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Immunological transitions in response to antigenic mutation during viral infection

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Abstract

Antigenic variation is an important factor in viral persistence and disease progression. We analyze immunological changes which occur in response to antigenic mutation during chronic viral infection. Using an established model of viral and immune system dynamics, we determine which qualitative shifts in the immune response can be elicited by the appearance of a new mutant. We find that antigenic mutation can cause dramatic shifts in the magnitude and type of anti-viral immune response. For example, the appearance of a mutant can elicit a new immune response which recognizes the original viral strain. We also find that novel strains of the virus which replicate more slowly than existing viral strains are able to invade and survive, even when the immune system is capable of mounting an immune response against the mutant.

Introduction

A typical viral protein contains several epitopes which may be recognized by cytotoxic T lymphocytes (CTL). Antigenic variation within the CTL epitope has been demonstrated for the human immunodeficiency virus HIV-1 (1–5, for review, see 6) and other viruses (7–12), and may be an important factor for viral persistence and disease progression (13).

We wish to investigate immunological changes which may occur in response to the appearance of antigenic mutations during viral infection. We are especially interested in qualitative shifts in the immune response which may be induced by the appearance of a new strain of the virus. Following on from (14), we determine which of these fixpoint transitions are likely to occur during the course of viral infection.

This investigation includes two important extensions of earlier work: we now explicitly include the effects of a limited population of healthy target cells and we consider situations in which at least some viral epitopes are variable, while others remain conserved across the viral population. Thus we are able to present a map of immune transitions which are theoretically likely, possible or forbidden in response to antigenic escape; we believe that an understanding of these transitions will become increasingly important as detailed characterizations of *in vivo* CTL specificity become available (13,15). Since most putative fixpoint transitions can be excluded on mathematical or biological grounds, our model predicts in detail the small number of immune transitions which are likely to occur in response to antigenic mutation. We therefore demonstrate which immunological changes during chronic infection are consistent with the current understanding of viral and immune dynamics.

The emergence of a novel mutation

In the absence of new mutations or other changes in the microenvironment, populations of virus, infected cells, target cells and lymphocytes interact in such a way that each population moves toward equilibrium; the possible equilibria and the conditions under which they exist have been described in detail (16). When a novel mutation arises, however, the conditions under which the new viral strain will survive are not obvious nor is the response of the immune system to this possible invasion.

It is clear that for the new strain to survive, each cell infected by the mutant must produce on average more than one newly infected cell. Intuitively, we expect that another condition for survival might be that the new strain replicates more quickly than existing viral sub-types or be able to escape immune recognition. In the latter case intuition suggests that the invading strain will out-compete and eventually replace the wild-type virus. In the former case we expect that the immune

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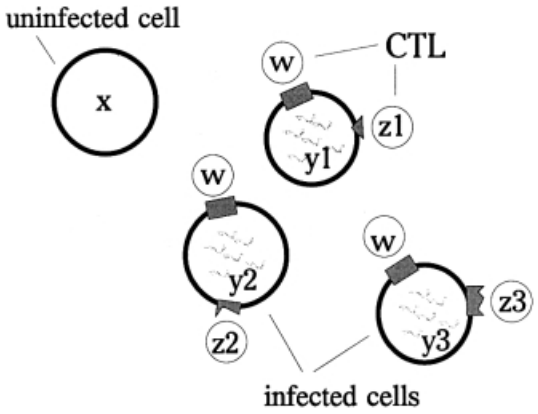

Fig. 1. Populations in the model: uninfected cells, infected cells and the immune response. Our model considers uninfected cells, x, and cells infected by antigenically heterogeneous viral strains, y_1, y_2, etc. The CTL response to the infection is either specific to each viral strain (z_1, z_2, ...) or is a 'cross-reactive' response (w) which recognizes an epitope from a conserved region of the viral genome.

system will respond by mounting a specific response to the invading viral strain.

In contrast, the analysis that follows demonstrates two counter-intuitive results: (i) viral sub-types which replicate more slowly than existing viral strains are able to invade and persist, even when the immune system is capable of mounting a response against them; (ii) viral sub-types which replicate more quickly than existing viral strains may co-exist with these precursors after invasion and may cause dramatic shifts in the magnitude or type of immune response elicited.

Throughout this paper we use a well-studied dynamic model of viral replication (16) which is explained in some detail in the Appendix. The key components of this model are:

- x, the population of healthy target cells.
- y_i, the population of cells infected by viral strain i. We note that viral strain i has (at least one) epitope which differs from every other viral strain in the system, but may also have epitopes in common with all other strains—we call these 'conserved' epitopes.
- z_i, the specific immune response against viral strain i. This immune response specifically recognizes cells infected by strain i, i.e. it responds to at least one of the epitopes which differentiate i from other viral strains.
- w, the 'cross-reactive' immune response. This immune response, if it exists, recognizes a conserved epitope. Such epitopes might exist, for example, in conserved regions of the viral genome where mutations are not possible or where mutations do not lead to immune escape.

A diagram illustrating this model is shown in Fig. 1. Please note that by convention we order the viral strains (i = 1, 2, 3 ...) by replication rate, such that strain 1 replicates faster than strain 2, etc. In general we then use y_2 to denote cells infected by the wild-type virus so that y_1 and y_3 denote cells infected by mutant strains which replicate more or less quickly than the wild-type respectively.

Finally, we note that the transitions described here will not be instantaneous; considerable time might be necessary for the system to reach the new equilibrium, particularly if immune memory is involved (17).

Transitions caused by the first antigenic mutation

In this section, we consider the simple case of an antigenic mutation which arises, and persists, in an otherwise antigenically homogeneous population of virus. We use 'wild-type' to refer to this population, but emphasize that the virus at this stage if antigenically, although not necessarily genetically, uniform. This is the first step towards antigenic viral diversity.

The wild-type virus can exist at steady state either with or without immune control; in the latter case the virus population is kept in check by the availability of healthy target cells. When an antigenic mutation occurs, two additional factors determine the range of outcomes: does the mutant replicate more or less quickly than the wild-type and are the epitopes which are currently recognized by the immune system conserved in the novel strain?

By examining the parameter ranges for each transition, we find that most putative transitions are forbidden; for completeness, they are summarized in Fig. 2. By 'forbidden' we mean that there are no possible values of the parameters in the model which would allow these transitions to occur. Our model predicts that none of the transitions illustrated in Fig. 2 will be observed during chronic viral infection. In general we see that it is rare for a viral strain which replicates more slowly than the existing viral population to be able to invade; the exceptions to this rule will be discussed in a later section (Fig. 5).

Each of the remaining, allowed transitions may occur for certain clearly defined parameter ranges in the model. The possibilities outlined below offer a detailed map of which mutants are able to emerge under what circumstances and the changes in the immune response which might occur in consequence.

Transitions when there is no previous immune response

First, if the wild-type is in equilibrium controlled only by target cell availability, only a viral strain which replicates more effectively can invade. This scenario occurs under the rather unlikely conditions that (i) the mutation confers a selective advantage to the virus and (ii) there is no immune response to the virus before the mutation arises.

When both of these conditions are met, the new strain may invade and either eliminate or co-exist with the wild-type. The two strains can only co-exist if an immune response specific to the new strain emerges. The four transitions which might occur under these conditions are illustrated in Fig. 3.

Transitions with a pre-existing immune response and a faster-replicating mutant

Second, we consider the transitions that are possible if (i) the mutation confers a selective advantage to the virus (enables faster replication), but in this case (ii) the wild-type is initially under immune control.

Under these conditions there are five transitions which can occur when the new mutant does not escape the current immune response, i.e. when the relevant epitopes are conserved; the same five transitions plus one additional transition are possible if the new mutant does escape the current immune

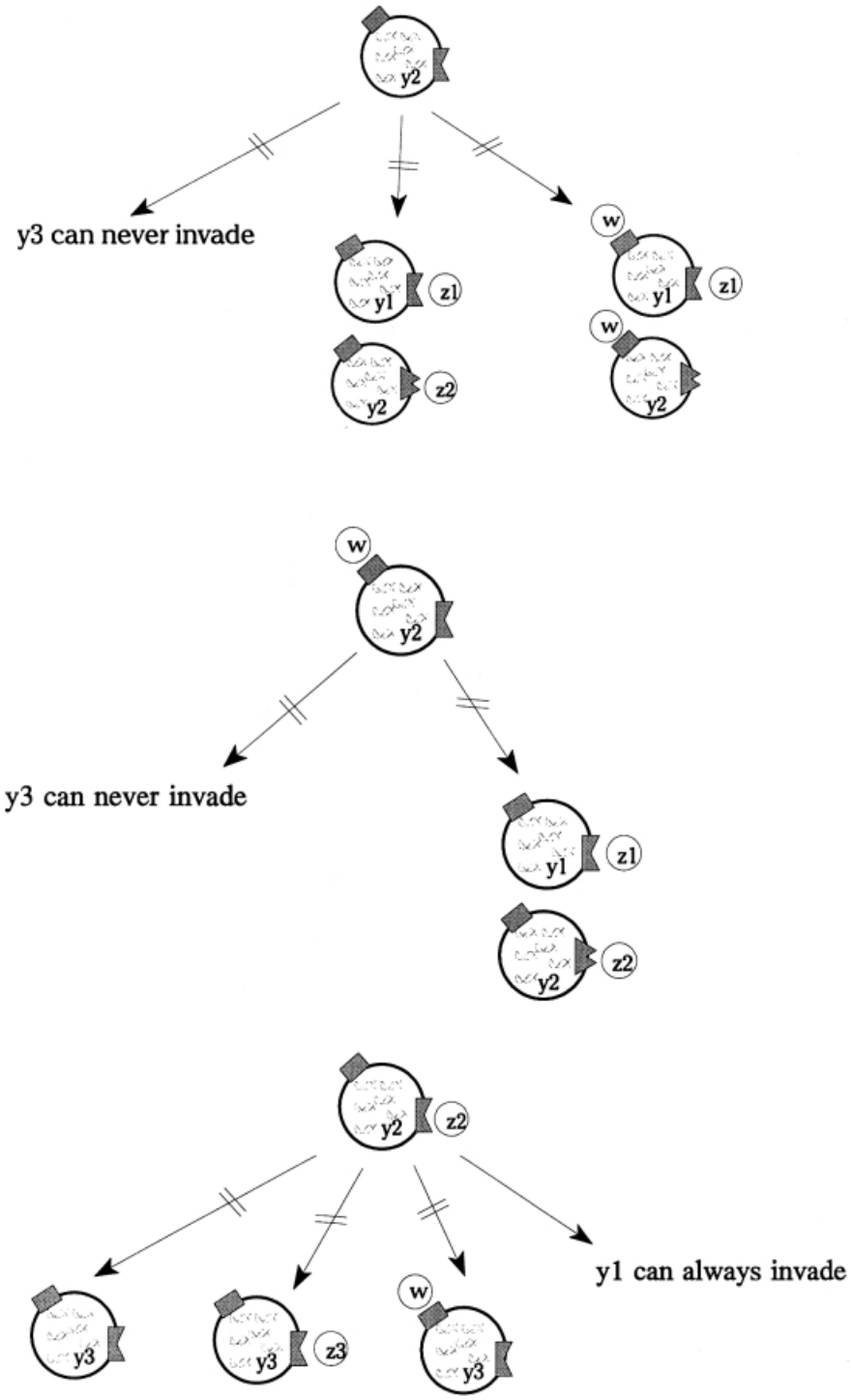

Fig. 2. Forbidden fixpoint transitions. This figure shows three distinct classes of transitions which cannot occur in response to the emergence of a new mutation. (y_2 represents the pre-existing virus; y_1 then represents a new viral strain which replicates more quickly than y_2 and y_3 represents a viral strain which replicates less quickly.) If the virus initially exists without immune control (top panel), a viral strain which replicates more slowly than the existing virus can never invade nor can a faster replicator invade and evoke two distinct immune responses (z_1 and z_2, or z_1 and w). If the virus initially exists with an immune response which recognizes the new viral strain (middle panel), once again a viral strain which replicates more slowly than the existing virus can never invade; in this case the transition illustrated, in which the cross-reactive immune response is replaced by two specific immune responses, can also not occur. Finally, if the virus initially exists with an immune response which does not recognize the new viral strain (lower panel), the only forbidden transitions are those in which a viral strain which replicates less quickly invades and replaces the initial viral strain. If a viral strain invades which replicates more quickly and is not recognized by the current immune response, the system may move to any equilibrium involving the co-existence of the two viral strains or the elimination of the original viral sub-type.

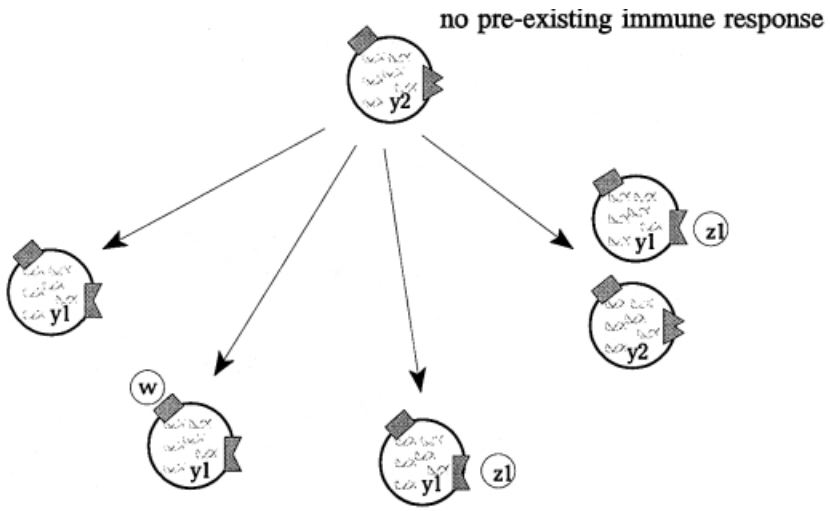

Fig. 3. Transitions from an antigenically homogeneous viral population—no pre-existing immune response. The figure illustrates the four possible transitions which might occur in response to a novel mutation, for an infection in which no immune response is present before the emergence of the mutant. For all transitions the new strain, y_1, must replicate more quickly than the existing viral population, y_2. In three cases the mutant out-competes and replaces the wild-type virus and an immune response to the mutant may emerge; in the additional case the two viral sub-types co-exist and an immune response specific to the mutant emerges.

response. Again we find that the mutant can either eliminate or co-exist with the wild-type at equilibrium. All of these possible transitions are illustrated in Fig. 4. We note an interesting feature of these transitions: the novel mutation may cause a qualitative shift in the immune response. Of particular interest here is the fact that CTL which recognize both viral strains may proliferate less well than those which recognize only the mutant; because of competition between immune responses, the cross-reactive immune response may therefore disappear when the mutant emerges. An example of such a change will be discussed in greater detail later (see Fig. 7).

We reiterate that for any of these transitions to occur, the invading mutant must replicate more quickly than the wild-type; while this is not the case for the vast majority of mutations, adaptations which enable faster replication may well occur in response to the specific microenvironment faced by the virus.

Transitions with a pre-existing immune response and a slower-replicating mutant

Finally, we consider the class of transitions which are most likely to occur: a new mutant arises which replicates less quickly than the existing virus. The three transitions which occur under these conditions, starting from y_2 and the specific immune response z_2, are:

- y_2, y_3 and z_2 (the invading strain emerges without changing the immune response)
- y_2, y_3, z_2 and z_3 (an immune response specific to the invader also emerges)
- y_2, y_3, z_2 and w (an immune response against a conserved epitope emerges)

Each of these transitions is illustrated in Fig. 5. We note that only three transitions are possible in response to a new mutant which replicates less quickly than the existing virus population. Each of these transitions is possible even though the immune system is capable of mounting a response against the mutant, as long as the new mutant is not recognized by the pre-existing immune response which is directed against the wild-type virus.

After the new slower-replicating mutant invades, the two viral types co-exist. In the transitions illustrated center and right, either an immune response specific to the new viral strain, or an immune response which recognizes both viral strains, will emerge.

On the left in Fig. 5 we illustrate the case when a new mutant emerges but an immune response which recognizes this mutant does not appear in the final equilibrium. This does not imply that this equilibrium will only exist if the mutant escapes the immune system; instead it implies that the immune response to the mutant is not required to keep the virus population in check. The availability of target cells sustains the mutant population at a frequency which is too small to elicit the proliferation of CTL in response to this mutant.

Mutations in an antigenically heterogeneous population

We would also like to examine the effects of the generation of an antigenic mutant in a heterogeneous population of viral sub-types. From our analysis of the simpler case, it seems likely that a shift towards a new steady state might occur, diversity might change and virus load (mirrored by the number of infected cells at equilibrium) might be adjusted. We also expect that shifts in the type of immune response elicited by the viral population might be possible.

Antigenic mutation can elicit an immune response against conserved epitopes

As an example, consider the situation illustrated in Fig. 6. The top panel illustrates one such fixpoint transition diagrammatically. On the left we see the population of virus which exists as two strains, y_2 and y_3, each of which is controlled by a specific immune response. A new mutant arises, y_1, which replicates more quickly than either of the two existing

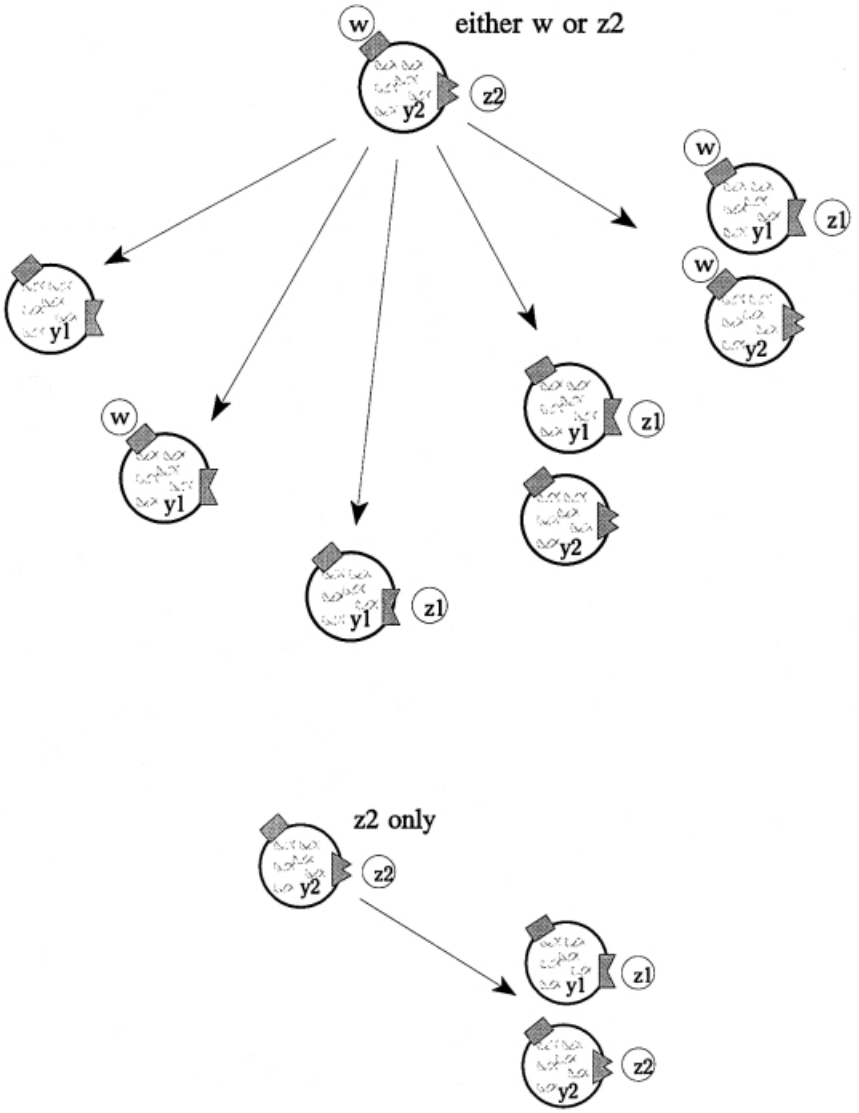

Fig. 4. Transitions from an antigenically homogeneous viral population—pre-existing immune response and faster-replicating mutant. At the top are illustrated the five transitions which can occur in response to a novel mutation, when an immune response pre-exists and the new mutant, y_1, replicates faster than the existing virus, y_2. In two cases the two viral sub-types co-exist, and an immune response specific to the mutant emerges. All of these transitions are possible whether or not the new mutant escapes the pre-existing immune response. One additional transition is illustrated below, which is possible only if the mutant escapes recognition by the pre-existing immune response; in this case the two viral sub-types can co-exist with a specific immune response directed against each.

strains. In this example, the emergence of the mutant elicits both specific (z_1) and cross-reactive (w) immune responses, i.e. immune responses emerge which recognize not only the new epitope but also recognize some epitope which is common to the original viral strains. This transition also causes the specific immune response against the slowest replicating viral strain (z_3) to disappear; the abundance of this strain is now kept at such low levels by the cross-reactive immune response that it does not elicit its own specific immune response effectively.

The lower two panels of Fig. 6 show a numerical simulation of this fixpoint transition. The appearance of the new mutant, y_1, causes a change in the equilibrium values of y_2 and y_3, but both survive the transition. The specific immune response z_3 disappears while the cross-reactive response w emerges. (For a discussion of the oscillatory dynamics of this system, refer to 18.)

From this example it is clear that a new, quickly replicating mutant may cause not only the emergence of an immune response which recognizes every viral strain in the system, but may also cause the disappearance of a specific immune response against another viral strain.

Antigenic mutation can cause the immune response against conserved epitopes to disappear

As a second example, let us consider an immunological system at an equilibrium with both specific and cross-reactive immune responses—both conserved and variable epitopes

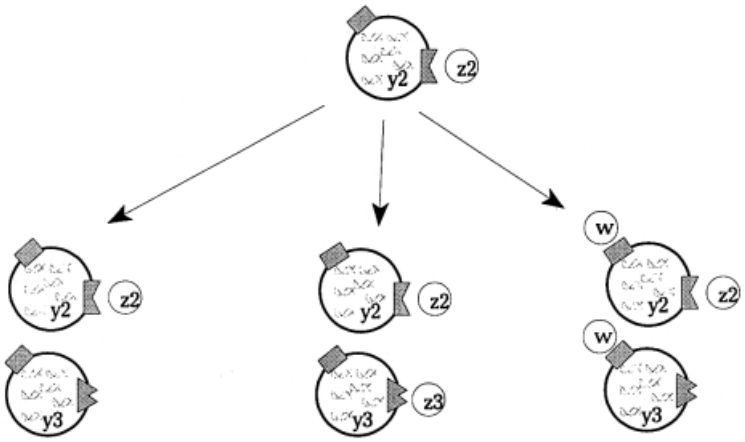

Fig. 5. Transitions from an antigenically homogeneous viral population—pre-existing immune response and slower-replicating mutant. If a new viral strain emerges by mutation and replicates more slowly than the existing viral strain, only three transitions are possible; in all three the new viral strain (y_3) must not be recognized by the current immune response (z_2). The new viral strain may emerge and elicit an immune response specific to itself (z_3, middle transition) or it may elicit an immune response which recognizes both itself and the previously existing virus (w, transition on the right). It is also possible that the new viral strain invades and co-exists with the initial viral population, without inducing any change in the existing immune response (transition on the left).

exist, and both are recognized by the immune system. A diagram illustrating this type of transition is shown in the top panel of Fig. 7. Initially (on the left) three viral strains exist under immune control. When a mutant appears which replicates more quickly than the existing viral strains (y_1), a specific immune response to the invading mutant appears (z_1). In this example, however, the appearance of the new mutant also causes the cross-reactive immune response (and the slowest replicating viral strain) to disappear. The lower two panels show a simulation of this transition; we see that y_4 disappears and after an interlude when the cross-reactive immune response w dominates, w also is eliminated from the equilibrium. Thus we find that the emergence of a novel mutation can cause the disappearance of other virus sub-types and the disappearance of an immune response, even if that immune response recognizes an epitope shared by every viral strain in the system.

'Escape' mutations

As a final example, we consider a system in which only specific immune responses are present at equilibrium (Fig. 8). In this case we simulate a situation in which a new mutant arises which replicates less effectively than the slowest replicating strain.

In the example illustrated, the new mutant emerges but is controlled by the availability of target cells (shown in the third panel, x; note the y-axis labels on the right). If this availability is small, the mutant strain may not grow to the frequency necessary to elicit its own specific immune response. Thus even though the immune system in our model has the capability to mount a specific (or cross-reactive) immune response against the new mutant, neither occurs and the mutant strain grows steadily towards its equilibrium value.

The conditions under which such a mutant is able to invade and survive can be deduced from the steady state conditions derived in (16). These conditions give a range of replication rates for which this phenomenon might be observed: the invading mutant must replicate sufficiently quickly in order to survive, but not so quickly that it elicits an immune response. A further note of interest is that the rate at which the abundance of the new 'escape' mutant grows may be very slow. This rate depends on the availability of target cells, a population which declines as the mutant population increases. Thus the growth rate of the mutant becomes progressively slower over time.

Discussion

Given the initial state of the viral population and immune specificity, a restricted set of immunological changes may occur in response to a novel mutation. We have addressed basic qualitative questions such as whether the new mutation will survive and co-exist with, or replace, the original viral population, and how the immune system may respond to the novel strain. As detailed *in vivo* characterization of CTL specificity becomes available, understanding these transitions will be increasingly important. Since each of these transitions is allowed only for certain well-defined parameter ranges, the experimental observation of any transition will set limits on quantitative variables such as viral replication rates and immunogenicities; conversely, *in vivo* measurement of these parameters will allow for detailed predictions of the immune changes to be expected during chronic infection.

Among this restricted set of possible fixpoint transitions, we note in particular three interesting possibilities:

(i) We predict that under certain conditions a new mutant viral strain may elicit an immune response which recognizes the original strain. This is possible when the cross-reactive epitopes are not expressed at levels high enough to elicit an immune response before the mutant emerges. To our knowledge, there are no experimental data yet available with which this question could be addressed, although the characterization of antigen specific T lymphocytes during infection is becoming feasible (13,15).

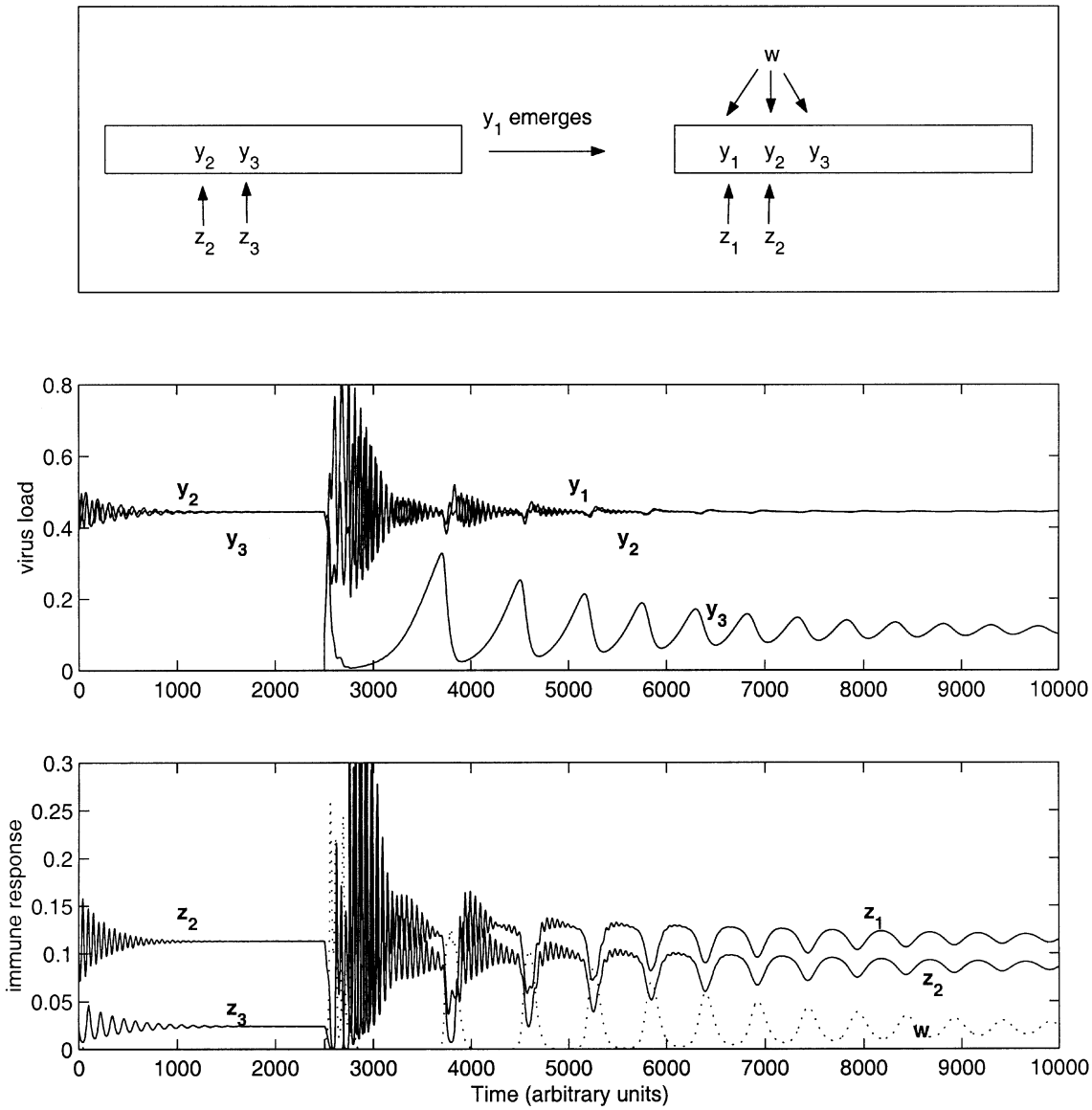

Fig. 6. Antigenic mutation can elicit an immune response against conserved epitopes. In the top panel we illustrate a population of virus which consists of two viral strains, y_2 and y_3. Each is controlled by an immune response which recognizes an epitope specific to that strain. A new mutant arises (y_1) which causes the emergence of both specific and cross-reactive immune responses (z_1 and w), as well as the disappearance of the specific immune response against strain y_3. The middle panel shows a simulation of this transition; the population is seeded with viral strains y_2 and y_3 initially, held in check by specific immune responses. At an arbitrary time (2500), a small amount of viral strain y_1 is injected, as well as small amounts of every possible immune response. All three viral strains survive the transition, along with immune response z_1, z_2 and w. Parameter values specific to this example are: $\beta_1 = 0.08$; $\beta_2 = 0.075$; $\beta_3 = 0.06$; $c_i = 0.9\ \forall i$; $k_i = 0.4\ \forall i$. Parameter values used for this and the following examples are $\lambda = 1$; $d = 0.5$; $a = 0.1$; $p = 0.3$; $q = 0.2$; $b = 0.4$.

(ii) Our analysis clarifies that mutations which reduce the viral replication rate are able to increase in abundance even when the immune system is capable of mounting a response against the novel strain. This can only occur, however, when the invading strain initially escapes recognition by the pre-existing immune responses, i.e. the immune responses which are elicited by the wild-type virus before the new mutation emerges. As the abundance of the mutant increases an immune response to the new strain may emerge; eventually the original viral population will co-exist with the novel strain and the novel immune response.

(iii) We also note examples in which no immune response is mounted against the invading strain, even though both cross-reactive and specific immune responses are available to the immune system. These latter examples are of particular interest because the abundance of the novel mutant will steadily increase without eliciting

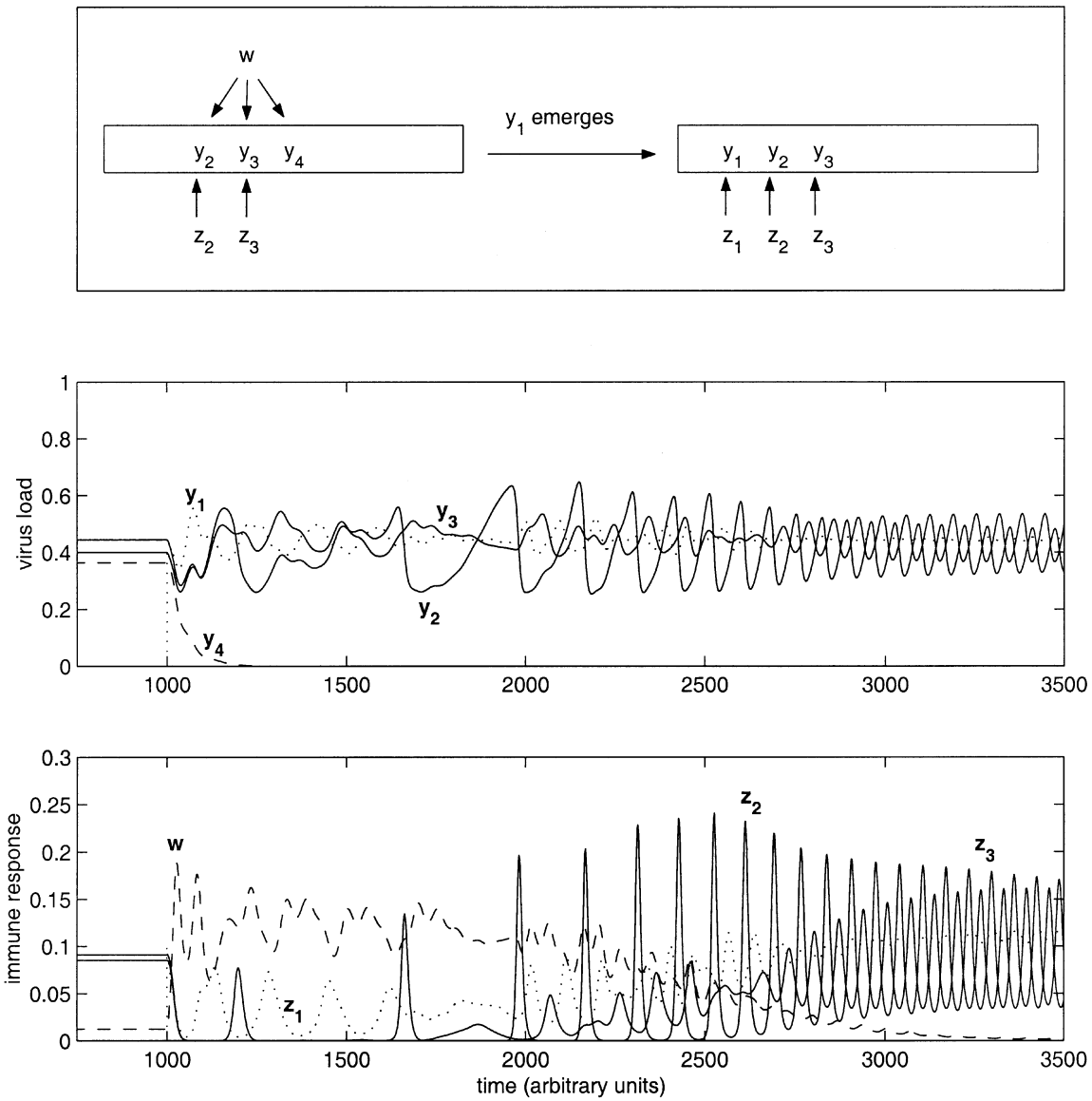

Fig. 7. Antigenic mutation can cause the immune response against conserved epitopes to disappear. In the top panel we illustrate a population of virus which consists of three viral strains, y_2, y_3 and y_4. Each is controlled by an immune response w against a conserved epitope; specific immune responses z_2 and z_3 also control y_2 and y_3 respectively. A new mutant arises (y_1), which causes the emergence of its own specific immune response, z_1, as well as the disappearance of the cross-reactive immune response; the viral strain y_4 also disappears. The middle panel shows a simulation of this transition; the population is seeded with viral strains y_2, y_3 and y_4 initially, as well as all specific and cross-reactive immune responses. At an arbitrary time (1000), a small amount of viral strain y_1 is injected, as well as (again) small amounts of every possible immune response. Only the three most infective viral strains survive the transition, along with the specific immune responses z_1, z_2 and z_3. Parameter values specific to this example are: $\beta_1 = 0.08$; $\beta_2 = 0.076$; $\beta_3 = .075$; $\beta_4 = 0.06$; c_1, c_3 and $c_4 = 0.9$; $c_2 = 1$; k_1 and $k_3 = 0.4$; $k_2 = 0.1$; $k_4 = 0.5$. All other parameters are as provided in the legend to Fig. 6.

an immune response—the viral strain appears to be an 'escape' mutant despite the fact that the immune system is capable of mounting a response against it. This phenomenon may occur when the replication rate of the mutant is slower than the replication rate of the existing virus (and is in fact too slow to elicit an immune response), but is just fast enough to invade. Under these conditions, the rate of increase of the mutant abundance may be very slow. Thus an apparent escape mutation grows slowly to a finite abundance, rather than rapidly out-competing the pre-existing virus [for an interesting clinical example see (13)]. A possible avenue for future study is to determine in greater detail the conditions under which this phenomenon can occur, and to derive realistic growth rates for such escape mutants. For a detailed experimental study of these phenomena, a method to measure the *in vivo* replication rate of virus mutants is required, as well as the detailed CTL specificity of patients against their own virus population.

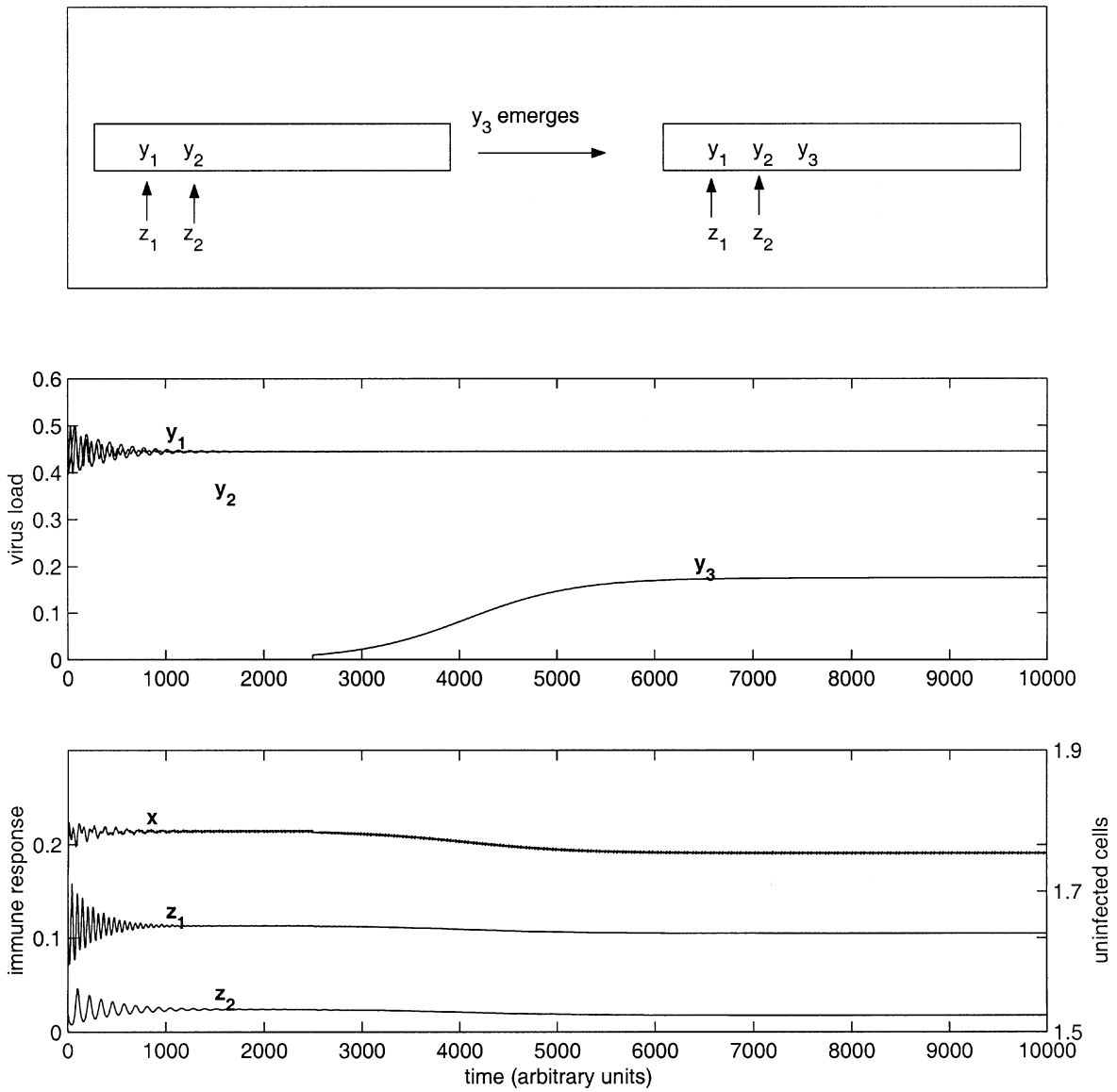

Fig. 8. 'Escape' mutations. In the top panel we illustrate a population of virus which consists of two viral strains, y_1 and y_2. Each is controlled by a specific immune response, z_1 or z_2 as illustrated. A new mutant arises (y_3) which does not cause the emergence of a specific or cross-reactive immune response; the new mutant enters the equilibrium and is controlled by the availability of target cells, x. The second panel shows a simulation of this transition; the population is seeded with viral strains y_1 and y_2 initially, as well as all specific and cross-reactive immune responses. At an arbitrary time (2500), a small amount of viral strain y_3 is injected, as well as (again) small amounts of every possible immune response. All three viral strains survive this transition, with only slight changes to the immune responses z_1 and z_2. The third panel also shows the abundance of uninfected cells (x); note the y-axis scaling on the right. Parameter values specific to this example are: $\beta_1 = 0.075$; $\beta_2 = 0.06$; $\beta_3 = 0.057$; $c_i = 0.9\ \forall i$; $k_i = 0.4\ \forall i$. All other parameters are as provided in the legend to Fig. 6.

The complex dynamics of viral escape from a co-evolving immune response contrast with the well-studied case of escape from anti-viral therapy (19–21), where the selection pressure remains constant.

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Abbreviations

CTL cytotoxic T lymphocyte.

References

1 Saag, M. S., Hahn, B. H., Gibbons, J., Li, Y., Parks, E. S., Parks, W. P. and Shaw, G. M. 1988. Extensive variation of human immunodeficiency virus type-1 *in vivo*. *Nature* 334:440.

2 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. and McMichael, A. J. 1991.

Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* 354:453.
3 Phillips, A. N. 1996. Reduction of HIV concentration during acute infection: independence from a specific immune response. *Science* 271:497.
4 Wolinsky, S. M., Korber, B. T. M., Neumann, A. U., Daniels, M., Kunstman, K. J., Whetsell, A. J., Furtado, M. R., Cao, Y., Ho, D. D., Safrit, J. T. and Koup, R. A. 1996. Adaptive evolution of human immunodeficiency virus-type 1 during the natural course of infection. *Science* 272:537.
5 Zinkernagel, R. M. 1996. Immunology taught by viruses. *Science* 271:173.
6 McMichael, A. J. and Phillips, R. E. 1997. Escape of human immunodeficiency virus from immune control. *Annu. Rev. Immunol.* 15:271.
7 Aebischer, T., Moskophidis, D., Rohrer, U. H., Zinkernagel, R. M. and Hengartner, H. 1991. *In vitro* selection of lymphocytic choriomeningitis virus escape mutants by cytotoxic T lymphocytes. *Proc Natl Acad Sci USA* 88:11047.
8 Klenerman, P., Rowland-Jones, S., McAdam, S., Edwards, J., Daenke, S., Lalloo, D., Koppe, B., Rosenberg, W., Boyd, D., Edwards, A., *et al.* 1994. Cytotoxic T cell activity antagonized by naturally occurring HIV-1 gag variants. *Nature* 369:403.
9 Niewiesk, S., Daenke, S., Parker, C. E., Taylor, G., Weber, J., Nightingale, S. and Banham, C. R. 1995. Naturally occurring variants of human T-cell leukemia virus type I Tax protein impairs its recognition by cytotoxic T lymphocytes and the transactivation function of Tax. *J. Virol* 69:2649.
10 de Campos Lima, P. O., Gavioli, R., Zhang, Q. J., Wallace, L. E., Dolcetti, R., Rowe, M., Rickinson, A. B. and Masucci, M. G. 1993. HLA-A11 epitope loss isolates of Epstein–Barr virus from a highly $A11^+$ population. *Science* 260:98.
11 Bertoletti, A., Sette, A., Chisari, F. V., Penna, A., Levrero, M., Decarli, M., Fiaccadori, F. and Ferrari, C. 1994. Natural variants of cytotoxic epitopes are T cell receptor antagonists for antiviral cytotoxic T cells. *Nature* 369:407.
12 Moskophidis, D. and Zinkernagel, R. M. 1995. Immunobiology of cytotoxic T-cell escape mutants of lymphocytic choriomeningitis virus. *J. Virol.* 69:2187.
13 Borrow, P., Lewicki, H., Wei, X., Horwitz, M. S., Peffer, N., Meyers, H., Nelson, J. A., Gairin, J. E., Hahn, B. H., Oldstone, M. B. A. and Shaw, G. M. 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat. Med.* 3:205.
14 Nowak, M. A., May, R. M. and Sigmund, K. 1995. Immune-responses against multiple epitopes. *J. Theor. Biol.* 175:325.
15 Altman, J. D., Moss, P. A. H., Goulder, P. J. R., Barouch,. D. H., McHeyzer Williams, M. G., Bell, J. I., McMichael, A. J. and Davis, M. M. 1996. Phenotypic analysis of antigen-specific T lymphocytes. *Science* 274:5284, 5294.
16 Bittner, B. and Wahl, L. M. 2000. Immune responses against conserved and variable viral epitopes. *J. Theor. Med.* In press.
17 Wodarz, D., May, R. M. and Nowak, M. A. 2000. The role of antigen-independent persistence of memory CTLp. *Int. Immunol.* 12:467.
18 Nowak, M. A., May, R. M., Phillips, R. E., Rowland-Jones, S., Lalloo, D. G., McAdam, S., Klenerman, P., Koppe, B., Sigmund, K., Banham, C. R. M. and McMichael, A. J. 1995. Antigenic oscillations and shifting immunodominance in HIV-1 infections. *Nature* 375:606.
19 McLean, A. R. and Nowak, M. A. 1992. Competition between zidovudine-sensitive and zidovudine-resistant strains of HIV. *AIDS* 6:71.
20 Bonhoeffer, S., May, R. M., Shaw, G. M. and Nowak, M. A. 1997. Virus dynamics and drug therapy. *Proc. Natl Acad. Sci. USA* 94:6971.
21 Ribeiro, R. M. and Bonhoeffer, S. 1999. A stochastic model for primary HIV infection: optimal timing of therapy. *AIDS* 13:351.
22 Perelson, A. S., Kirschner, D. E. and de Boer, R. 1993. Dynamics of HIV infection of $CD4^+$ cells. *Math. Biosci.* 114:81.
23 De Boer, R. J. and Perelson, A. S. 1998. Target cell limited and immune control models of HIV infection: a comparison. *J. Theor. Biol.* 190:201.
24 Regoes, R. R., Wodwarz, D. and Nowak, M. A. 1998. Virus dynamics: the effect of target cell limitation and immune responses on virus evolution. *J. Theor. Biol.* 191:451.
25 Ribeiro, R. M., Bonhoeffer, S. and Nowak, M. A. 1998. The frequency of resistant mutant virus before antiviral therapy. *AIDS* 12:461.

Appendix: The Model

The model we propose has been examined in detail elsewhere (16), and builds upon earlier models of in-host viral dynamics and the immune response to infection (14,17–25). In brief, the number of healthy cells is given by x, and these are produced at a constant rate λ and die at rate dx. The number of cells which are infected is given by $x\Sigma\beta_i y_i$ where y_i stands for the number of cells which are infected with virus type i, while β_i is the infectivity parameter. Without loss of generality the virus types are numbered so that $\beta_1 > \beta_2 > \ldots \beta_n$ (the higher the infectivity parameter the smaller the index). Infected cells dies at rate ay_i (here we allow a to be smaller than, equal to or larger than d). CTL z_i which are specific for mutants of type i kill infected cells at rate py_iz_i, while the cross-reactive immune response (w) is responsible for the death of qwy_i infected cells. The proliferation rate of CTL in response to each variable epitope is given by $c_iy_iz_i$; the response to the conserved epitope is evoked at rate $w\Sigma k_iy_i$ since all mutants carry this surface structure. The natural death rates of the immune cells—whether they are specific or cross-reactive—are by_i and bw respectively.

Further, we assume that there is a maximum number n different mutant strains. This number can be large. Given certain conditions (parameter values, immunological influences) neither all virus mutants will survive nor all types of immune response. Thus it is necessary to differentiate between the number of surviving mutants, m, and the maximum possible number of mutants, n.

This yields the following system of differential equations:

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

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

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

$$\dot{w} = w\,(\sum_{i=1}^{n} k_i y_i - b) \quad (4)$$

The possible steady states for this system, as well as the conditions under which they exist and are stable, have been enumerated in (16).

A clear limitation of this model, for antigenically heterogeneous viral populations, is that we do not include the effects of CTL which may recognize some, but not all, of the existing viral strains. We have instead made the simplifying assumption that any epitope that is conserved between some viral strains is conserved between all viral strains. We note, however, that for the simplest case this point is moot: the system reduces to two viral strains, and we consider both scenarios when the invading mutant does, and does not, escape immune recognition.