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Population Dynamics of Immune Responses to Persistent Viruses

Martin A. Nowak and Charles R. M. Bangham

Mathematical models, which are based on a firm understanding of biological interactions, can provide nonintuitive insights into the dynamics of host responses to infectious agents and can suggest new avenues for experimentation. Here, a simple mathematical approach is developed to explore the relation between antiviral immune responses, virus load, and virus diversity. The model results are compared to data on cytotoxic T cell responses and viral diversity in infections with the human T cell leukemia virus (HTLV-1) and the human immunodeficiency virus (HIV-1).

Molecular techniques have provided fundamental insight into the fine detail of the immune system. But many biologically important questions are not primarily concerned with the molecular mechanisms of immune recognition but with the population dynamics of the immune response. Such questions usually cannot be answered by experimental methods alone but require the help of mathematical models.

These questions arise particularly in the dynamics of host-parasite interactions (*1*). In HIV infection, for example, mathematical models have been devised to describe the slow decline in the numbers of CD4 cells over many years, the interaction between HIV and other opportunistic infections, the emergence of drug-resistant viruses, and the consequences of antigenic diversity and viral evolution during single infections (*2*, *3*). In HIV and hepatitis B virus (HBV) infection, mathematical models of drug treatment dynamics have provided estimates for the turnover rates of infected cells and free virus (*4*, *5*).

The strategy of successful mathematical modeling is akin to Ockham's razor: start with the smallest number of essential assumptions and follow the implications rigorously to their logical conclusions. An elegant model can often have greater intrinsic value than an accurate one overloaded with detail. Mathematical models differ from verbal theories in giving a precise and explicit connection between assumption and conclusion. The act of formulating a model forces one to ask questions that are often overlooked (*6*). Here a simple, but general mathematical framework is presented for viral replication and immune responses. We explore the basic dynamics of virus–host cell interaction and the consequences of immune responses on virus load and antigenic diversity.

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Parameters That Influence Infection Dynamics

Viruses are intracellular parasites that depend on the host cell to survive and replicate. The host cell can be damaged either directly by the virus or by immune responses to the virus; the balance of good and harm done by the antiviral immune response depends on the amount of virus present, the tissues infected, and the chronicity of the infection (*7*).

The abundance of virus—that is, the virus load—is an important determinant of the outcome of infection with many viruses: for instance, in HIV-1 and other lentivirus infections, virus load is correlated with pathogenicity, disease stage, and progression of disease (*8*, *9*); in HTLV-1, a large provirus load is associated with chronic inflammatory conditions (*10*); in HBV, the level of viremia is correlated with the risk of chronic infection (*11*); in cytomegalovirus infection, the amount of tissue damage is related to virus load (*12*); and in Lassa fever, mortality is correlated with the level of viremia (*13*).

Antibodies, cytokines, natural killer cells, and T cells are essential components of a normal immune response to a virus. But in most virus infections, cytotoxic T lymphocytes (CTLs) play a critical part in antiviral defense by attacking virus-infected cells. It is believed that they are the main host immune factor that limits the extent of virus replication in vivo and thus determines virus load. The clearest evidence for the role of these cells comes from passive transfer of immune CTLs to mice and humans (*14*). Using hu-

man volunteers, McMichael *et al.* (*15*) showed that virus-specific CTL activity was associated with reduced shedding of influenza virus. There is circumstantial evidence for the control of virus by CTLs in natural infections with HIV-1 (*16*), HTLV-1 (*17*), HBV (*18*), and Epstein-Barr virus (*19*).

An important concept discussed here is CTL responsiveness, which is defined as the rate at which an individual mounts a CTL response to a given virus. On a cellular level, CTL responsiveness is the average rate at which specific CTLs proliferate after encountering an infected cell. This rate will depend on factors such as the affinity of the T cell receptor for the combined viral peptide and major histocompatibility complex (MHC) molecules. The CTL responsiveness against a specific virus is likely to vary between individuals and depends, among other things, on the genes encoded by the MHC, which determine which epitopes of the virus are presented to the immune system. The CTL responsiveness of a patient can also vary over time: for example, in HIV infection it may vary as a result of antigenic variation or declining T cell help. Emergence of antagonistic variants may also reduce CTL responsiveness.

In contrast to the inherent property of CTL responsiveness, the term "CTL response" denotes the actual number of virus-specific CTLs present at a given time. It is this quantity that is measured by in vitro assays. The CTL response depends on the amount of stimulation provided by the virus and thus on virus load. CTL response and virus load are linked to each other in a density-dependent fashion: A strong CTL response may reduce virus load, but the resulting small virus load will provide less stimulation, and in time the CTL response will decline.

Here, we make the following argument: (i) Virus load is an important determinant of disease; (ii) CTLs limit virus load; (iii) therefore, individual variation in CTL responsiveness may account for much of the observed variation in the outcome of disease. The emphasis here is on CTLs because of their known importance in the defense against viruses, but we will show that identical principles apply to other host defense factors, including antibody responses. We will also discuss the following questions, all of which can be addressed by mathematical models: How do strong and weak CTL responders differ in their equilibrium virus load? What is the expected correlation between virus load and CTL activity in a cross-sectional study (that is, in a comparison between infected individuals)? What is the effect of a strong CTL response on virus diversity? How does antigenic variation in CTL epitopes affect virus load? Why is the CTL response to HTLV-1 equally strong in people whose HTLV-1 provirus loads differ by a factor of 10 to 100? And, finally, if CTLs limit HIV replication, why is there no correlation between the magnitude of the CTL response and virus load (or CD4 cell count) in HIV-infected patients?

Models for Infection Dynamics

Here, we will present three models: first, a simple model for the interaction between a replicating virus and host cells; second, a model that includes immune responses against infected cells; and, finally, a model in which the virus is allowed to mutate both in terms of replication ability and escape from immune responses. Our strategy is to consider the simplest possible models and to explore their implications.

Virus replication. The basic model of viral dynamics (*1–5*) contains three variables: uninfected cells x, infected cells y, and free virus particles v (Fig. 1). Infected cells are produced from uninfected cells and free virus at rate βxv and die at rate ay. Free virus is produced from infected cells at rate ky and declines at rate uv (*20*). Therefore, the average lifetime of an infected cell is $1/a$ and the average lifetime of a free virus particle is $1/u$; the total number of virus particles produced from one cell is k/a. Uninfected cells are produced at a constant rate, λ, from a pool of precursor cells and die at rate dx. This is the simplest possible host cell dynamics, which leads to a stable equilibrium of host cells in the absence of virus. These assumptions lead to the following system of differential equations:

$$\dot{x} = \lambda - dx - \beta xv$$
$$\dot{y} = \beta xv - ay$$
$$\dot{v} = ky - uv \qquad (1)$$

If the basic reproductive ratio (*1*) of the virus, $R_0 = \beta\lambda k/(adu)$, is smaller than 1, then in the beginning of the infection, each virus-infected cell produces on average less than one newly infected cell. Thus, the infection cannot spread, and the system returns to the uninfected state where $x_0 = \lambda/d$, $y_0 = 0$, and $v_0 = 0$. If R_0 is larger than 1, then initially each virus-infected cell produces on average more than one newly infected cell (exactly R_0 such cells). The infected cell population will increase, whereas the uninfected cell population will decline and therefore provide less opportunity for the virus to infect new cells. The system will converge (in damped oscillations) to the equilibrium

$$x^* = \frac{au}{\beta k} \quad y^* = \frac{\lambda}{a} - \frac{du}{\beta k}$$
$$v^* = \frac{\lambda k}{au} - \frac{d}{\beta} \qquad (2)$$

At equilibrium, further spread of the virus is limited by a reduced availability of uninfected cells. Each infected cell produces now, on average, exactly one newly infected cell.

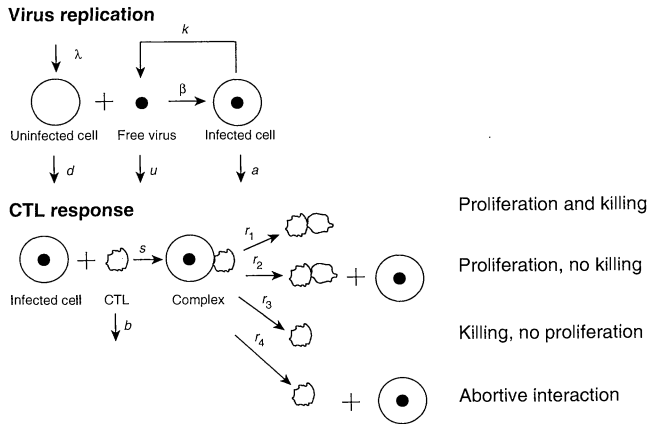

Fig. 1. A model for virus-CTL interaction. In virus replication, free virus particles and uninfected cells produce infected cells at rate β. Infected cells produce new virus particles at rate k. Uninfected cells are assumed to be generated at a constant rate λ from a pool of precursor cells. Free virus and infected and uninfected cells decline at rates u, a, and d, respectively. The population dynamics is described by Eq. 1. In the CTL response, infected cells and specific CTLs form a complex (at rate s). This complex dissociates in four pathways. (i) The target cell can be killed and the CTLs can be stimulated to divide (rate r_1). (ii) The CTLs can divide without killing the target cell (rate r_2). (iii) The target cell may be killed, but the CTLs may not divide (rate r_3). (iv) There may be no killing and no proliferation (rate r_4). The combined virus replication and CTL response-dynamics lead to the following kinetic equations: $\dot{x} = \lambda - dx - \beta xv$, $\dot{y} = \beta xv - ay - syz + (r_2 + r_4)w$, $\dot{v} = ky - uv$, $\dot{z} = (r + r_1 + r_2)w - bz - syz$, and $\dot{w} = szy - rw$, where x, y, v, z, and w denote uninfected cells, infected cells, free virus, CTLs, and complex, respectively. For this, $r = r_1 + r_2 + r_3 + r_4$. The system is equivalent to the simpler Eq. 3 if a steady-state approximation is valid for the dynamics of the complex. In any case, the system converges to the equilibrium values given in Eq. 4 and $\hat{w} = s\hat{y}\hat{z}/r$. The CTL responsiveness, c, is given by $c = sP$, where $P = (r_1 + r_2)/r$ is the probability of CTL proliferation after interaction with an infected cell. Thus, CTL responsiveness depends on the rate of complex formation and the probability of CTL proliferation after interaction with a target cell. The rate constant of CTL-mediated killing, p, is given by $p = sQ$, where $Q = (r_1 + r_3)/r$ is the probability that the infected cell is killed. The model can be expanded to include different subtypes of CTLs (effector and memory cells), which would differ primarily in the parameters r_1 to r_4.

Thus, it is not necessary to evoke an immune response to achieve a stable equilibrium level of virus in a persistent infection. But such an equilibrium has interesting limitations: (i) for a noncytopathic virus, most cells will be infected and (ii) for a cytopathic virus, the total abundance of cells will be greatly reduced. Note also that the more cytopathic a virus is (larger values of a), the smaller the steady-state abundance of free virus and infected cells and the larger the abundance of uninfected cells (if all other parameters are held constant). Thus, although it is possible to attain a stable equilibrium level of virus in the absence of an immune response, this will usually result in large virus load, severe tissue damage, or both (*21*).

Immune responses reduce virus load. We can now extend the basic system (Eq. 1) with an equation describing immune responses against infected cells:

$$\begin{aligned} \dot{x} &= \lambda - dx - \beta xv \\ \dot{y} &= \beta xv - ay - pyz \\ \dot{v} &= ky - uv \\ \dot{z} &= cyz - bz \end{aligned} \qquad (3)$$

The variable z denotes the magnitude of the CTL response—that is, the abundance of virus-specific CTLs. The rate of CTL proliferation in response to antigen is given by cyz. In the absence of stimulation, CTLs decay at rate bz. Infected cells are killed by CTLs at rate pyz. These simple dynamics can be derived from the kinetic interaction between CTLs and infected cells (Fig. 1). The parameter c denotes the CTL responsiveness, defined earlier as the growth rate of specific CTLs after encountering infected cells. The parameter p specifies the rate at which CTLs kill infected cells (*22*).

In the model, there is a minimum level of infected cells necessary to stimulate a CTL response. If $cy > b$, the CTL response will increase. The long-term outcome of the system depends on whether the equilibrium abundance of infected cells in the absence of a CTL response is above or below this threshold value. If $cy^* < b$ (where y^* is defined in Eq. 2), the CTL response may become only transiently activated, but eventually the system will converge to the equilibrium given by Eq. 2 without an active CTL response. If $cy^* > b$, the system shows damped oscillations (*23*) to the equilibrium

$$\hat{x} = \frac{\lambda cu}{(cdu + \beta bk)} \quad \hat{y} = \frac{b}{c} \quad \hat{v} = \frac{bk}{cu}$$

$$\hat{z} = \left(\frac{1}{p}\right)\left[\frac{\lambda\beta ck}{(cdu + \beta bk)} - a\right] \qquad (4)$$

There are two interesting aspects of this equilibrium. First, the equilibrium abundance of infected cells depends only on the immunological parameters b and c. Parameters determining the host cell dynamics enter only indirectly into the equation by means of the condition $cy^* > b$. Second, the condition $cy^* > b$ is equivalent to the conditions $x^* < \hat{x}$, $y^* > \hat{y}$, and $v^* > \hat{v}$. Thus, in the above model if there is an active CTL response, it will reduce virus load and increase the equilibrium abundance of uninfected cells. But the total abundance of infected and uninfected cells, $\hat{x} + \hat{y}$, can be increased or decreased by a CTL response compared to $x^* + y^*$.

At equilibrium, the average lifetime of an infected cell is $1/(a + p\hat{z})$, where a is the rate of cell death as a result of virus cytopathicity and $p\hat{z}$ is the rate of cell death as a result of the action of CTLs. We can ask how fast CTLs have to kill (compared to the virus-mediated death rate) in order to reduce the equilibrium virus load by a factor f compared to y^*. We find

$$p\hat{z} = \frac{a(R_0 - 1)(1 - f)}{[1 + (R_0 - 1)f]} \qquad (5)$$

where R_0 is the basic reproductive ratio of the virus, defined above as the number of newly infected cells arising from any one infected cell, in the absence of a CTL response. For example, if $R_0 = 10$, then to reduce the virus load to one-tenth of the levels without CTLs ($f = 0.1$), CTL-mediated killing has to be 4.3 times faster than virus-mediated killing, which means that 81% of infected cells have to be killed by CTLs.

Variation in immune responsiveness. The model can be used to study the relation between CTL responsiveness, c, CTL response, $\hat{z}$, and virus load, $\hat{y}$ or $\hat{v}$, in comparisons between different patients. The effects of individual variation in CTL responsiveness are shown in Fig. 2. Although CTL responsiveness determines virus load, it is not necessarily reflected in the magnitude of the CTL response at equilibrium. Depending on the detailed assumptions of how c (and p) vary among individuals, the equilibrium CTL abundance may increase at small values of c but saturate or decline for large values of c or may have no obvious correlation to c at all. Therefore, a strong responder is characterized by large values of c (and p), not necessarily by large values of $\hat{z}$. A strong responder limits the virus to low levels, where it provides only a weak stimulus for CTL proliferation. A weak responder allows a large virus population, which provides a stronger stimulus for CTL proliferation. Thus, strong and weak responders will differ in virus load but may have similar levels of CTL response. This seemingly nonintuitive result is a well-known feature of predator-prey dynamics. A similar result can be obtained for antibody responsiveness and virus load (*24*). This underlines the

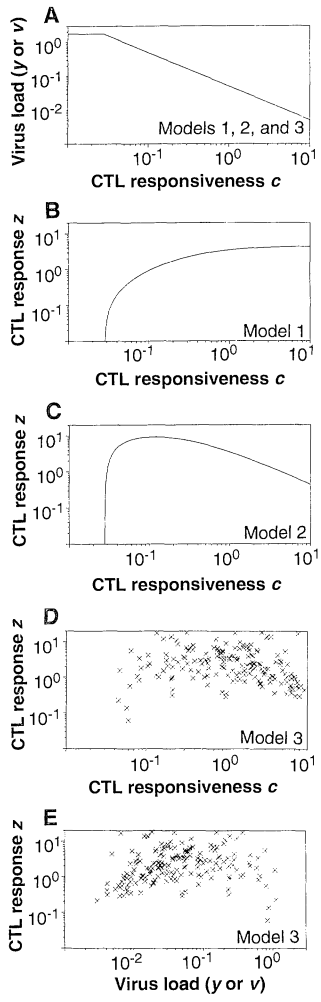

Fig. 2. The effect of individual variation in CTL responsiveness on virus load and CTL response. CTL responsiveness is defined as the rate at which viral-specific CTLs proliferate after encountering infected cells, whereas CTL response refers to the abundance of viral-specific CTLs in an infected individual. The virus-CTL model of Fig. 1 and Eq. 3 leads to an equilibrium (Eq. 4) that was used for this illustration. Model 1 assumes that variation between individuals is confined to CTL responsiveness c, whereas the rate of CTL-mediated killing, p, is constant. Model 2 assumes that individuals differ both in c and p but that the two parameters are linked to each other. Model 3 assumes that patients differ in both c and p but that these are (largely) uncorrelated. (**A**) For all three models, virus load is inversely correlated with CTL responsiveness c (except for small values of c, there is no CTL response and virus load is controlled only by target cell availability). (**B** and **C**) In models 1 (B) and 2 (C), the equilibrium CTL response, $\hat{z}$, increases for small values of c but saturates or even declines for large values of c. (**D** and **E**) Model 3 shows no correlation between CTL response and CTL responsiveness (D) or between CTL response and virus load (E). In all three scenarios, strong and weak responders will differ in their virus load but may not differ in their CTL response. Parameter values are as follows: $\lambda = 1$, $d = 0.01$, $a = 0.5$, $\beta' = \beta k/u = 0.05$, and $b = 0.05$; in model 1, $p = 1$; in model 2, $p = c$. From Fig. 1, we know $c = sP$ and $p = sQ$, respectively. Therefore, in model 1, P varies between individuals, whereas in model 2, s varies. For model 3, we take values for s from an exponential distribution (mean = 5) and values for P and Q from a uniform distribution between 0 and 1. More generally, the models can be interpreted as describing any type of specific immune response against a replicating pathogen; in this case, CTL responsiveness and response should be read as immune responsiveness and response, respectively.

generality of the idea that immune responsiveness determines virus load at equilibrium but that there need not be a simple correlation between immune responsiveness and the magnitude of the immune response in comparisons among different infected individuals.

Viral diversity and escape from immune response. Viral diversity is a consequence of mutation and selection. The short replication time of viruses gives them enormous potential for rapid genetic change in response to selection forces. Virus populations, even in single hosts, often consist of ensembles of many different genetic sequences, the so-called quasi species (*25*).

The immune response against a continuously replicating virus provides selection pressure for antigenic variation. There is evidence of CTL escape mutations in infections with HIV-1, HTLV-1, HBV, LCMV, and mouse retroviruses (*26*). Rare mutants that are not seen by ongoing immune responses can have a growth advantage and may therefore increase in abundance. But variation is opposed by functional constraints: virus mutants have to maintain sufficiently high replication rates to compete for available target cells. The following model illustrates the interplay between selection pressures for and against diversification:

$$\begin{aligned}\dot{x} &= \lambda - dx - x\sum_{i=1}^{n}\beta_i v_i \\ \dot{y}_i &= \beta_i x v_i - a y_i - p y_i z_i \\ \dot{v}_i &= k_i y_i - u v_i \\ \dot{z}_i &= c y_i z_i - b z_i \end{aligned} \qquad (6)$$

Here, y_i and v_i denote, respectively, the abundance of infected cells and free virus of type i. Viral variants differ in their antigenic specificity, in the rate at which they infect cells (β_i), and in the rate of virus production (k_i). The variable z_i denotes the magnitude of the specific CTL response against variant i. There are n virus variants in the system ($i = 1, \ldots, n$), only a fraction of which will survive at equilibrium. This "equilibrium diversity" depends on the CTL responsiveness, c, of the patient against the virus; a strong responder selects for higher diversity (*27*).

The model can simulate the dynamics of individual infections where new viral variants are continuously being produced (Fig. 3). The effect of viral diversity is to increase virus load. In this model, this leads to a positive correlation between load and diversity if a patient is followed longitudinally, provided that the immune responsiveness c is constant over time (*28*). The relation between load and diversity in comparisons among different infected individuals is more complicated. A strong responder (larger values of c) limits the virus to low abundance and selects for antigenic variation. A weak responder (smaller values of c) allows the virus to replicate to high abundance and provides little selection for variation. But antigenic diversity will tend to increase virus load. The model we describe above suggests that a negative correlation between virus load and diversity is the most likely outcome in cross-sectional studies (*27*).

Immune responses against multiple epitopes. An individual's immune system is able to mount responses against several epitopes of a virus. Mathematical models suggest that responses against different epitopes are not independent of each other but compete for antigenic stimulation (*29*). In an antigenically homogeneous virus population, the most immunogenic epitope will induce the dominant response (at equilibrium). Antigenic variation in the immunodominant epitope can shift the response toward other, less immunogenic epitopes (*29*). In antigenically diverse virus populations, there can be fluctuating responses against several epitopes simultaneously.

The consequence of multiple epitope responses on virus load and diversity can be seen when responses against variable and conserved epitopes are considered (Fig. 4). Responses against conserved epitopes enhance competition among viral mutants and thereby reduce diversity in variable regions of the genome (*30*). If patients differ mainly in their responsiveness to conserved epitopes, the model predicts a positive correlation between load and diversity: a weak responder will allow a large virus load and will provide little selection pressure against diversity, whereas a strong responder will select for low diversity and will also limit the virus population to low levels. If patients differ mainly in their responsiveness to variable epitopes, then the situation is as described previously; the model predicts a negative correlation between load and diversity in cross-sectional studies.

Applying the Model

HTLV. HTLV-1 causes a persistent infection that remains asymptomatic in 95% of infected individuals. The provirus load can differ by more than 100 times among infected people; those with a large load tend to

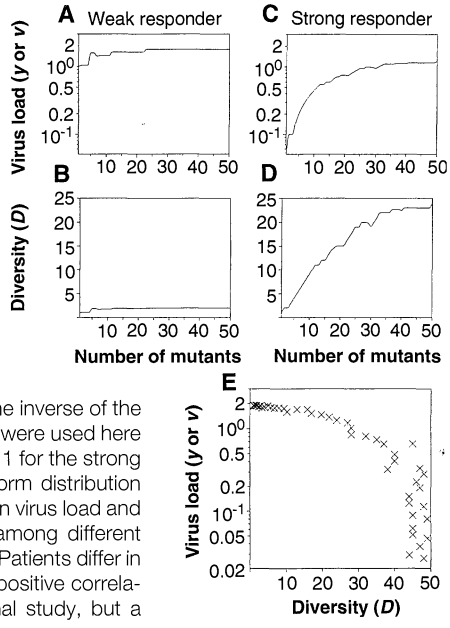

Fig. 3. (**A** through **D**) The sequential evolution of virus load and diversity in a weak and a strong immune responder. Antigenic mutants are continually generated over time (x axis). In the weak responder, the virus load is large. Adding new mutants has little effect on virus load (A) and virus diversity (B). Only a small fraction of the viral mutants can survive. The dominant selection force is competition for fast replication. In the strong responder, virus load is small initially but increases as new viral mutants are generated (C). Many of the mutants persist, and diversity increases over time (D). The dominant selection force is escape from the immune response. In both individuals, virus diversity increased virus load. The computer simulation is based on the system (*6*) and shows equilibrium values of virus load and diversity (measured by the inverse of the Simpson index: $y^2/\Sigma_i y_i^2$). The same parameter values were used here as in Fig. 2; $c = 0.05$ for the weak responder and $c = 1$ for the strong responder. The values for β_i were taken from a uniform distribution between 0 and 0.05. (**E**) An inverse correlation between virus load and diversity in a simulation of a cross-sectional study among different patients measured at equal time points after infection. Patients differ in their responsiveness c. Thus, the model generates a positive correlation between virus load and diversity in a longitudinal study, but a negative correlation in a cross-sectional study.

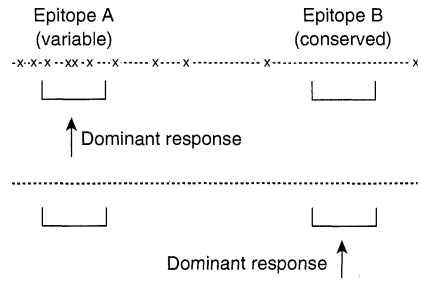

Fig. 4. Immune responses can provide selection pressure for or against viral diversity. A strong response to a variable epitope selects for escape mutations that may be inside or outside of the relevant epitope. These mutations can induce secondary mutations to maintain viral function. A strong response to a conserved epitope provides a selection pressure that enhances competition among possible virus variants and therefore reduces viral diversity. Whether an epitope is conserved will mostly depend on functional constraints acting on the viral sequence in this epitope. The figure shows diversity in various regions of the viral genome depending on the target epitope of the dominant immune response (arrow). Conserved positions are indicated by dashes, variable positions by "x."

develop inflammatory diseases, such as tropical spastic paraparesis–HTLV-I–associated myelopathy (TSP-HAM) (*10*). Most infected individuals have a chronically activated HTLV-1–specific CTL response, which exerts significant selection on the virus (*31*). However, we have found no significant difference in the magnitude of the fresh or memory CTL response between healthy carriers and TSP-HAM patients.

This paradox is resolved by our model, if we assume that people differ in their CTL responsiveness to HTLV-1. On this hypothesis, healthy carriers are strong CTL responders and therefore have a small virus load, whereas TSP-HAM patients are weak responders and therefore have a large virus load. But both groups can have similar levels of CTL response (Fig. 2).

What is the effect of a powerful CTL response on the sequence diversity of the virus population? Significant nucleotide and antigenic diversity exists in the dominant CTL target antigen Tax. Healthy carriers have more sequence diversity in Tax than TSP-HAM patients do (*31*). In the model, CTLs directed against variable epitopes select for increased sequence diversity; therefore, strong CTL responders should develop greater diversity. Thus, the model provides potential explanations for observations in HTLV-1 infection that were not previously available.

HIV. In HIV-1 infection, specific CTL responses arise early in primary infection and are lost in the final stages of the disease. They are believed to control virus replication during most of the asymptomatic phase (*16*) by killing infected cells and releasing chemokines that inhibit viral growth (*32*). Long-term survival of HIV-1 infection is usually correlated with good immune responses to the virus and small virus load (*8*). But in recent studies, no correlation was found between either a patient's virus load or CD4 cell count and the magnitude of the CTL response (*33*).

If patients differ in their CTL responsiveness against HIV, our model predicts that weak responders allow large viral loads, whereas strong responders limit the virus to low levels. But both groups of patients may have comparable amounts of HIV-specific CTLs. Assuming that CTL responsiveness, c, decreases over time of infection (because of CD4 cell depletion or shift of immune responses to less immunogenic epitopes), then according to the model this decrease will result primarily in increased virus load but may not reduce the amount of HIV-specific CTLs. Consequently, a patient with a low CD4 cell count and a large virus load can have an amount of HIV-specific CTLs similar to those in a patient with a high CD4 cell count and a small virus load.

In HIV-infected patients, viral diversity increases over the time since infection (*34*). But it has been reported that fast progression is normally associated with large virus load and low diversity, whereas slow progressors tend to have a small viral load but accumulate considerable diversity (*35*). Our model suggests that in individual infections, the effect of antigenic diversity is to increase virus load. In comparisons between different patients, however, the correlation between virus load and diversity can be positive or negative, depending on whether major immune responses are directed against conserved or variable regions of the virus. An inverse correlation between load and diversity is the theoretical expectation if patients mainly differ in their immune responsiveness to variable HIV epitopes.

Any theory of HIV disease progression has to explain how the rapid turnover of virus and cells (*4*) leads to a slow decline of CD4 cells over many years. Virus evolution can slowly shift the steady state between virus load and immune control. Increasing antigenic diversity can increase virus load (*3*). Antigenic escape can also divert immune responses to less immunogenic epitopes, thereby again increasing virus load (*29*).

Conclusions and Testable Consequences

We have explored the effects of individual variation in immune responsiveness on virus load and diversity. We analyzed the interaction between virus replication and CTL responses, but our findings also apply to antibody- or cytokine-mediated immunity.

The CTL responsiveness of a patient to a given virus is defined as the inherent rate of CTL proliferation after virus-infected cells are encountered. In simple mathematical models, CTL responsiveness determines virus load, but there may be no obvious correlation between virus load and the abundance of antiviral CTLs. Strong and weak responders may differ in virus load but can have similar levels of measurable CTL response. Therefore, a better indicator of CTL responsiveness is the equilibrium virus load, rather than the abundance of virus-specific CTLs. This result can explain the nonintuitive observation that in HTLV and HIV infection, virus load is not correlated with the magnitude of the CTL response, and yet CTLs are believed to play a major part in controlling virus replication in both cases. A testable consequence is that in population studies, polymorphic variants of genetic factors that control immune responsiveness (for example, MHC proteins) will be primarily associated with differences in virus load (*36*).

The mathematical models also show that immune responses can provide selection for or against diversity. Responses to conserved epitopes enhance competition among virus variants and therefore reduce diversity, whereas responses to variable epitopes can increase diversity. The relation between viral load and diversity depends on whether the dominant immune responses are directed against variable or conserved epitopes. If patients differ mainly in their immune responsiveness to variable epitopes, the model predicts a negative correlation between virus load and diversity in comparisons between patients. If individuals differ primarily in their immune responsiveness to conserved epitopes of the virus, a positive correlation between load and diversity is predicted. Virus sequence diversity will be lower in those patients with a dominant CTL response against a single conserved epitope. In a given individual, increasing viral diversity will, on average, increase virus load.

A quantitative understanding of the immune response to a virus requires experimental methods to measure the rates at which different effector mechanisms of the immune system are elicited by a given quantity of virus and the rates at which these mechanisms kill infected cells, inhibit virus replication, or eliminate free virus. Specifically for CTLs, we need to know the rate of proliferation after contact with an infected cell and the fraction of virus-infected cells that is eliminated because of CTL-mediated lysis as opposed to viral cytopathicity. Measurement of such quantities and virus load—and their variation between individuals—will provide a detailed understanding of viral pathogenesis and immune control.

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20. More accurately, the decay rate of free virus should also include a term for absorption of virus particles by host cells and should thus depend on host cell abundance. But if a large number of virus particles is produced, only a few of which will end up in host cells, then a constant death term is a reasonable approximation.
21. For a noncytopathic virus, where $a \approx d$, we find $y^*/x^* \approx R_0$. For cytopathic viruses, where $a \gg d$, we derive $(x^* + y^*)/x_0 \approx d/a$. These approximations are based on the natural assumption that R_0 is significantly greater than 1. In addition, for any virus we have $x^* = x_0/R_0$, which means that x^* will be much smaller than x_0.
22. In this model, we assume that the primary role of CTLs is to eliminate infected cells. Instead, we could also assume that CTLs release cytokines that inhibit infection of new cells. In both cases, we find similar results for the relation between equilibrium virus load, abundance of CTLs, and CTL responsiveness c.
23. For some parameter values, oscillations can go over several orders of magnitude and continue for a long time. But in more complicated, more realistic models, spatial heterogeneity or saturation effects in the rate of immune stimulation will lead to a rapid and efficient damping. We have checked this, for example, by using immune response equations of the type $\dot{z} = \eta cy + z[cy/(1 + \epsilon z + \delta y) - b]$, which are discussed in R. de Boer and A. S. Perelson, *J. Theor. Biol.* **175**, 567 (1995), and M. A. Nowak *et al.*, *ibid.*, p. 325.
24. Considering antibody responses, w, to free virus, the equations become $\dot{x} = \lambda - dx - \beta xv$, $\dot{y} = \beta xv - ay$, $\dot{v} = ky - uv - qvw$, and $\dot{w} = rvw - hw$. The parameter r describes the rate constant of stimulation of antibody responses (B cell proliferation) of a patient to a given virus—that is, the patient's antibody responsiveness. Solving for the equilibrium, we obtain similar relations between antibody responsiveness r, virus load v, and the magnitude of the antibody response w as in the CTL model.
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27. For a given choice of parameter values, the system (*6*) admits always one stable equilibrium that can be calculated as follows. We introduce the combined parameter $\beta_i' = \beta_i k_i/u$ and label the mutants such that $\beta_1' > \ldots > \beta_n'$. We define $\xi_i = \lambda/[d + (b/c)(\beta_1' + \ldots + \beta_i')]$ and use $v_i = k_i y_i/u$. *The first m* mutants will be present at equilibrium, where m is the largest index such that $\beta_m'\xi_{m-1} > a$. If in addition $\beta_m'\xi_m < a$, then the mth mutant will not induce a specific immune response z_m, but will be regulated by reduced target cell availability. The equilibrium is given by $x = a/\beta_m'$, $y_i = b/c$, and $z_i = a(\beta_i'/\beta_m' - 1)/p$, where $i = 1, \ldots, m-1$, $y_m = \lambda[1/a - 1/(\beta_m'\xi_{m-1})]$, and $z_m = 0$. If $\beta_m'\xi_m > a$, then the equilibrium is $x = \xi_m$, $y_i = b/c$, and $z_i = (\beta_i'\xi_m - a)/p$, where $i = 1, \ldots, m$. For a given value of c, increasing the number n of mutants will in general increase the number m of survivors at equilibrium and therefore increase virus load. Thus, there is a positive correlation between virus load, $y = \Sigma y_i$, and diversity, m, if an infected individual is followed over time (and c is constant). But for a cross-sectional comparison among patients with the same n but different c, we obtain a negative correlation: for a large number of strains, the index m is approximately given by the relation $\beta_m'\xi_m = a$. Using $y = mb/c$, we obtain $y = m\beta_m'\lambda/a - d)(\beta_1' + \ldots + \beta_m')$ which is for natural choices of β_i' a decreasing function of m.
28. This holds as long as there is an immune response acting on the virus. For example, in the final stages of AIDS (acquired immunodeficiency syndrome), we expect large viral loads with low antigenic diversity, because the immunological pressure against the virus has disappeared. Furthermore, newly emerging escape variants and oscillatory dynamics can make it difficult to verify the positive correlation between load and diversity in practice. It is also possible to construct situations where adding another mutant can indeed reduce virus load [S. Bonhoeffer and M. A. Nowak, *Immunol. Today* **16**, 131 (1995)].
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30. The simplest model to show this is $\dot{y}_i = y_i(r_i - pz_i - qw)$, $\dot{z}_i = z_i(cy_i - b)$, and $\dot{w} = w(ky - b)$, where y_i, z_i, and w denote infected cells, CTLs to the variable epitope, and CTLs to the conserved epitope, respectively. Viral growth is exponential and given by r_i with $r_1 > \ldots > r_n$. At the neutrally stable equilibrium, m viral variants will survive, where m is the smallest number larger than c/k. Thus, a large immune responsiveness against the conserved epitope (large values of k) will reduce viral diversity.
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