# AIDS pathogenesis: mathematical models of HIV and SIV infections



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## Introduction

There are still many questions about the detailed mechanisms whereby HIV induces AIDS in humans. Any potential explanation of HIV pathogenesis should provide answers to the following interlinked questions. (1) What causes the length and variability of the asymptomatic phase between infection and development of AIDS? (2) Why is HIV not cleared by the immune response? (3) What causes immunodeficiency? (4) What, if any, are the consequences of the genetic and antigenic variability of HIV? (5) Are opportunistic infections necessary for HIV to cause AIDS? (6) What are the differences among the several pathogenic or apathogenic infections of the primate lentiviruses HIV and simian immunodwficiency virus (SIV) in different species, and what might we learn from these differences?

A range of ideas have been put forward to explain how interactions between populations of HIV and populations of immune cells can lead to AIDS. From this population-level perspective, three broad classes of explanations may be distinguished.

### Immunological theories

These hypotheses generally assume (1) that the virus is suppressed by a (strong) immune response, and (2) that HIV induces a slow decline of the immune system during the asymptomatic phase. This may be caused by weakening CD4 cell function or reducing CD4 cell counts and possibly also by reducing the rate of peripheral replenishment of new CD4 cells (by infection of dendritic cells) or infection of precursor cells, or by autoimmune responses. The complexity of the immune system allows for a large variety of different specific hypotheses, and the longevity of CD4 cells may explain why there is an interval of several years between HIV infection and the complete breakdown of the immune system.

### Cofactor theories

The second class of models assumes that the virus is kept at low levels during the asymptomatic phase because of a lack of CD4 cell activation. There are not enough activated cells present to support high levels of virus replication. The progression to disease can then be explained by the stochastic arrival of other pathogens (or more general agents that increase CD4 cell activation). This leads to a 'vicious cycle': these other pathogens enhance HIV replication, which in turn weakens the immune response to yet other pathogens. The consequence is an ever increasing load of other pathogens, and an ever-increasing level of CD4 cell activation and hence HIV replication.

### Virological theories

The third class of models puts the virus itself in the centre, assuming that evolutionary processes within the virus population are responsible for the long and variable asymptomatic phase and eventual development of AIDS. The key assumptions are (1) that the immune response to HIV is an important factor in controlling virus growth during the asymptomatic phase, and (2) that antigenic variation enables the virus to escape from these responses.

We begin this review with a brisk summary of the main ideas that have been put forward under the first two of these headings. Some of these ideas have been presented in explicit, mathematical detail, while others have been presented more intuitively or in the form of verbal metaphors. We then shamelessly spend more time on the third, 'virological' category of explanation, giving a fairly detailed account of our ideas about diversity thresholds. We do this partly because we are enthusiastic about the work, and partly because we think it might be correct. HIV infections are complex, however, and it is likely that a full understanding of AIDS pathogenesis will eventually combine elements of all three approaches.

We conclude the review with suggestions for experimental tests that could help to measure the relative importance of the different mechanisms that have been proposed. We also sketch possible implications for immunotherapy, drug treatments, and the evolution of drug-resistant strains of HIV.

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## Immunological theories

It was originally thought that HIV destroyed the immune system simply by killing infected CD4 cells. This has been thrown into doubt by the finding that only a small proportion of CD4 cells in the periphery are infected with HIV in asymptomatic patients [1]. It is generally assumed that direct killing of these few infected CD4 cells is not sufficient to explain the large depletion of CD4 cells. Several mechanisms have been suggested to explain how HIV could induce killing of non-infected CD4 cells. This could result from syncytia formation, or from immune responses to uninfected CD4 cells that have bound to soluble gp120 molecules (which are shed by the virus). Extensive CD4 cell depletion could also be caused by the killing of dendritic cells, which are important for the production of new CD4 cells. (For a more detailed review see [2].)

In addition, several autoimmune models have been put forward. These are based on the assumption that there may be structural similarities between HIV proteins and major histocompatibility complex (MHC) class II molecules. The CD4 receptor recognizes a portion of the MHC II molecule. Since the HIV envelope protein, gp120, can also bind to the CD4 receptor, it may mimic the configuration of a certain portion of the MHC II molecule. Habeshaw *et al.* [3] suggest that gp120 induces a chronic graft-versus-host-like disease. They believe that certain homologies between gp120 and MHC (class II) molecules lead to a direct interaction between the T-cell receptor and gp120 (without MHC presentation). This results in increased immune activation and hence increased HIV replication.

A broadly similar idea has been proposed by Hoffmann *et al.* [4], who suggest an idiotypic network model where AIDS emerges as an autoimmune disease that is triggered by a combination of HIV and allogeneic stimuli. This is supported by the finding that alloimmune mice (i.e., mice that have been exposed to cells from another murine strain) produce antibodies against the Env and Gag proteins of HIV, although they have never encountered HIV before [5]. One consequence of these autoimmune models is that inducing tolerance to HIV should prevent the development of AIDS. The extent to which these autoimmune phenomena are involved in the cytopathicity of HIV infections is unclear at present.

Some immunologists believe that CD4 cells are inappropriately activated by HIV (via the CD4 receptor) and primed for apoptosis [6] or driven into clonal expansion, followed by anergy and death [7].

All these immunological models are qualitative, verbal descriptions of the complex interactions between populations of HIV and CD4 cells. They focus attention on the specific molecular mechanism of how HIV kills CD4 cells. This may be important for finding a cure, but these models do usually not attempt to explain the population dynamics of the infection, nor why there is such a long and variable incubation period; such details are left cloudy. Before we can gain a detailed understanding of what these various ideas imply for the interaction between HIV and the immune system, it will be necessary to express the assumptions in a precise (mathematical) form and explore the consequent dynamic behaviour. This is not an easy task, because the underlying immunological mechanisms and networks are both very complex and poorly understood. Nevertheless, it appears to us that the ideas we have just sketched must remain highly speculative until supported by specific models whose dynamical properties are clearly understood.

Perelson [8] developed a mathematical model for the interaction between HIV, CD4 cells, macrophages and other replicating antigens. In this rather complicated simulation, the development of AIDS over a long incubation period is modelled as a continuous rise in viral abundance and a fall in CD4 cell counts. Nelson and Perelson [9] explore the transfer of HIV from infected macrophages to HIV-specific CD4 cells. This may lead to a depletion of those CD4 cells that are specifically directed at HIV antigens and could represent a mechanism whereby slow-replicating strains of HIV escape from immune responses more efficiently than fast-replicating strains, thus tending to lengthen the incubation interval.

## Cofactor theories

Several mathematical models have been developed to explore the possible interactions between HIV and other pathogens [8,10–14]. The basis for these models is the observation that HIV can replicate in CD4 cells only if they have been activated (for example, by their specific antigen). This implies that infections with other pathogens, which result in CD4 cell activation, may enhance HIV replication. HIV, on the other hand, weakens the immune response to these other pathogens. The consequence of this synergistic interaction is a vicious cycle of increasing HIV levels and increasing loads of other infections. Clinical disease and death results from an overwhelming amount of such 'opportunistic infections'.

Using a deliberately oversimplified mathematical model, Anderson and May [12] have shown that the interaction between HIV and other infections can lead to chaotic fluctuations in HIV abundance, and to severe depletion of CD4 cell population levels.

McLean and Nowak [14] have developed the idea of a 'containment threshold', which is defined as the limit of the number of other pathogens that can be restrained by an immune system infected with HIV. This model also shows how immune modification by HIV can lead to the persistence of infections that would normally be cleared, and how such previously estab-

lished, but latent, persistent infections become most apparent in full-blown AIDS. This model does not assume that there are any *specific* cofactors necessary for the development of AIDS — it is simply a question of CD4 cell activation.

Such cofactor theories represent plausible mechanisms of AIDS pathogenesis. A number of empirical studies have confirmed that markers of immune activation (such as neopterin or $\beta_2$-microglobulin) generally correlate with the rate of disease progression [15–17]. One complication is that HIV itself appears to be a strong (unspecific) activator of CD4 cells and hence increasing immune activation may only be a consequence of increasing viral abundance.

## Virological theories

We will now give a much more detailed account of ideas that ascribe AIDS pathogenesis to some unusual features of the population biology of interactions between the HIV quasispecies and the immune system. A quasispecies is a well-defined ensemble of mutants that arise in a mutation selection process [18]. (For a recent review see [19].)

### The key assumptions

A remarkable feature of all lentiviruses is their extensive genetic and antigenic variation (also found in infections within individual hosts). This has been documented for HIV-1 [20–25], HIV-2 [26], several SIV strains [27–29], visna virus [30], equine infectious anaemia virus [31] and caprine arthritis encephalitis virus [32]. The large genetic variation of lentiviruses is generated by (1) the low replication accuracy of reverse transcription [33–35], (2) recombination during reverse transcription [36,37], (3) many virus generations in an individual host, with many virus particles produced from one infected cell, and (4) selective pressure for variation as exerted by the immune response [38].

Genetic variation is not uniform throughout the HIV genome. There is less variability in the *gag* and *pol* genes than in the *env* gene, within which there are five hypervariable regions. The third hypervariable region, the V3 loop, is of special interest, because it has immunodominant properties [39,40]. This region is approximately 30 amino acids long and contains epitopes for neutralizing antibodies, CD4 and CD8 cell responses. It also appears to play an important role in virus entry into the host cell and thereby influences cell tropism. A single amino-acid substitution can restrict recognition by neutralizing antibodies [41]. Such a point mutation may be within the V3 loop or somewhere else in the envelope protein (gp120), which could lead to a conformational change of the V3 loop and thus prevent antibody binding [42]. There is also variation and escape from cytotoxic T-cell binding in epitopes of the more conserved Gag protein [43].

These observations support the central assumption in our 'diversity threshold' model [44–49] that the virus is continuously producing 'escape mutants' that can escape from current immunological attack. These escape mutants spread in the virus population until specific immune responses against them have been mounted. They might then be suppressed, but new escape mutants might have arisen in the mean time. This antigenic drift can enable the virus population to persist, despite intensive and effective immune responses.

The second key assumption in our model is that the virus can impair immune responses. Large cohort studies have shown that CD4 cell counts decrease during infection in an almost linear fashion (perhaps slightly accelerated during the final stages of the disease) [50,51]. HIV can kill infected CD4 cells, but indirect mechanisms that lead to killing of uninfected CD4 cells have also been suggested (see below). The essential elements of our diversity threshold model do not depend on the detailed mechanisms; we require only that HIV can impair immune responses.

### The basic idea

With these two main assumptions, our 'diversity threshold' theory becomes an intuitive and simple concept. We may assume that the infection occurs with a heterogeneous inoculum of virus (this seems to be correct for all natural routes of transmission, such as sexual contact, needle-sharing, blood transfusion or mother-to-child transmission). During the earliest stages of infection we expect strong selection for the fastest growing variants, which would result in a fairly homogeneous population at seroconversion.

The immune response to HIV is likely to result in escape mutants, and thence in proliferating virus diversification. We expect variation to accumulate in those epitopes that are recognized by relevant immune responses. Such increasing antigenic diversity makes it more and more difficult for the immune system to down-regulate the various mutants simultaneously. The cause is the asymmetric interaction between immunological and viral diversity. Each virus strain can impair all immune responses by impairing their CD4 help, but individual strain-specific immune responses can attack only specific virus strains. In more heterogeneous virus populations the ratio between immune- response-induced killing of virus and virus-induced killing of immune cells is shifted in favour of the virus. This may lead to a complete breakdown of the immune system and uncontrolled virus replication (as seen in AIDS patients).

We call this phenomenon 'diversity threshold', because in the simplest mathematical model there is a critical number of antigenically distinct variants that can be controlled simultaneously by the immune system. In more realistic and more complicated versions of the model this 'diversity threshold' condition takes

a more general form, and indicates the point at which the immune system fails to control the virus population. These complications arise, for example, when one acknowledges that different virus strains have different replication rates or immunological properties, or that the basic parameters of the model are not constant but change during the course of infection (such as increasing virus replication rates, resulting from increasing CD4 cell activation).

Once the immune response has been overcome, there is no longer selection for variation. Again, as in the earliest stages, we may expect selection for the fastest growing strains. This effect may lead to a very low diversity in AIDS patients at advanced stages of the diseases. Fig. 1 summarizes the expected changes in viral diversity and their causes.

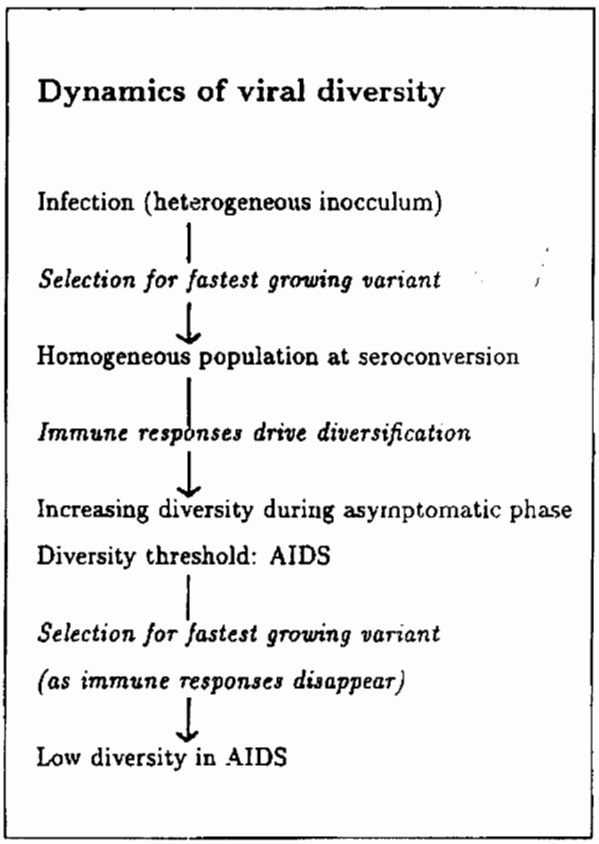


**Fig. 1.** The changes of viral diversity during individual HIV infections as predicted by the 'diversity threshold' theory.

**The essentials of the mathematical theory**

The mathematical model that makes these ideas explicit is designed to be as simple as possible, yet to capture those details that are essential for our understanding of the population dynamics of HIV infections. It has the following basic structure:

$$dv_i/dt = v_i(r - px_i - sz); \qquad i = 1,..,n \qquad (1)$$

$$dx_i/dt = kv_i - bx_i - uvx_i; \qquad i = 1,..,n \qquad (2)$$

$$dz/dt = k'v - bz - uvz \qquad (3)$$

The model has three types of variables, $v_i$, $x_i$ and z. Here $v_i$ denotes the population size of virus mutant labelled $i$; $x_i$ denotes the immune response (for example, the density of antibodies, B cells or T cells) directed specifically against virus strain $i$; and $z$ denotes the group-specific immune response directed against all different virus mutants. The total number of different virus strains is given by $n$; mutational events occur throughout the infection and therefore increase this number, $n$, as time goes by. We use the notation $v = \sum v_i$ which denotes the total population of virus.

The model has seven parameters, $r$, $p$, $s$, $k$, $k'$, $b$ and $u$. The parameter $r$ denotes the average rate of replication of all different virus strains; $p$ the efficacy of the strain-specific immune responses and $k$ the rate at which they are evoked; similarly, $s$ denotes the efficacy of group-specific immune responses and $k'$ the rate at which they are evoked. In the absence of further stimulation, the immune response decays at a (slow) rate given by the constant $b$. Lentiviruses can generally impair immune responses, either by killing infected CD4+ cells or by indirect mechanisms (such as syncytia formation, anergy or autoimmunity, as discussed above). These effects are summarized in the loss terms, $-uvx_i$ and $-uvz$. Thus the parameter $u$ characterizes the ability of the virus to impair immune responses. By impairing CD4 cell function, the virus indirectly impairs B-cell and cytotoxic T-cell-mediated immune responses.

This simple mathematical model represents a deliberately simplified concept of virus–immune system interactions. The individual virus strains are defined as being different with respect to strain-specific immune responses. In reality the situation is not so clear-cut, because viruses usually have several different epitopes, which can be recognized by either antibodies or T cells (in relation with MHC-I or MHC-II presentation). Some virus mutants may differ in one epitope, but coincide in others. This means that a given antibody or cytotoxic T-lymphocyte (CTL) may be able to recognize a number of different virus strains, but fail to recognize others. There is a variety of more or less group-specific and strain-specific immune responses. The model only considers the two extreme possibilities of completely group-specific and completely strain-specific immune responses. The whole spectrum of more or less cross-reactive responses is covered by assigning parameter values to balance the relative importance of the two extreme possibilities.

We also assume in equations 1–3 that the parameters $r$, $u$, $p$, $s$, $k$ and $k'$ are the same for all different virus strains. This means essentially that all virus strains have the same average replication rate and cytopathic effects, and are controlled by immune responses of equal strength. This simplification is not necessary, but it makes the mathematical analysis easier and more transparent. For a more general model with different rate constants for $r_i$, $u_i$, $p_i$ and $k_i$ for the different strains, see [47] and [49].

**Results: three kinds of behaviour**

From equations 1–3 we may obtain an equation for the rate at which the total virus population changes:

$$dv/dt = v(r - \frac{pkv}{b+uv}D - \frac{sk'v}{b+uv}) \qquad (4)$$

For this we have assumed that the individual $x_i$ converge to their steady-state levels $x_i^* = kv_i/(b+uv)$, and that $z$ converges to $z^* = k'v/(b+uv)$, on timescales short compared with those on which the total virus population changes. $D$ (denoting the Simpson index) $= (v_i/v)^2$, which is an inverse measure for viral diversity: if there is only one virus strain present then $D = 1$; if there are $n$ strains present, all of them at exactly the same abundance, then $D = 1/n$. $D$ is always between 0 and 1; it is the probability that two viruses chosen at random belong to the same strain. The concept of a virus strain, $v_i$, is well defined in the mathematical model. It is simply a subpopulation of viruses that are recognized by the same strain-specific immune response, $x_i$.

From equation 4 we see that $v$ converges towards the steady state:

$$v^* = rb/(sk' + pkD - ru) \qquad (5)$$

The product $sk'$ denotes the efficacy of the group-specific immune responses, such as antibodies or CTL directed at epitopes that are conserved between different virus strains. The product $pkD$ denotes strain-specific immune responses, such as antibodies or CTL directed at variable regions. The efficacy of these strain-specific responses depends on the antigenic diversity of the virus population. Equation 5 shows that increasing diversity (decreasing $D$) increases the total population size of the virus.

The model has three distinct parameter regions, which correspond to three qualitatively different courses of infection.

*(1) There is no asymptomatic phase, and the virus population immediately replicates to high levels*

This happens if

$$ru > sk' + pk \qquad (6)$$

Figure 2 shows a numerical simulation of the model for this parameter region. Essentially, viral replication, $r$, and/or cytopathic effects, $u$, are large compared with the combined effects of group-specific and strain-specific immune responses, $sk' + pk$. In this case the immune response is unable to control the viremia. The virus population replicates to high levels and may induce acute disease and death within a short time. No antigenic variation may be observed in this case because of selection for the fastest growing virus strain. The immune system does not have time to select for diversification.

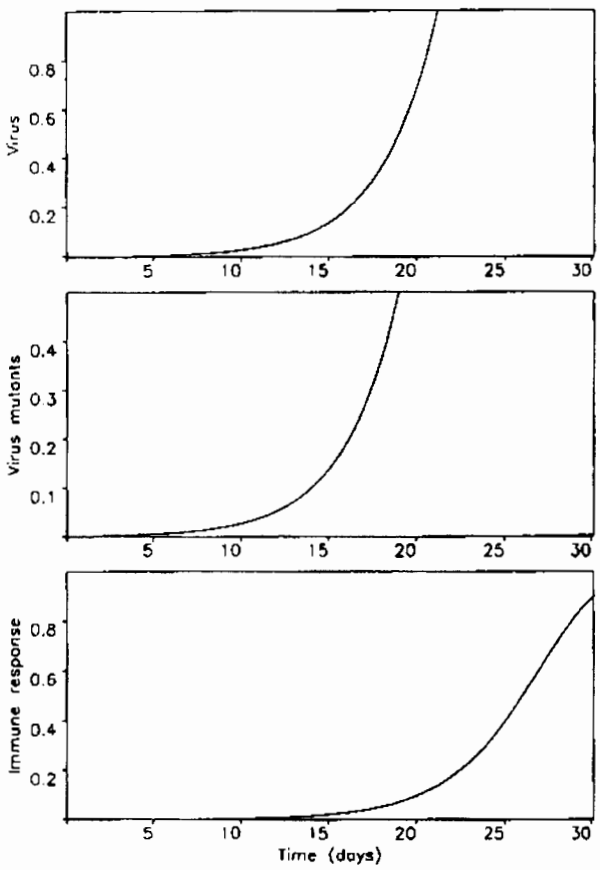


**Fig. 2.** Rapid progression to acute disease and death is one of the three possible solutions of the mathematical model (equations 1–3) of HIV and simian immunodeficiency virus (SIV) infections. This situation occurs if virus replication and cytopathic effects (against immune cells) exceed the combined effects of strain-specific (directed at variable epitopes) and group-specific immune responses (directed at conserved epitopes). The total virus concentration, the concentration of individual virus mutants and the strain-specific immune responses against the individual virus strains are shown here. Parameter values: $r = 7$, $p = 1.5$, $s = 2.4$, $k = k' = u = 1$, $b = 0.01$. The probability that a new mutant arises in the time interval $[t, t+dt]$ is given by $qv(t)dt$. Here $q = 0.8$.

*(2) Chronic infection, but no disease*

This happens if the group-specific immune responses by themselves are sufficiently large to control the virus

population. In mathematical terms this means:

$$sk' > ru \tag{7}$$

That is, the group-specific responses, $sk'$, are large compared with viral replication and killing of immune cells, $ru$. Here the immune response is able to suppress viral concentrations to very low levels. The time average of the virus population size during the chronic infection is given by equation 5 and depends on (1) the rate of virus replication, (2) the extent of impairment of immune functions, and (3) the efficacy of the immune responses. The effect of the strain-specific immune responses depends on the overall antigenic diversity. If there is a large amount of variation, then there are always some escape mutants that can replicate in the presence of an immune response, and there is an increase in the total virus population size. Thus, the virus level increases with increasing diversity, but in this parameter regime there is no critical diversity threshold beyond which equation 5 ceases to give sensible results. In principle the immune system is able to control the virus population indefinitely (virus levels increase with increasing diversity, but in a controlled manner). Figure 3 illustrates the dynamics of the infection for this parameter region.

*(3) Chronic infection and disease after a long incubation period*

The third, and most interesting, situation arises when the combined effects of group-specific and strain-specific immune responses are able to control viral replication (of the individual strains), but the group-specific responses alone are unable to do so (in contrast with chronic infection, but no disease). In mathematical terms this means:

$$sk' + pk > ru > sk' \tag{8}$$

Figure 4 shows the dynamics of the model for this parameter region. The strain-specific immune responses play an important role. If the virus diversity is low (and $D$ is high) then the total population size is regulated to some equilibrium value (given by equation 5). If viral diversity is high (and $D$ is low) then the denominator in equation 5 becomes very small, and hence the virus population size very large. The critical transition occurs when:

$$D < \frac{ru - sk'}{pk} \tag{9}$$

Beyond this point, the total virus population is no longer regulated by the immune system, and grows unboundedly (in this simple model). Equation 9 gives the diversity threshold. Once this viral diversity threshold is exceeded, the virus population escapes from control

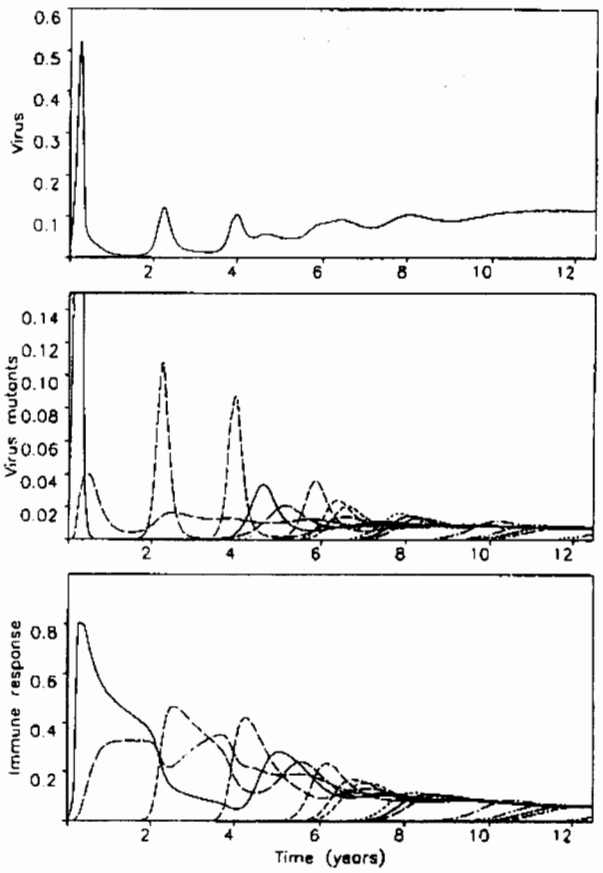


**Fig. 3.** A strong group-specific immune response (directed at the conserved epitopes of the virus) can lead to a chronic infection without development of disease. Parameter values as for Fig. 2, except: $r = 2.5$, $s = 2.6$. (Here, and in other figures, the units are arbitrary, although measured in years for illustrative purposes).

by the immune response and tends to arbitrarily high concentrations. This process may be interpreted as the development of immunodeficiency disease, which is characterized by high virus counts and depletion of CD4 cells. During the asymptomatic phase, on the other hand, the diversity is increasing, but the immune system is able to control viral densities and to maintain CD4 cell levels.

All the essentials of the above analysis — and in particular the identification of three distinct regimes of dynamic behaviour — remain true in more general models where the different virus strains have different rate constants (such as $r_i$, $u_i$ see [47,49]).

**Three kinds of SIV infections**

The closest relative of HIV is SIV. Multiple isolates of SIV have been obtained from a variety of non-human primates [52,53]. These include African green monkeys, pig-tailed macaques, cynomologous monkeys and sooty mangabeys. There are large variations in the pathogenesis of different SIV isolates from the same species of monkey, or in the same isolate from different monkey species [54]. It is tempting to compare the three qualitatively different solutions of our

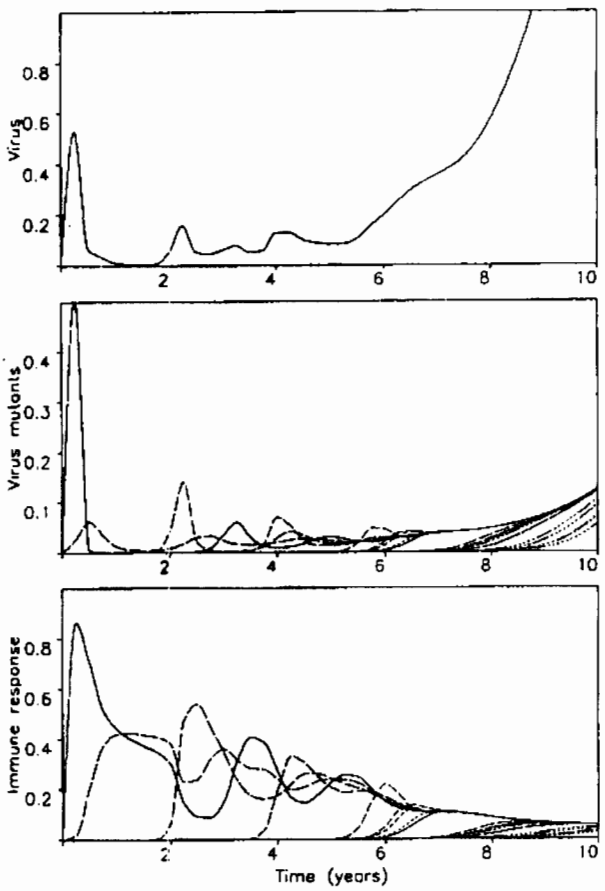


**Fig. 4.** A diversity threshold occurs when the group-specific immune response by itself is unable to control the virus population, but a combination between strain-specific and group-specific responses can control any one strain. Increasing antigenic diversity enables the virus population to escape from the immune response after a long incubation period. Parameter values are as for Fig. 2 except: $r = 2.5$, $s = 2.4$. Note that the small difference in parameter $s$, the efficacy of the group-specific immune responses, between Figs 3 and 4 leads to qualitatively different behaviour over time.

mathematical model with the three different patterns of SIV infections.

Rapid progression to disease and death has been observed in pig-tailed macaques infected with the acutely lethal variant $SIV_{smm\text{-}pbj14}$, which was isolated from sooty mangabeys [54]. The virus kills within 7–13 days after infection. The primary manifestation of disease, and the cause of death, is diarrhoea and its sequelae (rather than immunodeficiency). Our model only predicts that the immune responses are unable to control the virus, which in turn replicates to very high levels and thus causes disease. To validate our model in this particular example, one would have to check whether virus concentrations are very high in the sick animals. Another interesting test would be to construct an $SIV_{smm\text{-}pbj}$ variant with a reduced replication rate. This may not cause immediate disease and death, but a chronic infection (perhaps with slow development of immunodeficiency disease).

The second regime of our mathematical model corresponds, for example, to $SIV_{agm}$ in African green monkeys, the natural host of this strain. A large proportion of wild animals are infected (approximately 50%). The virus appears to cause a chronic infection, but no disease [53]. This apathogenicity is unexplained. The functional immune response of African green monkeys to $SIV_{agm}$ appears to be similar to the response of humans to HIV. There is also a productive infection of CD4 cells, and $SIV_{agm}$ has a viral load equivalent to that in asymptomatic HIV-1-infected humans [53]. There is a similar degree of genetic variation (although the SIV equivalent of the HIV V3 loop appears to be more conserved and probably not immunodominant) [29]. All these observations are consistent with our model. The parameter regions 2 and 3 can give rise to similar viral loads and similar antigenic diversities. The critical difference is that in case 2 the virus population is effectively controlled by group-specific immune responses, so there is no diversity threshold; in case 3, strain-specific responses are essential, resulting in the threshold phenomenon. The difference between SIV in African green monkeys and HIV in humans may be caused by a lower replication rate of $SIV_{agm}$ in the monkeys or (more likely) by a more efficient group-specific immune response. This is plausible: the long-established interaction between $SIV_{agm}$ and its natural host may have selected for a good group-specific immune response (i.e., relevant antibody and CTL responses may be directed against more conserved epitopes).

The third type of infection — the long asymptomatic phase followed by lethal immunodeficiency — is observed in macaques that are artificially infected with SIV [55].

### Variability of HIV infections

A similar variability of the course of infection is also apparent in HIV. Some patients have died within 6–12 months of infection [56]. This may be parameter region 1 of the model. Chimpanzees can be infected with HIV-1, but have not (as yet) developed AIDS. This may correspond to parameter region 2. The typical HIV infection in humans, however, corresponds to case 3. There is extensive variability in the length of the asymptomatic phase. Large cohort studies reveal that 50% of HIV-infected homosexual men develop AIDS within 10 years [56]. However, it is impossible at this time to estimate what fraction of infected people, if any, will never develop AIDS. Figure 5 shows the time distribution of the incubation period in a number of different groups of patients. From the data it is difficult to deduce what the true distribution function is, although gamma and Weibull distributions have been fitted [57].

This variability of incubation times is also a natural consequence of the diversity threshold model. The emergence of escape mutants is a stochastic process. The consequent statistics of early events — whether

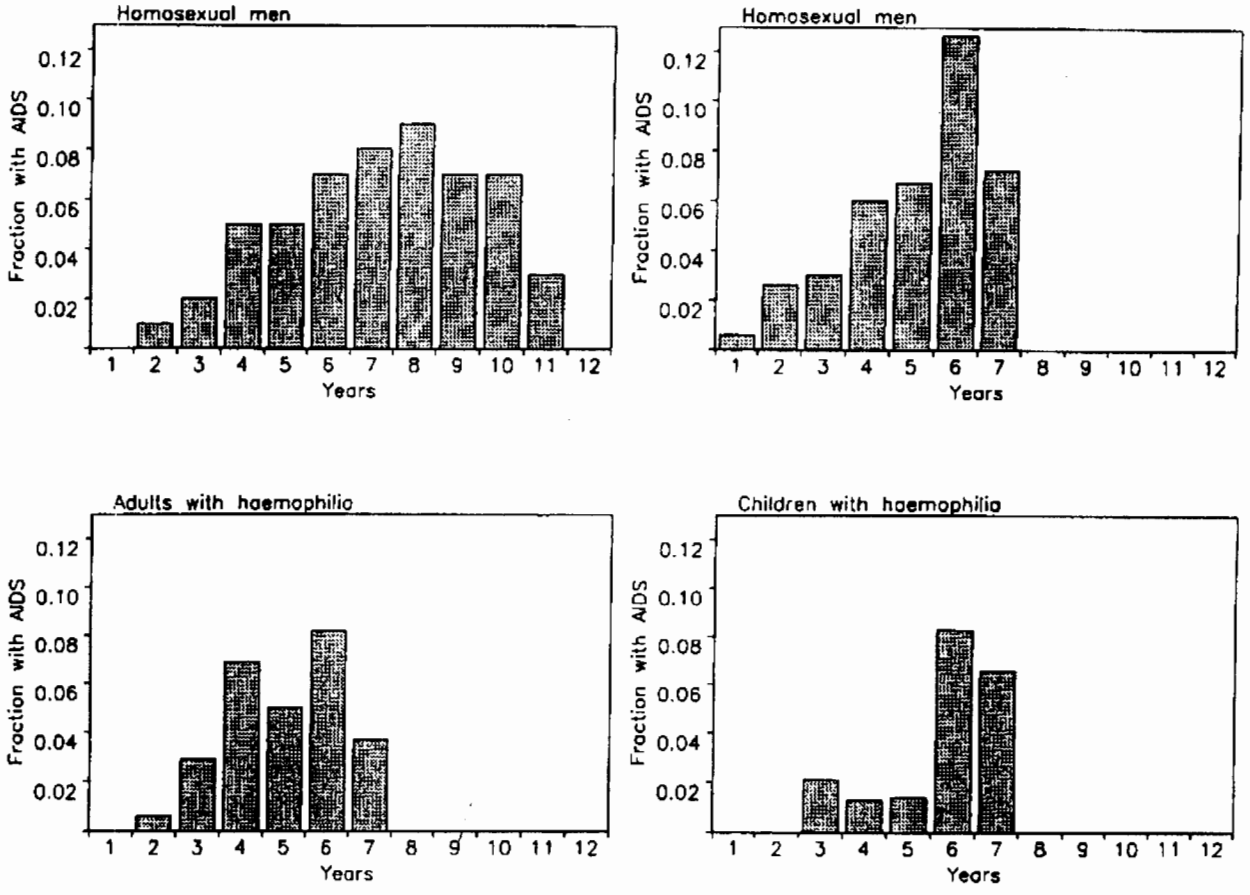


**Fig. 5.** Distribution of incubation times for the development of AIDS in different cohort studies. (a) 422 homosexual men; the cumulative risk of developing AIDS is approximately 13% within 5 years and 54% within 11 years; (b) 753 homosexual men; cumulative risk: 20% within 5 years, 40% within 7 years; (c) 190 adults with haemophilia; 10% within 5 years, 27% within 7 years; (d) 107 children ($\leq$ 18 years of age) with haemophilia; 5% within 5 years, 20% within 7 years. Homosexual men appear to progress faster than haemophiliacs because of the additional risk of Kaposi's sarcoma; when *Pneumocystis carinii* pneumonia is used as the end-point, the rate of progression is similar for both groups. It is not known why haemophiliac children progress significantly more slowly; possibly because of less exposure to opportunistic infections or a faster regenerating CD4 cell population or better immune responses? These data are from [56] and [75].

the first few esape mutants appear sooner or later than average — result in differences in the patterns of progression to disease. Figure 6 shows 100 computer simulations of the model, all with the same parameter values (in parameter region 3), but with different seeds for the random number generator (which determines when an escape mutant is produced). The variety of outcomes is notable. Figure 7 shows the distribution function for the incubation interval, using 1000 simulations. The theoretically derived distribution in Fig. 7 is similar to those actually observed, as shown in Fig. 5. If the probability of generating new mutants is (roughly) constant over time, then the diversity threshold model predicts a gamma distribution. The observed variability of the asymptomatic phase is both a consequence of the stochastic nature of the process that leads to exceeding the diversity threshold, and of differences in immune responses and levels of immune activation in different patients.

**CD4 cell decline in HIV infections**

One of the most important features of HIV infections is the continuous decline of CD4 cell counts. Some cohort studies suggest that this decline is constant (almost linear) with time [50], whereas others find an accelerated decline in the final phase of the infection [51]. The simplest way to model the population dynamics of CD4 cells is to assume that they are produced at a constant rate, $c$, that they proliferate and die, and that they are killed by HIV-induced mechanisms. This leads to an expression for the rate at which the total number of CD4 cells, $y$, is changing:

$$dy/dt = c - by - uvy \qquad (10)$$

Here $b$ is the difference between proliferation and 'natural' death rates (per cell), and $u$ denotes the HIV-induced killing. The steady state value of CD4 cells for a given viral load, $v$, is given by:

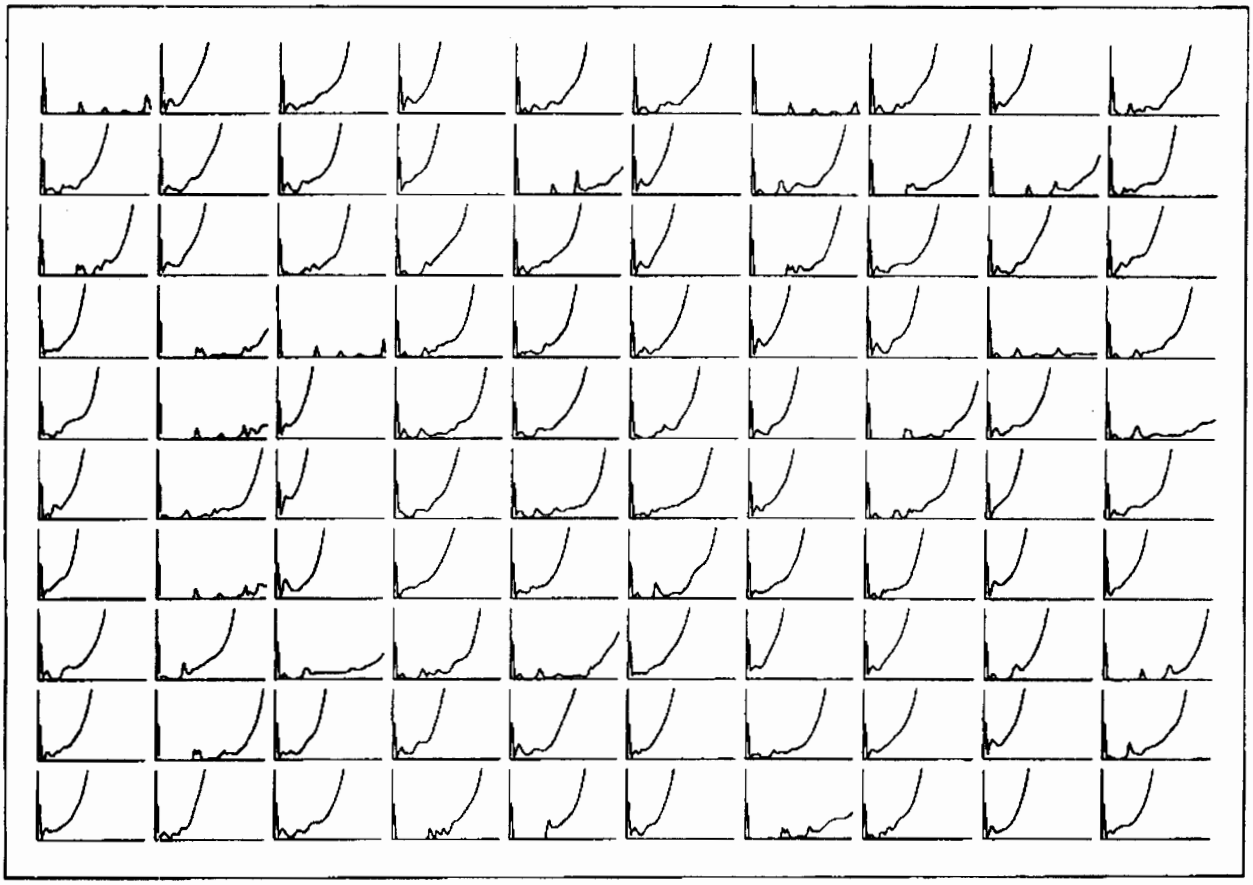

**Fig. 6.** Computer simulations of 100 different HIV infections. Each picture shows the total virus population size as a function of time. All 100 simulations were performed with exactly the same parameter values (those of Fig. 4). The only difference was the initial seed of the random number generator, which governs the stochastic process by which new escape mutants emerge. The simple model given by equations 1–3 can explain the large variability of the asymptomatic period.

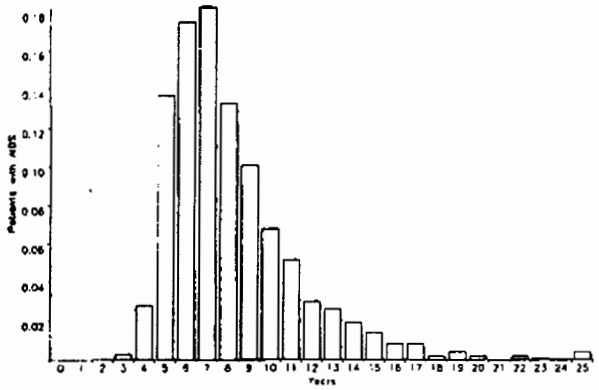

**Fig. 7.** Distribution of AIDS incubation times for 1000 computer simulations of HIV infections. Parameter values are as in Fig 4. The *x*-axis denotes years after infection, the *y*-axis the fraction of patients that developed AIDS within this year.

$$y^* = c/(b + uv) \qquad (11)$$

The rate of approach to this steady state may be very slow, according to the equation

$$y(t) = y^* - [y^* - y(0)]exp[-(b + uv)t] \qquad (12)$$

This equation describes the CD4 cell decline that we would expect in the diversity threshold theory. Our model predicts that the CD4 cell levels (and their rate of decline) are directly related to the viral load, via equations 11 and 12. This should be testable by obtaining accurate information on CD4 cell counts and total virus loads from a large number of patients.

The simplest of all explanations for the long delay between infection and the onset of AIDS is to assume that the slow, continuous decline in CD4 cell counts is simply caused by a *constant* HIV-induced killing of CD4 cells. To obtain the right time-scale for such dynamics, one has to assume that $1/(b + uv)$ is of the order of magnitude of 10 years. The CD4 cell counts in uninfected people are given by $y(0) = c/b$. This should be much larger than the CD4 cell count in AIDS patients, $y^* = c/(b + uv)$. Hence $b$ is much smaller then $b + uv$, but this implies that the characteristic time-scale for changes in CD4 cell population numbers, $1/b$, has to be much larger than 10 years. This seems unreasonable. (For example, zidovudine-treated patients recover their CD4 cell counts within

some months.) Thus we conclude that the length of the asymptomatic period is incompatible with a slow, continuous decline in CD4 cell numbers caused by a constant HIV-induced death rate.

Another important question is how HIV induces this depletion of CD4 cells. Is it necessary to evoke the indirect mechanisms discussed in immunological theories, above, or is it sufficient if HIV kills only infected CD4 cells? A simple mathematical analysis can put this question into qualitative terms: let $y$ and $w$ denote the concentration of uninfected and infected CD4 cells, respectively. Let us again assume that uninfected CD4 cells are produced at a constant rate, $c$, proliferate at the rate $gy$, and die at the rate $(b + g)y$, so that the per-cell difference between proliferation and death rates is $b$. Let us further assume that the virus is transmitted from infected to uninfected cells at the rate $\beta wy$. Infected cells die at the rate $aw$. This leads to the pair of ordinary differential equations:

$$dy/dt = c - by - \beta yw \quad (13)$$

$$dw/dt = \beta yw - aw \quad (14)$$

We expect that $a > b + g$ (i.e., infected cells die faster than uninfected cells) and $b > 0$ (i.e., the average proliferation rate of uninfected cells is smaller than their death rate), otherwise the population dynamics of CD4 cells would not be stable. The equilibrium concentrations are $y^* = a/\beta$ and $w^* = b(y_0 - y^*)/a$. Here $y_0$ is the CD4 cell concentration in the absence of an infection (i.e., $y_0 = c/b$). Now let us assume that 1 in 10000 CD4 cells are infected (in an asymptomatic patient) and that the level of CD4 cells is decreased to (only) 90% of its initial level. This means that $y^* = 0.9y_0$ and $w^* = 10^{-4}y_0$. Thus we obtain $a = 1000b$. If we assume that $1/b$ represents a time-scale of some months (as suggested above by zidovudine-related data), or even a few years, then the death rate of infected cells, $a$, would have to be unreasonably large. Thus the overall depletion of CD4 cell populations cannot be explained solely by increased death rates of HIV-infected cells (if the frequency of infected CD4 cells is indeed as low as suggested by the data on peripheral blood).

The arguments in this subsection may, however, be invalidated if non-linearities in the immigration from the thymus, proliferation and/or death rates of the CD4 cell population are important. For an ultimate answer a correct understanding of the factors that regulate the population size of CD4 cells *in vivo* is needed (see [58]). Another complication is that the frequency of infected CD4 cells in the circulating blood may not be an accurate reflection of the total pool of infected CD4 cells; Pantaleo *et al.* [59] have found that more CD4 cells are infected in lymphoid tissues.

## Experiments to distinguish between possible mechanisms of pathogenesis

We began by outlining three general classes of possible mechanisms of HIV pathogenesis: immunological, cofactor and virological theories. Having focused much of our exposition on the virological 'diversity threshold' mechanism and its consequences, we now turn more generally to survey actual and potential experiments, together with quantitative data, that could distinguish between the candidate theories.

The first and probably most important question is: what are the factors that control virus growth during the asymptomatic phase (and fail in the final phase of infection)? Is there extensive virus replication, but no increase in virus population size, because of effective immune responses? Or is there negligible viral replication because of a lack of activated CD4 cells? Answers to these questions would allow us to distinguish between the virological and cofactor models. The rapid turnover of different virus mutants and the (strong) selection pressure for variation in epitopes that are recognized by relevant immune responses may be read as favouring the view that the virus is suppressed by the immune system. It also appears more likely that the initial viraemia, which is observed in approximately 50% of patients, is down-regulated by immune responses, rather than a lack of CD4 cell activation [60,61]. However, more quantitative information is required.

A related question is whether the immune response against HIV (or SIV) is beneficial at all. Activation of CD4 cells may enhance virus replication, or autoimmune responses induced by HIV may accelerate the decline of the CD4 cell population. It would be revealing to discover whether an animal (for example, macaque) without immune response to SIV would develop disease. What is the effect of general immunosuppression of an asymptomatic SIV-infected macaque on the rate of disease progression? According to the 'diversity threshold' theory, the immune response to HIV (or SIV) is necessary to control virus levels during the asymptomatic phase. According to autoimmune response-based theories it would even be beneficial to induce tolerance to those HIV (or SIV) epitopes that share the structural similarity with MHC. According to cofactor theories, immunosuppression may reduce the rate of HIV replication and thus slow down disease progression.

It would be interesting to see whether a special SIV variant with lower levels of antigenic variation (due, for example, to lower mutation rates or functional constraints) can nevertheless cause a similar pattern of disease progression. If this happened, it would militate against the diversity threshold theory.

Quantitative data on virus replication rates and immune responses of different SIV strains in different monkey species could, in principle, confirm or disprove the diversity threshold result that pathogenicity is a simple function of the basic parameters (see *Results of the mathematical model*). It appears that cofactor models cannot account for the apathogenicity of SIV in African green monkeys. Why should wild African green monkeys have fewer other infections than macaques?

To improve our understanding of the population dynamics of HIV infections, longitudinal data on virus load (for both free virus and infected cells), CD4 cell counts, and percentage of activated CD4 cells are required. One of the basic questions is whether CD4 cell depletion is simply related to virus loads. Coombs *et al.* [62] find that plasma viraemia is associated with a marked decline in CD4 cell count. Schnittman *et al.* [63] relate CD4 cell counts and viral burden to the rate of disease progression (see Fig. 8).

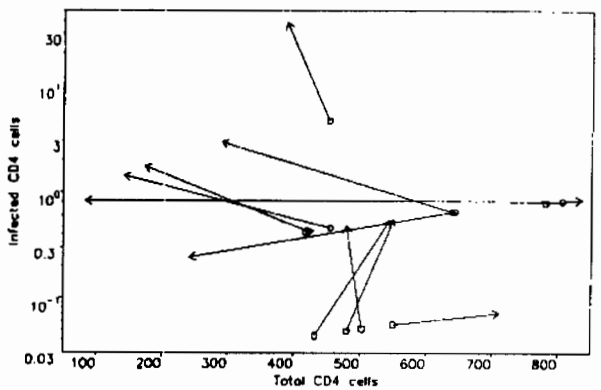


**Fig. 8.** What is the relationship between viral burden, CD4 cell counts and rate of disease progression? The figure shows changes in viral burden and CD4 cell count for 12 different HIV-infected patients. All patients were initially in Centers for Disease Control stage II (i.e., no history of opportunistic infections, no evidence of Kaposi's sarcoma, no symptoms such as fever, weight loss, lymphadenopathy or candidiasis). Data were obtained at two time-points for each patient. Each patient is represented by an arrow in the figure. The first time-point is at the start of the arrow, the second at the arrow head. There are approximately 14 months between these two time-points. Six patients remained asymptomatic; their first point is marked by a square. Six patients developed AIDS-related complex (ARC) or AIDS within this 14-month period; their initial point is marked by a circle. Patients who remained asymptomatic generally have a lower virus load, but the differences are not clear-cut in all cases. More data of this kind could reveal whether there is a simple relationship between CD4 cell depletion, virus load and the rate of disease progression. These data are from [63]. The *y*- and *x*-axes denote infected and total CD4 cell numbers per 1 μl blood, respectively.

From Ho *et al.* [1], we calculate that 1 ml blood of asymptomatic patients contains approximately 20 infectious free virus particles and 40 infected cells that harbour infectious virus. The same study reveals that patients with AIDS-related complex (ARC) or AIDS have 2100 free virus particles and 5400 infected cells and 2300 free virus particles and 4400 infected cells per 1 ml blood, respectively. These data are average values from 54 patients. Note that the ratio of free virus to infected cells is almost constant, regardless of disease stage (0.5, 0.39 and 0.52 in asymptomatic, ARC and AIDS patients, respectively). If AIDS is indeed caused by increased immune activation, it appears likely that this ratio should be much larger in ARC or AIDS patients than in asymptomatic patients (because increased immune activation should shorten the average life-span of infected CD4 cells and increase the rate at which they produce free virus).

Understanding of the evolutionary dynamics requires that we relate genetic variation to antigenic variation in a number of viral epitopes, including both neutralizing antibody [64,65] and CTL epitopes [66]. The ratio of silent to non-silent mutations within an epitope should be a quantitative measure of the selection pressure exerted by the immune system. This ratio should be obtained from a number of different epitopes to identify important targets of the immune responses.

Because of the extensive variation, it would be important to quantify a patient's immune response against the current virus population, rather than against an arbitrary virus strain. Genetic variation should also be studied with respect to variation in replication rates, cell tropism and cytopathicity. One of the big questions is whether the evolution of faster replicating strains causes progression to disease, or conversely is just a consequence of the failing immunological control (once the diversity threshold is exceeded).

Figure 9 shows the genetic diversity of the V3 loop in follow-up studies of three patients (see also [67]).

The idea that other pathogens are the main agents for disease progression in HIV has to be compared with epidemiological data. To date there appears to be little evidence for any systematic correlation between risk group and rate of disease progression, even though some risk groups show evidence of a larger variety of other infections [56]. According to the cofactor theories, patients with a higher load of other infections should progress faster. This is an important test, which may confirm the belief of many HIV researchers that avoiding other infections can increase the length of the asymptomatic phase. We believe that activation of CD4 cells is important to accelerate disease progression, because it increases viral replication, which in turn increases the rate at which new mutants are produced and lowers the diversity threshold. It is unclear at present what causes this activation: HIV itself, autoimmune responses, or opportunistic infections.

## Immunotherapy

Postexposure vaccination of HIV-infected people may be an important way to combat the spread of the AIDS

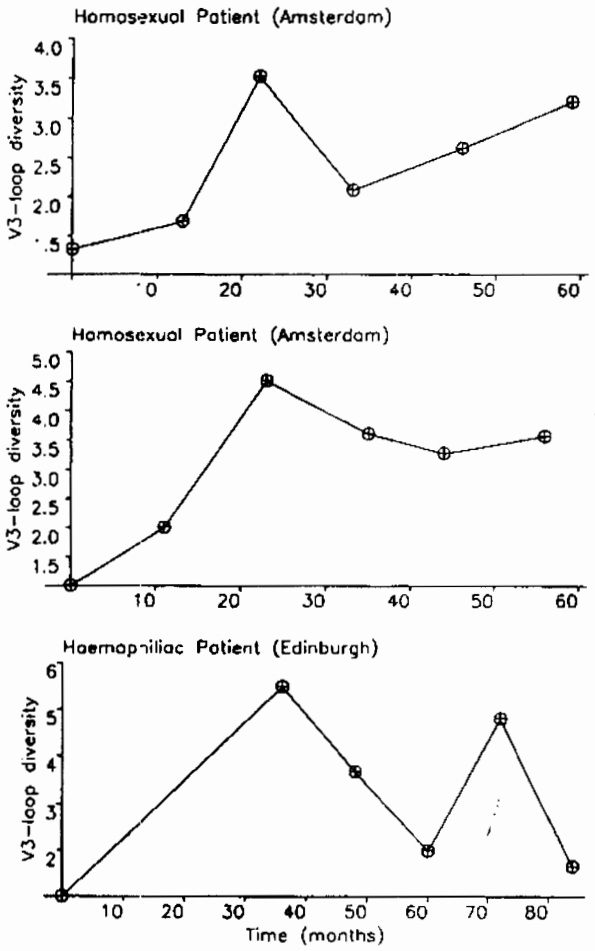


**Fig. 9.** Genetic diversity of the immunodominant and hypervariable V3 loop of the HIV envelope protein, gp120, in three patients who were followed for 5–7 years after seroconversion. Between 10 and 20 sequences have been collected at each time-point. $D$ (the Simpson index) = $(v_i/v)^2$ is used as a measure for viral diversity; here $v_i/v$ denotes the frequency of a given V3 loop sequence at a given time-point. Patients 1 and 3 remained asymptomatic throughout the study. Patient 2 developed AIDS after approximately 55 months. The observed pattern of viral diversity is in broad agreement with the prediction of the diversity threshold theory. The V3 loop sequences are almost completely homogeneous at seroconversion. Then the diversity appears to increase (with some fluctuations). More data are required to determine whether the diversity is indeed very low after full-blown AIDS has developed [25,47].

pandemic. However, it has been recognized that there might be a non-trivial risk with any vaccine that activates CD4 cells. The vaccine may not only enhance immune responses to HIV, but also increase viral replication.

In our model the success or failure of a vaccine that (1) induces strain-specific responses to HIV, and (2) enhances viral replication by CD4 cell activation, depends on the antigenic diversity of the virus population. In a patient with a rather homogeneous virus population (early in the infection) it may clear the virus, but in a patient with a more heterogeneous virus population, the same vaccine may accelerate disease progression. Figure 10 shows computer simulations of these two cases. To illustrate this effect, we must recognize that HIV replication rates may increase with increasing levels of activated HIV-specific CD4 cells. This is a small modification of our original model:

$$dv_i/dt = v_i[r(1+ax+az)-px_i-sz]; \qquad i = 1,..,n \quad (15)$$

$$dx_i/dt = kv_i - bx_i - uvx_i; \qquad i = 1,..,n \quad (16)$$

$$dz/dt = k'v - bz - uvz \quad (17)$$

This leads to a diversity threshold for immunotherapy. As long as $D > ar/p$, increasing the strain-specific responses reduces the total virus load. If the diversity is larger than this threshold value, increasing the strain-specific responses increases the total virus load. The effect of immunotherapy via group-specific responses is, of course, independent of viral diversity. If $s > ra$, then it is advantageous to stimulate group-specific responses. These complications are likely to cause problems when and if we get to the point of evaluating success and failure in early postexposure vaccination trials.

## Drug treatment and the evolution of resistant strains

Zidovudine treatment of AIDS patients leads to a rise in CD4 cell counts and a fall in circulating virus, as well as improvements in immune responses and weight gains [68]. Unfortunately, these improvements are only short-lived. After approximately 6 months of treatment virus titres rise again, and CD4 cell counts begin to fall. Several studies have drawn attention to the emergence of resistant HIV strains in individuals on long-term zidovudine therapy. A similar pattern may occur during dideoxyinosine (ddI) treatment, and more recent studies have reported HIV mutants with reduced sensitivity to ddI [69]. Both zidovudine and ddI work as chain-terminators during reverse transcription. HIV variants with reduced sensitivity to zidovudine or ddI have point mutations (up to five are known) within the otherwise rather conserved *pol* gene [70].

A quasispecies model of HIV can be used to study the emergence and population dynamics of these resistant mutants. Here we sketch a simple example of such a model:

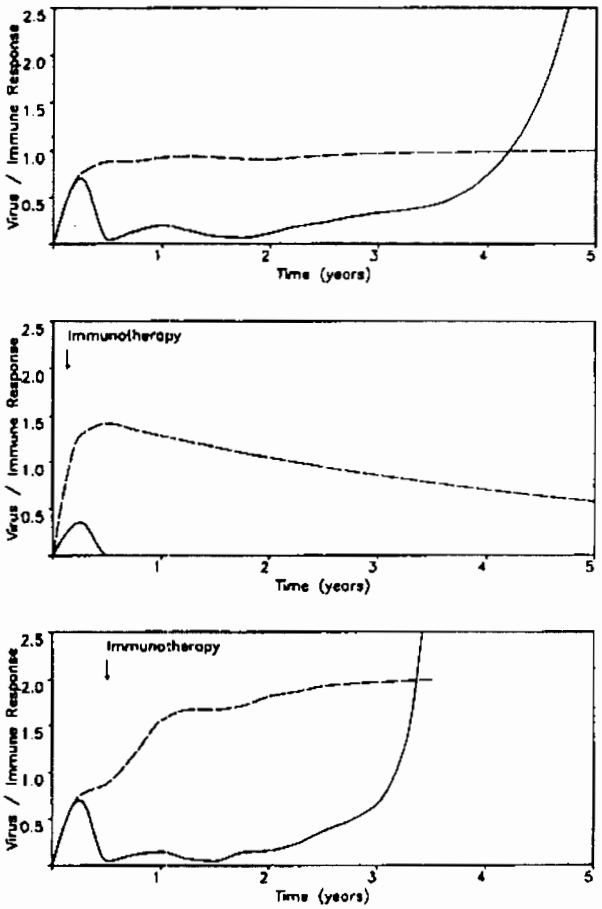


**Fig. 10.** Immunotherapy that activates CD4 cells can clear the infection or enhance disease progression. (a) Computer simulation of an HIV infection without treatment, using equations 13–15. (b) Immunotherapy is started 0.125 years after infection; the HIV population is almost completely eliminated. (c) The same immunotherapy can accelerate the development of disease if applied too late (here 0.5 years after infection). In these computer simulations immunotherapy enhances strain-specific immune responses to HIV. It is also assumed that this activates CD4 cells, which enhances HIV replication. The consequence is a diversity threshold for immunotherapy. If the virus diversity is below this threshold, then immunotherapy is beneficial; if the virus diversity is above this threshold, then the same immunotherapy is disadvantageous. This result suggests that immunotherapy is more likely to be efficacious in asymptomatic patients early after infection, when their viral diversity is still low. Virus population, $v$, (——) and total strain-specific immune responses, $x$ (- - - -), are shown. Parameter values are $r = 2$, $a = 0.15$; all other values are exactly as in Fig. 2. Immunotherapy was modelled by increasing the parameter $k$ from 1 to 2. This defines the rate (and hence the magnitude) at which strain-specific immune responses are mounted. The immunogen is assumed to be perfect, in the sense that it enhances strain-specific responses to all possible variants. This assumption is not necessary for the observed effects.

$$dy/dt = c - by - \sum_i r_i v_i y \qquad (18)$$

$$dv_i/dt = y \sum_j r_j Q_{ij} v_j - p v_i \qquad i = 1, \ldots, n \qquad (19)$$

Uninfected CD4 cells, $y$, are produced at a constant rate, $c$, and removed at rate $by$; $v_i$ denotes CD4 cells infected with virus mutant $i$; $r_i$ is the replication (or transmission) rate of mutant $i$; and $Q_{ij}$ is the probability that erroneous replication of mutant $j$ results in generation of mutant $i$. Infected CD4 cells are removed at the rate $pv_i$.

Figure 11 shows the evolution of drug resistance during treatment with zidovudine, as described by this model. The increasing virus concentration is caused both by the emergence of resistant virus and by the increased abundance of CD4 cells [71]. This is a consequence of the population dynamics of equations 18 and 19.

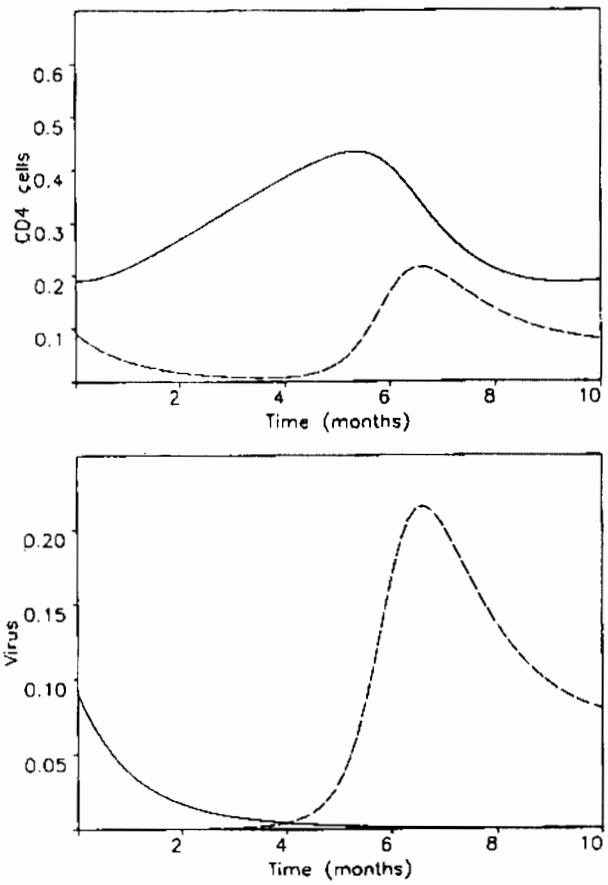


**Fig. 11.** The population dynamics of the evolution of drug-resistent strains during zidovudine therapy according to equations 19 and 20. (a) Total (——) and infected CD4 cells (- - - - -). (b) Zidovudine-sensitive virus (——) and zidovudine-resistant strain (- - - - -). The x-axis denotes time since beginning of zidovudine treatment. Parameter values are $c = 1$, $b = 0.1$, $p = 1$. In the absence of zidovudine the replication rate of the wild-type is $r_1 = 1$, and that of the resistant mutant is $r_2 = 0.9$. With zidovudine we choose $r_1 = 0.1$ and $r_2 = 0.8$.

To study the effect of alternating zidovudine and ddI treatment we distinguish three different mutants: (1) wild-type, which is sensitive to both zidovudine and ddI; (2) zidovudine-resistant virus (sensitive to ddI); and (3) ddI-resistant virus (sensitive to zidovudine).

Figure 12 shows a computer simulation of alternating treatment with zidovudine and ddI. The average CD4 cell count is maintained at much higher levels than under zidovudine therapy alone.

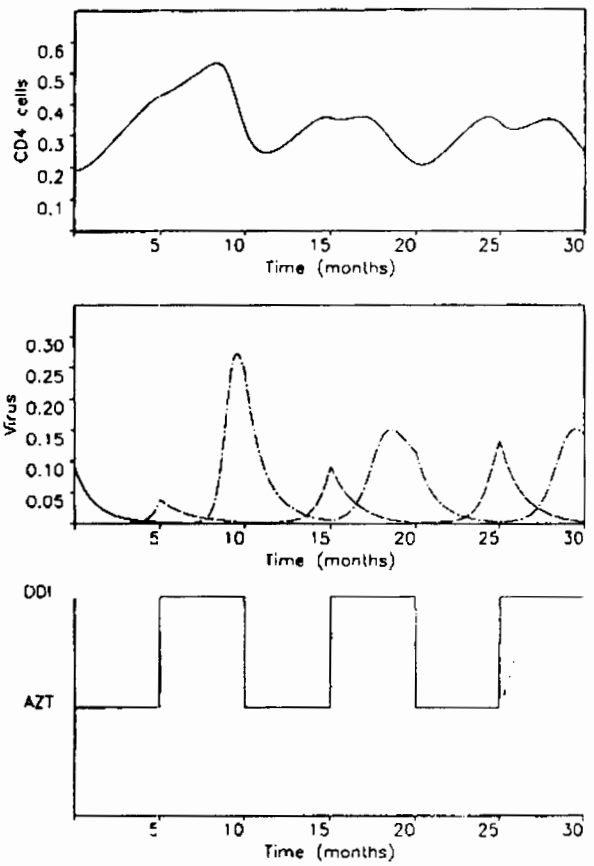


**Fig. 12.** Alternating treatment between zidovudine and dideoxyinosine (ddI) may maintain higher levels of CD4 cell counts. In this computer simulation it is assumed that HIV can produce mutants that are resistant to zidovudine or to ddI, but not completely cross-resistant to both. (a) CD4 cell counts. (b) Sensitive wild-type (——), zidovudine-resistant strain (- - - -), ddI-resistant strain (- - - -). (c) Switching between zidovudine and ddI. Parameter values as in Fig. 11. Without zidovudine and ddI, the replication rates are $r_1 = 1$, $r_2 = 0.9$, $r_3 = 0.9$. With zidovudine: $r_1 = 0.1$, $r_2 = 0.8$, $r_3 = 0.1$. With ddI: $r_1 = 0.1$, $r_2 = 0.1$, $r_3 = 0.8$.

This model is only a first step, and there is much scope for further work. More realistic models may include the genetic structure of the observed mutants, as well as mutants that have a slightly reduced sensitivity to both zidovudine and ddI. Parameter values can be assigned from the experimental data. The models should aim to find patterns of zidovudine and ddI treatment that maximize CD4 cell counts in patients.

Mathematical models have also been used to study the protocol for optimal treatment to minimize the toxicity of zidovudine on bone-marrow cells [72].

## Conclusions

The 'diversity threshold' theory suggests the following answers to the questions we asked at the beginning. (1) The length and variability of the asymptomatic phase is determined by evolution of the rapidly mutating HIV (or SIV) quasispecies under the various selection pressures during an infection. The immune response selects for increasing antigenic diversity, which in turn leads to its own defeat. This mutation selection process is likely to occur on the time-scale of several years [73]. The variability of the asymptomatic phase is caused by the stochastic nature of this process and differences in immune responses in different people. (2) Genomic integration and antigenic variation work in concert to ensure that HIV can establish a persistent infection. The diversity threshold model suggests that antigenic variation enables the virus to persist at much higher levels than would otherwise occur, which appears to be important in inducing immunodeficiency. (3) The depletion of CD4 cells is caused by direct or indirect mechanisms; at present none of the suggested mechanisms can be dismissed on theoretical grounds, but the recent finding of high viral loads in the lymphoid tissue [59] makes it more likely that most of the depletion of CD4 cells is caused by direct cytopathicity. (4) Genetic and antigenic variation is *necessary* for disease progression during a long asymptomatic phase. It is the basis for exceeding the diversity threshold. (5) Opportunistic infections are probably not necessary for HIV to induce immunodeficiency. From the evidence, it is not yet clear whether they accelerate disease progression. (6) Whether a given primate lentivirus is pathogenic in a given host species is a function of the basic rate parameters of the model. Sufficiently strong group-specific immune responses can lead to a chronic infection without disease. In this case there is no diversity threshold.

More generally, we emphasize that studies of theoretical models for the interactions between HIV and the immune system can be important not only in relation to particular experimental facts or in putting curves through data points, but more widely in exploring ideas, suggesting mechanisms and prompting experiments [74]. The need for creative interplay among theory, observation and experiment is fully recognized in mature disciplines, such as physics. Some molecular biologists and immunologists, however, occasionally take an oddly restricted view of the uses of theory. Ultimately, mathematical models are no more — but no less — than tools for thinking in a clear and unambiguous way. Such tools can be of special use for understanding phenomena like AIDS pathogenesis: the non-linearities inherent in the interactions between populations of pathogens and populations of immune cells can produce outcomes that defy any intuition on the interaction between individual cells and viruses.

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