shift of immunodominance. The parameters are: (a) $r_1 = 0.1$, $r_2 = 0.1$, $c_1 = 1$, $c_2 = 1.2$; (b) $r_1 = 0.1$, $r_2 = 0.05$, $c_1 = 1$, $c_2 = 0.1$; (c) $r_1 = 0.03$, $r_2 = 0.15$, $c_1 = 0.25$, $c_2 = 0.3$; (d) $r_1 = 0.04$, $r_2 = 0.06$, $c_1 = 1$, $c_2 = 0.1$; and $k = 0.2$, $b = 0.05$, $p_i = c_i$, $q = k$.

against the virus. This leads to

$$\dot{v}_{ij} = v_{ij}\left(r - p\sum_{l=1}^{n_1} c_{il}x_l - q\sum_{l=1}^{n_2} k_{jl}y_l\right)$$

$$\dot{x}_i = x_i\left(\sum_{l=1}^{n_1} c_{il}v_{l*} - b\right) \quad \text{with} \quad i = 1, \ldots, n_1$$

$$\dot{y}_j = y_j\left(\sum_{l=1}^{n_2} k_{jl}v_{*l} - b\right) \quad \text{with} \quad j = 1, \ldots, n_2. \qquad (39)$$

The outcome depends on the $n_1 \times n_1$ matrix $\{c_{ij}\}$ and the $n_2 \times n_2$ matrix $\{k_{ij}\}$; the earlier simpler model is recovered as the limit $c_{ij} \to c_i\delta_{ij}$, etc. As an example, let us assume a very simple form for these matrices: $c_{ii} = c$, $c_{ij} = cs_1$, $k_{jj} = k$ and $k_{ij} = ks_2$ for all values of i and j with $i \neq j$. The parameters c and k denote the immunogenicities of epitopes A and B as in the previous sections, and the parameters s_1 and s_2 specify the amount of cross-reactivity within epitopes A and B, respectively. If $s_1 = 1$ there is complete cross-reactivity, and if $s_1 = 0$ there is no cross-reactivity (for epitope A). By generalizing the arguments which lead to eqn (6), we find that the response against A will eventually dominate over the response against B if

$$\frac{c}{n_1}[1 + (n_1 - 1)s_1] > \frac{k}{n_2}[1 + (n_2 - 1)s_2]. \qquad (40)$$

This result shows that for the initial phase of an infection, when the diversity is low (i.e. n_1 and n_2

around one), the decisive parameter is immunogenicity (c versus k). At later stages of infection, when both n_1 and n_2 have increased, cross-reactivity becomes important. This means that during an HIV infection there may be a tendency to go from responses against highly immunogenic epitopes towards (possibly) less immunogenic, but more cross-reactive, epitopes. Here cross-reactivity is always defined within the various sequences of a given epitope. We do not consider cross-reactivity among different epitopes. These more general effects of cross-reactivity are, to some degree, captured by the cross-reactive immune response in our earlier papers (Nowak *et al*., 1990, 1991).

7. Immunogenicity and Intra-cellular Competition

The immunogenicity of an epitope may be affected by mutations that occur in the other epitopes. We can easily imagine a mutation that enhances a peptide's affinity for MHC binding. If there is some intra-cellular competition for MHC binding, then this could reduce the overall MHC presentation of another epitope. Thus in more general terms the immunogenicity of an epitope is not only a function of the particular peptide sequence, but also of the sequences of other epitopes (or the protein or pathogen as a whole). A model that takes this into account has the following form:

$$\dot{v}_{ij} = v_{ij}(r - x_i - y_j)$$

$$\dot{x}_i = x_i\left(\sum_{l=1}^{n_2} c_{il}v_{il} - b\right)$$

$$\dot{y}_j = y_j\left(\sum_{l=1}^{n_2} k_{lj}v_{lj} - b\right) \tag{41}$$

(For simplicity we present the case $\eta = 0$. Including a positive η term adds the usual complications.) We also restrict ourselves to the case where all virus mutants have the same replication rate, r. The $n_1 \times n_2$ matrix $\{c_{ij}\}$ has elements which denote the immunogenicity of sequence i in epitope A of the virus v_{ij}(with sequence j in eptiope B). Similarily k_{ij} is the immunogenicity of sequence j in epitope B given that there is sequence i in epitope A. The earlier, simpler model is recovered as the limit $c_{ij} \to c_i$ and $k_{ij} \to k_j$.

In general, eqn (41) admits three sets of equilibria: one set of interior equilibria with all x_i and y_j being positive and two sets of boundary equilibria with either all x_i or all y_j being zero. For the same argument as in Section 3, eqn (9), it is not possible to have a stable equilibrium with some y_j being zero and others positive. But contrary to system (4), interior equilibria with both all y_j and all x_i responses positive can exist. Without loss of generality we can set all $c_{ij} = 1$. This simply involves a rescaling of the individual v_{ij}. The three sets of equilibria are given by the relations:

1. *Interior equilibria*

$$x_i = \xi, \quad y_j = r - \xi,$$

$$\sum_{j=1}^{n_2} v_{ij} = b, \sum_{i=1}^{n_1} k_{ij}v_{ij} = b, \quad \forall i, j \tag{42}$$

Here ξ is some arbitrary number between 0 and r. There are $n_1 + n_2$ equations for the $n_1 \times n_2$ variables, v_{ij}. Thus in general such interior equilibria may exist. Our numerical simulations suggest that these equilibria are not stable, with the system exhibiting either collapse to a boundary equilibrium (see below) or heteroclinic cycles. In Appendix E we give analytic results, showing there cannot be convergence to an interior equilibrium; at best interior states can have locally neutral stability.

2. *Boundary equilibria with all* $y_j = 0$

$$y_j = 0, \quad \forall \text{j}; \quad x_i = r, \quad \sum_{j=1}^{n_2} v_{ij} = b, \quad \forall i. \tag{43}$$

In the face with all $y_j = 0$, these equilibria are surrounded by neutral oscillations. There is saturation with respect to invasion by the y_j if

$$\sum_{i=1}^{n_1} k_{ij}v_{ij} < b, \quad \forall j. \tag{44}$$

The unsolved problem is that some of the equilibria (depending on the particular v_{ij}-configuration) may be saturated while some may be unsaturated. Numerical simulations (and analytic investigations of special cases; see below) suggest that it depends on the initial conditions for the v_{ij}, whether or not there is convergence to this subset of saturated equilibria (with all $y_j = 0$).

3. *Boundary equilibria with all* $x_i = 0$

$$x_i = 0, \quad \forall \text{i}; \quad y_j = r, \quad \sum_{i=1}^{n_1} k_{ij}v_{ij} = b, \quad \forall j. \tag{45}$$

Saturation against invasion by the x_i requires

$$\sum_{i=1}^{n_1} < b, \quad \forall i. \tag{46}$$

Again some equilibria may be saturated and some may be unsaturated. Convergence appears to depend on the initial conditions.

We can derive a complete analytical understanding for a simple case with symmetric initial conditions. Let us consider eqn (41) with $n_1=n_2=2$, $c_{ij}=1$, $k_{11}=k_{22}=k$, and $k_{21}=k_{12}=\kappa$. Consider the initial conditions $v_{11}(0)=v_{22}(0)$ and $v_{12}(0)=v_{21}(0)$. Define $\beta\equiv v_{12}(0)/v_{11}(0)$. Because of symmetry we have $v_{11}(t)$ $v_{22}(t)$, $v_{12}(t)=v_{21}(t)$, $x_1(t)=x_2(t)$, $y_1(t)=y_2(t)$ and $v_{12}(t)/v_{11}(t)=\beta$. Thus the system collapses to only three independent dimensions:

$$\dot{v}=v(1-x-y)$$
$$\dot{x}=x[(1+\beta)v-b]$$
$$\dot{y}=y[(k+\beta\kappa)v-b]. \quad (47)$$

We have defined $v\equiv v_{11}$, $x\equiv x_1$, $y\equiv y_1$. Equation (47) has the solution

$$x^{k+\beta\kappa}y^{-(1+\beta)}=C\exp[(1-k+\beta(1-\kappa))bt]. \quad (48)$$

Here C is a constant specified by the initial conditions. Thus for $t\to\infty$, the x-responses die out if $1-k+\beta(1-\kappa)<0$; while the y-responses die out if the opposite inequality holds. Thus it depends on the initial ratio, $\beta=v_{12}(0)/v_{11}(0)$, whether the x- or y-responses become immunodominant. Coexistence occurs only for a set of initial conditions with measure 0, namely if $1-k+\beta(1-\kappa)=0$.

The important point of this section is that in situations where the immunogenicity of an epitope can be affected by changes in other epitopes, immunodominance may depend on the initial configuration of virus population and is thereby largely determined by chance events.

8. Immunotherapy

Several interesting hints can be given with respect to the design of a potential post-exposure vaccine. In general, immunotherapy should be directed at conserved epitopes. Remember that it is only necessary to control the virus population in a single epitope. But let us assume that there is a conflict in the sense that the highly immunogenic epitopes display antigenic variation, but the conserved epitopes are only weakly immunogenic. If the response against the weakly immunogenic but conserved epitope can be enhanced, such that this epitope becomes immunodominant, then the virus population will be controlled by the response against this epitope. Variation that may occur in other epitopes can then only reinforce the immunodominance of this conserved epitope. If immunotherapy is not potent enough to achieve immunodominance the response against the conserved epitope, then it may be advantageous to direct immunological attack at the more immunogenic but variable epitopes. Let us suppose that the immunogen will only stimulate responses against a certain fraction of the occuring variants. If these variants differ in their intrinsic immunogenicities, then it is always better to enhance the responses against the weakly immunogenic variants.

These points are illustrated by the following equations. Let us again consider the 2×1 case as given by eqn (28). Now suppose that immunotherapy against the variable epitope A can enhance the immunogenicity of variant 1 from c_1 to αc_1, with $\alpha>1$. Variant 2 is not recognized. Immunotherapy against the conserved epitope B enhances its immunogenicity from k to βk, with $\beta>1$. If $1/(\alpha c_1)+1/c_2<1/(\beta k)$ then the equilibrium virus load is $v=b[1/(\alpha c_1)+1/c_2]$. If conversely $1/(\alpha c_1)+1/c_2>1/(\beta k)$ then the equilibrium virus load is $v=b/(\beta k)$. Figure 6 gives an illustration of the equilibrium virus load as a function of the efficacies of the immunotherapies against the two epitopes. For high efficacy the virus population levels off if immunotherapy is directed at the variable epitope A, while it would still decline if the response is directed at B. For very low efficacy it may sometimes be advantageous to induce responses against the more immunogenic, but variable, epitope A. In general, it has very little effect to induce responses against the two epitopes simultaneously; it is better to concentrate on a single epitope.

9. What Next?

(i) *Recombination*. Like all retroviruses, HIV is diploid. During replication the reverse transcriptase binds to both RNA strands and can then switch from one strand to the other (Hu & Temin, 1990; Temin, 1994). If the virus particle contains two different templates (from a cell that was infected by more than one virus) then recombination can generate new mutants. In situations with two epitopes, recombination can in principle generate double escape mutants in both epitopes. Thus including the effects of recombination seems to be an important next step of expanding the present model.

We expect, however, that our results will be largely unaffected. Note that we studied selection dynamics, not mutation-selection dynamics. Thus the frequency of individual variants is determined by the different selection pressures (replication rates and immune responses) and not by the mutation rates at which they are produced. We also assumed that in the beginning all possible combinations of virus mutants are present. We only considered saturated equilibria, in the sense that virus mutants which are not present could not spread anyway. Recombination may shift some of

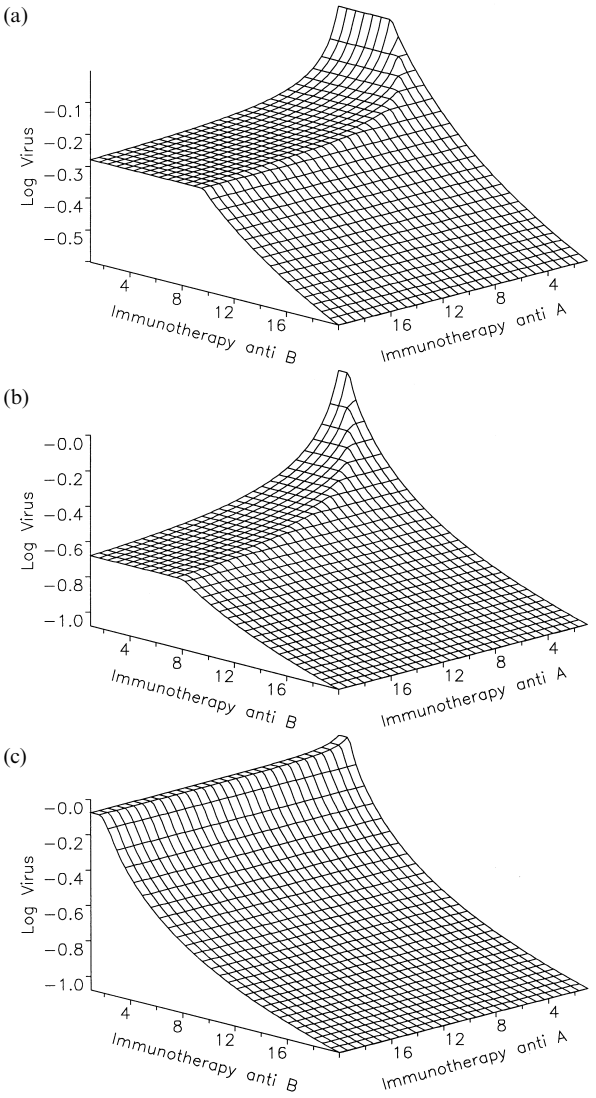


FIG. 6. The relative effect of immunotherapy directed at the variable epitope A, or the conserved epitope B. As a model we use the simple 2×1 case with $\eta = 0$, as described by eqn (28). Immunotherapy against A can only recognize sequence 1 and enhances its immunogenicity by a factor α. Immunotherapy against B enhances the immunogenicity of this peptide by a factor β. The figure shows the equilibrium virus load as a function of the efficacies of immunotherapy, α and β. If $1/(\alpha c_1) + 1/c_2 < 1/\beta k$ then the equilibrium virus load is $v = b[1/(\alpha c_1) + 1/c_2]$. If $1/(\alpha c_1) + 1/c_2 > 1/\beta k$ then the equilibrium virus load is $v = b/(\beta k)$. The basic message is that an effective control with immunotherapy can only be achieved by stimulating responses against conserved epitopes. If it is not possible to make the conserved epitopes immunodominant, than immunotherapy against the variable epitopes may be preferable, but here immunotherapy should always be directed at the weakly immunogenic sequences, too (compare 6b to 6c). Parameters: (a) $c_1 = 10$, $c_2 = 10$; (b) $c_1 = 2$, $c_2 = 10$; (c) $c_1 = 10$ $c_2 = 2$; $k = 1$. The replication rates are irrelevant.

these boundary equilibria into the interior of the phase space. But the overall effect will be very small, as long as recombination is not too frequent.

(ii) *Antagonism*. Recent studies (Klenerman *et al*., 1994; Bertoletti *et al*., 1994) have shown antagonistic effects of virus mutants in HIV and hepatitis B virus (HBV) infections, respectively. Antagonism works in the following way: a mutant in one epitope can impair the immune response against the original variant in this epitope. The detailed molecular mechanism is unclear, but it is conceivable that the mutant binds to the T-cell receptor without inducing a lethal hit. The T-cell remains engaged with the cell and is thereby prevented from killing cells infected with the original virus. Such interference with immune responses against other mutants should represent an important target for future modelling.

(iii) *Uniqueness and global stability*. For the system with different replication rates, r_{ij}, with $\eta = 0$, or $\eta > 0$ we conjecture that there is always a unique, globally stable fixed point, but we cannot prove it. Such a proof would be quite important, especially if it would give some characteristic properties of the stable equilibrium.

(iv) Appendix D outlines the population dynamics of a model with a different functional form of the immune response, which includes saturation of immune cell proliferation at high abundances of activated immune cells.

10. Conclusions

This paper develops a theory for immunodominance in simultaneous responses against several variable epitopes. The theory has been developed with respect to CTL responses against the HIV quasispecies, but has a much wider potential. It represents a mathematical framework for immunodominance in any kind of immune response (CD8+, CD4+, or antibody responses) against multiple epitopes of a replicating pathogen. The principal conclusions are as follows.

1. Antigenic oscillations can arise as a consequence of the dynamics of the immune response acting upon existing viral diversity. It is not essential that mutation continuously generates new antigenic material. Peaks consisting predominantly of different antigenic types can rise and fall as a consequence of the oscillatory dynamics: whenever the CTL response against a particular variant has fallen to lower levels, this variant may start to grow and cause a new peak.

2. Immunodominance is a function both of the immunogenicity and of the diversity of the epitopes, and also of the replication rate of the various mutants. If there is only a homogeneous virus population, then the generic situation is that there is exactly one immunodominant epitope. For an antigenically

heterogeneous virus population we may find coexisting responses against several epitopes. But if there is a heterogeneous virus population and all virus mutants have the same replication rate, then again generically there is always a single immunodominant epitope. Only the response against one epitope can survive; all other responses have to vanish. The epitope that minimizes $\Sigma_{i=1}^{n} 1/c_i$ is immunodominant (with c_i being the immunogenicity of sequence i, and n being the total number of variants in this epitope). If the virus mutants have different replication rates, then the conditions for immunodominance are more complex (see Section 3.1). If all responses are directed against a single epitope, then this must be the epitope which minimizes $\Sigma\, 1/c_i$. But this condition, although necessary, is not sufficient. A number of inequalities have to be fulfilled, and the replication rates of the individual mutants become important. As a rule of thumb, we may expect coexistence of immune responses against several epitopes, if the immunogenicities of the various epitopes are comparable and if the virus mutants differ in their overall replication rates.

In general this picture is also supported by observations of HTLV-I infections, where responses against several epitopes coexist, stimulated by an antigenically diverse virus population (Parker *et al*., 1994).

3. Antigenic variation (i.e. production of new antigenic material) can shift immunodominance. The emergence of a very weakly recognised sequence in an epitope does not necessarily lead to this sequence dominating the population, but will lead to a shift in immunodominance to another epitope. This possibly explains why it has been occasionally observed that escape mutants do not grow to dominate the population (Phillips *et al*., 1991).

4. One of the central points of this paper is a clear understanding of the events following the emergence of a new mutant. Consider a homogeneous virus population subject to immune repsonses against two epitopes, A and B. Suppose that the response against A is immunodominant. The emergence of an escape mutant in A can lead to four different outcomes, which depend on the relative replication rates and immunogenicities of the original virus and the new mutant: (i) the new mutant may induce a new specific response in epitope A, without affecting the response against B (this represents a simple diversification in epitope A); (ii) the new mutant may not induce a response in A against itself, but may enhance the response against epitope B (this corresponds to a partial shift in immunodominance); (iii) the new mutant may induce a response in A against itself, which outcompetes the original response in A (this always occurs together with an increase of the response against epitope B, thus representing a partial shift in immunodominance); (iv) finally, the new mutant may outcompete the original virus variant, and induce a complete shift in immunodominance to epitope B (the response against A essentially vanishes). This has important consequences for our understanding of the detailed escape dynamics with responses against multiple epitopes.

5. Shifting immunodominance to intrinsically weaker epitopes increases viral loads, and can thus represent a route to disease progression.

6. Clearly the models presented here are not limited to HIV, nor to any particular virus. Any (fast) replicating (variable) pathogen with several epitopes is a relevant target for our mathematical framework. Escape from CTL recognition has been demonstrated in a number of human virus infections, such as HIV-1 (Phillips *et al*., 1991), HTLV-1 (Parker *et al*, 1994), Hepatitis B Virus (Bertoletti *et al*., 1994) and Epstein-Barr Virus (Campos-Lima *et al*., 1993). Detailed *in vivo* and *in vitro* studies also exist for lymphocytic choriomeningitis virus (Aebischer *et al*., 1991). Antigenic diversity in CTL epitopes has also been found in human malaria (Hill *et al*., 1992). Our basic model has outlined the competitive dynamics of simultaneous immune (CTL) responses against multiple epitopes; it gives a quantitative concept of immunodominance. Furthermore, the model is not limited to CTL responses. Antibody or CD4+ T-helper responses are likely to obey the same underlying mathematical rules. We have concentrated on CTL responses simply because we think there here the biology is best understood.

7. With respect to HIV, our models reinforce the notion that viral diversity can be very important for understanding pathogenesis. There are obvious effects of antigenic diversity on viral levels, and hence on disease progression, even without invoking the viral-induced depletion of CD4 cells (which is essential for the diversity threshold theory: Nowak *et al*., 1990, 1991; Nowak & May, 1993). We have shown how diversification can shift immunological pressure towards weaker epitopes. Diversity matters.

Support from the Wellcome Trust, Keble College, and the Royal Society is gratefully acknowledged.

REFERENCES

Aebischer, T., Moskophidis, D., Hoffman Rohrer, U., Zinkernagel, R. M. & Hengartner, H. (1991). *In vitro* selection of lymphocytic choriomengitis virus escape mutants by cytotoxic T lymphocytes. *Proc. natn Acad Sci U.S.A.* **88,** 11047–11051.

Anderson, R. M. (1994). Mathematical studies of parasitic infection and immunity. *Science* **264,** 1884–1886.

Bertoletti, A., Sette, A., Chisari, F. V., Penna, A., Levrero, M., De Carli, M., Fiaccadori, F. & Ferrari, C. (1994). Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. *Nature* **369,** 407–410.

Campos-Lima, P., Gavioli, R., Zhang, Q., Wallace, L. E., Dolcetti, R., Rowe, M., Rickinson, A. B. & Masucci, M. G. (1993). HLA-A11 epitope loss isolates of Epstein-Barr virus from highly A11+ population. *Science* **260,** 98–100.

Carpenter, S., Evans, L. H., Sevoian, M. & Chesebro, B. (1990). *In vivo* and *in vitro* selection of equine infectious anemia virus variants. In: *Applied Virology Research*, *Vol*. 2, *Virus Variability*, *Epidemiology*, *and Control* (Kurstak, E., Marusyk, R. G., Murphy, F. A. & van Regenmortel, H. V., eds) pp. 99–115. NY: Plenum.

Domingo, E., Diez, J., Martinez, M. A., Hernandez, J., Holguin, A., Borrego, B. & Matteu, M. G. (1993). New observations on antigenic diversification of RNA viruses. Antigenic variation is not dependent on immune selection. *J. gen. Virol*. **74,** 2039–2045.

Hill, A. V. S., Elvin, J., Willis, A. C., Aidoo, M., Allsopp, C. E. M., Gotch, F. M., Gao, X. M., Takiguchi, M., Greenwood, B. M., Townsend, A. R. M., McMichael, A. J. & Whittle, H. C. (1992). Molecular analysis of the association of HLA-Bresistance to severe malaria. *Nature* **360,** 434–439.

Hofbauer, J. & Sigmund, K. (1988). *The Theory of Evolution and Dynamical Systems*. Cambridge: Cambridge University Press.

Holmes, E. C., Zhang, L. Q., Simmonds, P., Ludlam, C. A. & Leigh-Brown, A. J. (1992). Convergent and divergent sequence evolution in the surface envelope glycoprotein of HIV-1 within a single infected patient. *PNAS* **89,** 4835–4839.

Hu, W. S. & Temin, H. M. (1990). Retroviral recombination and reverse transcription. *Science* **250,** 1227–1233.

Klenerman, P., Rowland-Jones, S., McAdam, S., Edwards, J., Daenke S., Lalloo, D., Köppe, B., Rosenberg, W., Boyd, D., Edwards, A., Giangrande, P., Phillips, R. E. & McMichael, A. J. (1994). Cytotoxic T-cell activity antagonized by naturally occuring HIV-1 gag variants. *Nature* **369,** 403–407.

May, R. M. (1974). *Stability and Complexity in Model Ecosystems*, 2nd edn. Princeton: Princeton University Press.

McMichael, A. J. (1993). Natural selection at work on the surface of virus-infected cells. *Science* **260,** 1771–1772.

Nara, P. L., Smit, L., Dunlop, N., Hatch, W., Merges, M., Waters, D., Kelliher, J., Gallo, R. C., Fischinger, P. J. & Goudsmit, J. (1990). Emergence of virus resistant to neutralization by V3-specific antibodies in experimental HIV-1 IIIB infection of chimpanzees. *J. Virol*. **64,** 3779–91.

Nowak, M. A., May R. M. & Anderson, R. M. (1990). The evolutionary dynamics of HIV-1 quasispecies and the development of immunodeficiency disease *AIDS* **4,** 1095–1103.

Nowak, M. A., Anderson, R. M. McLean, A. R., Wolfs, T., Goudsmit, J. & May, R. M. (1991). Antigenic diversity thresholds and the development of AIDS. *Science* **254,** 963–969.

Nowak, M. A. & May, R. M. (1992). Coexistence and competition in HIV infections. *J. theor. Biol*. **159,** 329–342.

Nowak, M. A. & May, R. M. (1993). AIDS pathogenesis: mathematical models of HIV and SIV infections. *AIDS* **7,** Suppl. 1, S3–S18.

Nowak, M. A., May, R. M., Phillips, R. E., Rowland-Jones, S., Lalloo, D., McAdam, S., Klenerman, P., Köppe, B., Sigmund, K., Bangham, C. R. M. & McMichael, A. J. (1995). *Nature*, in press.

Parker, C. E., Nightingale, S., Taylor, G. P., Weber, J. & Bangham, C. (1994). Circulating antitax cytotoxic T lymphocytes from human T-cell leukemia virus type I infected people, with and without tropical spastic paraparesis, recognize multiple epitopes simultaneously. *J. Virol*. **68,** 2860–2868.

Pantaleo, G., Demarest, J. F., Soudeyns, H., Graziosi, C., Denis, F., Adelsberger, J. W., Borrow, P., Saag, M. S., Shaw, G. M., Sekaly, R. P. Fauci, A. S. (1994). Major expansion of CD8+ T cells with a predominant Vβ usage during the primary immune response to HIV. *Nature* **370,** 463–467.

Phillips, R. E., Rowland-Jones, S., Nixon, D. F., Gotch, F. M., Edwards, J. P., Ogunlesi, A. O., Elvin, J. G., Rothbard, J. A., Bangham, C. R. M., Rizza, C. R. & McMichael, A. J. (1991). HIV genetic variation that can escape Cytotoxic T cell recognition. *Nature* **354,** 453–459.

Salinovich, O., Payne, S. L., Montelaro, R. C., Hussain, K. A., Issel, C. J. & Schnorr, K. L. (1986). Rapid emergence of novel antigenic and genetic variants of EIAV during persistent infection. *J. Virol*. **57,** 71–80.

Sasaki, A. (1994). Evolution of antigen drift/switching: continuously evading pathogens. *J. theor. Biol*. **168,** 291–308.

Temin, H. M. (1993). Retrovirus cariation and reverse trabscription: abnormal strand transfers result in retrovirus genetic variation. *PNAS* **90,** 6900–6903.

Wahlberg, J., Albert, J., Lundeberg, J., von Gegerfelt, A., Broliden, K., Utter, G., Fenyö, E.-M. & Uhlen, M. (1991). Analysis of the V3 loop in neutralization-resistant human immunodeficiency virus type 1 variants by direct solid-phase DNA sequencing. *AIDS Res. Hum. Retrovir*. **7,** 983–990.

APPENDIX A

Remarks on Equation (11)

Consider the following Lotka–Volterra system

$$\begin{aligned}
\dot{v}_{ij} &= v_{ij}(r_{ij} - x_i - y_j) \\
\dot{x}_i &= x_i(c_i v_{i*} - b_i) \quad \text{with} \quad i = 1, \ldots, n_1 \\
\dot{y}_j &= y_j(k_j v_{*j} - d_j) \quad \text{with} \quad j = 1, \ldots, n_2.
\end{aligned} \tag{A.1}$$

This is the same as eqn (11) in Section 3. The only difference is that we also allow for different natural decay rates, b_i and d_j, of the immune cells.

The invariant of motion used in eqn (C.9) (Appendix C), a simple generalization of Volterra's function, works in a much more general context. Let us consider a subsystem of x_i's, y_j's and v_{kl}'s, and assume that all other species are not present. We call this subsystem Γ a *solvable array* if the corresponding system of linear equations fo the fixed point has some solution $\bar{x}_i$, $\bar{y}_j$ and $\bar{v}_{kl}$. More precisely, we require the following:

(i) for every x_i belonging to Γ, the set of all v_{ij} belonging to Γ is nonempty.

(ii) a corresponding condition for all y_j belonging to Γ.

(iii) for every v_{kl} belonging to Γ, there exists an x_k or an y_l belonging to Γ.

(iv) the corresponding set of linear equations

$$r_{kl} = x_k + y_l \tag{A.2}$$

$$b_i/c_i = \sum_j v_{ij} \tag{A.3}$$

$$d_j/k_j = \sum_i v_{ij} \tag{A.4}$$

has a solution $\bar{x}_i$, $\bar{y}_j$, $\bar{v}_{kl}$. (Here we consider only those variables belonging to Γ. If, for instance, x_k does not belong to Γ, then the first equation reads $r_{kl} = y_l$. Similarly, the sum in the second equation extends over

all those j for which v_{ij} belongs to Γ. We do not require that all these quantities are positive or uniquely determined). Whenever we have such a solvable array, the function

$$V=\sum(\bar{v}_{kl}\log v_{kl}-v_{kl})$$

$$+\sum\frac{1}{c_i}(\bar{x}_i\log x_i-x_i)+\sum\frac{1}{k_j}(\bar{y}_j\log y_j-y_j) \quad \text{(A.5)}$$

is an invariant of motion. To prove this, we note that

$$\dot{V}=\sum_{kl}(\bar{v}_{kl}-v_{kl})(r_{kl}-x_k-y_l)$$

$$+\sum_i(\bar{x}_i-x_i)\left(\sum_j v_{ij}-\frac{b_i}{c_i}\right)$$

$$+\sum_j(\bar{y}_j-y_j)\left(\sum_i v_{ij}-\frac{d_j}{k_j}\right) \quad \text{(A.6)}$$

Upon replacing r_{kl} by $\bar{x}_k+\bar{y}_l$, b_i/c_i by $\Sigma\bar{v}_{ij}$ and d_j/k_j by $\Sigma\bar{v}_{ij}$, we obtain

$$\dot{V}=\sum_{kl}(\bar{v}_{kl}-v_{kl})(\bar{x}_k-x_k+\bar{y}_l-y_l)$$

$$+\sum_i(\bar{x}_i-x_i)\sum_j(v_{ij}-\bar{v}_{ij})$$

$$+\sum_j(\bar{y}_j-y_j)\sum_i(v_{ij}-\bar{v}_{ij}). \quad \text{(A.7)}$$

That is,

$$\dot{V}=\sum_i(\bar{x}_i-x_i)\sum_j(\bar{v}_{ij}-v_{ij}+v_{ij}-\bar{v}_{ij})$$

$$+\sum_l\left((\bar{y}_l-y_l)\sum_k(\bar{v}_{kl}-v_{kl}+v_{kl}-\bar{v}_{kl}\right), \quad \text{(A.8)}$$

which reduces to $\dot{V}=0$. QED.

If the fixed point given by the ($\bar{x}_i$, $\bar{y}_j$ and $\bar{v}_{kl}$) has all components positive, then the function V attains its unique maximum at this point. Hence this equilibrium is neutrally stable and all eigenvalues are purely imaginary. All populations originally present (i.e. belonging to the array Γ) persist forever. However, the system is not permanent: a sequence of random perturbations can send the state from one level-set to another, and thus eventually to the boundary of the positive state space. We have seen that we can have at most n_1+n_2-1 viral species present in a solvable array. If, on the other hand, some components of the fixed point are negative, the corresponding populations have to vanish (possibly after an initial phase of growth).

APPENDIX B

Local Dynamics of Equation (20)

In this Appendix, we give a linearized analysis of the dynamics of the system in which activated CTLs arise from inactivated precursors, eqn (20) in the main text, in the biologically interesting limit when all r_{ij} have the same value, $r_{ij}=r$ (see Section 4.1).

In the usual way, we begin by writing

$$x_i(t)=\xi+v_i(t), \quad \text{(B.1)}$$

$$y_j(t)=(1-\xi)+\phi_j(t), \quad \text{(B.2)}$$

$$v_{ij}(t)=v_{ij}^*+\chi_{ij}(t). \quad \text{(B.3)}$$

Here v_i, ϕ_j, and χ_{ij} represent small perturbations about the interior equilibrium defined by eqns (22) and (23). As discussed in the main text, the individual equilibrium values of v_{ij} (here denoted by v_{ij}^*) can take arbitrary values within the envelope set by the equilibrium values of v_{i*} and v_{*j}, as given by eqn (23). We now substitute eqns (B.1)–(B.3) into eqns (20), Taylor expand to first order (discarding all terms of second or higher order in v_i, ϕ_i, and χ_{ij}), and factor out the time-dependence in the ensuing set of linearized differential equations as $\exp(\Lambda t)$:

$$\Lambda\chi_{ij}=-v_{ij}^*(v_i+\phi_j), \quad \text{(B.4)}$$

$$\Lambda v_i=c_i(\eta p_i+\xi)\sum_{k=1}^{n_2}\chi_{ik}+(c_iv_{i*}-b)v_i, \quad \text{(B.5)}$$

$$\Lambda\phi_j=k_j(\eta q_j+1-\xi)\sum_{k=1}^{n_1}\chi_{kj}+(k_jv_{*j}-b)\phi_j. \quad \text{(B.6)}$$

Using eqn (23) to substitute for the equilibrium values of v_{i*} and v_{*j}, and using eqn (B.4) to substitute for χ_{ij} in eqns (B.5) and (B.6), we arrive at a set of

n_1+n_2 linear equations for the (small) perturbations to x_i and y_j, namely v_i and ϕ_j, respectively:

$$\left(\Lambda+\frac{\eta p_i b}{\eta p_i+\xi}\right)v_i+\frac{c_i}{\Lambda}(\eta p_i+\xi)\times\sum_{l=1}^{n_2}v_{il}^*(v_i+\phi_l)=0, \quad \text{(B.7)}$$

$$\left(\Lambda+\frac{\eta q_j b}{\eta q_j+1-\xi}\right)\phi_i+\frac{k_j}{\Lambda}(\eta q_j+1-\xi)\times\sum_{h=1}^{n_1}v_{hj}^*(v_h+\phi_j)=0. \quad \text{(B.8)}$$

Rearrangement, and further use of eqn (23), gives:

$$\left[\Lambda^2+\Lambda\frac{\eta p_i b}{\eta p_i+\xi}+b\xi\right]v_i+c_i(\eta p_i+\xi)\sum_{l=1}^{n_2}v_{il}^*\phi_l=0, \quad \text{(B.9)}$$

$$\left[\Lambda^2+\Lambda\frac{\eta q_j b}{\eta q_j+1-\xi}+b(1-\xi)\right]\phi_j+k_j(\eta q_j+1-\xi)\sum_{h=1}^{n_1}v_{hj}^*v_h=0. \quad \text{(B.10)}$$

Equations (B.9) and (B.10) represent a homogeneous, linear set of equations for the n_1+n_2 variables $\{v_i\}$ and $\{\phi_j\}$. The corresponding $(n_1+n_2)\times(n_1+n_2)$ matrix of coefficients must therefore have a vanishing determinant (note that this matrix paritions into two purely diagonal sub-matrices, $n_1\times n_1$ and $n_2\times n_2$, and two other sub-matrices, $n_1\times n_2$ and $n_2\times n_1$, whose elements depend upon the arbitrary (subject to constraints) values of v_{ij}^*). The requirement that this overall determinant be zero leads to values for the quantities Λ which characterize the time dependence, and hence to elucidation of the system's local stability properties. We have not succeeded in showing that $Re(\Lambda)\leqslant 0$ for the general case of eqns (B.9) and (B.10), but we can make some progress in the special case when p_i and q_j are constants ($p_i=p,\ p_j=q$).

In this case, eqns (B.9) and (B.10) can be reduced to the form

$$\sum_{h=1}^{n_1}[A_{ih}-F(\Lambda)\delta_{ih}]v_h=0. \quad \text{(B.11)}$$

Here δ_{ih} is the Kronecker delta, and the $n_1\times n_1$ matrix A has elements

$$A_{ih}=c_i\sum_{l=1}^{n_2}k_l v_{il}^* v_{hl}^*. \quad \text{(B.12)}$$

The function $F(\Lambda)$ is defined as

$$F(\Lambda)=\left[\Lambda^2+\Lambda\frac{\eta p b}{\eta p+\xi}+b\xi\right]\times\left[\Lambda^2+\Lambda\frac{\eta q b}{\eta q+1-\xi}+b(1-\xi)\right]\times[\eta p+\xi)(\eta q+1-\xi)]^{-1}. \quad \text{(B.13)}$$

Denote the eigenvalues of the matrix A as $\lambda_i (i=1, 2, \ldots, n_1)$. The stability-determining quantities Λ are then found by solving the quartic equations

$$F(\Lambda)=\lambda_i. \quad \text{(B.14)}$$

Notice that A is symmetric, up to the row-constants c_i, whence it follows that all the eigenvalues λ_i are real (see e.g. May, 1974).

In general, the eigenvalues of the matrix A defined by eqn (B.12) depend on the values of $\{v_{ij}^*\}$, and cannot be obtained analytically. We can, however, get exact solutions in two limiting cases, which are likely to "bracket" more general cases.

One limiting case arises when all v_{ij}^* are equal (this is, the asymptotic result which seems to occur in most of our numerical simulations with $p_i=p$, $q_i=q$ and $r_{ij}=1$). In this case, we can use eqn (23) to write $c_i v_{il}^*=b\xi/[n_2(\eta p+\xi)]$ and $k l v_{hl}^*=b(1-\xi)/[n_1(\eta q+1-\xi)]$. Then all elements of the matrix A have the same value, $A_{ik}=a\equiv b^2\xi(1-\xi)/[n_1(\eta p+\xi)(\eta q+1-\xi)]$. Such an $(n_1\times n_2)$ matrix has n_1-1 eigenvalues $\lambda_i=0$ $(i=2, 3, \ldots, n_1)$, and one eigenvalue $\lambda_1=n_1 a$. Returning to eqn (B.14), we see that there are n_1-1 internal modes whose dynamics are characterized by Λ-values which obey $F(\Lambda)=0$, with $F(\Lambda)$ the product of two quadratics, defined by eqn (B.13). All four Λ-values for each of these n_1-1 (identical) internal nodes then clearly lie in the left-half plane. Moreover, for small values of η, these two quadratics each correspond to weakly damped oscillations, two with frequency $\sqrt{b\xi}$ and characteristic damping time $2\xi/(\eta p b)$, and two with frequency $\sqrt{b(1-\xi)}$ and characteristic damping time $2(1-\xi)/$

(ηqb). The remaining Λ-values correspond to the dynamics of the system as a whole, and are given by $F(\Lambda)=n_1 a$, which reduces to

$$\left[\Lambda^2+\Lambda\frac{\eta pb}{\eta p+\xi}+b\xi\right]$$

$$\times\left[\Lambda^2+\Lambda\frac{\eta qb}{\eta q+1-\xi}+b(1-\xi)\right]$$

$$=b^2\xi(1-\xi). \tag{B.15}$$

The constant terms cancel, giving a cubic in Λ, of the form $\Lambda^3+\alpha\Lambda^2+\beta\Lambda+\gamma=0$; it is easy to see that $\alpha>0$, $\beta>0$, $\gamma>0$, and $\alpha\beta>\gamma$, so that all Λ-values lie in the left-half plane. In the limit of very small η, we get weakly damped oscillations with frequency $\sqrt{b}$ and characteristic damping time $2/[\eta b(p+q)]$; there is also a monotonically damped mode with damping time, τ, of around $\tau^{-1}=\eta b\{[p(1-\xi)/\xi]+[q\xi/(1-\xi)]\}$.

An opposite limiting case arises when $n_1=n_2$ and each row and column of the v_{ij}^*-matrix has only one non-zero entry. In this case we can bring the v_{ij}^*-matrix into diagonal form, and thence write the eigenvalues of the matrix A as

$$\lambda_i=\frac{b^2\xi(1-\xi)}{(\eta p+\xi)(\eta q+1-\xi)}, \quad \forall i. \tag{B.16}$$

Substituting this into eqn (B.14) leads again to eqn (B.15) for all the Λ-values in this case. As above, all these stability-determining Λ-values lie in the left-half plane. Again we have weakly damped oscillations with frequencies $\sqrt{b}$ and characteristic damping times of order $1/\eta$ if η is very small.

It seems reasonable to assume that other assignments of $\{v_{ij}^*\}$, within the overall constraints set by the v_{i*} and v_{*j} of eqn (23) in the main text, will lead to dynamics whose qualitative behaviour is bracketed by these two limiting cases. We thus expect the interior equilibrium of Section 4.1 generally to be locally stable.

APPENDIX C

The 2×2 System

A3.1. $\eta=0$

Let us now consider the system

$$\begin{aligned}
&\dot v_{ij}=v_{ij}(r_{ij}-x_i-y_j)\\
&\dot x_i=x_i(c_i v_{i*}-b) \quad \text{with} \quad i=1,2\\
&\dot y_j=y_j(k_j v_{*j}-b) \quad \text{with} \quad j=1,2.
\end{aligned} \tag{C.1}$$

Note that the ratio $\rho=v_{11}v_{22}/(v_{12}v_{21})$ is a Lyapunov function:

$$\dot\rho=\rho(r_{11}+r_{22}-r_{12}-r_{21}). \tag{C.2}$$

If $r_{11}+r_{22}>r_{12}+r_{21}$ then $\rho\to\infty$ which implies that v_{12} or v_{21}(or both) have to converge to zero. Of course, this excludes the possibility of an interior equilibrium. Note that $\rho\to\infty$ does not exclude the possibility that also v_{11} or v_{22} may converge to zero.

We shall now give a full classification of system (C.1) for a generic choice of parameters. We shall show that the system always admits a unique saturated equilibrium P, which lies on some boundary face (either one or two of the four viral species, and the same number of the CTL species have to vanish). Within the corresponding boundary face, however, we know from Appendix A that P is neutrally stable: all eigenvalues are on the imaginary axis, and the orbits do neither converge toward P nor diverge away from P. For every initial condition, the orbit converges towards the face defined by P. Those components which do not vanish will exhibit undamped oscillations. Their time averages will be given by P.

Let us start the classification by assuming that

$$\frac{1}{c_1}+\frac{1}{c_2}<\frac{1}{k_1}+\frac{1}{k_2}. \tag{C.3}$$

This is no restriction of generality (if the converse inequality is valid, we just have to interchange x and y), and it implies, as we have seen, that at least one of the y-responses converges to 0. Next, we assume that

$$r_{12}+r_{21}<r_{11}+r_{22}. \tag{C.4}$$

Again, this can be achieved without restricting generality: if the converse inequality holds, we just have to exchange v_{11} with v_{12}, and v_{21} with v_{22}. These conditions imply that at least one of the viral species v_{12} and v_{21} vanishes. The remaining part of the parameter space will be divided into three mutually exclusive cases:

(A) $r_{11}<r_{12}$ (which implies $r_{21}<r_{22}$);
(B) $r_{12}<r_{11}$ and $r_{22}<r_{21}$;
(C) $r_{12}<r_{11}$ and $r_{21}<r_{22}$.

Each of these can be subdivided into three cases in turn.

(A1) $(1/k_2)<(1/c_2)$. In this case $y_1=v_{12}=0$.
(A2) $(1/c_2)<(1/k_2)<(1/c_2)$. In this case $y_1=v_{21}=0$.
(A3) $(1/c_1)+(1/c_2)<(1/k_2)$. In this case $y_1=y_2=v_{11}=v_{21}=0$.
(B1) $(1/k_1)<(1/c_1)$. In this case $y_2=v_{21}=0$.
(B2) $(1/c_1)<(1/k_1)<(1/c_1)+(1/c_2)$. In this case $y_2=v_{12}=0$.

(B3) $(1/c_1)+(1/c_2)<(1/k_1)$. In this case $y_1=y_2=v_{12}=v_{22}=0$.
(C1) $(1/k_2)<(1/c_2)$ (which implies $(1/c_1)<(1/k_1)$). In this case $y_1=v_{12}=0$.
(C2) $(1/c_2)<(1/k_2)$ and $(1/k_1)<(1/c_1)$. In this case $y_2=v_{21}=0$.
(C3) $(1/c_2)<(1/k_2)$ and $(1/c_1)<(1/k_1)$. In this case $y_1=y_2=v_{12}=v_{21}=0$.

All other components of P are strictly positive and can easily be computed. It is a straightforward, but rather tedious, task to check that in each case no equilibrium other than P is saturated.

As an example, consider the fixed point in the interior of the face $(y_2=0, v_{22}=0)$ which is given by $v_{21}^*=(b/c_2)$, $v_{11}^*=(b/k_1)-(b/c_2)$, $v_{12}^*=(b/c_1)+(b/c_2)-(b/k_1)$, $x_1^*=r_{12}$, $y_1^*=r_{11}-r_{12}$ and $x_2^*=r_{21}+r_{12}-r_{11}$. Since these quantities have to be positive, we must have

$$r_{12}<r_{11}<r_{12}+r_{21} \tag{C.5}$$

and

$$\frac{1}{c_2}<\frac{1}{k_1}<\frac{1}{c_1}+\frac{1}{c_2}. \tag{C.6}$$

If the parameters are chosen properly, none of the missing species y_2 and v_{22} can invade. Indeed, the fixed point is saturated in the sense that the two transversal eigenvalues $\dot{y}_2/y_2$ and $\dot{v}_{22}/v_{22}$ are negative. These eigenvalues are given by $r_{22}-x_2^*=r_{22}+r_{11}-r_{12}-r_{21}$ and by $k_2v_{12}^*-b_2$, which is a positive multiple of $(1/c_2)+(1/c_2)-(1/k_1)-(1/k_2)$. Thus we have to choose

$$r_{11}+r_{22}<r_{12}+r_{21} \tag{C.7}$$

and

$$\frac{1}{c_1}+\frac{1}{c_2}<\frac{1}{k_1}+\frac{1}{k_2}. \tag{C.8}$$

If these conditions are satisfied, and we start with the full system (i.e. all eight populations positive), then y_2 and v_{22} vanish and the remaining species will persist.

Furthermore, note that the function

$$\begin{aligned} V=&(v_{11}^*\log v_{11}-v_{11})+(v_{12}^*\log v_{12}-v_{12})\\ &+(v_{21}^*\log v_{21}-v_{21})+\frac{1}{c_1}(x_1^*\log x_1-x_1)\\ &+\frac{1}{c_2}(x_2^*\log x_2-x_2)+\frac{1}{k_1}(y_1^*\log y_1-y_1) \end{aligned} \tag{C.9}$$

is a constant of motion. All orbits lie on the constant level sets in V. This implies that within its face the fixed point is neutrally stable.

A3.2. $\eta>0$

If we now consider the case of small $\eta>0$, we see that its saturated equilibria points must, by continuity, converge (for $\eta\to 0$) to saturated quilibria of the $h=0$ case. Hence P is the only possible candidate; it follows that at least for small for $\eta>0$, the system has a unique saturated fixed point. This point differs from P by having small, but positive values for those CTLs which, in the $\eta=0$ equilibrium, were not present but have viral species which stimulate their replication. The pattern of virus distribution, on the other hand, remains unchanged, since the v-equations do not depend on η. Hence with small $\eta>0$, the number of CTL species will be higher, by 1 or 2, than the number of viral species.

A complete classification of the 2×2 case for general $\eta>0$ is not possible, but below we give a complete analysis for the interesting limit of large η. There we find ten mutually exclusive parameter regions which cover the whole parameter space like a jigsaw puzzle. Each parameter region specifies exactly one stable fixed point. Therefore by continuity we conjecture that also the general η system admits always a single stable fixed point.

A3.3. THE LIMIT OF LARGE η

In this 2×2 case, we give a complete listing of all ten saturated fixed points, and we show that one, and only one, of these ten states exists for any specific choice of the parameters. Table C.2 shows how to determine which state, dependent upon 13 inequalities among the parameters, as defined below. This illustrative example requires only that η be large, in the sense defined below; a completely general analysis of the 2×2 case is not feasible, although intuition backed by numerical studies suggests that for any specified set of parameter values there will in general be a unique saturated fixed point.

For the 2×2 case of the system given by eqns (25) in Section 4.3, equilibrium values of the four variables v_{ij} are found by putting $\dot{v}_{ij}=0$, which—as discussed in the main text—gives *either* $r_{ij}=x_i+y_j$ or $v_{ij}=0$ (along with the condition $r_{ij}<x_i+y_j$). In the remaining eqs (25), setting $\dot{x}_i=0$ and $\dot{y}_j=0$ leads to the further conditions $v_{i*}=bx_i/(\eta c_ip_i+c_ix_i)$ and $v_{*j}=by_j/(\eta k_jq_j+k_jy_j)$, which in combination with the earlier equations involving r_{ij} lead to a complete specification.

There are thus two possible solutions for each v_{ij}, leading to $2^4=16$ possible solutions in total. But, as discussed in the main text, we cannot have saturated fixed points for which an entire row or column of the

v_{ij} matrix vanish: this rules out the single solution where all $v_{ij}=0$, and the four where only one $v_{ij}\neq 0$. Also, as noted in Section 4.2, the solution with all four of the $v_{ij}\neq 0$ is not generically possible. This leads to ten cases to be examined, four with one $v_{ij}=0$ and the other three non-zero, and six with two of the $v_{ij}=0$ and the other two non-zero.

As an example, we sketch the derivation of the condition for the existence of a saturated fixed point with $v_{11}=0$ and $v_{ij}\neq 0$ otherwise. These conditions correspond to particular inequalities that the parameters $\{r_{ij}\}$, $\{p_ic_i\}$, and $\{q_jk_j\}$ must satisfy. Without discussing the other nine cases in detail, we then set out conditions for the existence of each of the ten possible saturated fixed points. This is done in Table C.2.

Finally, we emphasize that for a specified set of parameter values, one and only one of the ten states of Table C.2 will arise. That is, the patchwork of inequalities summarized in Table C.2 fits together like a jigsaw puzzle. This result is not immediately obvious, and we conclude this appendix by sketching the proof.

The illustrative case of $v_{11}=0$. For $v_{11}=0$ and saturated, we require $\dot{v}_{11}<0$, which implies $r_{11}<x_1+y_1$. From $v_{ij}\neq 0$ for $i, j\neq 1, 1$, we have the three equations $r_{ij}=x_i+y_j$ when $i, j\neq 1,1$. There are also four relations among v_{ij} and x_i, y_j, as follows:

$$v_{12}=bx_1/(\eta c_1p_1+c_1x_1), \tag{C.10}$$

$$v_{21}+v_{22}=bx_2/(\eta c_2p_2+c_2x_2), \tag{C.11}$$

$$v_{21}=by_1/(\eta k_1q_1+k_1y_1), \tag{C.12}$$

$$v_{12}+v_{22}=by_2/(\eta k_2q_2+k_2y_2). \tag{C.13}$$

As emphasized above, the analysis in this appendix depends (only) on the limiting assumption that η is large, in the sense that the second term in the brackets in each of the eqns (C.10)–(C.13) can be ignored; effectively, this means $\eta \gg$ (terms of order of $r_{ij}/p_i, q_j$). In this limit, we have a set of linear relations between

TABLE C.1
Saturated fixed points of the 2×2 *system with* $\eta=0$, *as specified by eqn* (*C*.1)

v_{ij} matrix	(x_1, x_2)	(y_1, y_2)	Case		Conditions of existence and stability
$\begin{pmatrix}+ & 0\\ + & +\end{pmatrix}$	$(+, +)$	$(0, +)$	A1	$r_{11}<r_{12}$ $r_{21}<r_{22}$	$1/k_2<1/c_2$
			C1	$r_{11}>r_{12}$ $r_{21}<r_{22}$	$1/k_2<1/c_2$
$\begin{pmatrix}+ & 0\\ + & +\end{pmatrix}$	$(+, +)$	$(+, 0)$	B2	$r_{11}>r_{12}$ $r_{21}>r_{22}$	$1/c_1<1/k_1<1/c_1+1/c_2$
$\begin{pmatrix}+ & +\\ 0 & +\end{pmatrix}$	$(+, +)$	$(0, +)$	A2	$r_{11}<r_{12}$ $r_{21}<r_{22}$	$1/c_2<1/k_2<1/c_1+1/c_2$
$\begin{pmatrix}+ & +\\ 0 & +\end{pmatrix}$	$(+, +)$	$(+, 0)$	B1	$r_{11}>r_{12}$ $r_{21}>r_{22}$	$1/k_1<1/c_1$
			C1	$r_{11}>r_{12}$ $r_{21}<r_{22}$	$1/k_1<1/c_1$
$\begin{pmatrix}0 & +\\ 0 & +\end{pmatrix}$	$(+, +)$	$(0, 0)$	A3	$r_{11}<r_{12}$ $r_{21}<r_{22}$	$1/c_1+1/c_2<1/k_2$
$\begin{pmatrix}+ & 0\\ + & 0\end{pmatrix}$	$(+, +)$	$(0, 0)$	B3	$r_{11}>r_{12}$ $r_{21}>r_{22}$	$1/c_1+1/c_2<1/k_1$
$\begin{pmatrix}+ & 0\\ 0 & +\end{pmatrix}$	$(+, +)$	$(0, 0)$	C_3	$r_{11}>r_{12}$ $r_{21}<r_{22}$	$1/c_1<1/k_1$ and $1/c_2<1/k_2$

Without loss of generality we have assumed $1/c_1+1/c_2<1/k_1+1/k_2$ (which implies that at least one y_i has to converge to zero) and $r_{12}+r_{21}<r_{11}+r_{22}$ (which implies that v_{12} or v_{21}-or-both have to converge to zero). There are seven stable fixed points, characterized by nine parameter regions. Each parameter region admits exactly one stable fixed point. The conditions A3, B3, and C3 specify the interesting situation of complete immunodominance (i.e. $y_1=y_2=0$). Note that this lack of y-responses can either occur with homogeneity (A3, B3) or heterogeneity (C3) in the y-epitope.

v_{12}, v_{21}, v_{22} and x_i, y_j. Combining these with the three equations $r_{ij}=x_i+y_j(i,j\neq 1,1)$, we have:

$$br_{12}=\eta(c_1p_1+k_2q_2)v_{12}+\eta k_2q_2v_{22}, \qquad \text{(C.14)}$$

$$br_{21}=\eta(c_2p_2+k_1q_1)v_{21}+\eta c_2p_2v_{22}, \qquad \text{(C.15)}$$

$$br_{22}=\eta k_2q_2v_{12}+\eta c_2p_2v_{21}+\eta(c_2p_2+k_2q_2)v_{22}. \qquad \text{(C.16)}$$

Solving this set of linear equations gives explicit expressions for v_{12}, v_{21}, v_{22}, and thence for x_1, x_2 and y_1, y_2.

By tedious but routine algebraic manipulations, it can be seen that the conditions $v_{12}>0$, $v_{21}>0$, $v_{22}>0$ lead, respectively, to the requirements:

$$r_{22}<r_{12}\left[1+\frac{v_2\rho_1}{\rho_2(v_2+\rho_1)}\right]+r_{21}\left(\frac{v_2}{v_2+\rho_1}\right), \qquad \text{(C.17)}$$

$$r_{22}<r_{12}\left(\frac{\rho_2}{v_1+\tau_2}\right)+r_{21}\left[1+\frac{v_1\rho_2}{v_2(v_1+\rho_2)}\right], \qquad \text{(C.18)}$$

$$r_{22}>r_{12}\left(\frac{\rho_2}{v_1+\rho_2}\right)+r_{21}\left(\frac{v_2}{v_2+\rho_1}\right). \qquad \text{(C.19)}$$

Here we have, for notational convenience, defined

$$v_i=c_ip_i \quad \text{and} \quad \rho_j=k_jq_j. \qquad \text{(C.20)}$$

Clearly x_i and y_j are all positive if v_{12}, v_{21}, v_{22} are. The remaining requirement is that $x_1+y_1>r_{11}$ (so that $\dot{v}_{11}<0$), which immediately implies the inequality

$$r_{11}+r_{22}<r_{12}+r_{21}. \qquad \text{(C.21)}$$

TABLE C.2

Saturated fixed points of the system of equations (10), *for the case* $n_1=n_2=2$, *in the limit of large* η

State specified by v_{ij} matrix (+ represents a positive value of v_{ij})	Number labelling the state	Inequalities to be satisfied for this state to be saturated fixed point
$\begin{pmatrix}0 & + \\ + & +\end{pmatrix}$	1	$\bar{A}$, B_{22}, C_{22}, D_{22}
$\begin{pmatrix}+ & 0 \\ + & +\end{pmatrix}$	2	A, B_{21}, C_{21}, D_{21}
$\begin{pmatrix}+ & + \\ 0 & +\end{pmatrix}$	3	A, B_{12}, C_{12}, D_{12}
$\begin{pmatrix}+ & + \\ + & 0\end{pmatrix}$	4	$\bar{A}$, B_{11}, C_{11}, D_{11}
$\begin{pmatrix}0 & + \\ + & 0\end{pmatrix}$	5	$\bar{A}$, $\bar{B}_{11}$, $\bar{B}_{22}$
$\begin{pmatrix}+ & 0 \\ 0 & +\end{pmatrix}$	6	A, $\bar{B}_{12}$, $\bar{B}_{21}$
$\begin{pmatrix}+ & 0 \\ + & 0\end{pmatrix}$	7	$\bar{C}_{11}$, $\bar{C}_{21}$
$\begin{pmatrix}0 & + \\ 0 & +\end{pmatrix}$	8	$\bar{C}_{22}$, $\bar{C}_{12}$
$\begin{pmatrix}+ & + \\ 0 & 0\end{pmatrix}$	9	$\bar{D}_{11}$, $\bar{D}_{12}$
$\begin{pmatrix}0 & 0 \\ + & +\end{pmatrix}$	10	$\bar{D}_{22}$, $\bar{D}_{21}$

The symbols A, B_{ij}, C_{ij}, D_{ij} stand for the inequalities defined by eqns (D.13)–(D.16), and the "bars" denote the opposite inequality.

Listing the ten possible states. These calculations can obviously be repeated, mutatis mutandis, for the other three possible states with a single $v_{ij}=0$, and for the six states with two of the $v_{ij}=0$. These ten states, and the inequalities which must be satisfied for each of them to be a saturated fixed point, are catalogued in Table C.2. In this table, the symbols A, B_{ij}, C_{ij}, $D_{ij}(i, j=1, 2)$ refer to the following inequalities:

$$A: r_{11}+r_{22}>r_{12}+r_{21}, \tag{C.22}$$

$$B_{ij}: r_{ij}>r_{Ij}[\rho_j/(v_I+\rho_j)]+r_{iJ}[v_i(v_i+\rho_J)], \tag{C.23}$$

$$C_{ij}: r_{ij}<r_{Ij}[\rho_j/(v_I+\rho_j)] + r_{iJ}[1+v_{I\rho j}/\{v_i(v_I+\rho_j)\}], \tag{C.24}$$

$$D_{ij}: r_{ij}<r_{Ij}[1+v_i\rho_J/\{\rho_j(v_i+\rho_J)\}] + r_{iJ}[v_i(v_i+\rho_J)]. \tag{C.25}$$

Here the capital letter subscripts denote $i=2$ if $i=1$, and conersely. If the inequalities do not hold (i.e., if we have the opposite), we write $\bar{A}$, $\bar{B}_{ij}$, etc.

Uniqueness. It can be seen that the set of inequalities listed in the rightmost column of Table C.2. imply that one, and only one, state will ensue for any arbitrary choice of the underlying parameters $\{r_{ij}\}$, $\{v_i\}$, $\{\rho_j\}$ which determine the inequalities A, B_{ij}, C_{ij}, D_{ij}.

The proof is straightforward, but a bit intricate, and depends on relations among the inequalities themselves. Thus it can be seen that $\bar{A}\wedge B_{11}\Rightarrow\bar{B}_{22}\Rightarrow C_{22}, D_{22}$; $A\wedge B_{12}\Rightarrow\bar{B}_{21}\Rightarrow C_{21}, D_{21}$; and so on ($\wedge$ stands for AND). Likewise, although with a bit more difficulty, it can be shown that $\bar{C}_{22}\wedge\bar{D}_{22}\Rightarrow r_{22}>r_{12}+r_{21}\Rightarrow A$; $\bar{C}_{12}\wedge\bar{D}_{12}\Rightarrow\bar{A}$; etc. Finally, it can also be shown that $\bar{A}\wedge\bar{C}_{ii}\Rightarrow\bar{D}_{\hat{i}\hat{i}}$, and $A\wedge D_{\hat{i}\hat{i}}\Rightarrow\bar{D}_{ii}$. Threading our way through the resulting maze, we find there is one, and only one, state for each specified set of parameters (and consequent inequalities).

We conclude with one example, to make these ideas more concrete. Suppose the values of $r_{ij}, c_i, k_j, p_i, q_j$ are such that $r_{12}+r_{21}>r_{11}+r_{22}(\bar{A})$, and also that B_{22} is satisfied. This implies $\bar{B}_{11}$, and thence C_{11} and D_{11}. Working down the right-hand column of Table C.2. we see that the only possible states are then 1, 8, 10. If C_{22} and D_{22} are both satisfied, then we have the unique answer of state 1. If $\bar{C}_{22}$, then the interrelations listed above imply $\bar{C}_{12}$ (also D_{22}), whence state 8 is the only answer. Conversely, if $\bar{D}_{22}$ then $\bar{D}_{21}$ (and also C_{22}), so that state 10 is the unique answer.

Systematic elaboration of these lines of argument leads to the conclusion that any choice of parameters leads (in the limit of large η) to one, and only one, of the ten states catalogued in Table C.2.

APPENDIX D

A Saturation for Proliferation Rates of the Immune Cells

In this Appendix we analyse a system that includes some saturation of immune cell proliferation for high abundances of activated immune cells.

$$\begin{aligned}
\dot{v}_{ij} &= v_{ij}(r-px_i-py_j)\\
\dot{x}_i &= x_i\left[\frac{cv_{i*}}{1+\epsilon(x+y)}-b\right], \quad \text{with} \quad i=1,\ldots,n_1\\
\dot{y}_j &= y_j\left[\frac{kv_{*j}}{1+\epsilon(x+y)}-b\right], \quad \text{with} \quad j=1,\ldots,n_2.
\end{aligned} \tag{D.1}$$

Here ϵ is a small, positive parameter. The underlying biological assumption is that, for high abundances of activated CTLs (high $x+y$), the rate of proliferation declines. This has a certain saturation effect. The activated CTLs may produce some interleukin, which generates a feedback loop to prevent the immune response from overshooting.

System (D.1) does not have an interior equilibrium, i.e. both responses, x and y, cannot coexist. Again if $c/n_1>k/n_2$ then all y_j converge to zero, which leads to

$$\begin{aligned}
\dot{v}_{i*} &= v_{i*}(r-px_i)\\
\dot{x}_i &= x_i\left[\frac{cv_{i*}}{1+\epsilon x}-b\right] \quad \text{with} \quad i=1,\ldots,n_1.
\end{aligned} \tag{D.2}$$

This system shows a peculiar behaviour. The total viral load and the total amount of immune cells converge to their equilibrium values

$$v=\frac{bn_1}{c}\left(1+\frac{\epsilon n_1 r}{p}\right) \quad \text{and} \quad x=\frac{n_1 r}{p}, \tag{D.3}$$

but the individual v_{i*} and x_i continue forever in neutral oscillations (again with a period of roughly $T\approx 2\pi/\sqrt{rb}$.) The damping of the overall system occurs at the long time scale $2/(\epsilon b n_1)$. Note that the equilibrium virus abundance, v, increases at first linearly with n_1, and for large values of n_1 with the square of the diversity n_1. Again the individual v_{ij} are not specified, byond the constraints implied by the values of $v_{i*}=v/n_1$.

We now sketch a derivation of these results.

For this system, the indeterminism of the v_{ij}^* causes no problems, because we need to deal only with the

dynamics of (x_i, v_i*). As usual, we expand $x_i(t)$, $v_i*(t)$, and $x(t)=\Sigma_{i=1}^{n_1}x_i(t)$ about their equilibrium values:

$$x_i(t)=(r/p)+v_i(t), \tag{D.4}$$

$$v_i*(t)=(v/n_1)+\chi_i(t). \tag{D.5}$$

Here v is given by eqn (D.3), and $x(t)=x^*+\Sigma_{i=1}^{n_1}v_i(t)$. We now substitute these expressions into eqn (D.1), Taylor-expand, discard terms of second or higher order, and factor out the time-dependence in the ensuing linear equations as $\exp(\Lambda t)$:

$$\Lambda\chi_i=-(v/n_1)pv_i, \tag{D.6}$$

$$\Lambda v_i=(r/p)\left[\frac{c\chi_i}{1+\epsilon x}-\frac{\epsilon cv}{n_1(1+\epsilon x)^2}\sum_{j=1}^{n_1}v_j\right]. \tag{D.7}$$

Using eqn (D.6) to eliminate χ_i in eqn (D.7), and substituting for x and v from eqn (D.3), we arrive at

$$\sum_{j=1}^{n_1}[\alpha+\beta\delta_{ij}]v_j=0. \tag{D.8}$$

Here α and β are defined as

$$\alpha=\Lambda\epsilon rb/(p+\epsilon n_1 r), \tag{D.9}$$

$$\beta=\Lambda^2+rb. \tag{D.10}$$

Following along lines similar to those in Appendix B, we see that this set of n_1 homogeneous, linear equations have consistent solutions if, and only if, the determinant of B equals 0, where B is the matrix with elements $B_{ij}=\alpha+\beta\delta_{ij}$. This will be true if $\beta=0$ (n_1-1 solutions), and if $\beta=-n_1\alpha$ (once); the former $(n-1)$ solutions correspond to internal modes of the corresponding dynamical system, and the single solution corresponds to the collective mode.

For the internal modes, $\beta=0$ leads to

$$\Lambda^2+rb=0. \tag{D.11}$$

That is, we get undamped, neutrally stable oscillations; for small amplitudes, the frequencies are $\sqrt{rb}$, as stated in the text.

For the collective mode, $\beta+n_1\alpha=0$ leads to a quadratic for the stability-determining Λ-values:

$$\Lambda^2+\Lambda\frac{b(\epsilon n_1 r/p)}{1+(\epsilon n_1 r/p)}+rb=0. \tag{D.12}$$

Both roots must lie in the left-half plane, corresponding to local stability. For small values of ϵ (specifically, $\epsilon n_1 \ll p/\sqrt{rb}$), we have weakly damped oscillations, with frequency again $\sqrt{rb}$ and with a characteristic damping time of $\tau\approx 2p/(\epsilon bn_1 r)$.

APPENDIX E

"The Knitting Done"

This Appendix shows that the set of equations (41) of Section 8, which describe the dynamics of immunogenicity when intra-cellular effects are significant, cannot have a locally stable interior equilibrium.

As in Appendix B [eqns (B.1)–(B.3)], we expand the variables about the interior fixed point [given by eqn (42)]: $x_i(t)=\xi+v_i(t)$, $y_j(t)=\xi'+\phi_j(t)$, $v_{ij}(t)=v_{ij}^*+\chi_{ij}(t)$ (here $\xi'\equiv r-\xi$). We now Taylor-expand eqns (41) to first order, and factor out the time dependence in the ensuing set of linear equations as $\exp(\Lambda t)$, to get:

$$\Lambda\chi_{ij}=-v_{ij}^*(v_i+\phi_j), \tag{E.1}$$

$$\Lambda v_i=\sum_{l=1}^{n_2}\chi_{il}, \tag{E.2}$$

$$\Lambda\phi_j=\xi'\sum_{m=1}^{n_1}k_{mj}\chi_{mj}. \tag{E.3}$$

Here we have, as in Section 8, put $c_{ij}=1$.

Using eqn (E.1) to substitute for χ_{ij} in eqns (E.2) and (E.3), we have

$$\Lambda^2 v_i+\xi\sum_{l=1}^{n_2}v_{il}^*(v_i+\phi_l)=0, \tag{E.4}$$

$$\Lambda^2\phi_j+\xi'\sum_{m=1}^{n_1}k_{mj}v_{mj}^*(v_m+\phi_j)=0. \tag{E.5}$$

Using the equilibrium expressions given by eqn (42), we can reduce this pair of equations to

$$(\Lambda^2+\xi b)v_i+\xi\sum_{l=1}^{n_2}v_{il}^*\phi l=0, \tag{E.6}$$

$$\xi'\sum_{m=1}^{n_1}k_{mj}v_{mj}^*v_m+(\Lambda^2+\xi' b)\phi_j=0. \tag{E.7}$$

Using eqn (E.7) to substitute for ϕ_l in eqn (E.6) leads us finally to a set of n_1 equations for the perturbations v_i:

$$\sum_{j=1}^{n_1}[A_{ij}-F(\Lambda)\delta_{ij}]v_j=0. \tag{E.8}$$

Here A is the $n_1 \times n_1$ matrix with elements

$$A_{ij} \equiv \xi\xi' \sum_{l=1}^{n_2} v_{il}^* v_{jl}^* k_{jl}, \tag{E.9}$$

and $F(\Lambda)$ is a fourth-order polynomial in the stability-determining quantities Λ

$$F(\Lambda) = (\Lambda^2 + \xi b)(\Lambda^2 + \xi' b). \tag{E.10}$$

That is, $F(\Lambda)$ is a quadratic in Λ^2:

$$F(\Lambda) = (\Lambda^2)^2 + br(\Lambda^2) + \xi\xi' b^2. \tag{E.11}$$

Here we have used $\xi + \xi' = r$.

The expression (E.8) is reminiscent of eqn (B.11) of Appendix B. We again observe that, if λ_i are the eigenvalues of the matrix A of eqn (E.9) (with $i = 1, 2, \ldots, n_1$), then Λ^2 are given from the quadratic equations $F(\Lambda) = \lambda_i$, or

$$(\Lambda^2)^2 + br(\Lambda^2) + (\xi\xi' b^2 - \lambda_i) = 0. \tag{E.12}$$

Unless both roots of all n_1 such quadratic equations for Λ^2 are real and negative, there will be at least one stability-determining rate Λ with positive real part, implying that the interior fixed point is unstable. But if *all* Λ^2 are real and negative, then the interior fixed point will be (locally) neutrally stable. This latter event requires $b^2 r^2 > 4(\xi\xi' b^2 - \lambda_i) > 0$, for all i. From the definition of the matrix elements of A, eqn (E.9), we can rescale $\lambda_i = \xi\xi' b^2 \lambda_i^*$ (rescaling the v_{ij}^* to v_{ij}^*/b), so that the constant term in eqn (E.12) reads as $\xi\xi' b^2(1 - \lambda_i')$. For many matrices k_{ij}, the equilibrium values v_{ij}^* will indeed lead to $\lambda_i' < 1$, so that the conditions for a (locally) neutrally stable equilibrium (namely, $r^2 > 4\xi(r - \xi)(1 - \lambda_i') > 0$) are satisfied, provided ξ or $(r - \xi)$ is sufficiently small relative to r.