Mathematical Biology of HIV Infections: Antigenic Variation and Diversity Threshold

MARTIN A. NOWAK AND ROBERT M. MAY
Department of Zoology, University of Oxford, OX1 3PS, Oxford, England

Received 25 October 1990; revised 20 December 1990

ABSTRACT

Infection with the human immunodeficiency virus (HIV) results in severe damage to the immune system and consequent disease (AIDS) after a long and variable incubation period (on average 8–10 years). Why the incubation period should be so long is a puzzle. We outline an explanation based on the dynamics of the interplay between the immune response and antigenic variation in the virus population. The essential idea is that AIDS results when the diversity of antigenic variants of HIV in an infected patient exceeds some threshold, beyond which the immune system can no longer cope. The paper develops a simple mathematical model for this process, based on experimental observations, and explores several ramifications.

1. INTRODUCTION

Antigenic variation seems to be a major property of many parasitic infections. In response to the ability of the immune system to attack foreign antigens, parasites have evolved the capacity to escape immunological surveillance by mutating their immunodominant epitopes continuously during the time of an infection. The immune response is thus continually confronted with new targets. No sooner has the immune system generated cellular or humoral attack against these targets than the parasite has escaped with mutated antigens. This antigenic variation with the subsequent development of neutralizing antibodies to each emergent subpopulation of the pathogen is a common feature of lentivirus infections in particular. In this paper we stress the potential importance of antigenic drift for the pathogenesis of AIDS.

The human immunodeficiency virus (HIV) is the etiological agent of the acquired immunodeficiency syndrome (AIDS). Despite intensive study, it is still unclear how the virus induces immunodeficiency and which basic principles determine the development, and time scale to onset, of disease.

©Elsevier Science Publishing Co., Inc., 1991
655 Avenue of the Americas, New York, NY 10010 0025-5564/91/$03.50

1.1. *NATURAL HISTORY OF HIV-1 INFECTIONS*

Primary HIV-1 infection is typically associated with viremia, and for a short but variable period (weeks to months) after infection, virus replication can be detected either via virus isolation or by viral antigens in blood. Seroconversion follows, but there-after virus isolation becomes difficult, and viral antigens are often undetectable during the asymptomatic phase between primary HIV-1 infection and the occurrence of AIDS-related complex (ARC) or persistent generalized lymphadenopathy (PGL). This long and variable incubation period is characterized by low-level viral replication and constant or slowly decreasing $CD4^+$ T-cell numbers. As symptoms of disease develop, the ease of virus isolation increases; the fraction of infected cells in peripheral blood (estimated by isolation or the polymerase chain reaction) appears to be 100–1000-fold higher in AIDS patients than in asymptomatic individuals [18].

1.2. *HIV AND THE IMMUNE SYSTEM*

The interaction between the virus and the immune system appears to be a process of extraordinary complexity [16].

HIV can directly infect and kill the $CD4^+$ T cells that play a central role in organizing immune responses. In addition to this direct cytopathic effect, HIV seems to induce the depletion of these cells by indirect mechanisms, such as the killing of precursor cells, or by anti-gp120 antibody attack against cells that have bound to soluble gp120 (the viral envelope protein) shed by HIV particles or infected cells.

The depletion of CD4 cells is probably not the only adverse effect of HIV on the immune system. The CD4 receptor, which is used for viral binding and entry into the host cell via interaction with the envelope protein gp120, is the natural receptor for MHC class II molecules. The reported sequence homology between a region in gp120 and a region in MHC II molecules may result in the generation of cross-reactive antibodies that suppress natural immune function.

The interaction between the CD4 receptor and gp120 has also led to the hypothesis that this contact might deliver inappropriate signals that turn the CD4 cell into an uncontrolled state of useless activation [1].

In short, HIV appears to disrupt and confuse the immunoregulatory network.

However, immune responses against the virus can be observed in infected patients. The existence of neutralizing antibodies specific to particular HIV-1 antigens has been well documented [14, 21]. The development of humoral and cell-mediated responses soon after infection may be responsible for the suppression of the early viremia and the establishment of the

asymptomatic period. Unfortunately, it seems impossible at the moment to evaluate the effect of the different immune responses against HIV. Whereas antibodies can induce neutralization, cytotoxic killer cells appear to be more oriented against conserved epitopes.

1.3. *GENETIC VARIATION*

A striking feature of infection and the development of disease is the high genetic variability in virus isolates obtained either sequentially from the same infected patient or from different patients [2, 5, 9, 17, 19]. During the genetic metamorphosis of the RNA genome of HIV-1 into a DNA provirus upon cell infection, reverse transcription errors produced during DNA strand synthesis become fixed, and new "quasi-species" (populations of closely related but distinct viral genomes) of virus are created. The rate of nucleotide misincorporation is of the order of $> 10^{-4}$ per base per cycle, and for a genome of 10^4 bases this implies a reverse transcription error rate of more than one base per genome per metamorphic cycle.

Sequence variation is not uniform throughout the genome. The *gag* and *pol* genes are more conserved than the *env* gene. Within the *env* gene there is a pattern of five hypervariable regions, V1–V5. Of particular interest is the immunodominant V3 loop, a region of about 30 amino acids within the envelope protein (gp120), which appears to trigger neutralization phenomena in infected humans or chimpanzees [6, 10]. However, this part of the envelope protein mutates rapidly. For example, viral isolates derived from infected chimps soon after infection were resistant to sera that were able to neutralize the viral isolate used to first infect the host [10]. The change of a single amino acid in gp120 can apparently account for such clonal restriction of neutralizing activity [8].

Extensive sequencing of the V3 region in infected hemophiliac patients has made possible an estimate of the rates of synonymous and nonsynonymous nucleotide substitutions [2]. The ratio thus obtained, $K_s / K_a = 0.67$, was the lowest ever found. This study suggests strong positive selection for sequence change in the immunodominant V3 loop. In this context the observed viral variability represents an adaptive response by HIV to evade the immune system. The high mutation rate of HIV seems to maximize the rate at which new resistant mutants are produced [12].

Recent studies have also demonstrated sequence variation in epitopes of the conserved *gag* gene that are recognized by cytotoxic killer cells [13].

Infected individuals appear to harbor a quasi-species of the virus, with a broad distribution over the sequence space. Within this quasi-species, many immunologically different mutants are found, so that sera from such patients generally neutralize a broad range of isolates. In general, longitudinal observations on patients from the point of infection suggest specific re-

sponses early on and a gradual broadening of the immune response during the long incubation period of AIDS [3].

The reported continuous genetic variation during the asymptomatic phase suggests that viral replication takes place throughout the "silent" phase. This would contradict speculations that the silent phase is caused by true latency.

1.4. *VARIATION IN CELL TROPISM, CYTOPATHIC PROPERTIES, AND REPLICATION RATE*

A growing body of evidence reveals that the biological characteristics of the various quasi-species differ in such attributes as cell tropism and cytopathic properties. Interestingly, viral replication properties appear to be associated with cytopathic effects (with respect to both direct killing and the ability to induce syncytia formation). Recent work suggests an association between the presence of quasi-species with low replicative ability and no or mild disease and, conversely, the presence of quasi-species with high replicative ability and advanced disease [20]. Furthermore, it has been demonstrated that a change from low to high replicative ability occurs in viral isolates obtained from patients during progression from an asymptomatic state to a state of immunodeficiency and disease [4]. Such changes have been interpreted as signs of increased viral virulence in the host (high replication being associated with severe cytopathicity), but it is unclear whether the described changes are a cause or a consequence of the deterioration of the immune system.

1.5. *MATHEMATICAL MODELING FROM A SIMPLISTIC PERSPECTIVE*

In the subsequent sections we describe the development and analysis of a simple mathematical model of the interaction of HIV-1 with the human immune system [11]. Our aim is to outline some basic ideas that have the potential to explain observed patterns of change in viral abundance and diversity, and thence to explain the observed clinical picture in relation to the development of immunodeficiency and disease in infected patients during the long and variable incubation period of AIDS.

The model is based on three key assumptions:

(1) The continual evolution of new resistant viral mutants (via errors in viral replication) enables the total viral population (formed from the summation of all the quasi-species) to evade elimination by the immune system (selection by the immune system giving rise to new variants in the presence of neutralizing antibodies has been observed in vitro [15]).

(2) Immunological responses to the virus are characterized by a specific response to individual strains and a nonspecific general response that acts

against all strains (subpopulations of CD4-positive T-helper cells specific to a particular viral strain direct immunological attack against that strain).

(3) Each mutant can kill all $CD4^+$ T-helper cells regardless of their specificity to a particular mutant.

Assumptions 2 and 3 characterize the asymmetric interaction between CD4 cells and HIV particles. We consider subpopulations of CD4 cells that can mount immunological attack against specific viral epitopes. If this epitope is conserved among different mutants, then the resulting immune response is cross-reactive. If this epitope is within a hypervariable region, then only some viral variants are recognized by this immune response. For HIV it seems that most immunodominant epitopes mutate rapidly. Therefore, most of the relevant immune response seems to be based on strain-specific reactions. On the other hand, there appears to be no limitation for HIV strains to kill CD4 cells regardless of their specificity for certain epitopes.

As a consequence, the central hypothesis in our investigation is that the human immune system is able to mount an effective immune response only against a viral quasi-species whose diversity is below some threshold value. As the total population of viral quasi-species exceeds this "diversity threshold," the immune system is liable to "collapse," being unable to regulate viral replication and CD4 cell destruction.

2. THE BASIC MODEL

In this section we present a mathematical model that explores the quantitative consequences of the antigenic drift of HIV. The basic set of equations is as follows.

$$\dot{v}_i = v_i(r - sz - px_i), \qquad i = 1,\ldots,n; \tag{1}$$

$$\dot{x}_i = kv_i - uvx_i, \qquad i = 1,\ldots,n; \tag{2}$$

$$\dot{z} = k'v - uvz. \tag{3}$$

Here v_i denotes the population size of virus strain i. The replication rate r (including the whole cycle of infection and assumed to be the same for all strains in this initial model) might be thought of as the difference between birth and death rates: $r = bQ - d$, where the birth term includes the probability Q that replication is done without error. At first we assume that the replication rate is independent of the number of host cells (i.e., the number of potential host cells is roughly constant); d is the natural death rate of the virus (e.g., due to shedding of the envelope protein). The total virus population density is given by $v = \Sigma v_i$.

The terms sz and px_i represent unspecific (i.e. cross-reactive) and strain-specific immune reactions, respectively. These can be justified as follows. Let us assume that each mutant i induces the production of certain immune agents (CD4 cells), a fraction of which are specifically directed only against that particular mutant strain (e.g., via the immunodominant loop), while the other fraction is directed against more conserved sites (e.g., the *gag* or *pol* gene products or conserved regions within the *env* protein) and hence able to react with several different mutant strains. z is the number of immune agents directed against conserved regions, x_i is the number of cells directed specifically against a particular strain, and $x = \Sigma x_i$ denotes the total density of these "specific" immune cells.

The immune cells x_i and z are produced (recruited from a precursor cell population with roughly constant density) at the rates kv_i and $k'v$, proportional to the density of antigens. The killing of immune cells by viral mechanisms is denoted by the terms uvx_i and uvz; specific immune cells can be evoked only by specific viral strains (v_i) but can be killed by all strains (v).

Note that in this simplest model all viral mutants are assumed to have the same replicative capacity r; the same immunological parameters s, p, k, k'; and the same cytophatic capacity u. The mutants differ only in their specific (immunodominant) antigens. However, the results will remain essentially unchanged if the parameters are not equal for different strains.

Equations (1)–(3) describe the population dynamics for n different virus strains. The number of different strains, n, is not constant during infection. We assume that new mutants, which escape from the current strain-specific responses, are produced continuously during infection. To be specific, we assume that the probability that a new mutant is created in the time interval $(t, t+h)$ is given by $bQ'v(t)h$ (as $h \to 0$), where Q' is the probability that mutation yields an escape mutant. We neglect the possibility that mutation leads to mutants that are already present in the system, because the number of immunologically different mutants appears to be very large. (The combinatorial possibilities of the 19 variable amino acids in the immunodominant loop is 19^{20}, and furthermore the shape of the loop can also be altered by mutations in other parts of the envelope protein.)

Note that, summing Equation (2) over all strains, we get

$$\dot{x} = v(k - ux).$$

Hence the total number of specific and unspecific immune cells converge monotonically toward the equilibrium values $\hat{x} = k/u$ and $\hat{z} = k'/u$.

The parameter space can be divided into three regions:

(1) If $r - s\hat{z} < 0$, then the unspecific immune response is sufficiently strong in relation to viral replication rates and, by itself, able to suppress

the growth of all viral strains. There will be a rise in viral abundance following the initial infection, but once the unspecific immune response has been mounted the initial strain and all subsequently evolved strains will be suppressed by this generalized response.

(2) If $r - s\hat{z} - p\hat{x} > 0$ then the initial viral strain has a replication rate so fast that it can overwhelm both the specific and unspecific immune responses. The immune response will not be able to cope with this initial infection, and v goes to infinity (in this simplest model).

(3) Between these two extremes—that is, when $p\hat{x} > r - s\hat{z} > 0$—lies the interesting region of dynamical behavior, with its viral diversity threshold, that we now explore in more detail. This situation corresponds to individual viral strains having replication rates that can outrun the unspecific immune response but not the combined effect of unspecific and specific immune responses.

We can now establish the potential existence of a viral diversity threshold. The immune system can control strain i if $\dot{v}_i < 0$, which is to say if

$$r - sz - px_i < 0.$$

The immune system can thus control each individual strain only if this holds for all i ($i = 1, \ldots, n$), which implies the restriction that

$$n < n_c(x, z) = p\hat{x}/(r - s\hat{z}).$$

Hence there exists an upper limit, n_c, of different strains that can be suppressed simultaneously by the immune system, given by

$$n_c = pk/(ru - sk').$$

It is straightforward to show that once the viral diversity exceeds the threshold value ($n > n_c$), then the total amount virus ($v = \Sigma v_i$) will increase on average. This can be done by considering the product $P = v_1 v_2 \cdots v_n$ and noting that $\dot{P} > 0$ if and only if $n > n_c$. For a given value of P, the total amount of virus v thus has to be larger than $v_{\min} = n(P)^{1/n}$. Therefore v is bounded from below by $v_{\min}$, which increases monotonically.

Figure 1 shows typical computer simulations of Equations (1)–(3). Initially we observe high levels of virus, but the immune response is slowly increasing and finally manages to suppress the most abundant strain. In the meantime, however, new mutants have been created. The mini outbreaks of higher virus levels correspond to the occurrence of newly arisen neutralization-resistant mutants. During the course of infection the number of mutant strains continually increases and finally exceeds the threshold value n_c, which results in the sudden and continuous rise in viral abundance.

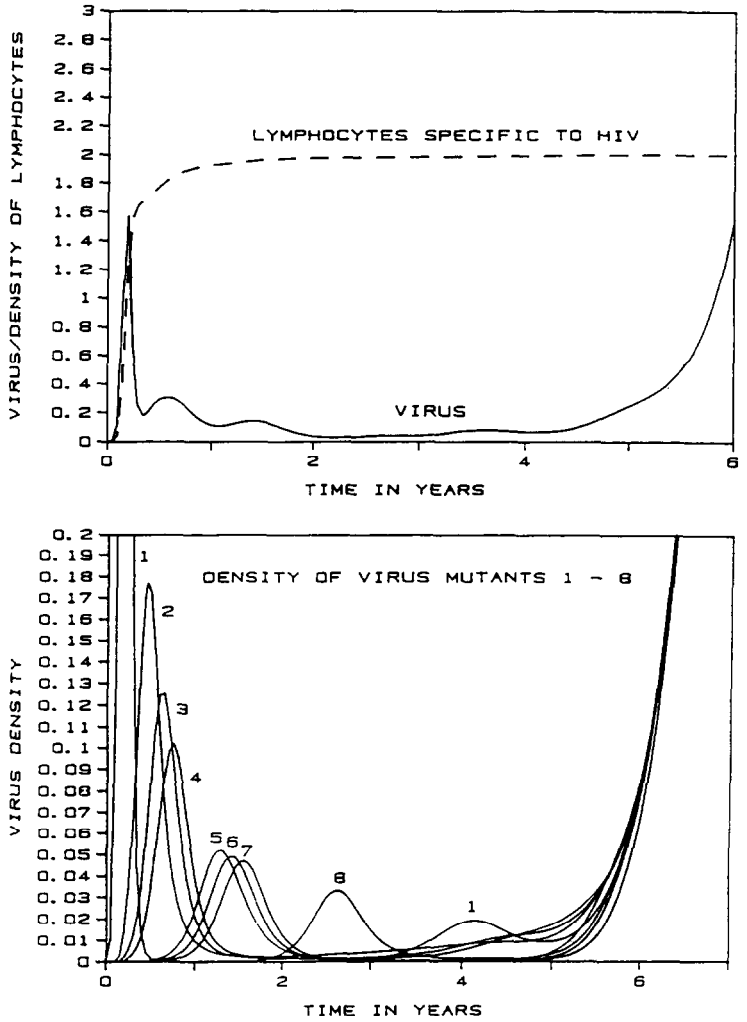


FIG. 1. Numerical simulation of HIV infection, as described by Equations (1)–(3). (Top) The solid curve shows the total virus concentration v, and the dashed curve shows the concentration of lymphocytes specific to HIV (the immune response, $x + z$). (Bottom) The sequence of antigenic drift. The individual mutants are downregulated by the immune response, but new mutants have been generated in the meanwhile. In the final phase we observe a simultaneous rise of all the strains present. In this figure, the parameters have the values $r = 5$, $s = 4.5$, $p = 5$, $k = k' = u = 1$, and $bQ' = 2$, implying a diversity threshold $n_c = 10$.

The unspecific immune response in our model is responsible for the fact that the initial strains grow to higher levels than the following escape mutants. Roughly speaking, the higher the effect of the unspecific response the higher the difference between the initial peak and the mini outbreaks in the silent phase. A stronger unspecific (cross-reactive) immune response is therefore correlated with lower viral abundance in the incubation period and with an increased length of this period.

In this simple model the immune response against HIV (the sum $x + z$) increases steadily during the course of infection. This reveals an interesting feature: It is not the constant depletion of immune cells that finally allows the virus to escape immune control, but rather the highly diverse virus population that manages to escape from an activated immune system. Beyond the threshold level of viral density, as the uncontrolled virus replicates to higher and higher levels, our model becomes unrealistic, because it has the feature that arbitrarily high virus concentrations can induce an arbitrarily high rate of immune cell production. This oversimplification will be corrected in Section 8.

In the final phase, when the threshold is exceeded and the virus escapes control by the immune system, strains that earlier were outcompeted can

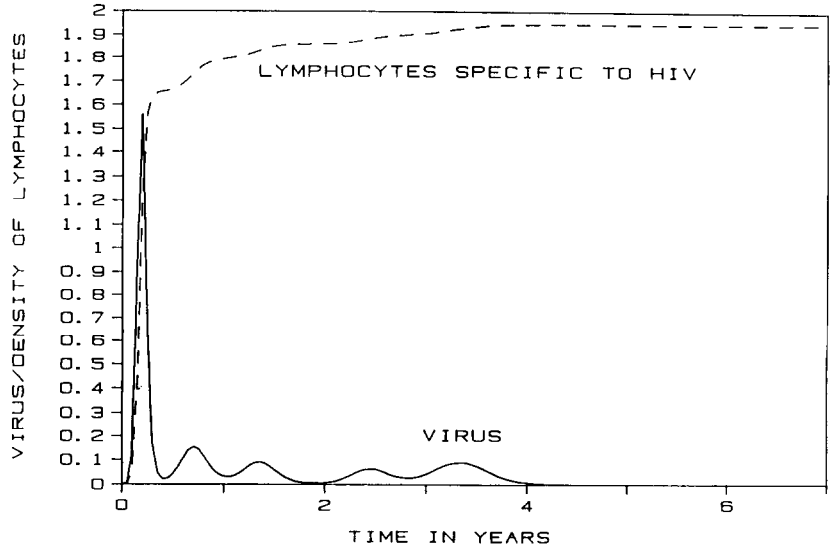


FIG. 2. The virus population is eventually eliminated by the immune response if the production rate of new resistant mutants is too low. In this example the parameters have the values $r = 5$, $s = 4.5$, $p = 5$, $k = k' = u = 1$, and $bQ' = 1.75$. For this mutation rate the virus population fails to reach the diversity threshold $n_c = 10$.

rise again. This happens as follows. After the immune system has suppressed a strain completely (v_i very close to zero) there is no further induction of specific immune cells directed toward that particular strain. After some time the frequency x_i has dropped below the threshold ($x_i < r - sz/p$) and $\dot{v}_i$ becomes positive again. This point seems to be important for the accumulation of disease in the final phase of infection.

Within our model it can also happen that the infection is cleared during the period of low virus concentration following the initial peak. This happens if the mutation rate to produce new resistant mutants is too low. On average we require that each virus strain has to produce at least one new escape mutant before being suppressed by the immune system.

Figure 2 shows a simulation where the immune system manages to kill the virus population. This would explain some observations that once-infected patients have lost the virus and recovered completely.

3. THE MINIMAL MUTATION RATE: THE ANTIGENIC DRIFT CONDITION

In this section we derive the minimal production rate of new resistant mutants that is necessary for the antigenic drift to continue.

The number of escape mutants produced by one viral strain in the time interval $[t, t+dt]$ is given by $bQ'v_i(t)\,dt$. The average number of escape mutants produced from one viral strain is then

$$R = bQ' \int_0^\infty v_i(t)\,dt.$$

We try to estimate the value of the integral $\int_0^\infty v_i(t)\,dt$. If we assume that the whole virus population at a given time point is dominated by only one strain, we may write

$$\dot{v}_i = v_i(r' - px_i), \qquad \dot{x}_i = v_i(k - ux_i),$$

where $r' = r - s\hat{z}$. Here we find the invariant

$$v_i(t) = v_i(0) + \int_0^{x_i(t)} \frac{r' - px}{k - ux}\,dx,$$

which leads to

$$v_i(t) = v_i(0) + \frac{p}{u}x_i - \frac{pk - r'u}{u^2} \log \frac{k}{k - ux_i}.$$

As $t \to \infty$ we expect $v_i(t) \to 0$, and therefore $x(\infty)$ is given by the solution of

the transcendental equation

$$\frac{px_i}{pk - r'u} = \frac{1}{u} \log \frac{k}{k - ux_i} \tag{4}$$

in the limit of very small $v_i(0)$. For reasonable (= larger) values of n_c (which means that $pk \gg r'u$), the solution converges quickly to $x_i(\infty) = 2r'/pk$. We obtain

$$\int_0^\infty v_i(t)\,dt = \int_0^{x_i(\infty)} \frac{dx}{k - ux} = \frac{1}{u} \log \frac{k}{k - ux_i(\infty)},$$

and together with Equation (4) we find

$$\int_0^\infty v_i(t)\,dt = 2\frac{r'/k}{pk - r'u} = \frac{2}{ku}\left(\frac{r - s\hat{z}}{p\hat{x} + s\hat{z} - r}\right).$$

Hence the mean number of new escape mutants produced by one parental strain is given by

$$R = \frac{2bQ'}{ku}\left(\frac{r - s\hat{z}}{p\hat{x} + s\hat{z} - r}\right).$$

The antigenic drift can be maintained only if each viral strain produces on average at least one resistant offspring. Hence we obtain the criterion $R > 1$.

4. ANTIGENIC DRIFT AS BRANCHING PROCESS

Next we consider the antigenic drift condition, $R > 1$, in a stochastic context.

Let $P_i(t)$ denote the probability that a certain virus strain produces i resistant offsprings in the time interval $[0, t]$, where time 0 represents the origin of the considered strain. The probability that no resistant mutant is produced can be decomposed as

$$P_0(t + h) = P_0(t)P_0(h) = P_0(t)[1 - bQ'v(t)h].$$

Taking the limit $h \to 0$, we obtain the differential equation

$$\dot{P}_0(t) = -bQ'v(t)P_0(t).$$

For the initial condition $P_0(0) = 1$, we have

$$P_0(t) = \exp\left[- bQ' \int_0^t v(\tau)\, d\tau \right].$$

The probability that a given strain does not produce any resistant offspring at all is then

$$P_0 = \exp\left[- bQ' \int_0^\infty v(\tau)\, d\tau \right] = e^{-R}.$$

It can be shown that the total number of escape mutants produced from one strain follows a Poisson distribution,

$$P_i = R^i e^{-R} / i!$$

Each strain produces i offspring with probability P_i.

If there are n_t different strains present at time t, the number of strains in the next "generation," $t + 1$, is given by

$$n_{t+1} = \sum_{j=1}^{n_t} k_j,$$

where each k_j is a random variable with distribution $\Pr(k_j = i) = P_i$. This generates a branching process, where the number of offspring is Poisson-distributed. The probability-generating function of this process is given by

$$F(s) = \sum_{i=0}^{\infty} P_i s^i = e^{R(s-1)}.$$

The probability of eventual extinction is represented by the smallest positive root, s^*, of the equation $s = F(s)$. In other words, $1 - s^*$ denotes the probability that the antigenic drift continues forever, that is, reaches any arbitrarily high diversity threshold. Table 1 shows this probability for some values of R.

A detailed discussion of branching processes is given by Karlin and Taylor [7].

5. IMMUNIZATION AGAINST MUTATING EPITOPES

In this section we ask the question, What is the fraction of HIV variants that must be recognized by an immunogen (or vaccine) to prohibit the development of disease (AIDS)?

TABLE 1

Reproduction Rate of Escape Mutants, R, versus the Extinction Probability of the Branching Process, s^*

R	s^*
1	1.0
1.001	0.9980
1.01	0.9803
1.1	0.8239
1.2	0.6863
1.5	0.4172
2	0.2032
3	0.0595
4	0.0198
10	4.54×10^{-5}
20	2.06×10^{-9}
30	9.36×10^{-14}
$1+\epsilon \gg 1$	$1-2\epsilon+\frac{8}{3}\epsilon^2\cdots$ $e^{-R}(1+Re^{-R}+\cdots)$

We assume that AIDS is developed if the virus has reached the diversity threshold. In the context of the described branching process, we simply sum up the total number of strains that are produced in each "generation" (starting with initial diversity n_0):

$$n = n_0 + n_1 + n_2 + \cdots .$$

If the sequence n_i tends to infinity, then n is clearly larger than n_c, and AIDS is developed. If the sequence n_i goes to zero, then we have two possibilities: Either $n > n_c$ and disease is induced, or $n < n_c$, which means that the infection was cleared before the diversity threshold was reached and therefore the patient remains healthy. This implies that the probability that no disease is developed is smaller than s^*, the extinction probability of the branching process.

Now suppose that infected patients are treated with an immunogen that can, for example, neutralize as many as 80% of all possible HIV variants. The effect of this treatment depends on the value of R, the average number of escape mutants produced from one parental strain. This can be illustrated by looking at Table 1, which shows, for example, that reducing the parameter R from 10 to 2 increases the probability of recovery from 0.000045 to 0.2. But if originally $R = 20$, then the 80% immunogen increases the chances from essentially zero to only 0.02 (for $R = 4$), which is still rather poor.

6. SELECTION BETWEEN STRAINS OF DIFFERENT REPLICATIVE CAPACITY

It has been observed that fast replicating strains are favored in the final phase of the infection. Within the framework of our model this is an obvious conclusion.

Let us assume that there are two strains, v_1 (fast replicating) and v_2 (slow). We have

$$\dot{v}_{1,i} = v_{1,i}(r_1 - s_1 z - p_1 x_{1,i}),$$

$$\dot{v}_{2,i} = v_{2,i}(r_2 - s_2 z - p_2 x_{2,i}),$$

$$\dot{x}_{1,i} = k v_{1,i} - u v x_{1,i}, \qquad \dot{x}_{2,i} = k v_{2,i} - u v x_{2,i},$$

$$\dot{z} = v(k' - uz).$$

We use the notation $v_1 = \sum_i v_{1,i}$, $v_2 = \sum_i v_{2,i}$, $v = v_1 + v_2$, $x_1 = \sum_i x_{1,i}$, $x_2 = \sum_i x_{2,i}$, and $x = x_1 + x_2$.

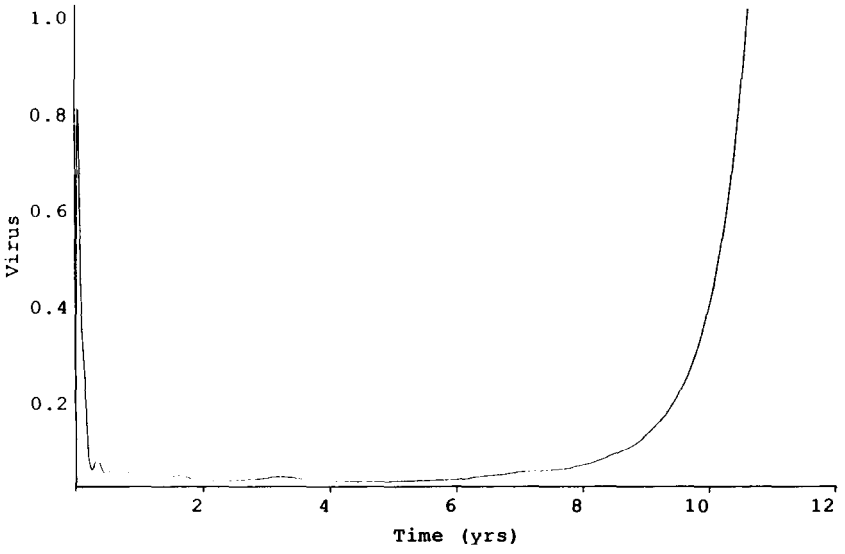
Fast replication (r_1 large) is correlated with high immunosuppression (p_1, s_1 large), while the slow replicating strains are assumed to be worse targets for neutralization (p_2, s_2 low). Fast replication means that new virus particles rupture quickly from their host cell. These strains are therefore more exposed to immunological attack than slow replicating strains that spend a larger fraction of their generation time inside the host cell, more effectively hidden from antibody attack.

Figure 3 shows a computer simulation of this equation. The infection is induced by equal amounts of fast and slow replicating viruses. In the initial phase of infection ($z = 0, x_1 = x_2 = 0$), strain 1 will grow faster (because $r_1 > r_2$). The average replicative capacity $\bar{r} = (v_1 r_1 + v_2 r_2)/v$ is driven toward r_1.

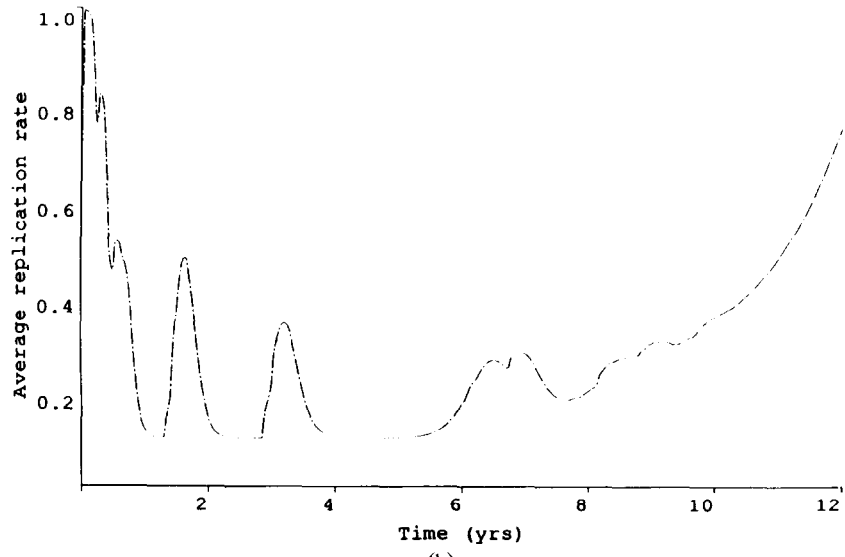
In the following phase, the immune system ($z = 1, x = 1$) suppresses v_1 more than v_2 because $p_1 > p_2$ and $s_1 > s_2$. During this phase strain 1 is present in much lower frequency than strain 2. The average replication rate $\bar{r}$ decreases toward r_2. Therefore, the immune system can select for slow replicating strains that cause the long period with low viral activity.

In the final phase of infection, as viral diversity increases, the breakdown of the specific immune response will again result in strain 1 replicating faster than strain 2 (if $r_1 - s_2 > r_2 - s_2$).

According to this argument, the mechanism underlying the observed change from slowly to rapidly replicating strains during the development of AIDS is simply selection. Our argument would also imply that in the very beginning of infection, when the immune system has not kicked in, fast replication should be of selective advantage.



(a)



(b)

FIG. 3. A simulation of HIV infection in the presence of fast and slow replicating strains according to the model described in Section 7. (a) The virus concentration v as a function of time. (b) The average replication rate of the virus population. During the initial viremia, fast replication is favored. In the following asymptomatic period a strong immune response selects for slow replicating strains, which spend a larger fraction of their generation time inside the cell hidden from antibody responses to the free virus particle. From time to time, new fast replicating escape mutants are produced that cause the peaks in replicative capacity. Correlated with progression to disease and the dilution of the specific immune response is the increase in the abundance of faster replicating (more cytopathic) cells. The parameters have the values $r_1 = 10$, $r_2 = 1$, $s_1 = 9.7$, $s_2 = 0.9$, $p_1 = 20$, $p_2 = 1$, $k = k' = u = 1$.

7. DYNAMICS OF THE CD4 CELL POPULATION

In the preceding sections we have analyzed the dynamics of the virus population and of CD4 positive lymphocytes that are specifically directed against HIV antigens. In this section we include the population dynamics of the total number of CD4 positive cells, including those not directed against HIV, denoted by X. Let us write

$$\dot{X} = \Lambda - \mu X - uvX - kvX - k'vX, \tag{5}$$

$$\dot{v}_i = v_i[r(X + x + z) - sz - px_i], \qquad i = 1, \ldots, n, \tag{6}$$

$$\dot{x}_i = kv_iX - uvx_i, \qquad i = 1, \ldots, n, \tag{7}$$

$$\dot{z} = k'vX - uvz. \tag{8}$$

We have assumed that CD4 lymphocytes are produced at a constant rate Λ and removed at rate μX. The virus can kill CD4 cells at the rate uvX. CD4 cells are activated by exposure to viral antigens into cells that trigger the immunological attack against the virus, x_i and z. This happens at the rates kvX and $k'vX$, respectively. HIV replicates in CD4 cells; therefore, the viral replication rate is proportional to the number of CD4 cells present, $X + x + z$.

The basic model (Section 2) is obtained as a special case of this more general model by assuming that X is constant and always much larger than $x + z$.

The diversity threshold is given by

$$n_c = \frac{px}{r(X + x + z) - sz}.$$

For a certain virus density v, the number of CD4 cells converges to

$$X \to \Lambda/(\mu + vu')$$

with $u' = u + k + k'$; in the same way, $x \to kX/u$ and $z \to k'X/u$. We obtain

$$n_c = pk/(ru' - sk').$$

This is essentially the same as for the basic model if X is much larger than $x + z$ or $u \gg k + k'$.

The model also allows us to express the total number of CD4 cells as a function of the virus population size,

$$X + x + z = \frac{\Lambda}{\mu + vu'}\left(\frac{u'}{u}\right).$$

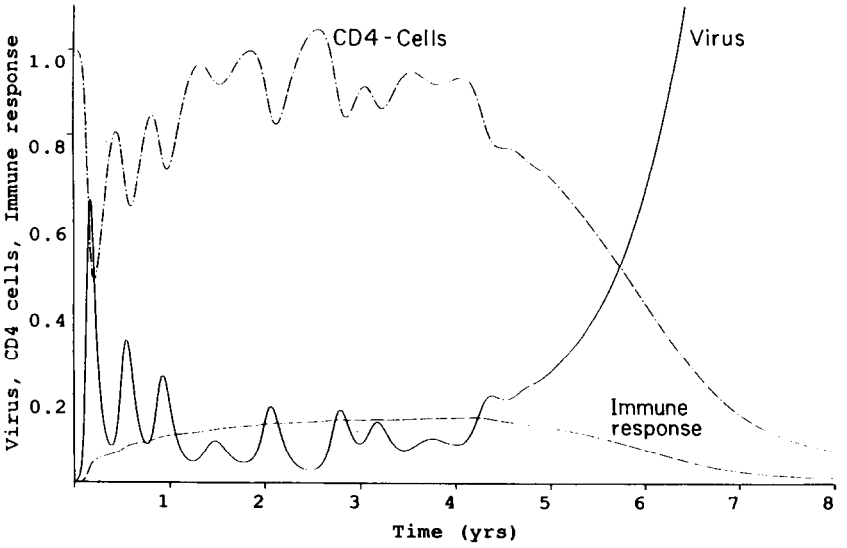


FIG. 4. Simulation of the model described in Section 8 [Eqs. (5)–(8)]. The virus population density, CD4 cell density, and strength of the immune response against HIV (the sum $x + z$) are shown as a function of time. We observe fluctuations in the total CD4 cell count and the virus density during the incubation period. As the virus escapes from immunosurveillance (by breaching the diversity threshold), the increasing virus population induces depletion of CD4 cells and therefore severe damage to the immune system.

In Figure 4, a typical simulation of Equations (5)–(8) is shown. The increasing virus population in the final phase of the infection destroys almost the whole CD4 cell population, which induces the breakdown of the immune response against HIV epitopes.

8. DISCUSSION

The models described in the preceding sections are obviously very simple caricatures of the true complexity of the interaction between coexisting HIV-1 quasi-species and the human immune system. This approach seems to be justified if we consider the need for a mechanistic interpretation and understanding of the accumulating data. Furthermore, the developed models can serve as a starting point for adding further biological realism as knowledge improves, both of the molecular and population genetics of viral replication and persistence and of the factors that determine the interaction with the immunoregulatory network.

Our mathematical analysis clearly reveals how antigenic variation can establish a number of the observed features of HIV infection and AIDS disease.

(1) A two-peaked pattern of viral abundance during the course of infection, with high total viral abundance in the early stages of infection (the primary HIV-1 infection stage) and a high and increasing viral load during the late stage of infection when the disease AIDS is manifest

(2) A long and variable asymptomatic phase of infection with low viremia

(3) The coexistence of many (immunologically) different mutants throughout the incubation period of AIDS

(4) A humped pattern in specific immunological responsiveness to viral antigens [specific CD4 cells that target the immunological response, via the stimulation of antibody attack directed against the envelope protein (gp120) of the virus], with low responsiveness during the asymptomatic stage and a decline in measurable responses as ARC and AIDS develops

(5) The dominance of slowly replicating viruses during the asymptomatic phase of infection

(6) The dominance of rapidly replicating quasi-species in ARC or AIDS patients

A crucial point in our theory is that the virus is able to generate new resistant virus strains that can escape from the strain-specific immune response (exerted, for example, by neutralizing antibodies against the V3 loop of the envelope protein gp120). In our model this mechanism is responsible for the survival of the virus population in the presence of strong immunological attack. Antigenic variation must occur at a rate fast enough that each virus strain produces on average one resistant offspring strain before its own eventual extinction. This generates the antigenic drift condition in Section 4. Depending on the rate of antigenic variation, we have calculated the probability for the virus population to reach any arbitrarily high antigenic diversity threshold and how this probability could be altered by a potential immunotherapy that can induce immunity against a certain fraction of HIV variants.

Central to our theory is the concept of the viral diversity threshold. Each subset of specific CD4 positive T-helper cells is directed against only one quasi-species, but each quasi-species is cytopathic to all subsets of CD4 positive cells. This assumption creates the "diversity threshold" where the immune system can control a limited diversity of viral types but is unable to constrain viral population growth when too many quasi-species are present. This notion is speculative at present, but the high mutation rate (created by errors in transcription and reverse transcription, and possibly by recombination) of HIV-1 will certainly facilitate the likelihood of ever-increasing

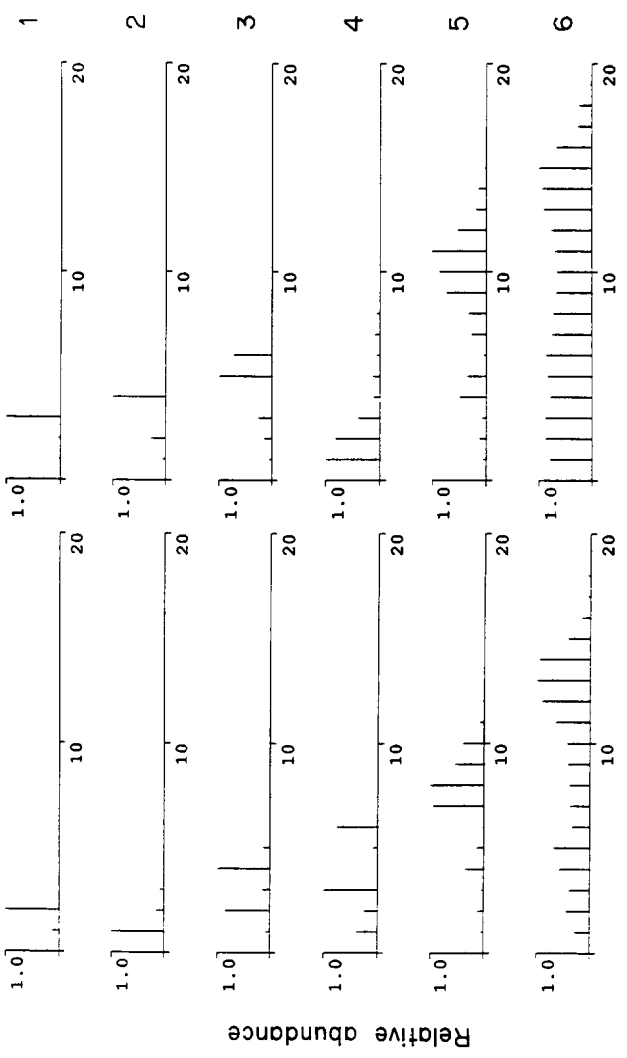


FIG. 5. Sampling viral diversity at sequential time points (0.5 year) for the simulation illustrated in Figure 4. The horizontal axes denotes different HIV strains; their relative abundance is indicated on the vertical axes (always scaled such that the most abundant strain has relative abundance 1).

genetic diversity in the viral population during the course of the incubation period. It is important to note that the diversity threshold does not arise because there might be only a limited variety of antibodies or cellular immune responses, but is the consequence of the opposed forces between the virus population (with its cytopathic effect on the immune system) and the immune cell population (with its adverse effects on the virus population.)

Although the concept of a diversity threshold arises clearly from our mathematical arguments, the real importance of the described phenomena in infected patients remains obscure. Studies are currently in progress to look for stronger evidence of increasing antigenic diversity during the course of infection. Such studies are made difficult by the fact that sampling from the highly "overdispersed" distribution of the relative abundances of different viral strains can result in discrepancies between the actual number of HIV variants present and the number obtained from repeated virus isolation. This discrepancy is illustrated in Figure 5. On average, however, we would expect that a strong immune response selects for variation in the recognized epitopes and therefore increases the diversity of the virus quasi-species. Parallel to this increasing viral diversity (with its proposed fatal consequences for the immune system), a slow overall weakening of the immune system may occur even during the asymptomatic period. This can happen, for example, because of a depletion of dendritic cells (Stella Knight, personal information, 1990) or a general disruption of immunoregulatory networks. Whereas the theoretical diversity threshold might be larger for a healthy person, it might decrease during the time of infection.

In the context of our model, however, an overall weakening of the immune system during the asymptomatic incubation period is not necessary for the virus to escape from immunosurveillance finally. Overcoming the diversity threshold by increasing its antigenic diversity allows the virus population to replicate to higher levels, which in turn induces severe damage to the CD4 cell population and the immune system in general.

This work was supported by an Austrian 'Erwin Schrödinger' scholarship (M.A.N.), by a research grant from the MRC, and by the Royal Society (R.M.M.).

REFERENCES

1 M. S. Ascher and H. W. Sheppard, AIDS as immune system activation, *J. AIDS* 3:177–191 (1990).

2 P. Balfe, P. Simmonds, C. A. Ludlam, J. O. Bishop, A. J. Leigh Brown, Concurrent evolution of HIV-1 in patients infected from the same source, *J. Virol.* 64:6221 (1990).

3 D. P. Bolognesi, Vaccine strategies for AIDS, in *Retrovirus Biology and Human Disease*, R. C. Gallo and F. Wong-Staal, Eds., Marcel Dekker, New York, 1990, pp. 383–400.
4 C. Cheng-Mayer, D. Seto, M. Tateno, J. A. Levy, Biological features of HIV-1 that correlate with variation in the host, *Science* 240:80–82 (1988).
5 M. Eigen, The AIDS debate, *Naturwissenschaften* 76:341–350 (1989).
6 J. Goudsmit, Immunodominant B-cell epitopes of the HIV-1 envelope, *AIDS* 2 (Suppl 1):S41–S45 (1988).
7 S. Karlin and H. M. Taylor, *A First Course in Stochastic Processes*, Academic, New York, 1975.
8 D. J. Looney, A. G. Fisher, S. D. Putney et al., Type restricted neutralization of molecular clones of HIV, *Science* 241:357–359 (1988).
9 A. Meyerhans, R. Cheynier, J. Albert et al., Temporal fluctuations in HIV quasispecies in vivo are not reflected by sequential HIV isolations, *Cell* 58:901–910 (1989).
10 P. Nara, AIDS viruses of animals and man, *Los Alamos Sci.* 18:55–89 (1988).
11 M. A. Nowak, R. M. May, and R. M. Anderson, The evolutionary dynamics of HIV-1 quasispecies and the development of immunodeficiency disease, *AIDS* 4:1095 (1990).
12 M. Nowak, HIV mutation rate, *Nature* 347:522 (1990).
13 R. E. Phillips, D. F. Nixon, F. M. Gotch, J. G. Elvin, J. A. Rothbard, A. J. McMichael, Epitope sequence variation and changes in cytotoxic T-cell recognition of HIV in vivo, preprint.
14 M. Robert-Guroff, M. Brown, R. C. Gallo, HTLV III neutralizing antibodies in patients with AIDS and AIDS related complex, *Nature* 316:72–74 (1985).
15 M. Robert-Guroff, M. S. Reitz, W. G. Robey, R. C. Gallo, In vitro generation of an HTLV III variant by neutralizing antibody, *J. Immunol.* 137:3306–3309 (1986).
16 Z. F. Rosenberg and A. S. Fauci, Immunopathogenic mechanism of HIV infections: cytokine induction of HIV expression, *Immunol. Today* 11:176–180 (1990).
17 M. S. Saag, B. H. Hahn, J. Gibbons et al., Extensive variation of HIV-1 in vivo, *Nature* 334:440–444 (1988).
18 S. M. Schnittman, M. C. Psallidopoulos, H. C. Lane et al., The receiver for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4, *Science* 245:305–308 (1989).
19 P. Simmonds, P. Balfe, C. A. Ludlam, J. O. Bishop, A. J. Leigh Brown, Analysis of sequence diversity in hypervariable regions of the external glycoprotein of HIV-1, *J. Virol.* 64:5840 (1990).
20 M. Tersmette, R. A. Gryters, F. deWolf et al., Evidence for a role of virulent HIV variants in the pathogenesis of AIDS: studies on sequential HIV isolates, *J. Virol.* 63:2218–2125 (1989).
21 R. A. Weiss, P. R. Clapham, R. Cheingsong-Popov et al., Neutralization of HTLV III by sera of AIDS and AIDS risk patients, *Nature* 316:69–72 (1985).