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Antigenic Diversity Thresholds and the Development of AIDS

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Longitudinal studies of patients infected with HIV-1 reveal a long and variable incubation period between infection and the development of AIDS. Data from a small number of infected patients show temporal changes in the number of genetically distinct strains of the virus throughout the incubation period, with a slow but steady rise in diversity during the progression to disease. A mathematical model of the dynamic interaction between viral diversity and the human immune system suggests the existence of an antigen diversity threshold, below which the immune system is able to regulate viral population growth but above which the virus population induces the collapse of the CD4$^+$ lymphocyte population. The model suggests that antigenic diversity is the cause, not a consequence, of immunodeficiency disease. The model is compared with available data, and is used to assess how the timing of the application of chemotherapy or immunotherapy influences the rate of progress to disease.

MUCH UNCERTAINTY STILL SURROUNDS THE PROCESSES governing the development of acquired immunodeficiency syndrome (AIDS), after an individual is infected with the human immunodeficiency viruses (HIV-1 and HIV-2). There is a long and highly variable incubation period for AIDS, with roughly 50 percent of male homosexuals developing the disease within 10 years after infection (1), and a slow but steady depletion of CD4$^+$ T-helper or inducer lymphocytes over this period in those who develop AIDS (2). The interaction between the viral population and the host's immune and other systems is very complex, with the virus being able to infect not only cells within the immune system but also a wide variety of other cell types in the brain, the gastrointestinal tract, the kidney, and other tissues (3).

Various explanations have been offered for the slow impairment of immune functions and the increased susceptibility of AIDS patients to opportunistic infections. These range from those based on the ability of the virus to kill CD4$^+$ cells, to those that invoke the presence of other infectious agents, such as mycoplasms, as necessary cofactors for the development of disease (4).

Understanding what is going on might seem to have been made more difficult by the discovery of great genetic diversity in viral isolates obtained either sequentially from the same infected individual or from different individuals (5). As a retrovirus, HIV lacks mechanisms that correct errors during replication, and the result is an error rate of about 10^{-4} per base, or one misincorporation per genome per replication cycle (6). Thus, each viral genome must be viewed as being different from any other, and viral isolates must be

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thought of as populations of closely related genomes. Such ensembles of genomes are called quasispecies (*7*). The different mutants within such a quasispecies may exhibit marked differences with respect to biological properties such as cell tropisms, cytopathic properties, replication rates, and surface antigen characteristics (notably, for HIV, those associated with the V3 hypervariable region of the viral envelope protein gp120) (*8*). This antigenic variability is particularly significant in helping a viral quasispecies to persist under immunological attack; many infectious agents [and, in particular, many lentivirus infections (*9*)] exhibit the ability to vary their dominant surface antigens, often by mutation events in replication, which helps them escape surveillance and destruction by the host's immune system (*10*).

We propose that the genetic variability of HIV is not so much a complication, as the key to understanding the development of AIDS. In particular, we examine a mathematical model for viral multiplication that explicitly describes the interplay between the total diversity of viral strains (which in general will increase over time) and the suppressing capacity of the immune system. The model shows that the human immune system is only able to mount an effective response against HIV quasispecies whose diversity is below some threshold value; once the population of viral strains exceeds this "diversity threshold" the immune system is no longer able to regulate viral replication, with consequent destruction of $CD4^+$ cells.

These ideas are used to interpret new data from longitudinal studies of two infected patients (*11*). The model also helps explain many puzzling aspects of infection and the development of disease, including the variable likelihood of transmission between infected and susceptible sexual partners or infected mothers and unborn infants, the variety of cell types that the virus appears able to infect, the variable duration of the incubation period of AIDS, and the great diversity of symptoms of disease exhibited by patients. Moreover, if the ideas encapsulated in the model are basically correct, they have implications for the way different treatments (chemotherapy or immunotherapy) may alter the dynamic interplay between viral diversity and immune suppression, and thence the rate of development of AIDS.

Genetic Change in Viral Populations During the Incubation Period of AIDS

For a short but variable period—a few weeks to a few months—after an individual is infected with HIV-1, virus is typically found in the blood (viremia), and high levels of virus replication can be observed. Antibodies then appear in blood serum (seroconversion), after which it becomes difficult to isolate the virus; viral antigens are often undetectable during the long but variable asymptomatic or incubation period between primary HIV-1 infection and the occurrence of AIDS. This incubation period is characterized by low viral replication (interspersed with minor and short-lived upsurges of viremia in some patients), and by constant or slowly decreasing numbers of $CD4^+$ cells. As AIDS develops, viral isolation becomes easier; the proportion of infected cells in peripheral blood is 100 to 1000 times higher in AIDS patients than in asymptomatic individuals (*12*). In AIDS patients, viremia can be reduced, and $CD4^+$ cell counts raised, by treatment with Zidovudine (AZT), but such changes may be transient, only lasting 6 to 12 months (*13*).

These temporal trends in virus antigen (P24) and $CD4^+$ cell counts were observed in two homosexual men in Amsterdam who seroconverted in 1985 (*14*). One individual developed AIDS 55 months after becoming infected with HIV-1 (specifically, from the first p24 antigenemia peak), and chemotherapy was begun in late 1989 (Fig. 1A). The second individual remained asymptomatic (Fig. 1C). Infected individuals appear to harbor a quasispecies of the virus, with a broad distribution of molecular sequences. Sequence variation is not uniform over the viral genome; the *gag* and *pol* genes, for example, show less variability than the *env* gene. Within the *env* gene, there are five hypervariable regions, V1 to V5. The immunodominant V3 loop is a region of about 30 amino acids within the envelope protein gp120; the V3 region contains an epitope that elicits isolate-specific neutralizing antibodies. This V3 region exhibits high mutation rates, and the change of one amino acid in this V3 region can restrict recognition by neutralizing antibodies (*15*).

Some evidence indicates that the biological properties of HIV-1

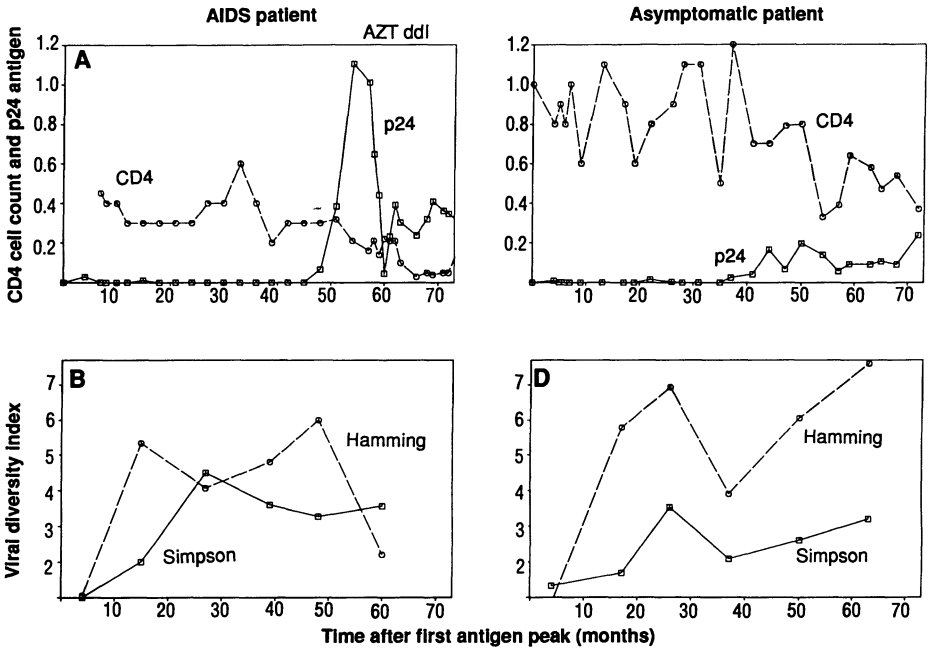

Fig. 1. (**A** and **C**) $CD4^+$ cell counts ($\times 10^9$ per liter) and p24 antigen ($\times$ 2000 pg/ml) for two homosexual male patients, 1 and 495 (*14*). For both men, the first serum sample was taken in 1985 at the moment of p24 antigen conversion, 0.5 to 3 months before the development of antibodies to HIV. Successive samples were obtained 13, 22, 34, 46, and 59 months later for patient 1, and 12, 24, 36, 45, and 57 months later for patient 495. Patient 1 remained asymptomatic during follow-up (only nonsyncytium-inducing (NSI) viruses could be isolated) and received no anti-viral therapy. Patient 495 developed AIDS (CDC-IV C1) 55 months after antigen conversion, began AZT treatment in month 56, and was switched to ddI treatment in month 64 (a change from NSI to SI viruses was observed between 1988 and 1989). (**B** and **D**) The genetic diversity of the V3 loop of the envelope protein gp120 was extremely low at time of seroconversion (11 of 11 sequences identical for the V3 loop from patient 495, and 6 of 7 sequences identical from patient 1), and increased during the asymptomatic period. Nucleotide sequences of a region containing the V3 loop were obtained by repeated isolation of virus particles from the serum, transcription of the viral RNA into cDNA, and double PCR (polymer chain reaction) amplification (*32*). From 8 to 12 sequences were analyzed at six time points. The viral population diversities for both patient 1 (D) and patient 495 (B) were calculated from the amino acid sequences by two different methods: the mean Hamming distance (*21*) for the entire sequence of 93 amino acids and the Simpson index (as defined in the text), for only the 36 amino acids in the V3 loop.

may vary significantly from clone to clone (*16*). There is, for example, measurable variation among the replication rates of HIV-1 strains in $CD4^+$ cells (*17*). In general, more virulent strains tend to appear later in the incubation period (with virulence defined in terms of cytophatic properties and high replication rates) (*3, 18*). Thus HIV-1 isolates from asymptomatic carriers tend to grow slowly and to have low titers of reverse transcriptase (RTase) activity, whereas isolates from patients with "AIDS related complex" (ARC) or with AIDS grow rapidly, induce cell syncytia more frequently, and show high RTase activity (*18*). Mathematical models of the interaction of HIV-1 and $CD4^+$ suggest that evolutionary forces may drive selection for more virulent strains (*19*).

To assess how genetic variation within the HIV quasispecies infecting a given patient changes over time, we studied viral isolates from serum samples taken at intervals of 10 to 12 months from the two homosexual males referred to above (Fig. 1, A and C). Variation in the V3 domain was examined by cloning sequences derived from the virus's genomic RNA, which had been isolated from serum, amplified by the polymerase chain reaction (PCR), treated with RTase, and cloned into PGem. The first serum sample was taken during the first peak in p24 antigenemia. An appropriate inverse measure of the quasispecies diversity is given by the ecologists' Simpson index, $D = \Sigma_i (\nu_i/\nu)^2$, where ν_i denotes the number of type i sequences in the sample, and ν denotes the total number of sequences; the index takes into account the frequency of sequence i in relation to the total sample of sequences. For the two patients described earlier, we plotted the parameter $1/D$ and the mean Hamming distance (*20*) for each sample as a function of time (Fig. 1, B and D). In both patients the genetic diversity was extremely low at the time of seroconversion and it increased during the asymptomatic period. In the patient who developed AIDS, diversity reached a peak before the onset of AIDS and seemed to decline thereafter (Fig. 1B). The pattern of decline in diversity as AIDS develops may appear counterintuitive. The observed pattern was, however, what would be expected from sampling theory (which underlies the definition of the Simpson's index) when isolates are drawn from a large population of different mutants during the AIDS phase; in this phase, which is characterized by relatively unregulated growth of the viral population, strains with faster replication rates will be most abundant and will be most likely to be detected (*21*). The apparent decline in diversity is thus caused by selection for fast replicating mutants once the immune system can no longer regulate the viral population (*22*).

The observations that the number of different strains increases over time (provided that sampling biases are taken into account), and that virulent strains occur more frequently once symptoms of disease develop, lead us to ask whether this pattern is a cause, or a consequence, of immunodeficiency. To pose this question more sharply, we used a simple mathematical model of how the evolutionary dynamics of HIV-1 quasispecies interact with the immune system.

Mathematical Model of Virus Evolution During the Course of This Infection

The model, an extension of an earlier one (*23*), keeps track of the changes, over time, in the densities of the populations of the various viral strains and different kinds of $CD4^+$ cells. The model consists of a system of ordinary differential equations, whose structure reflects what we hypothesize are three key features distinctive to HIV infections. (i) The continuing appearance of new antigenic variants, or "escape mutants," of the virus enables the overall virus population to evade elimination by the immune system. (ii) Immunological responses directed against the virus involve a specific response to individual strains (subpopulations of $CD4^+$ cells specifically directed toward immunological attack against that strain) along with a cross-reactive response that acts against all strains. (iii) Each viral strain can infect and subsequently kill all $CD4^+$ cells, regardless of their specificity to a particular mutant. More precisely, the assumption (ii) deals with subpopulations of $CD4^+$ cells that can mount immunological attack against specific viral epitopes; if an epitope is conserved among mutants then the resulting immune response is cross-reactive, but if it is within a hypervariable region (such as the V3 loop) then only some viral variants are recognized by this particular response.

The model resulting from these assumptions has four kinds of

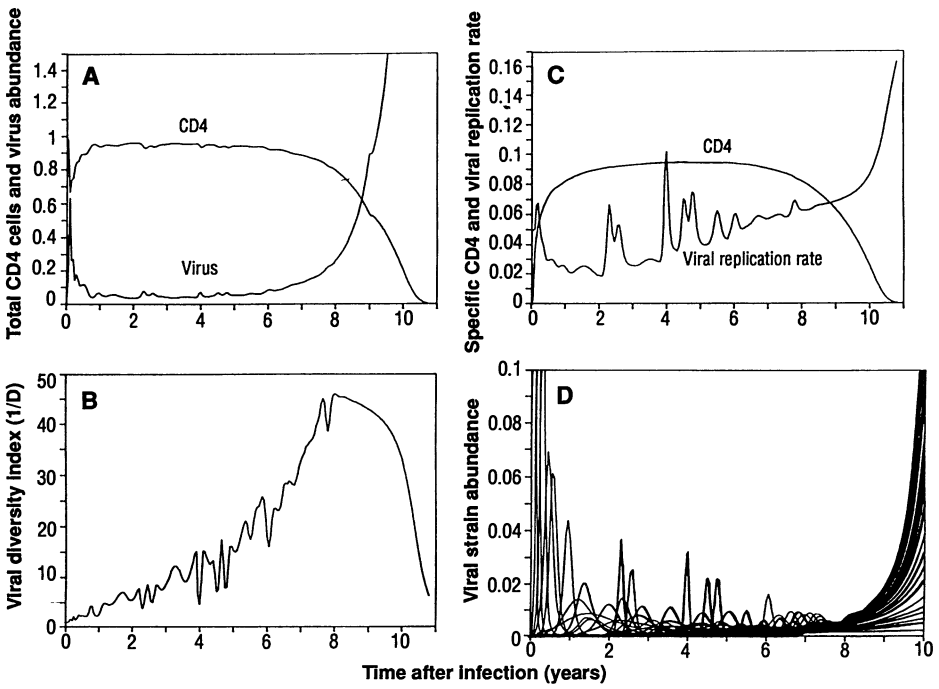

Fig. 2. A computer simulation of changes over time in HIV abundance and $CD4^+$ cell counts in an individual patient, as described by Eqs. 1 to 4. (**A**) Total virus and $CD4^+$ cell abundance in arbitrary units. (**B**) Antigenic diversity of the virus population measured by the Simpson index, D; the plot shows $1/D$ as a function of time. (**C**) The average virus replication rate $r = \Sigma\ r_i\ \nu_i/\nu$ "switches" from low to high as AIDS develops (the unit of the replicative rate is yr^{-1}); superimposed is the total density of $CD4^+$ cells (arbitrary units) directed to HIV antigens. (**D**) Abundance of 40 different HIV variants (arbitrary units) that evolve during the course of infection. The parameter values in Eqs. 1 to 4 are taken to be: $K = 100$; $d = 1$; $k = k' = 0.1$; $u = 1$; $r_i' = 3\ r_i$; $s_i = 9.5\ r_i$; $p_i = 20\ r_i$ (all rates have dimension yr^{-1}). The variable r_i was taken from an exponential distribution, with mean 0.05. This implies that the diversity threshold is $n_c = 25$.

variables: v_i, y, x_i, and z, which denote the densities of virus strain i, total CD4$^+$ cells, CD4$^+$ cells specific to strain i, and CD4$^+$ cells that mount cross-reactive responses to all strains, respectively. The total virus population is represented by $v = \Sigma v_i$. The rates of change of each variable with respect to time ($t = 0$ being the point when the host acquires infection) are then:

$$\text{Virus population } dv_i/dt = f_i(v_i,y) - v_i(s_i z + p_i x_i)\ i = 1, 2, \ldots, n \quad (1)$$

$$\text{Total CD4}^+ \text{ cells } dy/dt = K - dy - uvy \quad (2)$$

$$\text{Strain-specific CD4}^+ \text{ cells } dx_i/dt = kv_i y - uvx_i\ i = 1, 2, \ldots, n \quad (3)$$

$$\text{Cross-reactive CD4}^+ \text{ cells } dz/dt = k'vy - uvz \quad (4)$$

The variables x_i and z are some fraction of the total CD4$^+$ cell population. In Eq. 1 the term $f_i(v_i,y)$ denotes the reproductive rate of viral strain i. We define this function as $f_i(v_i,y) = (r_i' + r_i y)v_i$ to denote replication of strain i at a per capita rate $r_i y$, arising from infection of CD4$^+$ cells (y), along with a low but constant background replication rate r_i' to denote replication of the virus in cells other than of the CD4$^+$ type (such as macrophages or monocytes). The term $s_i z v_i$ represents the killing of the strain i by cross-reactive CD4$^+$ cells, and the term $p_i x_i v_i$ denotes the killing by strain-specific CD4$^+$ cells. In Eq. 2, K is the recruitment rate of CD4$^+$ cells (from the thymus), d is the per capita death rate, and uvy denotes the rate at which cells are killed by any member of the total virus population. Equivalently, the recruitment terms in Eqs. 3 and 4 ($kv_i y$ and $k'vy$, respectively) denote activated cells joining the strain-specific and cross-reactive CD4$^+$ cell populations. Activated strain-specific and cross-reactive lymphocytes are killed by the virus at net rates uvx_i and uvz, respectively, in Eqs. 3 and 4. The total number of virus strains, n, is not constant, because replication errors generate new mutants that escape the current strain-specific immune responses and persist in the presence of the cross-reactive responses. This introduces a stochastic element, where the probability that a new strain is generated in the time interval between t and $t + dt$ is given by $Pv(t)dt$. The constant P convolves the replication rate of the virus population and the probability that mutation generates a new strain that is not recognized by the current strain-specific responses.

The main properties of the above model can be understood from analytic and numerical studies of the set of equations. One such simulation is presented in Fig. 2. Initially we see high levels of viremia (characteristic of primary HIV infection), but the immune response soon suppresses the strains of the infecting inoculum and of early mutants (Fig. 2A). However, over time there arise new mutants that are not recognized by the current strain-specific immune responses (escape mutants), and these mutants generate mini-outbreaks of viremia (Fig. 2, A and D). These mini-outbreaks are in turn suppressed by a combination of specific and cross-reactive responses. As the total number of antigenically distinct viral strains increases over time (Fig. 2B), the total population of CD4$^+$ cells begins to decline (*24*) (Fig. 2A). After a long period of low viremia (with sporadic small blips), viral abundance begins to rise rapidly and, concomitantly, CD4$^+$ cell abundance declines to low levels. This final phase represents the collapse of the immune system and the development of AIDS. Over this long period, the average replication rate of specific CD4$^+$ cells initially rises, attains a plateau, and then declines as the total virus population escapes regulation by the immune system (Fig. 2C). Viral diversity, as measured by the inverse of the Simpson's index, initially increases, but in the later stages declines as the faster replicating strains predominate (even though the total number of strains continues to increase) once immunological regulation breaks down (Fig. 3). The patterns generated by the model are quite similar to those observed in infected patients (compare Figs. 1 and 2).

Insight into the nonlinear mechanisms that generate the slow development of immunodeficiency and the final rise in viral population abundance can be gained from analytic investigations of a simplified version of the above equations (*23*). Specifically, assume that all viral strains have the same replication rate, r (regardless of the total density of CD4$^+$ cells), and that the parameters s and p in Eqs. 1 to 4 are also constant and independent of strain type. With these assumptions, we can see that the total abundances of specific and unspecific CD4$^+$ cells converge quickly, as infection progresses, to steady levels x and z, respectively. Equation 1, describing how the density of viral strain i changes over time, now becomes $dv_i/dt = v_i(r - sz - px_i)$. In the parameter region where the strain replication rate, r, can outrun the unspecific immune response, but not the combined effects of unspecific and specific responses (that is, when $px > r - sz > 0$), only the continuous generation of new escape

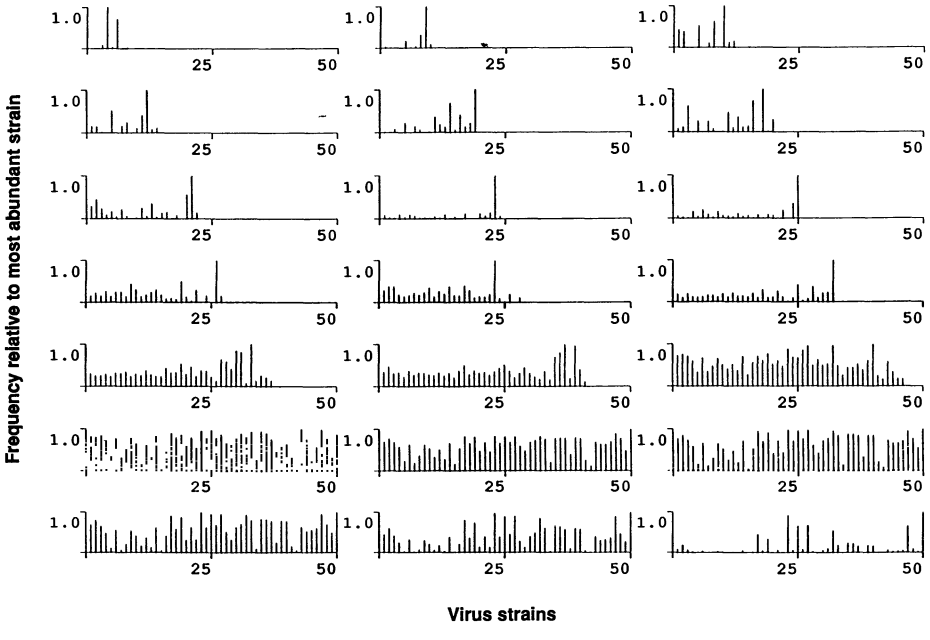

Fig. 3. Diversity plot for the simulation shown in Fig. 2. Viral diversity is sampled every 0.5 year (starting from the top left line). Each graph shows the different strains (labeled 1-50 on the x axis) and their abundance relative to the predominant strain (on the y axis). This figure illustrates the fluctuating, nonmonotonic increase in viral antigenic diversity. The number of different strains increases continuously, but the diversity of the population (as measured by the Simpson index) does not (Fig. 2B).

mutants enables the virus population to persist under immunological attack. The immune system should then be able to control the strain labeled i if dv_i/dt is eventually negative, that is if $r - sz - px_i < 0$. The immune system can therefore control the total viral population only if this inequality holds for all n values of i, where n is the total number of strains. This implies the restriction that (*23*)

$$n < n_c = px/(r - sz) \quad (5)$$

Thus, the total virus population can only be regulated by the immune system provided that the number of antigenically distinct strains present, n, is less than a critical antigenic diversity threshold, n_c. A generalized version of the threshold criterion, Eq. 5, can be derived when each escape mutant has a different value for the biological parameters r_i, s_i, u_i, p_i, and k_i in the modified Eqs. 1 to 4 (*25*). This threshold criterion is a peculiarly nonlinear property of a system in which the virus can kill any of the cells that are directing the immunological attack against it, but in which different viral strains require specific immune responses for effective control.

In short, as antigenic diversity increases over the course of HIV-1 infection (as new mutants accumulate), the diversity itself enables the virus population to escape control by the immune system and, concomitantly, results eventually in the destruction of this system. More specifically, the virus population can be seen to escape regulation once the inverse of the Simpson's diversity index, $1/D$, exceeds the value of the antigenic diversity threshold, n_c. The theory therefore provides an explanation of the slow progression of HIV infection to AIDS based on the slow accumulation of immunologically distinct HIV strains via the generation of escape mutants (over the asymptomatic stage); it ends with the breaching of the diversity threshold, upon which the virus population escapes regulation and induces the destruction of the immune system.

The model provides other insights. For example, the generation of escape mutants by replication errors is a chance process. Hence it can happen that, in some of the simulations with a given set of parameters, the infection is cleared from the host (viral abundance is reduced effectively to zero). More generally, the average infected individual develops AIDS only if each virus strain produces at least one new escape mutant before being suppressed by the immune system; the average number of escape mutants is measured by R_0, the basic reproductive number (*26*). The prediction that the viral population may sometimes be eliminated during the asymptomatic phase of infection is of interest in that some infected individuals (both adults and infants born to infected mothers) remain antibody positive but convert to a state where antigen is undetectable (*27*). It could be that some fraction of seropositive individuals have been able to clear the viral infection because of chance effects in the timing of the appearance of new strains.

Another prediction deals with temporal patterns in the diversity of fast replicating strains in the viral population. Once the diversity threshold is exceeded, strains with faster replication rates would predominate in the rapidly growing viral population, although slower growing strains would also expand their population sizes. The model therefore suggests that the increased frequencies of fast-replicating strains observed in patients with symptoms of disease is a consequence of the antigenic diversity threshold being exceeded, and not the cause of the severe immunodeficiency (Fig. 2C). Also Eq. 5 has the implication that a "weaker" immune system (smaller x or z) implies a lower diversity threshold, and hence faster progression to AIDS in infants and, to a lesser extent, in older people; this is in accord with the facts.

The qualitative agreement between a range of predictions and the patterns observed in infected patients engenders confidence in the biological assumptions crudely captured by the model and prompts us to use this template to assess (i) the potential impact of immunotherapy and chemotherapy in delaying the onset of disease and (ii) the problems in vaccine development.

Vaccination and Immunotherapy

How many of the large number of different antigenic strains of HIV-1 must be recognized by a vaccine if it is to prevent the progression from infection to AIDS? To address this question, we consider a simple branching process that caricatures the emergence and loss of escape mutants (*28*). This leads to an expression for the probability, μ, that infection with a single strain of HIV-1 will eventually lead to AIDS (that is, to the number of strains, n, exceeding the diversity threshold, n_c); this expression for μ involves only the single parameter R_0 defined as the average number of escape mutants produced by each strain. A successful vaccine must reduce R_0 below unity. If R_0 is originally large, it will be difficult for a vaccine that stimulates strain-specific immunity to bring it below unity. Alternatively a vaccine could stimulate the production of cross-reactive $CD4^+$ cells (to increase the magnitude of z in Eq. 1), which could suppress the virus if its replication rate is unable to outrun the unspecific immune response ($f_i < s_i v_i z$ for all i in Eq. 1).

Similar principles apply to immunotherapy, which aims to enhance the specific *or* non-specific responses in patients who are already infected. The success of an immunogen that can neutralize, say, 80 percent of all possible HIV variants, depends on the magnitude of R_0. If $R_0 = 10$, then an 80 percent reduction in its magnitude decreases the probability of developing AIDS from 0.9999 to 0.8; but if $R_0 = 20$, then an 80 percent immunogen decreases the chances from essentially unity to only 0.98, which is still rather poor (*28*). As yet we have little idea for the magnitude of R_0 for HIV-1.

Another relevant question is how the rate of progression to AIDS is affected by when immunotherapy starts. We have explored the dynamical behavior of Eqs. 1 to 4 when immunotherapy is begun at a defined time, t, from the point of infection and continues thereafter (the basic parameter values are as in Fig. 2). Figure 4, A to C, shows the simulated system with immunotherapy begun 4, 8, and 9 years, respectively, after the initial infection. The immunogen used in the treatment was assumed to stimulate immunological recognition of 80 percent of all strains present, and to enhance the strain-specific response to these variants by a factor 5 (in Eq. 3, $k = 5$ for 80 percent of the strains, and $k = 1$ for 20 percent of the strains). When treatment was started at year 9, it had essentially no effect; when started in year 8, it prolonged the incubation period of AIDS by roughly 3 years; and when treatment started in year 4, viral abundance was still low 15 years after the original infection. Thus, Eqs. 1 to 4 suggest that much may be gained by treatment early in the course of infection. This conclusion is, however, tentative, given the many uncertainties about how potential therapies may stimulate the immune system.

Chemotherapy and Drug-Resistant Strains of the Virus

A number of studies, both in vivo and in vitro, have demonstrated the ability of HIV-1 to evolve resistance to the drug AZT (*29*). Indeed, the observation that AZT suppresses viremia in AIDS patients only for periods of roughly 6 to 12 months is probably associated with the emergence of resistant strains, a finding that raises several questions. For example, does the timing of chemotherapy affect the speed at which resistant strains emerge; and does

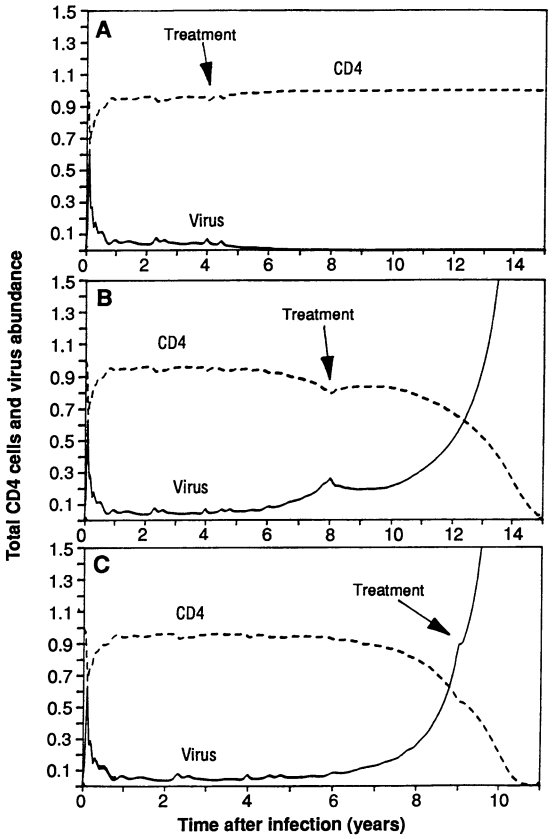

Fig. 4 (left). Simulation, with immunotherapy starting at different time points after infection. (**A**) At 4 years; (**B**) at 8 years, (**C**) at 9 years. The immunogen increases the strain-specific response to 80 percent of all viral variants (that is, $k = 5$ for recognized strains and $k = 1$ for nonrecognized strains). This simulation suggests that early treatment is more effective because the virus diversity is lower then. The parameter values are as defined in Fig. 2. Virus abundance and CD4 cell count in arbitrary units.

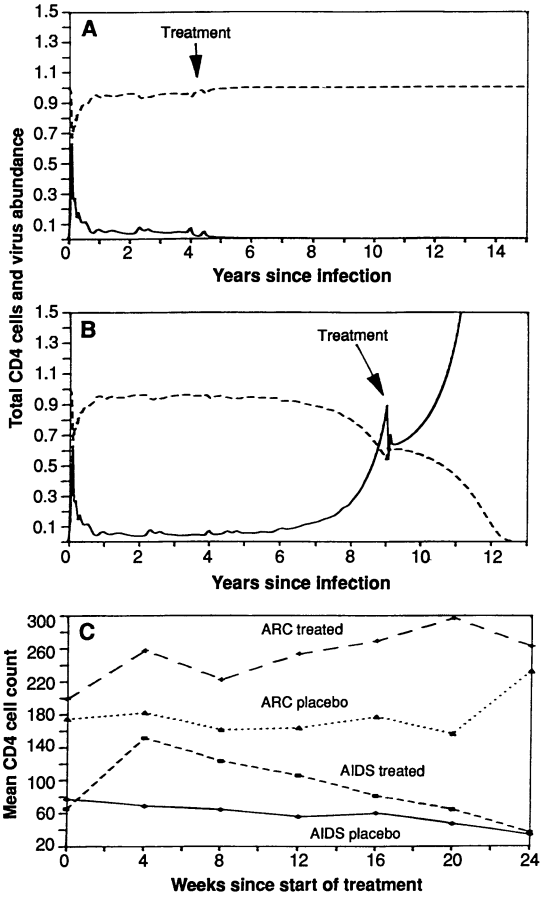

Fig. 5 (right). Chemotherapy (for example, AZT treatment) may reduce the viral replication rate. In these simulations chemotherapy is started (**A**) 4 years and (**B**) 9 years after infection; once begun, chemotherapy is applied continuously throughout the duration of the simulation. This leads to an increase in the disease-free period, which is more pronounced if treatment begins earlier [compare (A) and (B)]. The evolution of drug-resistant strains is modeled by the assumption that 20 percent of all HIV strains are less sensitive to the drug. Treatment reduced the replication rates of AZT-sensitive strains to one-tenth of their original value; replication rates of AZT-resistant strains remained unchanged. All other parameter values are as defined in Fig. 2. Virus abundance and CD4 cell count are given in arbitrary units. (**C**) Data for the population dynamics of $CD4^+$ cells ($\times 10^6$ per liter) in ARC and AIDS patients under AZT treatment (*13*); initially the $CD4^+$ cell count increases, but it decreases as resistant strains are selected. The observed dynamics are similar to those recorded in the simulations.

treatment with AZT in the asymptomatic phase of infection significantly delay the onset of symptoms of disease? Equations 1 to 4 can be used to reflect the impact of chemotherapy, thus indicating answers to these questions.

In Fig. 5 an example is presented that records numerical simulations in which the drug dose acts to reduce the replication rate of 80 percent of the strains by a factor of 10, while the remaining 20 percent of strains are drug resistant (having the same replication rate as before treatment, as in Fig. 2). In the first simulation treatment was started at year 4, and in the second at year 9, from the onset of infection. The principles that emerge are similar to those for simulated immunotherapy. Treatment late in the course of infection has modest impact (delaying rapid viral population growth by about 1 to 1.5 years in this example), whereas early treatment can significantly lengthen the incubation period. The patterns of change in viremia and $CD4^+$ cell abundance are similar to those observed in treated patients (Fig. 5C).

Future Directions

The ideas presented here (and summarized by Eqs. 1 to 4) suggest that viral antigenic diversity is the cause, not a consequence, of the development of AIDS. The theory rests on three main assumptions, each supported by data. (i) Replication errors produce antigenically distinct strains of HIV-1 at a high rate; (ii) among these strains are "escape mutants," whose control requires additional strain-specific immune responses; and (iii) all strains of the viral quasispecies can kill any of the $CD4^+$ cells that orchestrate both specific and unspecific immunological responses. The nonlinear dynamics of this peculiar system of interacting population of cell types can generate an antigenic diversity threshold: the immune system is able to regulate a viral population whose diversity is below a threshold value, but is unable to constrain growth once diversity becomes too high. Thus, for this particular system, the action of strain-specific immunity in creating antigenic diversity paradoxically ends up triggering the destruction of the immune system itself.

The mathematical model leads to clearly testable (and falsifiable) hypotheses, which survive comparison with the admittedly limited data that are available from longitudinal studies of patients over the incubation period of AIDS. These observations include a two-peaked pattern of viral abundance (with peaks during the initial HIV infection, and as ARC and AIDS develop), small sporadic upsurges in the intervening asymptomatic phase, the coexistence of an increasing number of antigenically distinct viral strains over the incubation period, and the dominance of fast replicating strains in ARC or AIDS patients (with the last 2 features resulting in a "humped" pattern of viral diversity, as measured by Simpson's index or other such measures). The model also makes testable predictions about control strategies, including the suggestion (subject to the caveats above) that immunotherapy and chemotherapy are likely to be more effective in delaying the onset of symptoms if they are begun early in the course of the infection.

The model contains parameters whose values must be assigned.

Although predicted patterns are in qualitative agreement with observed trends, the quantitative details depend on the precise values of the parameters. Accurate information about these basic parameters is lacking, because the population biology of cell infection and death, of viral population growth rates, or of the rate of production of "escape mutants" during replication require further studies and, if possible, studies in vivo (*30*).

In general, we need more studies of the population biology of the human immune system and its interaction with infectious agents. The population dynamics of such systems are typically highly nonlinear, so that changes in cell populations over time cannot be inferred simply from descriptions of the interactions between different types of individual cells and viral variants, no matter how detailed these may be (*19, 31*). Our model of antigenic change in HIV-1 infection shows that a mathematical description of the biological processes can help us interpret observed trends and define what needs to be measured (*33*).

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24. Note also the initial blip in $CD4^+$ density in Fig. 2A, induced by the primary HIV infection; see H. Gaines *et al.*, *AIDS* **4**, 995 (1990).
25. When the different escape mutants have different values of the parameters r_i, s_i, u_i, p_i, and k_i, then the simple threshold criterion of Eq. 5, namely $n(r - sz)u/pk > 1$, is replaced by
$$\sum_{i=1}^{n} (r_i - s_i z)\, u_i/p_i k_i, > 1$$
(M. A. Nowak, in preparation). A few strains with very high reproductive rates, or high pathogenicity in $CD4^+$ cells, can now have disproportionate influence on whether the diversity threshold is breached.
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28. The number of escape mutants produced by one virus strain follows a Poisson distribution with mean value R_0 (as defined in the text). The number of strains in each generation (if we assume discrete time) can be described by a branching process. Starting with one strain, the probability of extinction of the virus population is given by the root of the equation $S = \exp[R_0(S - 1)]$. For $R_0 >> 1$, we have approximately $S = \exp(-R_0)$; the probability that the virus population survives by antigenic variation (and develops AIDS after some time) is given by $\mu = 1 - \exp(-R_0)$. The average time to exceed the diversity threshold can be roughly estimated by the following argument: after t generations there are approximately R_0^t strains on average; therefore $t \simeq \log(n_c)/\log(R_0)$ generations are required to produce n_c strains. For this branching process, the minimal fraction of strains that must be recognized by a successful vaccine is given by $f = 1 - 1/R_0$. There is also a critical time for successful immunization given by $t_c = -\log(1 - p)/\log R_0$, where p is the probability that immunization from the beginning of infection leads to the extinction of the virus infection. This means that immunization before t_c can be successful, but not after t_c. This should be compared with Fig. 4.
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32. *Molecular cloning.* Isolation of virus particle (genomic) RNA from 50 μl of serum proceeded according to Boom *et al.* Viral RNA was converted to cDNA with AMV RT (5U) (Boehringer) and 10 pmol of the 3′V3 primer (described below) in RT-buffer containing 75 mM KCl, 50 mM tris-HCl (pH 8.3), mM $MgCl_2$, 10 mM DTT (dithiothreitol), and 0.25 mM each of dATP, dCTP, dGTP, and dTTP (Pharmacia, Woerden) in a total volume of 20 μl. A sample without AMV RT was used as a negative control. cDNA was subjected to a double polymerase chain reaction (PCR). Primers used for the first PCR were 5′V3 (5′-ATAAGCT-TCAATGTACACATGGAATT-3′, HXB2 position 6506–6523, Los Alamos 1990), and 3′V3 (5′-ATGAATTC ATTACAGTAGAAAAATTCCC-3′, HXB2 position 6909–6928) bracketing the primers J-5′-2-KSI (5′-ATAAGCTTG-CAGT- CTAGCAGAAGAAGA-3′, HXB2 position 6558–6576) and J-3′-2-KSI-2 (5′-ATGAATTC TGGGTCCCCTCCTGAGGA-3′, HXB2 position 6860–6877) which were used for the second PCR. To allow subsequent cloning, a Hind III (AAGCTT) restriction site was incorporated in the J-5′-2-KSI primer and an Eco RI (GAATTC) restriction site in the J-3′-2-KSI-2 primer. The final PCR reaction mixture (100 μl) consisted of 53 mM KCl, 25 mM tris-HCl (pH 8.4), 3.3 mM $MgCl_2$, BSA at 75 μg/ml, each dNTP at 0.24 mM, 10 pmol of each oligonucleotide primer, and 3 U of Taq polymerase (Perkin-Elmer Cetus). The reaction was performed for 35 cycles. Each cycle consists of a 1-minute denaturation step at 95°C, a 1-minute annealing step at 55°C, and a 2-minute elongation step at 72°C. A subsequent reamplification of 10 μl out of the first PCR in a second PCR with the KSI-primers was performed under the same conditions. PCR products were purified by preparative agarose gel electrophoresis and digested with Eco RI and Hind III. The digested fragment was cloned into Eco RI and Hind III digested plasmid pGEM-7 (Promega Biotec) and transformed into *E. coli* strain HB 101. For sequencing, plasmid DNA from 50-ml cultures was extracted with the Qiagen plasmid kit according to the manufacturer's recommendation (Qiagen). Sequencing was performed with an automatic sequencer (Applied Biosystems, CA) with the use of Sequenase (USB, Ohio).

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