

Immune responses against multiple epitopes: a theory for immunodominance and antigenic variation



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A simple and natural model for the nonlinear interaction between the immune system and multiple epitopes of a genetically variable pathogen can explain the main features of the complex phenomenon of immunodominance. In this model, antigenically homogeneous populations of pathogens stimulate an immunodominant response against a single epitope. In contrast, a heterogeneous pathogen population induces a dynamically complicated array of fluctuating responses against multiple epitopes. Antigenic escape in one epitope can shift immunodominance to other, potentially weaker, epitopes, thereby altering the selective pressures on the pathogen population as a whole. These ideas are compared with detailed studies of the shifting patterns of antigenic variation and cytotoxic T-cell responses seen in HIV-1 infected patients.

Key words: immunodominance / antigenic variation / HIV / cytotoxic T lymphocytes / mathematical model

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Immunodominance

Cytotoxic T lymphocytes (CTL) can identify and remove virus-infected cells. Within infected cells viral proteins are continuously degraded, and the resulting oligopeptides bind to major histocompatibility complex (MHC) molecules. The resulting MHC–peptide complex is brought to the surface of the infected cell where it can interact with the T-cell receptor on the surface of a CTL. If the interaction between the MHC–peptide complex and the T-cell receptor is of sufficient affinity then the CTL may be stimulated to kill the virus infected cell and to proliferate. Whereas MHC molecules are generally rather unspecific and can bind a large number of different peptides (including ‘self-peptides’ that derive from ordinary cellular proteins), the interaction between the T-cell

receptor and the peptide–MHC complex is highly specific thereby ensuing specific immune responses against infected cells and avoiding reactivity against uninfected cells.

A T-cell epitope is usually around 10 amino acids long, and a virus like HIV may have about 10–20 potential epitopes that can be seen by a patient’s CTL.¹ But a typical anti-viral CTL response *in vivo* is only directed against one (or a few) of these epitopes. This phenomenon is known as immunodominance.^{2–6} There is no clear explanation for immunodominance. But it is, of course, well understood that epitopes differ in their ability to induce CTL responses. Let us define the immunogenicity of an epitope as the rate at which it induces CTL proliferation. An epitope is likely to be highly immunogenic if it binds the presenting MHC molecule with high affinity, if the resulting MHC–peptide complex is present in abundance on the surface of the infected cell, and if this complex has a high affinity for the T-cell receptor. The abundance of an epitope will be influenced by the concentration of the source protein, by its intrinsic stability and cellular localization, and by the presence of appropriate proteolytic cleavage sites that lead to efficient antigen processing. Moreover, the immunogenicity of an epitope may also depend on the frequency of specific precursor CTL.

Competition between CTL epitopes: the competitive exclusion principle

Based on these underlying principles, the intuitive expectation is that the immune system mounts a variety of CTL responses against each potential epitope proportional to its intrinsic immunogenicity. But in recent papers^{7,8} we have shown that CTL responses against different epitopes are likely to be in competition with each other and that in a persistent infection a ‘competitive exclusion principle’ leads to a situation where the most immunogenic epitope induces a significant CTL response and all other responses become extinct. The competitive exclusion principle originates from ecological theory and states

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essentially that two (or more) predator species cannot survive on one prey species (as the only food supply). If one predator eats prey faster than its competitor, then it will reduce the prey population size below a threshold level which is necessary to ensure survival of the other predator.

The analogy to CTL and viruses is straightforward: viruses are prey, and CTL are predator. CTL kill viruses and are stimulated by viruses to proliferate. In the absence of stimulation the (active) CTL response declines. The rate of overall CTL proliferation will depend on the viral population size. For a large virus population, the specific CTL population may grow faster than it decays. Conversely for a small virus population, the CTL population will tend to decay. There is a specific virus population size at which the CTL response is at a steady-state. The virus reproduces in the absence of CTL and is killed by CTL. Now imagine two CTL responses reactive against two epitopes. If one response is stimulated faster than the other then it will increase in abundance and reduce the virus population below a threshold level necessary to maintain the other response. Therefore in a persistent virus infection only one CTL response can survive. Competitive exclusion explains immunodominance.

In ecology it is understood that competitive exclusion is not a hard-and-fast rule. Many factors are known which can facilitate survival of both predators, such as spatial heterogeneity, or different predator growth rates at different prey abundances. (One predator may outcompete the other predator at low prey abundance, while the other predator wins at high prey abundance.) Similar factors may operate on CTL-virus interactions, and one cannot rule out situations where different CTL responses coexist in a persistent viral infection. But the important point is that the most natural, straightforward models for CTL-virus interaction will have the feature that CTL responses against different epitopes are in competition with each other, and therefore it is likely that (in an anti-viral immune response) one CTL response will predominate. The model also does not rule out the possibility that in an acute viral infection, which is cleared by CTL, a range of responses against different epitopes are activated simultaneously. This reflects a transient, non-steady-state situation where competitive exclusion does not apply.

Antigenic variation can change the pattern of immunodominance

The discussion of the previous section is based on the assumption of an antigenically homogeneous virus population. In this section I will discuss the consequences of antigenic variation in multiple epitope responses.

Antigenic variation in CTL epitopes has been shown for HIV,^{9,10} and other viruses,¹¹⁻¹⁵ but it has been said that antigenic escape in a single epitope cannot be of great significance given that a typical virus contains a large number of potential epitopes. The mathematical model (see next section), however, will make it clear that antigenic escape in one epitope can lead to important changes in the genetic structure of the virus quasispecies and also in the specificity of the immune responses. The unseen escape mutant will initially have a certain (small) selective advantage and will increase in frequency, but then there is a complex range of dynamical possibilities, and the major consequence of escape in one epitope is a shift of the immunodominant response to another epitope. This shift of immunodominance to another epitope alters the selection pressure on the virus population as a whole. We find situations where the new mutant never induces a specific response against the mutated epitope and yet comes to equilibrium (steady-state) at some arbitrary (high or low) frequency. Thus in multiple epitope responses antigenic variation can lead to unseen escape mutants which do not grow to fixation (100% frequency). There are also complex fluctuations of immune reactivity to various epitopes, and the predominant response can fluctuate backwards and forwards among different epitopes, accompanied by oscillations in viral abundance with individual peaks dominated by single viral genotypes (antigenic oscillations).

The model

Assumptions

The model keeps track of the population sizes of virus mutants and of activated CTLs. Each virus variant has several epitopes which can stimulate different CTL responses. In each epitope there can be a number of variant peptide sequences inducing different lines of immune cells. The immunogenicity of an epitope sequence may be defined as the rate of production of CTL specific to that epitope, by activation of resting

memory CTL and proliferation of activated CTL. Activated CTLs are derived from a pool of precursor cells by stimulation with their specific MHC–peptide complex. Already-activated cells are also stimulated to proliferate by interaction with their specific MHC–peptide complex. (In our model precursor cells include both virgin CTL and resting memory CTL. Activated cells include dividing memory CTL and effector CTL.) Individual virus mutants may replicate at different rates, and so mutations in the epitopes may affect the overall replication rate. With these basic assumptions, the model takes the following form (for simplicity, we discuss a model with two epitopes, but our results can be generalized to several epitopes).

The basic model

Virus dynamics:

$$dv_{ij}/dt = v_{ij}(r_{ij} - p_i x_i - q_j y_j), \quad i = 1, \dots, n_1 \text{ and } j = 1, \dots, n_2. \quad (1)$$

Immune responses against epitope A:

$$dx_i/dt = \eta c_i v_{i^*} + x_i(c_i v_{i^*} - b), \quad i = 1, \dots, n_1. \quad (2)$$

Immune responses against epitope B:

$$dy_j/dt = \eta k_j v_{*j} + y_j(k_j v_{*j} - b), \quad j = 1, \dots, n_2. \quad (3)$$

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 $n_1 \times n_2$ possible 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 lines directed at the various A variants and n_2 against the B variants. The virus variant v_{ij} reproduces at the (average) rate r_{ij} . Different virus mutants can have different reproduction rates. Virus-infected cells are killed by CTL responses at the rates $p_i x_i v_{ij}$ and $q_j y_j v_{ij}$, where p_i and q_j are rate constants specifying the kinetics of removal of infected cells. CTLs are stimulated by their specific epitope sequence (in association with MHC presentation). They are either produced by activation from a pool of precursor cells (at the rates $\eta c_i v_{i^*}$ and $\eta k_j v_{*j}$) or by proliferation of already activated cells (at the rates $c_i x_i v_{i^*}$ and $k_j y_j v_{*j}$). The factor η describes the ratio of the rate at which CTLs are activated from precursor cells over the rate of proliferation of already-activated CTLs. Thus a

small η implies that most activated CTLs arise from proliferation of already-activated cells; this will usually be what actually happens. We have used the notation $v_{i^*} = \sum_j v_{ij}$ and $v_{*j} = \sum_i v_{ij}$. Thus a particular CTL clone recognizes all viruses that have the specific sequence in the appropriate epitope; i.e. x_i is directed at (and is stimulated by) v_{i^*} , whereas y_j recognises v_{*j} . The constants c_i and k_j describe the immunogenicities of sequence i in epitope A and sequence j in epitope B, respectively. In this simplest model, it is assumed that immunogenicity is a property of the particular epitope sequence and is unaffected by mutations in the other epitope, but the model can be generalized to include such interactions. Finally we assume that in the absence of antigenic stimuli the activated CTLs decline at the rates $b x_i$ and $b y_j$. The main properties of the above model can be understood by a combination of analytic and numerical studies.

Results of the model

Virus variability determines the pattern of immunodominance

For an antigenically homogeneous virus population, the model predicts ‘complete immunodominance’, i.e. eventually the whole response will be directed against one epitope. The epitope with highest immunogenicity is immunodominance. Thus for a homogeneous virus population, immunogenicity determines immunodominance.

In a heterogeneous virus population, we will either find complete immunodominance or coexistence of responses against different epitopes. If all virus variants have the same replication rate (the ‘neutral’ approximation), then there is always complete immunodominance, and a simple inequality determines the competition: in the system described by eqs (1–3) the responses against epitope A will eventually win if

$$\sum_{i=1}^{n_1} 1/c_i < \sum_{j=1}^{n_2} 1/k_j. \quad (4)$$

If all variants of a given epitope have the same immunogenicity, i.e. $c_i = c$ and $k_j = k$, then inequality (4) becomes $c/n_1 > k/n_2$. Thus immunodominance is a function both of the immunogenicity and of the diversity of the individual epitopes. Antigenic diversity effectively reduces the immunodominance of an epitope. The above result strictly holds only for $\eta = 0$, i.e. neglecting the recruitment of activated CTLs from inactivated precursor cells. In effect, this corresponds

to the realistic assumption that *most* of the activated CTLs arise from proliferation of already-activated cells. For $\eta > 0$ there is always a coexistence between responses against different epitopes, but for small η the response against the non-immunodominant epitopes will be weak, and may be below experimental detection thresholds.

If individual virus mutants have different replication rates, then it is possible, but unlikely, to have complete immunodominance. The conditions for such complete immunodominance are complex,⁸ but if complete immunodominance does arise then it can only be directed at the epitope with the smallest $\sum 1/c_i$. But in general we may expect coexistence of responses against several epitopes in antigenically heterogeneous virus populations. Thus a lack of immunodominance may occur in systems with a large number of different virus mutants where the mutations in the epitopes alter the replication rates, and where different epitopes have comparable immunogenicity.

Partial immune escape

The model can be expanded to include cross-reactivity among the individual peptide sequences of an epitope. Suppose there are n_1 sequences in epitope A (all with immunogenicity c) and n_2 sequences in epitope B (all with immunogenicity k). Let us further assume that any sequence in epitope A cross-stimulates the response against any other sequence in epitope A at the rate c_1 (with the s_1 denoting the cross-reactivity parameter of epitope A, $0 \leq s_1 \leq 1$). Similarly we define s_2 for epitope B. Then immunodominance is decided (in favour of epitope A) by the inequality

$$\frac{c}{n_1} [1 + (n_1 - 1)s_1] > \frac{k}{n_2} [1 + (n_2 - 1)s_2]. \quad (5)$$

This result shows that for a homogeneous virus population (maybe during the initial phase of an infection), where n_1 and n_2 are one, the decisive parameter is simply immunogenicity (c versus k). For more heterogeneous virus populations, with larger n_1 and n_2 , the amount of cross-reactivity within the peptides of an epitope becomes important and maybe decisive for immunodominance. This means that during an HIV infection, for example, there may be a tendency to go from responses against highly immu-

nogenic epitopes toward less immunogenic, but more cross-reactive, epitopes.

Antigenic oscillation in persistent virus infections

The model can generate very complicated time series, with distinct peaks in viral abundance containing different antigenic variants. We call this phenomenon ‘antigenic oscillation’: such oscillations arise as a consequence of the non-linear dynamics of the immune responses acting on existing viral diversity. The peaks are often dominated by single viral genotypes. Such peaks occur whenever the response against a particular virus variant has declined to low levels (because of a lack of stimulation). For these antigenic oscillations to occur, *it is not essential that mutation continuously generates new antigenic material.*

Antigenic oscillations are different from the usual concept of antigenic drift, where the emergence of escape mutants is thought to cause peaks of viral abundance.¹⁶⁻²¹ For antigenic drift continuous production of escape mutations is essential, whereas for antigenic oscillations the diversity can be there from the beginning, and the peaks arise as a consequence of the oscillatory dynamics.

Antigenic oscillation causes fluctuation in the CTL response

Antigenic oscillation can also happen in situations with a predominating response against a single epitope that contains a number of antigenic variants. If there are several epitopes of comparable immunogenicity, then the antigenic oscillations are generally accompanied by oscillations in the size and specificity of the CTL responses against the individual epitopes. Sometimes the response against one epitope predominates, sometimes the response against another epitope. In this sense, antigenic oscillations may be accompanied by fluctuating immunodominance.

Figure 1 is a computer simulation of eqs (1-3) with two variants in each of the two epitopes. Thus in total there are four virus variants, two specific responses against epitope A, and two specific responses against epitope B. The figure shows oscillations in the total viral abundance, in the immune responses against the two epitopes, and in the genetic composition of the two epitopes. All the virus mutants are already present at the beginning of the simulation, and there is no generation of new mutants. Peaks of viral abundance consist largely of individual mutants. The oscillations are weakly damped, on a time scale of the order of

magnitude $1/\eta$ for small η the oscillations persist for a very long time. The asymptotic equilibrium state may never be reached once we also allow for the emergence of escape mutants.

Consequences of the emergence of a new virus mutant: four different outcomes

We now extend our analysis of the dynamics of the basic model by asking: how does the emergence of

such a mutant affect the immune responses against the whole (heterogeneous) virus population? What are the selective advantages of the new mutant? Will the new mutant grow to fixation, or may we expect a coexistence among different virus mutants? Will there be a shift in immunodominance to other epitopes?

One of the central points of the model is an understanding of the events following the emergence of a new mutant, in situations with several potential epitopes. Consider a homogeneous virus population

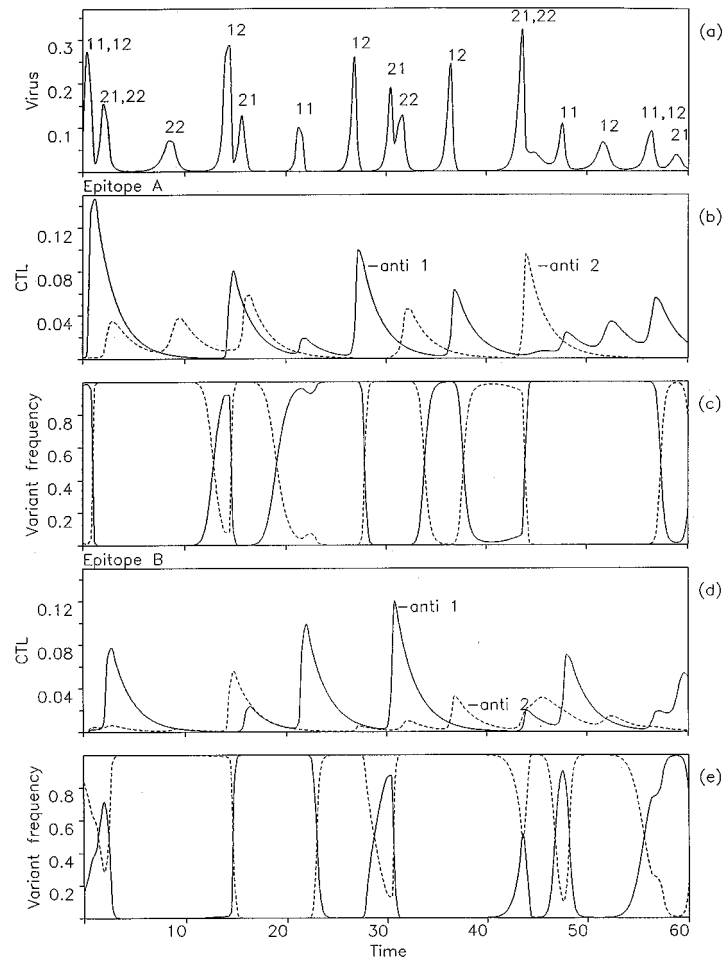


Figure 1. Antigenic oscillations and fluctuating immunodominance in a model with two epitopes. Peaks of viral abundance that consist of antigenically different variants are accompanied by oscillations in the size and specificity of the CTL responses. All virus variants are present at the beginning of the simulation; there are no additional mutational events. In each epitope we have two antigenically different variants that induce specific CTL responses. The four peptide sequences have different immunogenicities, and the four virus variants have different replication rates. The figure shows the transient dynamics; eventually the oscillations will settle down to an equilibrium. (a) Total virus abundance, (b) CTL response against epitope A, (c) frequency of the two different peptides in epitope B, (d) CTL response against epitope B, (e) frequency of the two different peptides in epitope B. The parameters of the simulation are: $r_{11} = 0.2$, $r_{12} = 0.15$, $r_{21} = 0.16$, $r_{22} = 0.1$, $c_1 = 1$, $c_2 = 1.1$, $k_1 = 1.9$, $k_2 = 0.8$, $p_i = q_j = 5$, $b = 0.02$, $\eta = 0.0001$. Reprinted with permission from *Nature* 375: 606-611 (1995) Macmillan Magazines Limited.

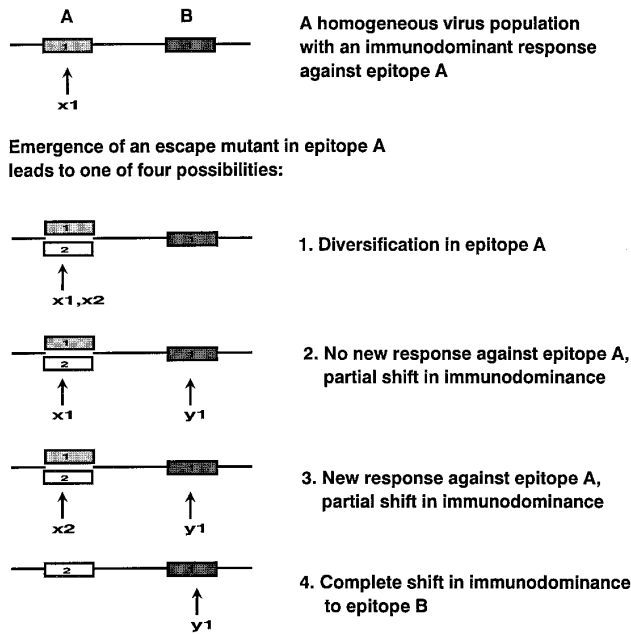


Figure 2. Antigenic variation can shift immunodominance. Consider a homogeneous virus population, v_{11} , which is exposed to CTL responses against two epitopes, A and B. The immunogenicity of epitope A is c_1 and of epitope B is k_1 . Assume that $c_1 > k_1$. Hence the response against epitope A is immunodominant. Suppose an escape mutant, v_{21} , emerges in epitope A. The immunogenicity of the variant sequence in epitope A is denoted by c_2 , and the replication rates of the original variant and the escape mutant by r_{11} and r_{21} , respectively. The CTL responses against peptides 1 and 2 of epitope A are denoted by x_1 and x_2 , the response against peptide 1 of epitope B by y_1 . For the following considerations we use the limiting case $\eta = 0$. The emergence of the escape mutant leads to one of four outcomes: (i) It can simply lead to a diversification in epitope A without stimulating an immune response against epitope B. This happens if $1/c_1 + 1/c_2 > 1/k_1$. The system converges to oscillations around an equilibrium with $v_{11}, v_{21}, x_1, x_2 < 0$ and $y_1 = 0$. (ii) The new mutant may not elicit a specific immune response against itself in epitope A, but may induce a partial shift in immunodominance to epitope B. This happens if $1/c_1 + 1/c_2 < 1/k_1$ and $r_{11} > r_{21}$. The system converges to oscillations around an equilibrium with $v_{11}, v_{21}, x_1, y_1 > 0$ and $x_2 = 0$. (iii) The new mutant may induce a specific response against itself in epitope A, which outcompetes the response against the original virus, and induces a partial shift in immunodominance. The conditions for this behaviour are $1/c_1 + 1/c_2 > 1/k_1 > 1/c_2$ and $r_{11} < r_{21}$. The system converges to oscillations around an equilibrium with $v_{11}, v_{21}, x_2, y_1 > 0$ and $x_1 = 0$. (iv) Finally, the new mutant can induce a complete shift in immunodominance to epitope B. This happens for $1/c_2 > 1/k_1$ and $r_{11} < r_{21}$, and brings us eventually to oscillations around an equilibrium with $v_{21}, y_1 > 0$ and $v_{11}, x_1, x_2 = 0$. For case (i) and (iii) a homogeneous population of the mutant, v_{21} , would induce an immunodominant response against epitope A, whereas for case (iv) it would induce immunodominance of epitope B. For case (ii) it is not specified.

subject to immune responses against two epitopes, A and B. Suppose that the response against epitope A is immunodominant. The emergence of an escape mutant in A can lead to one of four different outcomes (Figure 2), 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 simply a 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 is necessarily accompanied by an increase of the response against epitope B, thus again 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). Figure 2 gives a schematic illustration of these possibilities, together with the relevant conditions for the rate constants.

Figure 3 shows shifting immunodominance in a model with responses against seven epitopes. Originally there is a homogeneous virus population which induces a dominant response against epitope 5. An escape mutant appears in epitope 5, and the new heterogeneous virus population induces a predominant response against epitope 2. When antigenic variation occurs in epitope 2, epitope 4 becomes immunodominant. Note that the relative degree of immunodominance declines as the virus population becomes more heterogeneous.

Summary

Thus antigenic variation in models with multiple epitopes is qualitatively different from the simple escape dynamics of single epitope models. In multiple epitope models we can find situations where the escape mutant does not reach genetic fixation or dominate the population. The mutant may fail to induce a specific response against the new peptide

Thus shifting immunodominance does not simply reflect the immunogenicity of the escape mutant. (Note that the peptide sequences in epitopes A and B are generally assumed to be different. The numbers 1 and 2 only count the peptide sequence in the relevant epitope.) Reprinted with permission from *Nature* 375: 606-611 (1995) Macmillan Magazines Limited.

even if the peptide is potentially immunogenic. The new mutant may induce a shift in immunodominance to another epitope, even if the homogeneous population of the mutant induces a response against the peptide where the mutation occurred. In multiple epitope models the most important consequence of antigenic variation is a shift of the immunodominant response to other (weaker) epitopes. This can

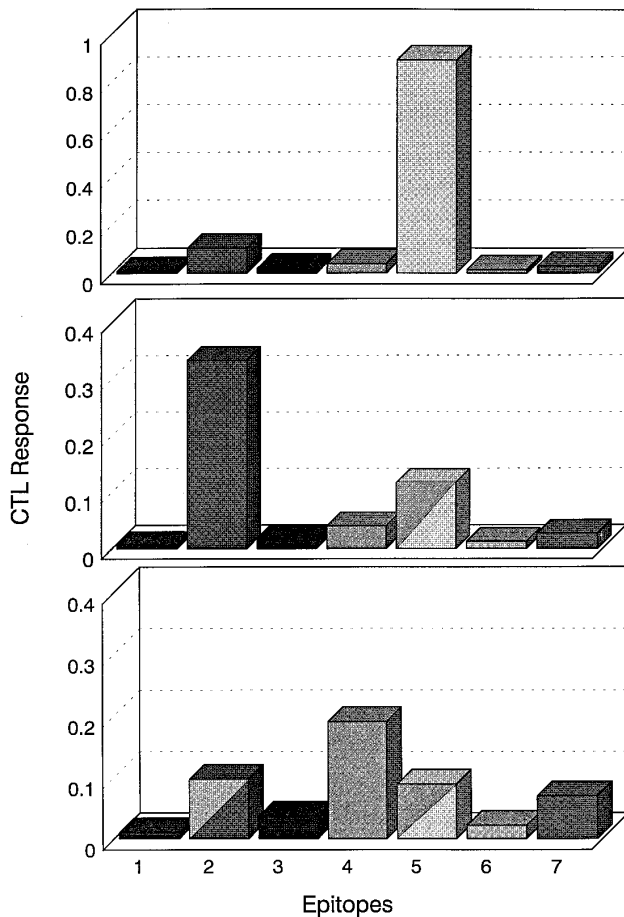


Figure 3. Antigenic variation and shifting immunodominance in a computer simulation of responses against seven epitopes. The three panels represent the relative magnitudes of the CTL response against the 7 epitopes at three successive stages of the persistent infection. Epitope 5 is most immunogenic, followed by epitopes 2 and 4. Originally the virus population is homogeneous and induces a dominant response against epitope 5 (top). Following the emergence of an escape mutant in epitope 5, the dominant response is diverted against epitope 2 (middle). An escape mutant in epitope 2 shifts the major response to epitope 4 (bottom). As the virus population becomes more heterogeneous the degree of immunodominance decreases and in the bottom panel there are essentially coexisting responses of similar magnitude.

increase the viral load and can thus represent a route to disease progression in persistent viral infections such as HIV.

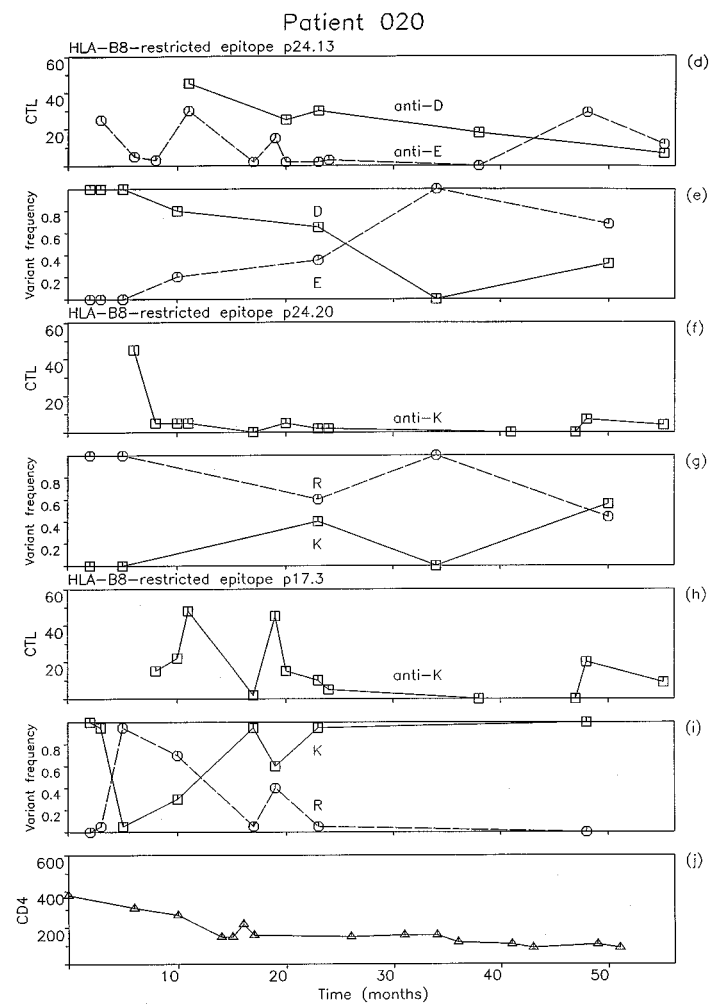
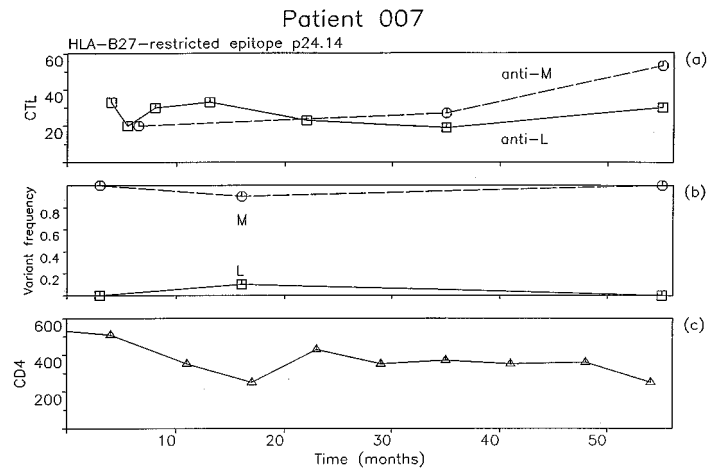
HIV

CTL are believed to play an important role in controlling HIV infections,²² but it has not been possible to quantify the contribution of CTL mediated killing to the decay of infected cells. In acute seroconversion illness, specific CTL directed against the HIV proteins gag, pol and env are detectable as early as two days after the onset of symptoms.²³ The rise in HIV-specific CTL is associated with a fall in viraemia and occurs weeks before neutralizing antibodies are detectable.²⁴ Long-term survivors have persistent CTL responses while the development of AIDS is associated with a loss of CTL activity.²⁵ Antigenic variation and escape from CTL responses may contribute to viral persistence.

Figure 4 shows longitudinal studies from HIV infected patients followed for 55 months. Patient 007 has a sustained CTL response to a single HLA B27 restricted epitope in Gag, and both the major and minor variant are seen by his CTL with comparable efficiency. A weak CTL response against Pol was seen on one occasion only and no responses have been found to Nef or Env. This individual remains well 10 years after acquiring the infection. Patient 020 shows antigenic variation within three HLA-B8-restricted Gag epitopes. In all three epitopes there were variants which at certain times were not seen by the patients' CTL. Some of these 'unseen' escape mutants apparently increased in abundance to about 100% whereas others lingered at low frequencies. There were also fluctuations in the predominant CTL response among the three Gag epitopes. No CTL responses were found to other epitopes. The patient had a progressive loss of CD4 cells, developed AIDS within 8 years of infection, and died from a gastrointestinal haemorrhage.

Conclusions and implications

1. *Antigenic oscillations, that is, fluctuations in the abundance of a particular antigenic variant, 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 variants. 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 in the immune response. This is a consequence of our model assumption that the CTL pressure against a certain peptide sequence declines if this sequence is no longer presented to the immune system in sufficient amount.

2. *Immunodominance is a function both of the immunogenicity and of the antigenic diversity of the epitopes*; it also depends on the *replication rate* of the various mutants. In a homogeneous virus population we expect a predominant response against one epitope. In a heterogeneous virus population in which all virus mutants have similar replication rates, then again there should be a single immunodominant epitope; only the response against one epitope can survive in the long run, and all other responses have to vanish. The epitope that maximizes the ratio of immunogenicity over diversity is immunodominant. If the virus mutants have different replication rates and if the immunogenicities of the various epitopes are comparable, then in general we expect coexistence of immune responses against several epitopes.
3. *Antigenic variation can shift immunodominance to other*

Figure 4. Serial studies of cytotoxic T-lymphocyte recognition of epitope variants in two HIV-1 positive haemophilic patients.⁷ Patient 007: This individual has an HLA B27 restricted response to a single epitope in *gag* (p24–14).¹⁷ (a) Percentage of specific lysis of HLA matched target cells treated with either the M variant (KRWIIMGNK), indicated by the circles, or the L variant (KRWIILGNK), indicated by the squares. (b) Relative frequency of M and L variants in the proviral DNA extracted from peripheral blood mononuclear cells. (c) Serial CD4 counts. Patient 020 has an HLA B8 restricted CTL response to three epitopes in HIV-1 *gag* (p24–13, p24–20, and p17–3). (a) Percentage specific lysis, as for 007, of HLA matched target cells treated with either the D variant (GDIYKRWII), indicated by squares, or the E variant (GEIYKRWII), indicated by circles. (b) Relative frequency of D and E variants in proviral DNA. (c) Percentage specific lysis of HLA matched target cells treated with the K variant (DCKTILKAL), indicated by squares. The R variant (DCRTILKAL) was never recognized by this patient's CTL. (d) Relative frequency of the K and R variants of p24–20 in the proviral DNA. (e) Percentage specific lysis of HLA matched target cells coated with the K variant peptide (GGKKKYKLLK), indicated by squares. The R variant (GGRKKYKLLK) was never seen by this patient's CTL. (f) Relative frequency of the K and R variants of p17–3 in proviral DNA. (g) Serial CD4 counts. For methods see refs 7 and 9. Reprinted with permission from *Nature* 375: 606–611 (1995) Macmillan Magazines Limited.

epitopes. The emergence of an escape mutant in one epitope can induce a partial or complete shift of immunodominance to another epitope. Thereby the selective advantage of such an escape mutant may become negligible. The escape mutant may not reach genetic fixation even if it fails to induce a specific response against the variant peptide. More generally, this argument predicts that strongly immunogenic epitopes are more likely to be found in relatively conserved regions since immunodominance is determined by immunogenicity and antigenic diversity. The emerging picture is one where CTL responses exert selection pressure on virus variants, and conversely virus variants select which immune responses are dominant.

4. The present model has been developed with respect to CTL responses against HIV, but has a much wider potential. It represents a mathematical framework for any kind of immune response (CD8+, CD4+, or antibody responses) against multiple epitopes of a replicating pathogen.²⁶ In HTLV-1 infection antigenic variation in CTL epitopes have been observed, together with coexisting CTL responses against several different epitopes.^{14,15}
5. With respect to HIV, *our models reinforce the notion that viral diversity and evolution during infection can lead to AIDS.* In models with multiple epitopes, antigenic diversification can shift immunological pressure towards weaker epitopes, which may increase overall viral loads. Assuming that CTL proliferation needs some help from CD4+ T cells, and recognizing that HIV can impair CD4+ cell function, we obtain a model for disease progression with an antigenic diversity threshold.^{7,20} Diversity may accumulate in the relevant epitopes until the immune system can no longer control the HIV population.
6. Understanding the interaction between immune responses and antigenically varying epitopes has *implications for the design of a vaccine against HIV.* Because of the intrinsic competition among CTL responses against different epitopes, it may be best to boost the response against a single, *conserved* epitope (even if it is not the natural immunodominant epitope), thereby hoping to induce a stable pattern of CTL recognition.

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