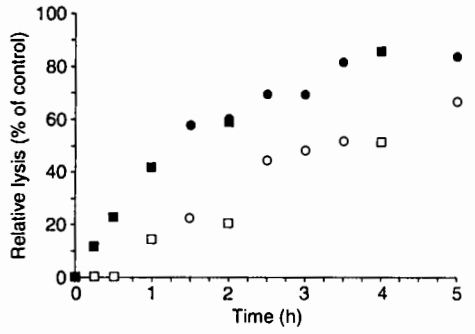

FIG. 4 Kinetics of H3 and M1 presentation. The human B-cell line 721.45 was pulsed for the indicated times with a 10^{-5} dilution of ultraviolet-inactivated influenza virus[8] and tested for lysis by the H3-specific clone E1.9 (filled symbols) and by the M1-specific clone C3.5 (open symbols) at an effector to target ratio of 8. Squares and circles represent data obtained in two separate experiments. Data are expressed as relative lysis as described in Fig. 2. Specific lysis with synthetic peptide was 81.5% and 78.2% for H3, 55.3% and 53.9% for M1, in the two experiments, respectively.

may interact jointly with other molecules that control internalization.

To test whether the presentation pathway involving internalization of DR molecules can be used by other antigens, cells were pulsed with myelin basic protein (MBP) that was purified in its native lipid-bound form[21], and were assayed for lysis by HLA-DR1-restricted MBP-specific T cells (Fig. 3*c*). Presentation was greatly reduced in 8G9-αΔβ and 8G9-βΔα cells, even though these two cells presented a synthetic MBP peptide as efficiently as 8G9-DR cells. H3 (ref. 8) and, as reported previously[22], MBP presentation did not require Ii (Fig. 3*c*). The requirement for internalization was also observed in the absence of Ii (Fig. 3*c*). Thus the new presentation pathway described here applies to different types of immunologically relevant antigen.

Complexes of class II molecules with high-affinity peptides do not detectably dissociate[23,24]. However, a fraction of class II molecules is occupied by lower affinity peptides[25] and by Ii-derived CLIP peptides[26] that may exchange with other peptides after internalization of class II molecules. The compartment for peptide loading on recycling class II molecules is probably distinct from the one where newly synthesized class II molecules are transported, because H3 failed to be presented by cells expressing Ii with truncated DR molecules, in which M1 presentation was normal. The pathway described here may be useful for the presentation of antigens that are rapidly degraded after uptake by antigen-presenting cells. □

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Antigenic oscillations and shifting immunodominance in HIV-1 infections

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A TYPICAL protein antigen contains several epitopes that can b recognized by cytotoxic T lymphocytes (CTL), but in a character istic antiviral immune response *in vivo*, CTL recognize only a sma number of these potential epitopes, sometimes only one[1,2], thi phenomenon is known as immunodominance[1–10]. Antigenic varia tion within CTL epitopes has been demonstrated for the humai immunodeficiency virus HIV-1 (ref. 11) and other viruses[12–17] an such 'antigenic escape' may be responsible for viral persistence Here we develop a new mathematical model that deals with th interaction between CTL and multiple epitopes of a geneticall variable pathogen, and show that the nonlinear competition amon CTL responses against different epitopes can explain immuno dominance. This model suggests that an antigenically homogen eous pathogen population tends to induce a dominant respons against a single epitope, whereas a heterogeneous pathogen popula tion can stimulate complicated fluctuating responses against multi ple epitopes. Antigenic variation in the immunodominant epitop can shift responses to weaker epitopes and thereby reduce immuno logical control of the pathogen population. These ideas are consist ent with detailed longitudinal studies of CTL responses in HIV-i infected patients. For vaccine design, the model suggests that th major response should be directed against conserved epitopes ever if they are subdominant.

In HIV-1 infection, there are strong CTL responses to severa epitopes, and evidence is mounting that CTL are important i controlling the virus[18–23]. HIV persistence and disease progres sion may rely partly on evasion of CTL responses by antigeni variation. We have followed CTL responses in two HIV-infected haemophiliac patients for 55 months (Fig. 1) and fin that their responses are very different. Patient 007, who remain well, has a sustained CTL response to a single HLA-B27 restricted gag epitope. A weak response to pol was found or only one occasion and no CTL responses have been found t nef or env, nor to any other epitope in gag. In the gag epitop we found one predominant and one rare variant, but both

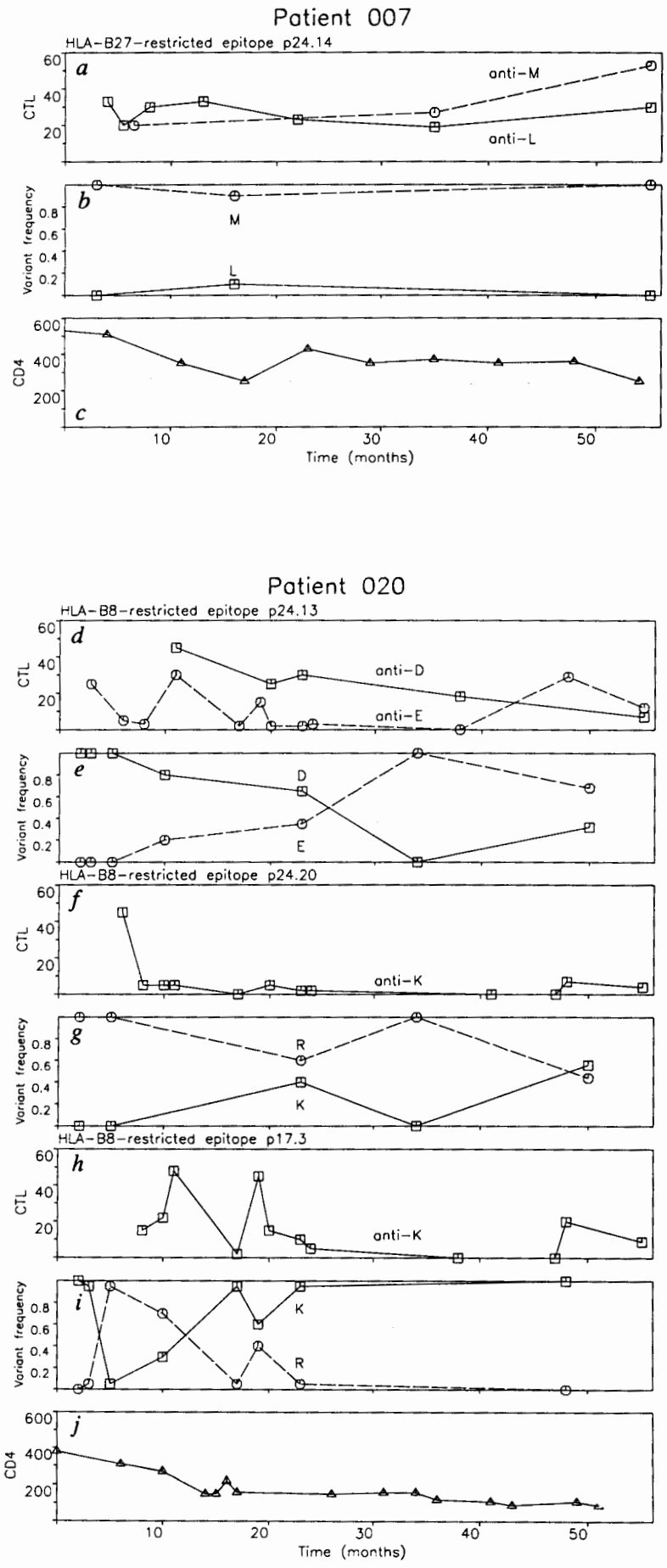

FIG. 1 Serial studies of CTL recognition of epitope variants in two HIV-1 positive haemophiliac patients. Patient 007 has a sustained response to the HLA-B27-restricted epitope gag p24.14(263–272). *a*, Percentage of specific lysis of HLA-match target cells treated with the peptides KRWIIMGNK (single-letter amino-acid codes, M variant) or KRWIILGNK (L variant). *b*, Sequencing of proviral DNA revealed that virtually all virus in peripheral blood mononuclear cells (PBMC) consists of the M variant rather than the L variant, which represents the database consensus sequence. c, Patient 007 is a slow progressor, with a slow decline in CD4 T-cell count to 300 cells per μl. Patient 020 made responses to three HLA-B8-restricted epitopes in gag, designated p24.13(259–267), p24.20(329–337) and p17.3(24–32). *d*, The patient's CTL fluctuate in their capacity to recognize the two variants of epitope p24-13, GEIYKRWII (E variant) and GDIYKRWII (D variant), probably because of changes in the relative abundance of specific CTL clones. Both peptides bind HLA B8 with similar efficiency (S.McA. *et al.*, manuscript submitted). e, Changes in frequency of the p24.13 D and E variants. Initially only the D variant was detected in proviral DNA. Loss of the CTL response against the E variant at around 20 months was associated with its emergence as the only detectable variant. This appears to be an example of escape from a CTL response at a time when the circulating CTL clones fail to recognize the variant; later a crossreactive response returned and the selection pressure altered. *f*, CTL response to p24.20 variants. This response must be considered in conjunction with the response to p24.13, 60 amino acids upstream in HIV gag. The CTL response against the K variant was initially present and then lost. *g*, Frequency of p24.20 variants. The peptide DCRTILKAL (R variant), which cannot be recognized by the patient's CTL although it binds to HLA B8, (S. McA. *et al.*, submitted), predominated. Later, the recognizable variant, DCKTILKAL (K variant), dominated but there were no specific CTL detectable. The linkage of variants in p24.13 and p24.20 changed: at 23 months the D + R variants were linked, whereas at 34 months the E + R variants were the majority species. *h*, The CTL response against p17.3 was variable as the predominant sequence shifted from GGKKKYKLK (K variant) to GGRKKYKLK (R variant) and back. CTL never recognized the R variant[11], although it binds to HLA B8 (S.McA. *et al.*, submitted). *i*, Changing frequency of the p17.3 variants. The K variant became more abundant when the CTL response to this epitope was low. *j*, Serial CD4 counts. Patient 020 had a progressive loss of CD4 T cells, developing AIDS with a CD4 T-cell count of <200 per μl, and pneumocystis pneumonia. He died of a gastrointestinal haemorrhage.

METHODS. CTL cultures were induced by incubating PBMC with autologous phytohaemagglutinin(PHA)-activated PBMC for 10–14 days (ref. 11, and S.McA. *et al.*, submitted). Killer-to-target ratios were between 30:1 and 50:1. Standard errors of the CTL responses were less than 10% of the specific lysis values. Peptides were synthesized by f-moc chemistry on an automated Zinsser peptide synthesizer. Proviral DNA was extracted from lymphocytes after brief (5–7 d) culturing with PHA and subjected to nested polymerase chain reactions (PCR)[11]. At each time point we sequenced at least 20 M13 recombinant clones. Amplification was carried out with positive and negative controls. Sequence data from the adjacent epitopes p24-13 and p24-20 were obtained from a PCR reaction which amplified both epitopes simultaneously.

variants are seen by the patient's CTL. Patient 020, who progressed to AIDS in 8 years, showed variation within three HLA-B8-restricted gag epitopes[11]. We found variants in all three epitopes that were not seen at certain time points by the patient's CTL. There is some evidence for selective increase of such escape variants: the p24.13E variant increased in frequency from 0 to 100% at a time when specific CTL failed to recognize this epitope. Similarily, a poor CTL response to the p17.3K variant is associated with accumulation of this variant. But the pattern is complex overall, and we observe changes in the frequency of variant viruses with shifting immunodominance in the CTL responses.

To explain these observations and to develop a general framework for understanding immunodominance and antigenic variation in several epitopes, we designed a new mathematical model for the dynamic interaction between virus mutants and activated CTL. Each virus mutant has several epitopes, and in each epitope there can be a number of variant peptide sequences inducing different lines of CTL. For simplicity, we discuss a model with two epitopes, but our results can be generalized to several epitopes. Virus dynamics can be represented as:

$$dv_{ij}/dt = v_{ij}(r_{ij} - p_i x_i - q_j y_j) \quad (1)$$

where $i = 1 \ldots n_1$ and $j = 1 \ldots n_2$; immune responses against epitope A as:

$$dx_i/dt = \eta c_i v_{i*} + x_i(c_i v_{i*} - b) \quad (2)$$

where $i = 1 \ldots n_1$; and immune responses against epitope B as:

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

where $j = 1 \ldots n_2$. 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 sequences for epitope A and n_2 for epitope B. The variables x_i and y_j denote CTL directed at sequence i of epitope A and sequence j of epitope B, respectively. The virus variant v_{ij} reproduces at the rate r_{ij}. Virus-infected cells are killed by CTL responses at the rates $p_i x_i v_{ij}$, where p_i and q_j are rate constants specifying the kinetics of removal of infected cells. CTL are stimulated by their specific epitope sequence (in association with HLA presentation). They are either produced by activation

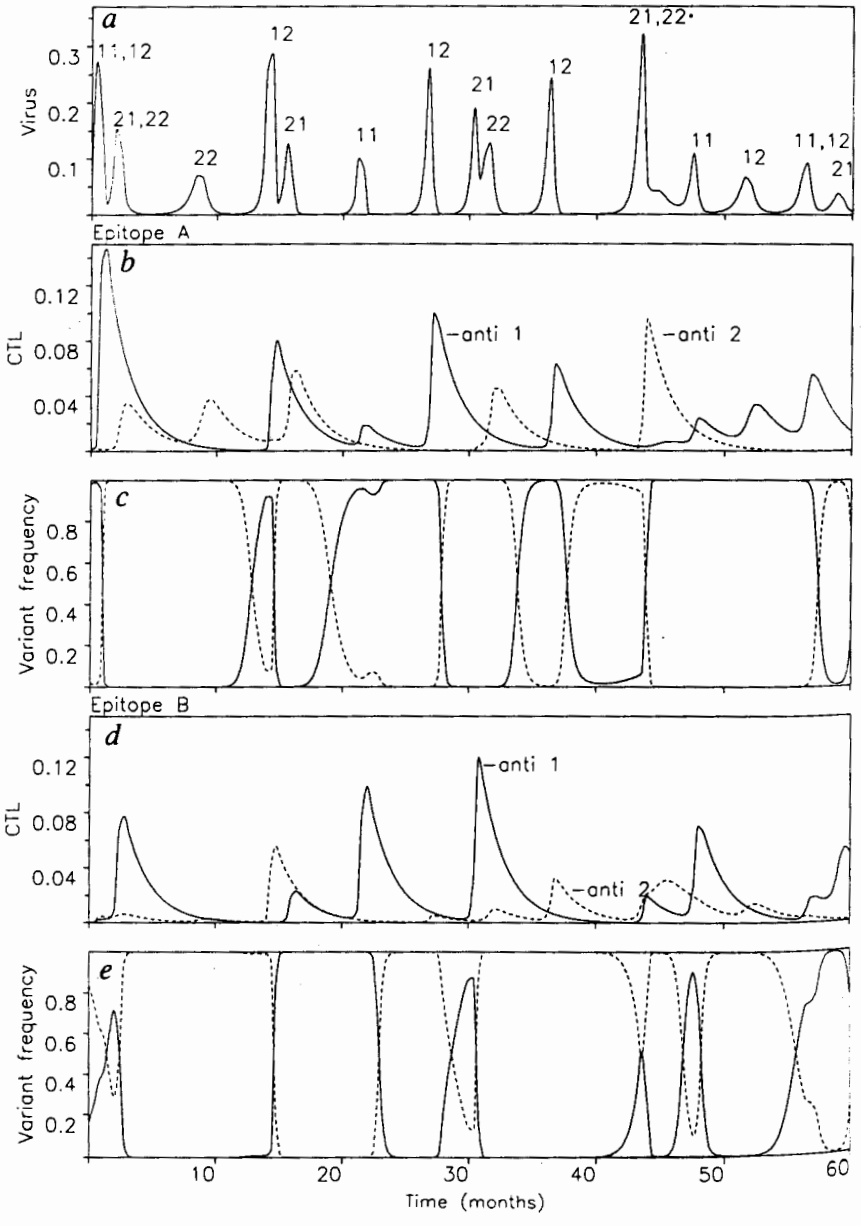

FIG. 2 Antigenic oscillations and fluctuating immunodominance in a model with two epitopes. Peaks of viral abundance that consist of antigenically different variants are accompanied by oscillations in the size and specificity of the CTL responses. All virus variants are present at the beginning of the simulation; there are no additional mutational events. In each epitope we have two antigenically different variants that induce specific CTL responses. The 4 peptide sequences have different immunogenicities, and the 4 virus variants have different replication rates. The figure shows transient dynamics; the oscillations are damped on a time scale of $1/\eta$. *a*, Total virus abundance; *b*, CTL response against epitope A; c, frequency of the two different peptides in epitope A; *d*, CTL response against epitope B; *e*, frequency of the two different peptides in epitope B. The parameters of the simulation are: $r_{11} = 0.2$, $r_{12} = 0.15$, $r_{21} = 0.16$, $r_{22} = 0.1$, $c_1 = 1$, $c_2 = 1.1$, $k_1 = 1.9$, $k_2 = 0.8$, $p_i = q_j = 5$, $b = 0.02$, $\eta = 0.0001$.

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 $k_j y_j v_{*j}$), where $v_{i*} = \sum_{j=1}^{n_2} v_{ij}$ and $v_{*j} = \sum_{i=1}^{n_1} v_{ij}$. Thus a particular CTL clone recognizes all viruses that have the specific sequence in the appropriate epitope; that is, x_i is directed at (and is stimulated by) v_{i*}, whereas y_j recognizes v_{*j}. The rate constants c_i and k_j describe the immunogenicities of sequence i in epitope A and sequence j in epitope B, respectively. The factor η describes the ratio of the rate at which CTL are activated from precursor cells over the rate of proliferation of already activated CTL. A small η implies that most activated CTL arise from proliferation of already activated cells. In the absence of antigenic stimulation, the activated CTL decline at the rates bx_i and by_j, where b represents the decay constant.

In our model, the immunogenicity of a peptide is defined as the rate at which it stimulates specific CTL. In molecular terms, several factors contribute to immunogenicity: a peptide is likely to be highly immunogenic if it binds the presenting HLA molecule with high affinity[6,7], if the resulting HLA–peptide complex is present in abundance on the surface of the infected cell, if this complex has a high affinity for the T-cell receptor, and if the reacting T-cells have high precursor frequency. The abundance of an epitope will be influenced by the concentration of the source protein, by its intrinsic stability and localization, and by the presence of appropriate proteolytic cleavage sites that lead to efficient antigen processing[5].

An intrinsic feature of our model is competition among immune responses against different epitopes. Immunodominance arises naturally as a consequence of this competition. Consider a homogeneous virus population with two epitopes that have different immunogenicities and therefore induce CTL responses at different rates. Suppose that both CTL responses have equal decay rates in the absence of antigenic stimulation. An equilibrium with a persistent virus population arises when the CTL have reduced the virus population to a level where CTL stimulation equals CTL decay. As the two epitopes have different immunogenicities, such an equilibrium is only possible for one of the two CTL responses. The same argument applies to situations with immune responses against many potential epitopes. In a homogeneous virus population there is competitive exclusion among the responses against different epitopes and eventually only a single response will persist. For a heterogeneous virus population, the situation is more complex[24] and antigenic variation generally leads to coexisting responses against several epitopes.

In situations with antigenic variation in multiple epitopes, the model generates complex oscillatory dynamics. There are distinct peaks in viral abundance, which are often dominated by single viral genotypes and occur whenever the responses against particular variants have declined to low levels because of a lack of stimulation (Fig. 2).The timescale of such oscillations is roughly determined by the time it takes for an activated CTL response to decline in the absence of further stimulation. For acute virus infections, activated CTL decline within 2–4 weeks after infection[25]. For these 'antigenic oscillations' to occur, it is not essential for mutation continuously to generate new antigenic material. Antigenic oscillations are different from the previously described 'antigenic drift', where the emergence of a new variant gives rise to a peak in viral abundance[26]. Antigenic oscillations arise as a consequence of the nonlinear dynamics of the immune responses acting on existing viral diversity.

A central point here is an understanding of the events following the emergence of a new mutant, in situations with several potential epitopes. All earlier experimental or theoretical studies of escape dynamics are essentially based, explicitly or implicitly, on models with single epitopes. In such models, immunological escape by an epitope confers a selective advantage, if the phenotype of the mutant is unaffected; such an escape mutant will increase in abundance and dominate the population until it induces a new specific response. If it fails to induce such a response, the escape mutant grows to fixation.

But antigenic variation in models with multiple epitopes is qualitatively different from the simple escape dynamics of single epitope models. We find situations where the escape mutant does not reach fixation or dominate the population. The immune system may not respond to the new peptide even if it 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 in which 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 increase the viral load and can thus represent a route to disease progression.

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. If $c_1 > k_1$, 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 equations (1–3) in the limiting case $\eta = 0$. The emergence of the escape mutant leads to one of four outcomes (Fig. 3). (1) 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 $y_1 = 0$. (2) The new mutant may not elicit a specific immune response to epitope A, but may induce a partial shift in immuno-

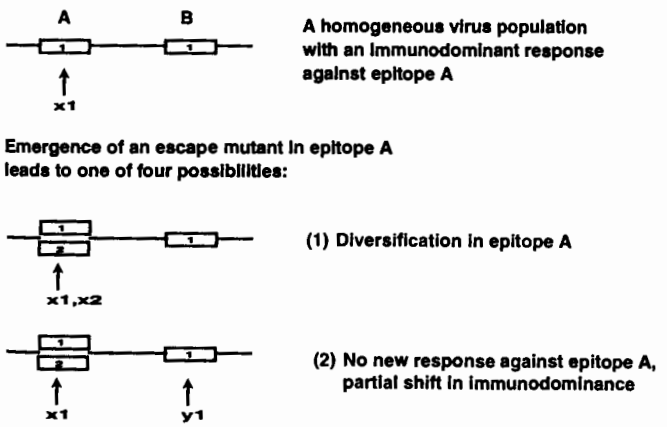

FIG. 3 Antigenic variation can shift immunodominance. The emergence of an escape variant in an immunodominant epitope, A, leads to one of 4 possible outcomes. (1) Diversification in epitope A, leading to a virus population with 2 epitope variants in A, both of which are seen by specific CTL. (2) Partial shift of immunodominance to epitope B, no specific response against the escape variant in A. (3) Partial shift of immunodominance to epitope B, together with a change in specificity of the response to epitope A. (4) Complete shift of immunodominance to epitope B, together with a complete replacement of the original variant in A by the escape mutant. Specific CTL to the original variant in epitope A, the escape variant in epitope A, and epitope B are denoted by x_1, x_2 and y_1, respectively.

dominance 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 $x_2=0$. (3) The new mutant may induce a new specific response to epitope A, which outcompetes the response against the original virus, and induce 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 $x_1=0$. (4) 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 cases (1) and (3), a homogeneous population of the mutant, v_{21}, would induce an immunodominant response against epitope A, whereas for case (4) it would induce immunodominance of epitope B. For case (2) it is not specified. Thus shifting immunodominance does not simply reflect the immunogenicity of the escape mutant.

Patient 007 appears to represent an example of case (1). There are two variants in epitope p24.14, both of which are seen by the patient's CTL. There is no indication of a shifted response to another epitope. Patient 020 provides examples of case (2). In all three epitopes there are variants that are not seen at some time points by the patient's CTL. There are shifts in the dominant response from one epitope to another.

It has often been asked how escape from CTL can be important if there are responses against several epitopes; our models show that persistent CTL responses against multiple epitopes are caused by immune escape. Furthermore, it is generally believed that patients who recognize a variety of different epitopes have better immune control than patients who only respond to a single epitope. Our theoretical and empirical studies suggest, however, that the opposite may be true, because persistent multiple-epitope responses are a consequence of antigenic variation.

The concept of shifting immunodominance in response to antigenic variation can also explain why not all escape mutants increase in abundance. They may remain at low levels, because the selection pressure on the virus population has changed with the altered immune response. Therefore, it is obvious that a conclusive demonstration of escape from immune response and selective increase in abundance is much more difficult than

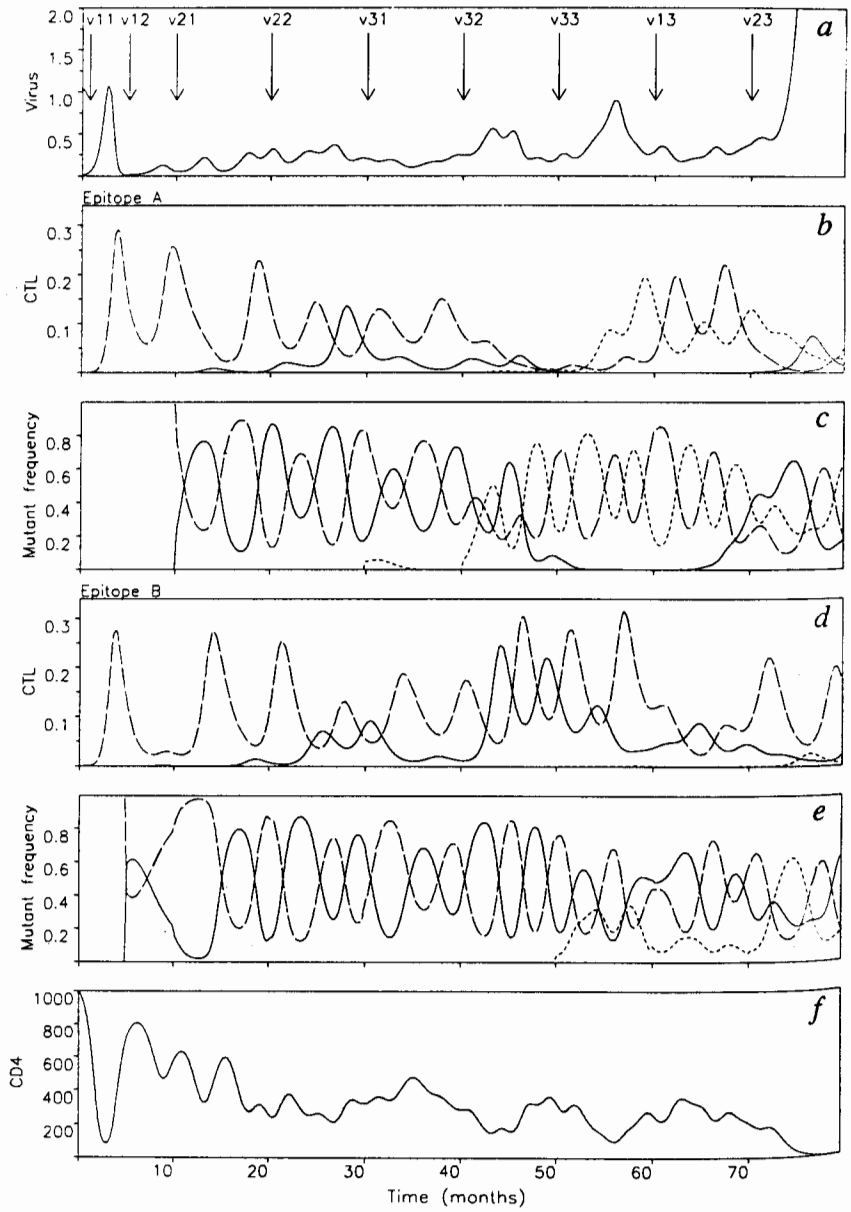

FIG. 4 HIV disease progression can be modelled with two additional assumptions: (1) CTL proliferation requires $CD4^+$ help; and (2) HIV impairs $CD4^+$ cell function. This creates an upper limit of viral diversity beyond which the immune system can no longer control HIV replication. In this schematic simulation we consider CTL responses against two epitopes, A and B. With time we introduce escape mutants in both epitopes. This leads to a sequence of antigenic fluctuations and shifting immunodominance from one epitope to the other. Throughout most of the infection there are strong responses against both epitopes. The prevalence of individual virus mutants fluctuates and so does the specificity of the CTL response. Virus load increases with antigenic diversity. $CD4^+$ cells are declining in an almost linear fashion (subject to fluctuations that reflect fluctuations in viral load). The final mutant v_{23} breaches the diversity threshold, and the virus population escapes and destroys the last $CD4^+$ cells. The differential equations for this simulation are: $dv_{ij}/dt = v_{ij}(r_{ij}-px_i-qy_j)$, $dx_i/dt=\eta czv_{i*}+x_i(czv_{i*}-b)$, $dy_j/dt=\eta kzv_{*j}+y_j(kzv_{*j}-b)$, and $dz/dt=\lambda-dz-uvz$. The variable z denotes $CD4^+$ cell population size. For simplicity we assume that $CD4^+$ cells immigrate from precursor cells at a constant rate, λ, die at rate dz, and are killed by HIV at rate uvz. These population dynamics simply reflect the assumption that $CD4^+$ cell numbers and function are inversely correlated with virus load. All other variables and parameters are defined as in equations (1–3). a, Total virus abundance; b, CTL response against the variants of epitope A; c, frequency of the variants of epitope A; d, CTL response against the variants of epitope B; e, frequency of the variants of epitope B; f, $CD4^+$ T-cell count. We used the parameters: $p=1$, $q=1$, $c=2.98$, $k=2.96$, $b=0.1$, $\lambda=0.1$, $d=0.1$, $u=1$, $\eta=0.001$; the r_{ij} were chosen randomly between 0 and 0.2.

escape from drug treatment. Escape from drug treatment is the consequence of a constant selection pressure on the virus population, whereas escape from immune responses alters the selection pressure exerted by the immune system.

The model provides a theory for immunodominance and antigenic variation for variable pathogens. It is the first theoretical formulation of the escape dynamics of a pathogen from immune responses directed at multiple epitopes. Although the ideas have been developed in the specific context of HIV, our intention is to shed light on the general problem of immunodominance and antigenic variation with implications for broader classes of host–parasite systems.

With respect to HIV, we have shown that it is possible to explain much of the observed complexity of viral escape from CTL responses by a simpler nonlinear model. The model suggests that those patients who recognize fewer epitopes of the virus have a more stable CTL response and thus control the virus more effectively. This is consistent with the contrasting data for, and clinical history of, patients 007 and 020. It remains to be determined whether these patients are typical of slow or more rapid progressors towards AIDS. The model makes the testable prediction that a stable CTL response to an invariant epitope may lead to slow progression. Conversely, viral diversity and evolution during infection can lead to AIDS[27,28]; the shift in CTL response towards weaker epitopes should increase overall viral loads and hence drive disease progression (Fig. 4). One testable route to immunotherapy would be to boost the CTL response to single conserved epitopes, making them immunodominant by increasing the frequency of reacting T cells, thereby aiming to induce a stable pattern of immunological recognition. □

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A DNA metalloenzyme with DNA ligase activity

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SINGLE-STRANDED DNA can fold into well-defined sequence-dependent tertiary structures that specifically bind a variety of target molecules[1–10], raising the possibility that some folded single-stranded DNAs might exhibit catalytic activities similar to those of ribozymes and protein enzymes. Derivatives of the hammerhead ribozyme that contain a majority of deoxyribonucleotides retain the ability to cleave RNA[11], and a 'deoxyribozyme' was generated by leaving all essential ribonucleotides of the hammerhead on the RNA 'substrate'[12]. Recently *in vitro* selection has been used to isolate a DNA sequence that shows Pb^{2+}-dependent RNA-cleaving activity[13]. Here we report the isolation by *in vitro* selection[14–17] of a small single-stranded DNA that is a Zn^{2+}/Cu^{2+}-dependent metalloenzyme. The enzyme catalyses the formation of a new phosphodiester bond by the condensation of the 5′-hydroxyl of one oligodeoxynucleotide and a 3′-phosphorimidazolide on another oligodeoxynucleotide, and shows multiple turnover ligation.

Oligodeoxynucleotides can be non-enzymatically ligated on either single-stranded[18] or duplex[19] DNA templates. We were interested in determining whether some DNA sequences might catalyse DNA ligation more efficiently than simple templating. We therefore designed an *in vitro* selection strategy for the isolation of such sequences from a large pool of random sequences (Fig. 1), based on their ability to condense their own 5′-hydroxyl group to the chemically activated 3′-phosphate group of a substrate oligodeoxynucleotide. After nine cycles of selection and amplification, the DNA pool displayed efficient ligation activity (Fig. 2*a*). Incubation of pool 9 DNA with the activated substrate yielded a ligated product with the correct molecular mass and the expected sequence at the ligation junction. To analyse the selected sequences, DNA from pool 9 was cloned and sequenced. Most of the clones contained a common consensus sequence, consisting of two small domains separated by a spacer of variable length and sequence (Fig. 2*b*). These domains were embedded in entirely different flanking sequences, implying that several independent sequences in the original pool were carried through the selection process. Inspection of the consensus sequence suggests a secondary structure that is more complex than a simple template but nevertheless brings the 5′-hydroxyl and the 3′-phosphorimidazolide into close proximity (Fig. 3*a*).

Based on the consensus sequence, we designed the small catalyst E47, a 47-nucleotide single-stranded DNA that ligates two separate DNA substrates S1 and S2 (Fig. 3*b*). Incubation of radioactively labelled S2 with activated substrate S1 and the catalyst E47 resulted in the appearance of the expected ligated product (Fig. 3*c*). Product formation required all three components to be present in the reaction, and required activation of the 3′-phosphate group of S1. E47 catalysed the ligation reaction twice as fast as pool 9. Small deletions within E47 resulted in severe losses of catalytic efficiency (Fig. 3*d*), indicating that the central consensus sequence is necessary for catalysis. The initial rate of ligation of S1 and S2 by E47 was 3,400-fold greater than the rate of the same reaction catalysed by a single complementary template under the same conditions, and was at least 10^5-fold faster than the untemplated background ligation (Fig. 3*d*). This rate enhancement is comparable with values obtained for *in vitro* selected ribozymes[14–17] and catalytic antibodies[20].

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