

Evolutionary dynamics of HIV-induced subversion of the immune response

Authors' address

Dominik Wodarz, Martin A. Nowak,
Institute for Advanced Study, Princeton,
New Jersey, USA.

Correspondence to:

Dominik Wodarz
Institute for Advanced Study
Olden Lane
Princeton NJ 08540
USA
Fax: 1 609 951 4438
e-mail: wodarz@ias.edu

Summary: Human immunodeficiency virus (HIV) disease progression is characterised by a slow but steady decline in the number of CD4⁺ T cells. It results in the development of AIDS when the immune response collapses and the virus grows uncontrolled. Pathogenicity of HIV may be due to viral escape from cellular immune responses as well as virus-induced immune impairment. Here we discuss how the dynamic interactions between the virus population and the immune response may lead to the development of AIDS. In particular we argue that *in vivo* evolution of HIV may be the driving force successively weakening the immune system. This may lead to increased levels of viraemia as well as to the evolution of more virulent phenotypes which indicate progression to AIDS. These insights are important for understanding the disease process itself and for designing effective treatment regimes.

In vivo dynamics of HIV

Recent quantitative virological data from HIV-1-infected patients receiving antiviral therapy, in conjunction with mathematical models, have for the first time provided precise information on the kinetics of virus turnover *in vivo* (1–8). It was shown that the half-life of productively infected cells is around 2 days (3, 4). Free virus particles in the plasma have an even shorter half-life. The current estimate is about 6 h, but it is likely that the actual figure is lower than this, which suggests that close to 100% of the free virus population in the plasma of a patient turns over every day (6). These studies provide conclusive evidence that the asymptomatic phase of HIV-1 infection is not a period of latency but a dynamic process involving continuous rounds of *de novo* replication and infection, and that HIV is not a “slow virus” in terms of its daily replication potential. These findings are crucial for understanding the driving forces underlying disease progression in HIV-1-infected patients: the rapid viral turnover during the asymptomatic phase indicates a great potential for the virus to evolve in response to selection pressures exerted either by the immune system or by drug treatment (3, 4, 9, 10). The potential for rapid viral evolution is demonstrated by findings that wild-type virus can be re-

placed by drug-resistant strains within 14–28 days of drug therapy (4, 11).

Such infection dynamics involve non-linear interactions of many different components, and this makes the use of mathematical models necessary to provide a correct interpretation of empirical results, to generate new insights and hypotheses and to guide further experimental work. In this review we will demonstrate how mathematical models have been used to identify evolutionary mechanisms that may drive disease progression in HIV-infected patients and as to achieve a better understanding of the principles underlying antiviral therapy.

HIV pathogenesis and subversion of cellular immunity

In the primary phase of the disease, after an individual has been infected with HIV, the virus replicates to relatively high levels for a short but variable period of time. The subsequent rise of the immune response suppresses this primary viraemia and the patient enters the asymptomatic phase. During this phase, the virus is kept at low levels and one observes a slow decline in the CD4⁺ cell count of the patient. The duration of the asymptomatic phase varies widely between individuals. Fast progressors develop AIDS within a few years, whereas slow or non-progressors remain without symptoms for 10–15 years. The final development of AIDS is often characterised by an upsurge in virus load accompanied by a fall in the number of CD4⁺ cells found in the blood. It is now common to define AIDS disease if the number of CD4⁺ cells has fallen below 200 per mm³ (a healthy, uninfected person has about 1,000 CD4⁺ cells per mm³).

At the beginning of the infection, there is little sequence variation in the HIV population isolated from a patient (12–15). In the course of infection, HIV mutates in the cytotoxic T lymphocytes (CTL) and antibody epitopes, resulting in an increase in antigenic diversity until the patient develops AIDS (16–24).

Virus strains isolated in the asymptomatic phase tend to use the CCR5 co-receptor (R5 viruses) (25–26). Therefore, they not only infect primary T cells, but also have the ability to infect macrophages. These isolates tend to show a slow rate of replication (27) and a low degree of cytopathogenicity and are usually associated with the non-syncytium-inducing (NSI) phenotype (14, 28–34). During the asymptomatic phase of the infection, HIV evolves to show stronger tropism for T cells (32). These virus strains also tend to replicate at a faster rate (27), and are characterised by higher degrees of cytopathogenicity. (33–34). Such isolates may either use both the CCR5 and the CXCR4 co-receptors (termed R5X4 strains) (25, 26) or, in

about 50% of the patients, become specialised to use the CXCR4 receptor only (termed X4 viruses) (25, 26). X4 strains have lost the ability to infect macrophages, are associated with the syncytium-inducing phenotype and are markers of disease progression (30, 32, 35, 36).

Central to the understanding of HIV pathogenesis is the concept of immune impairment. During the course of the infection, HIV evolves towards phenotypes that are characterised by increased virulence, especially in T-helper cells. Loss of T-helper cell function reduces the efficacy of the CTL response. This allows the virus to replicate more efficiently and to switch to more pathogenic phenotypes, which in turn increases virus load. Therefore, the observed negative correlation between virus load and CTL in HIV-infected patients (37) indicates that subversion of the CTL response by the virus is an important factor governing progression of the disease (D. Wodarz, S. Hall, G. Ogg, A. J. McMichael, M. A. Nowak, C. R. M. Bangham, submitted; D. Wodarz, S. Hall, C. R. M. Bangham, M. A. Nowak, submitted).

In the following we will show how mathematical models may explain this subversion of the CTL response, and how impairment of the immune system favours the evolution of viral phenotypes that indicate progression to AIDS.

Dynamics of immune impairment by HIV

Mathematical models suggest that impairment of the immune response may result from the dynamical interactions of the virus population with both T-helper cells and CTL. *In vivo* evolution of HIV may shift the balance between HIV replication and the immune response successively in favour of the virus, eventually resulting in the collapse of the immune system and uncontrolled viral replication. We have identified two evolutionary forces which may result in similar patterns of CTL impairment: evolution towards increased antigenic diversity and evolution towards increased rates of replication. These will be discussed in turn.

Antigenic diversity and immune impairment

Since there is no proof-reading mechanism during reverse transcription, HIV is characterised by a relatively high mutation rate of about one base per genome per replication cycle (38, 39). This high mutation rate, together with the selection pressure exerted by the antiviral immune response, provides ideal conditions for the evolution of antigenic escape mutants (19, 22, 23). In fact, the error rate of the reverse transcriptase of HIV seems to maximise the chance of producing antigenic escape mutants (40). These escape mutants may in turn be controlled

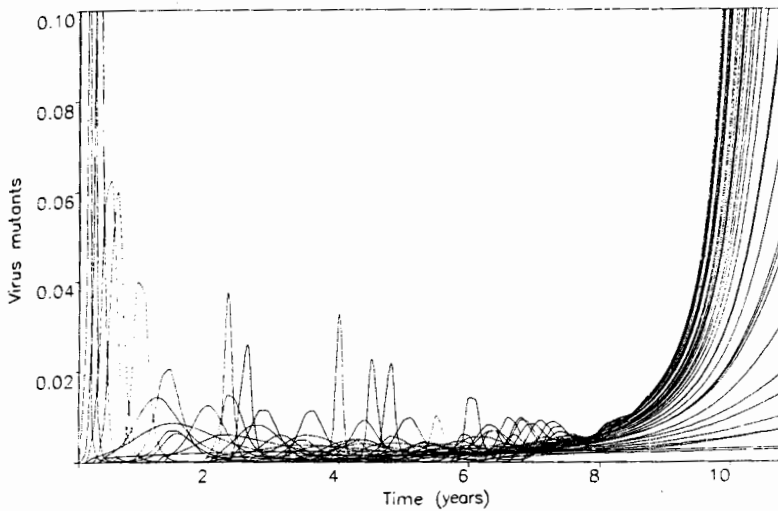


Fig. 1. Evolution of many different virus strains during the time of infection. Initially, the strains grow to high levels, which may cause the clinical symptoms observed during primary HIV infection. The escape mutants subsequently emerging are suppressed at a faster rate, because of the action of cross-reactive immune responses. Different virus strains grow to different levels according to their growth rates. The accumulation of viral diversity breaches the diversity threshold after approximately 7 years at this simulation. In the final phase, the fastest growing strains dominate the virus population. The y-axis indicates the relative concentration of different virus mutants.

by rising immune responses specific for these newly evolved virus strains. However, there is an inherent asymmetry in the interaction between the evolving virus population and the antiviral immune response. While a given immune response may have to be specific for a given virus strain in order to kill it, each virus strain can infect and kill any T-helper cell regardless of its specificity. These basic assumptions can be captured in simple mathematical models (9, 41–44). Such models take into account the evolving virus population, strain-specific immune responses as well as cross-reactive immune responses. The evolutionary dynamics also involve a stochastic process where the probability that a new mutant emerges is either constant or more realistically proportional to the total virus load, since the number of mutations is proportional to the number of replication events.

Such models are characterised by three parameter ranges, depending on the relationship between viral replication and/or immune impairment and the strength of the strain-specific and cross-reactive immune responses. i) If viral replication together with immune impairment out runs the combined effect of strain-specific and cross-reactive immune responses, the immune system immediately collapses, resulting in uncontrolled viral replication and the development of AIDS without the presence of a prolonged asymptomatic period. No antigenic variation will be observed and the fastest replicating strain will emerge to dominate the virus population. Such dynamics are observed in rapid progressors (45). ii) If the cross-reactive immune response alone is sufficiently large compared to virus replication and the amount of immune impairment, the immune system may effectively control the virus population without the development of disease. In this case, the level of virus

load controlled by the immune response depends on the rate of viral replication, the amount of immune impairment by the virus, the efficacy of the immune response and the number of antigenic variants present. However, uncontrolled viral replication is not possible in this parameter region. Although some HIV-infected patients are long-term non-progressors and may fall in this parameter region, it is not clear whether they will develop symptoms at a later stage. Chronic infection without development of disease is, however, the rule for simian immunodeficiency virus infections in their natural hosts (46). The lack of disease progression in this case may be due to strong cross-reactive immune responses as well as slower replication rates of the virus. iii) The most interesting case predicting the course of HIV infection in humans arises when the combination of cross-reactive and strain-specific immune responses may control the virus, but the strain-specific response alone is unable to do so. In this case, the dynamics depend on the amount of antigenic diversity. If antigenic diversity is low, the virus is controlled, corresponding to the asymptomatic phase of the infection. Increasing antigenic diversity results in an increase in virus load. The immune system keeps the virus population in check as long as the antigenic diversity lies below a threshold. Crossing the diversity threshold leads to uncontrolled virus growth accompanied by a collapse of the immune system. A typical course of disease progression predicted by the model is illustrated in Fig. 1. The length of the asymptomatic period is determined by the magnitude of the diversity threshold, which in turn depends on the efficacy of the immune response as well as on the amount of viral replication and immune impairment. Variation in these parameters among patients may therefore account for the variability observed in the

disease process. In addition, the stochastic nature of the emergence of escape mutants may also contribute to the variability of the disease process. Whether the first few escape mutants arise sooner or later than average may result in marked differences in the pattern of disease progression (43). In summary, viral evolution towards increased antigenic diversity may be the cause rather than the consequence of disease progression and may contribute to the transition from the asymptomatic period of the disease to AIDS.

Wolinsky et al. (45) interpreted their finding that rapid progressors can have low genetic variation as being at variance with the diversity threshold theory. This conclusion is incorrect. As explained in the first papers on the diversity threshold idea, rapid progression is expected to occur with low antigenic diversity if the patient has weak anti-HIV immune responses.

In most viral infections, including HIV, there are immune responses against several epitopes; this complicates viral dynamics and the effect of antigenic variation. Therefore, a theoretical framework was developed to explore the dynamics of an antigenically variable virus population and an array of CTL responses directed against multiple epitopes (47–49). This multiple epitope theory has also been applied to other infectious diseases (50, 51). Central to the understanding of the effect of CTL responses against multiple epitopes is that these CTL clones are in competition with one another. The different CTL clones can be viewed as species of predators that proliferate, with different efficiencies, in response to a common food source (the virus population).

Consider first the simple assumption of an antigenically homogeneous virus population. In such a scenario, the model predicts that the CTL response with the strongest immunogenicity will outcompete all other CTL responses. This is analogous to the competitive exclusion principle in ecology: the strongest competitor will reduce virus load to levels that are not sufficiently high to stimulate the competitively inferior CTL responses. This may explain the concept of immunodominance i.e. that *in vivo* the CTL response is predominantly directed against one or only a few epitopes (52–56).

For a heterogeneous virus population, the situation is more complicated than this. One has to distinguish between two cases. Either all antigenic variants have the same replication rate, or their replication kinetics differ. Consider the simpler case of equal replication rates first. The model still predicts the occurrence of immunodominance, but now the competitive ability of a CTL response against a given epitope not only depends on its immunogenicity but also on viral diversity in this epitope. Viral diversity essentially reduces immunogenicity. Assume that CTL proliferate equally well in response to all

variants of a given epitope. Denoting the immune responsiveness against epitope A as c , the immune responsiveness against epitope B as k , and the number of antigenic strains in the respective epitopes as n_A and n_B , the immune response against epitope A will dominate if $c/n_A > k/n_B$.

On the other hand, if the antigenic variants replicate at different rates, the model predicts the co-existence of CTL responses directed against the different epitopes. In other words, a heterogeneous virus population characterised by concomitant differences in the viral replication kinetics induces the lack of immunodominance.

Antigenic variation in multiple epitopes can lead to complicated 'antigenic oscillations' (Fig. 2) (47), that is, distinct peaks in viral abundance, often dominated by a single genotype, that arise when the CTL response against a given variant declines to low levels due to temporary lack of stimulation. This leads to concomitant fluctuations in immunodominance. In contrast to the occurrence of peaks in viral abundance upon the emergence of new variants (antigenic drift), antigenic oscillations are the result of the non-linear dynamics between the existing heterogeneous virus population and CTL responses against multiple epitopes and do not require the emergence of new mutants.

Next, consider the emergence of a new mutant in a given epitope. Suppose the existence of a homogeneous virus population and an immunodominant CTL response against one of two epitopes (epitope A, Fig. 3). The emergence of an escape mutant in epitope A may lead to one of four possible outcomes depending on the replication rates and the immunogenicities of the mutant relative to the wild type (Fig. 3). Denote the replication rate of wild type and mutant as r_w and r_m , respectively, and denote the immunogenicity of wild-type and mutant epitope A as c_w and c_m , respectively. The immunogenicity of epitope B is described by k . The four outcomes are as follows: i) A new specific response against epitope A is induced by the mutant without affecting epitope B. This represents diversification in epitope A and will be observed if $1/c_w + 1/c_m > 1/k$. ii) No new response against epitope A is induced by the mutant, but the response against epitope B is enhanced. This is a partial shift in immunodominance and will occur if $1/c_w + 1/c_m < 1/k$ and $r_w > r_m$. iii) The mutant may induce a new response against epitope A which outcompetes the response against the wild type, consequently causing a partial shift in immunodominance. The condition for this outcome is given by $1/c_w + 1/c_m > 1/k$ and $r_w < r_m$. iv) Finally, if $1/c_m > 1/k$ and $r_w < r_m$, the mutant virus outcompetes the wild type, resulting in a complete shift in immunodominance. Thereby, the selective advantage of the escape mutant becomes negligible, and it may not

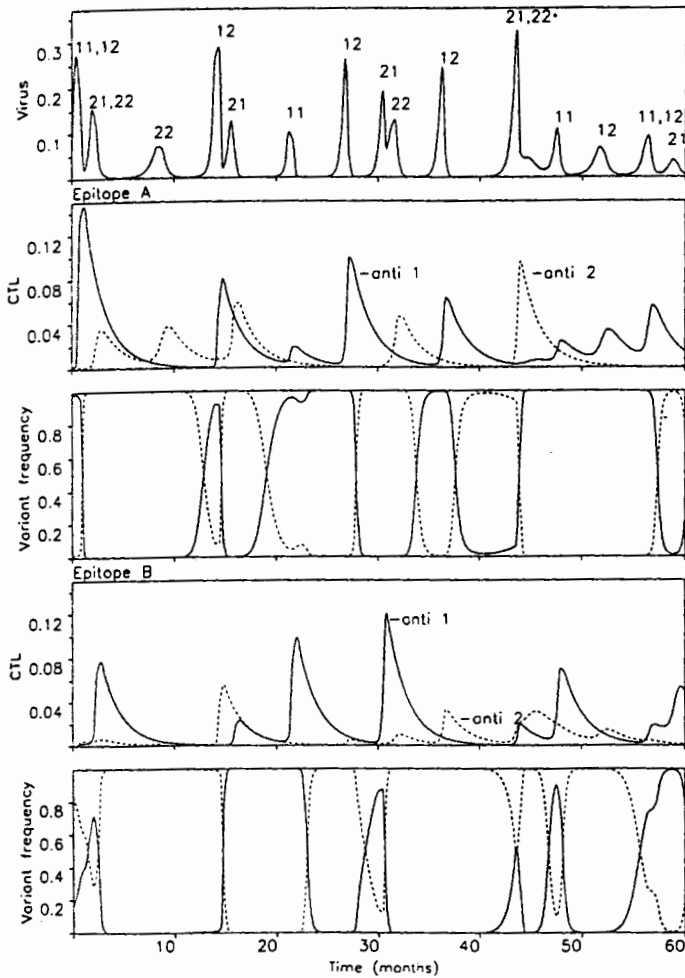


Fig. 2. Antigenic oscillations and fluctuating immunodominance in a model with two epitopes. Peaks of viral abundance that consist of antigenically different variants are accompanied by oscillations in the size and specificity of the CTL responses. All virus variants are present at the beginning of the simulation and there are no additional mutational events.

reach fixation even if there is no CTL response against the variant peptide.

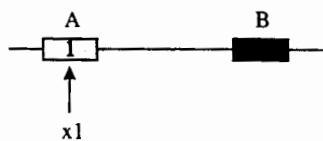
In terms of HIV infection, this analysis extends and reinforces the idea that viral evolution towards increased antigenic diversity may drive progression of the disease. A stable response against an invariant epitope will lead to efficient control of the virus and slow progression. Viral diversity may lead to unstable dynamics, and the evolution of new antigenic variants may result in a shift in immunodominance to weaker epitopes, leading to less efficient control of HIV and an increase in virus load. These notions have been confirmed experimentally by Borrow et al. (22), Goulder et al. (23) and Price et al. (24).

Replication rate and immune impairment

The notion that changes in the replication rate of HIV may be important for disease progression has often been pointed out (28, 36, 57–59). Connor & Ho (27) analysed sequential HIV-1

isolates from a patient who progressed to AIDS within 5 years. Virus strains isolated at the beginning of the infection were characterised by relatively slow replication kinetics. During the asymptomatic phase they observed a strong and steady increase in the replication rate of the virus until the onset of AIDS. The increasing abundance of faster replicating variants during disease progression has been described mathematically by Nowak & May (41, 60), deBoer & Boerlijst (61) and Schenzle (62).

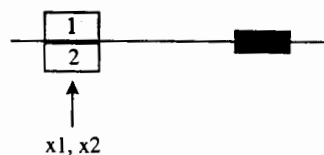
More specifically, Wodarz et al. (63) showed how viral evolution towards increased replication kinetics in the asymptomatic phase may contribute to the transition to full-blown AIDS. They considered a mathematical model where the target cells are antigen presenting cells or T-helper cells (which are both infected by HIV). They distinguished between precursor and effector CTL and assumed that the proliferation of precursor CTL requires the help of uninfected CD4⁺ T cells e.g. via cytokine production. These assumptions lead to the result that the



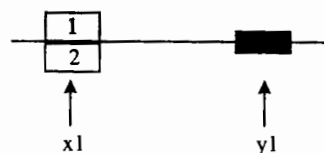
A homogeneous virus population with an immunodominant response against epitope A

Fig. 3. Antigenic variation may shift immunodominance. The emergence of an escape variant in an immunodominant epitope (A) leads to one of four possible outcomes. For details see text.

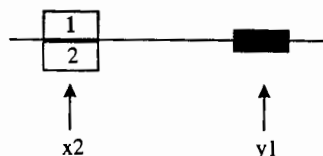
Emergence of an escape mutant in epitope A leads to one of four possibilities:



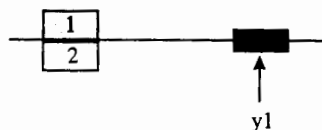
(i) Diversification in epitope A



(ii) No new response against epitope A; partial shift in immunodominance



(iii) New response against epitope A; partial shift in immunodominance



(iv) Complete shift in immunodominance to epitope B

virus may induce the exhaustion of the virus-specific CTL response. In terms of HIV, two parameters are important in determining the fate of the CTL response. There is a replication rate threshold beyond which the HIV-specific CTL response will be exhausted. The higher the immune responsiveness of the host, the higher the replication rate of the virus required to induce CTL exhaustion. CTL exhaustion has been proposed as a possible mechanism for the eventual breakdown of the immune system upon progression to AIDS (e.g. 64–66).

Fig. 4 demonstrates how evolution towards increased replication rates of HIV may lead to a pattern of disease progression similar to that observed in HIV-infected patients. As long as the

replication rate of the virus is sufficiently low for the CTL response to persist, evolution towards faster replication results in a relatively slow decline of the uninfected T-helper cells and a slow rise in virus load. When the virus evolves beyond the replication rate threshold, it induces the exhaustion of the specific CTL response, allowing virus load to shoot up to relatively high levels and causing a sharp drop in the number of T-helper cells found in the patient. As was the case with the diversity threshold model, variation in the immune responsiveness of the host may account for variability in the duration of the asymptomatic phase of the infection, with a stronger responder remaining asymptomatic for a longer period of time. As can be

seen in Fig. 4, the model also predicts that the CTL precursor population starts to decline earlier and declines at a faster rate than the CTL effector population. Thus, when the CTL precursors have largely vanished, one might still expect to see considerable CTL effector activity. Such observations have been made by Rinaldo et al. (66, 67). They found that there was a correlation between CTL effector activity and time, and that towards the end stage of the disease, a significant CTL effector response was still present, possibly arising from residual memory.

Evolution towards higher replication rates is related to evolution of antigenic diversity in that mutation in the CTL epitopes may allow effectively faster viral replication. This may occur either through simple escape mutations or through the evolution of altered peptide ligands, which may act as TCR antagonists (68–71) or promote responses with inappropriate specificities (22, 72–76). Moreover, evolution of resistance to immune responses inhibiting the overall replication kinetics of the virus, such as interferon or antibodies, may also contribute to the exhaustive process.

Immune impairment and phenotype switching

The differential tropism of HIV for macrophages and T cells may be a key element of HIV pathogenesis (32, 33, 77–85). Strains using the CCR5 co-receptor (R5 viruses) infect primary T cells and macrophages. They are slowly replicating and relatively non-virulent (NSI phenotype). As disease progresses, HIV becomes increasingly T-cell tropic, using the CXCR4 co-receptor. Virus strains may use both the CCR5 and the CXCR4 co-receptors (termed R5X4 virus) (25, 26), or they evolve to specialise on the CXCR4 co-receptor (X4 virus) (25, 26), as happens in about 50% of the patients. Such isolates have lost the ability to infect macrophages, replicate at a faster rate and tend to show the syncytium-inducing phenotype. The evolution of virulent X4 strains is associated with progression to AIDS and mathematical models can help us identify factors that lead to the emergence of virulent CXCR4 tropic mutants.

Mathematical models have so far focused on two basic concepts concerning cell tropism in HIV infection. i) First, there is a difference in the biology of macrophages and T cells: while HIV can only replicate in activated T cells, activation and cell division does not seem to be required for macrophage infection (86, 87). The reason for this difference rests in the mechanism underlying the transport of the HIV genome into the nucleus of the two cell types (86–92). While the HIV preintegration complex may reach the macrophage nucleus by active transport, this mechanism does not work in T cells, where breakdown of the nuclear envelope is required for infection. ii) Second, there

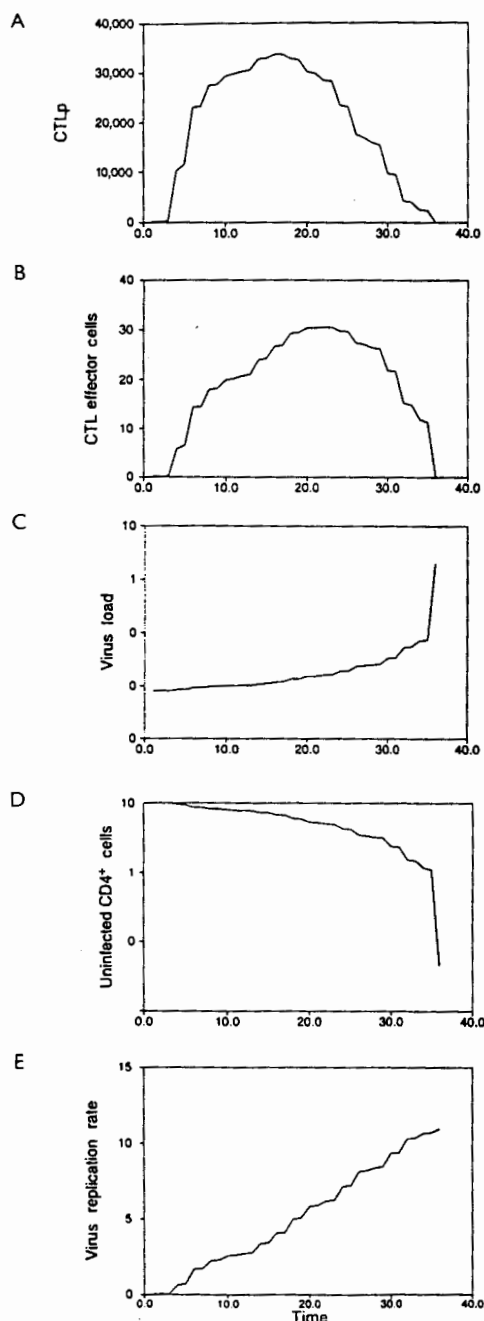


Fig. 4. Implications of CTL exhaustion for HIV disease progression.

The graphs show the effect of virus evolution towards higher replication kinetics on the number of (A) CTL precursor cells, (B) CTL effector cells, (C) virus load and (D) the number of uninfected target cells. At each time interval the viral replication rate (E) is increased by a random amount and the equilibrium values of the respective variables calculated. See text for details.

is a difference in the viral phenotypes between strains using the CCR5 and the CXCR4 receptor. While R5 viruses are supposed to be non-cytopathic and slowly replicating, X4 viruses are thought to be strongly cytopathic and faster replicating.

Macrophage versus T-cell infection

Macrophage infection by HIV can be modelled by the basic equations describing virus dynamics set out at the beginning of this review. However, in order to study T-cell infection in a more realistic way, Wodarz et al. (93) modified the basic virus infection model to take into account resting target cells that cannot be infected, and susceptible target cells that are generated from the resting cell population through activation in response to antigen. These assumptions complicate the dynamics of HIV infection. If the rate of background activation of T cells due to other pathogens being present at the time of HIV infection is relatively low, the outcome may depend on a complex interplay between host and viral parameters as well as the initial conditions. An important host factor is the immune responsiveness. If the immune responsiveness lies above a certain threshold, the infection is cleared. The reason for this is that a relatively strong CTL response will depress the virus load to very low levels, so that the virus load is not enough to maintain a significant number of activated T-helper cells. Since these are the target cells of the virus, the infection will vanish.

Among the viral parameters, the replication rate of the virus is required to lie above a certain threshold for infection to be possible. In the parameter region where the virus may establish a persistent infection, the outcome of the dynamics depends on the initial abundance of virus and virus-specific CTL (Fig. 5). The initial virus load must lie above a certain threshold level in order to activate a sufficient number of target cells for invasion to be possible. Given that the initial virus load is sufficiently high to allow invasion, the CTL response may either clear the infection or control a persistently replicating virus. If the initial virus load lies above a threshold, the CTL response can grow fast enough to reduce the virus population quickly by a significant amount. This in turn leads to a decline in the number of activated target cells, making it impossible to support persistent replication. Consequently, the virus population cannot recover and becomes extinct. On the other hand, intermediate initial values of virus load allow virus replication to outrun the CTL response, which can only grow at a slower rate. Thus, persistent replication can be established. The higher the initial abundance of CTL, the lower the threshold level of virus load required to achieve clearance of the infection. Above a certain initial CTL abundance, the establishment of persistent infection becomes impossible.

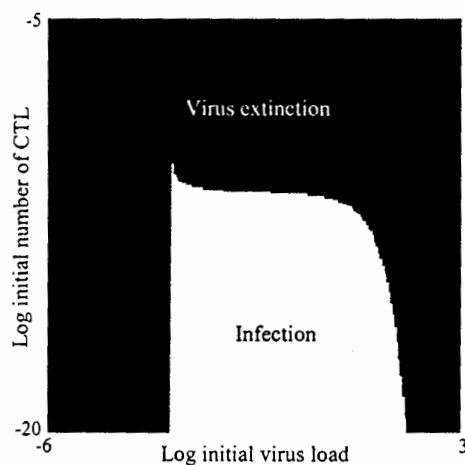


Fig. 5. Dependence of the outcome of T-helper cell infection on the initial conditions. Initial virus load (y_0) needs to lie above a threshold for persistent infection to be possible. However, in the presence of a CTL response, the virus population becomes extinct if virus load lies above a certain threshold. This is because a high initial virus load increases the initial growth rate of the CTL response, which in turn quickly suppresses viral abundance. This reduces the number of activated target cells to below the level required for persistent replication. Therefore, the initial virus load needs to be intermediate for establishment of persistent infection to be successful. The initial number of CTL (z_0) also plays an important role. The higher the initial levels of CTL, the lower the upper virus load threshold leading to virus extinction. Above a certain initial abundance of CTL, the establishment of persistent infection becomes impossible.

This model has been extended to take into account both types of virus strains: R5 viruses capable of infecting macrophages and X4 viruses capable of infecting T cells only. We assumed that T-helper cells are activated by both types of virus. The presence of strains capable of infecting macrophages significantly enhances the invasion of CXCR4 tropic strains, eliminating the complex dependence on initial conditions. The model identifies three parameter regions (93). If the immune responsiveness to macrophage tropic HIV is above a certain threshold, CXCR4 tropic strains cannot invade the host. On the other hand, if the immune responsiveness to macrophage tropic strains is sufficiently low, so that invasion of CXCR4 tropic mutants is possible, one has to distinguish between two situations (Fig. 6). If the immune responsiveness to both virus strains is not low enough, CXCR4 tropic strains may initially grow to a peak; this rise is only temporary, since the CTL response is still strong enough to suppress these mutants to very low levels, indicating extinction. If CXCR4 tropic strains

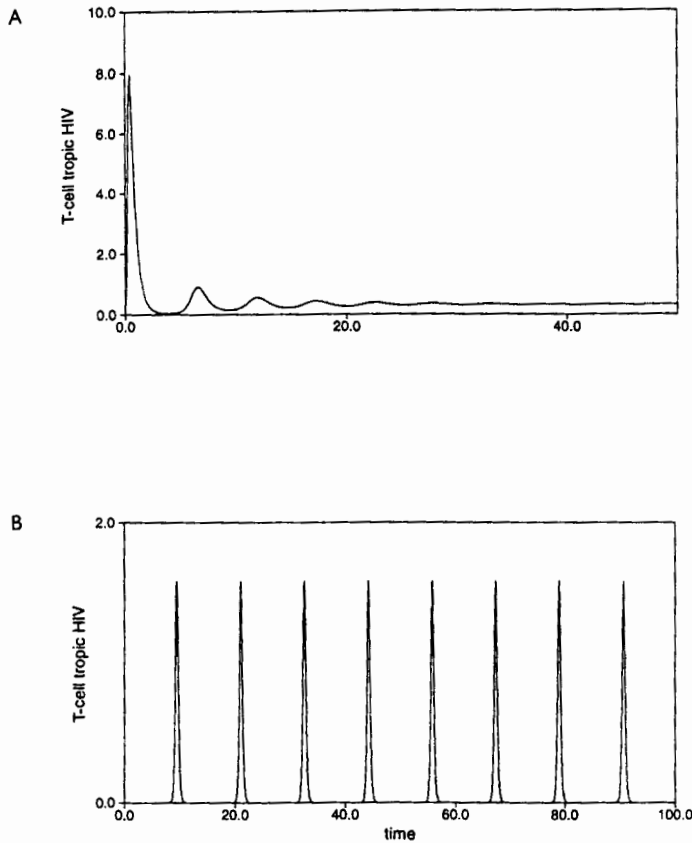


Fig. 6. Two types of dynamic behaviour can occur if CXCR4 tropic HIV has a positive initial growth rate. Either (A) stable equilibrium is reached by damped oscillations, or (B) stable limit cycles occur. The limit cycles are characterised by very low troughs. This indicates that, in the parameter space where limit cycles occur, the emergence of a CXCR4 tropic mutant leads to strong initial growth up to a peak. Subsequently, the immune response suppresses these mutants towards extinction. If CXCR4 tropic HIV is continuously generated, this would lead to the occurrence of 'blips' of such mutants, which rapidly go extinct.

are produced continuously, this may result in the appearance of 'blips' of CXCR4 tropic HIV, which immediately goes extinct (Fig. 6). Finally, if the immune responsiveness to both strains is reduced below a threshold, the immune system is not strong enough to suppress these HIV variants, leading to the permanent rise of CXCR4 tropic HIV (Fig. 6).

To summarise, these studies indicate that infection of macrophages may be essential for maintaining a persistent infection and for facilitating the eventual rise of CXCR4 tropic mutants once the immune system has been sufficiently weakened by the virus, e.g. due to antigenic variation. These results are in line with the observation that individuals with a deletion in the CCR5 co-receptor, making macrophage infection impossible, are unlikely to become infected by HIV (94–102).

R5 versus X4 viruses

While CCR5 tropic HIV persists throughout the infectious process, the virus specialises on the use of the CXCR4 receptor in

about 50% of the patients. This is associated with the syncytium-inducing phenotype and marks progression to the end stage of the disease. Mathematical models suggest a possible reason for the rise of such specialist HIV in only about half of the patients. Wodarz & Nowak (manuscript submitted) devised an evolutionary model describing the dynamics of cell tropism. They considered n virus strains that could infect two alternative target cell types with different efficiencies. The rate of target cell entry of the successive mutants was assigned in such a way that it increased monotonically in one cell type while it decreased monotonically in the alternative cell type (Fig. 7). As can be seen in Fig. 7, the change in the rate of target cell entry in the successive mutants can either be greater than linear or less than linear. In biological terms, a greater than linear change in the viral replication kinetics may occur if, for example, the accumulation of mutations leads to an increasing effect on viral replication. Similarly, a less than linear change may occur if the initial mutations have a relatively large effect on viral replica-

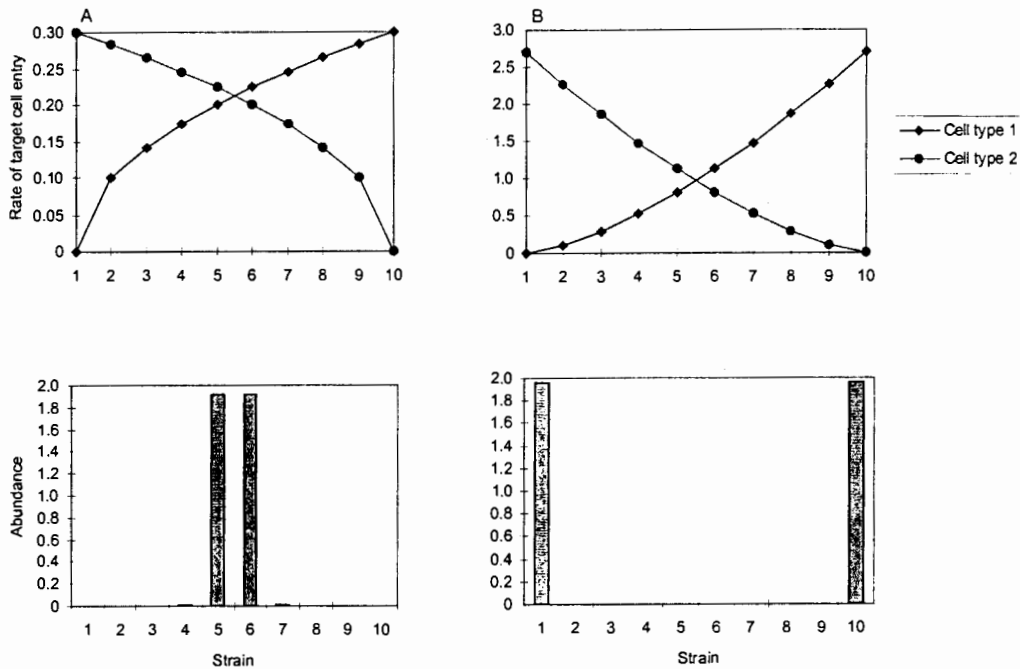


Fig. 7. Evolution of generalism versus specialism assuming the existence of two target cell types. We also assume that the viral replication kinetics increase monotonically from strain 1 to n in one cell type, while they decrease monotonically in the alternative cell type. The resulting fitness landscape of the virus mutants can be divided into two categories.

The change in the replication rate of the successive mutants may be (A) less than linear or (B) greater than linear. If the change is less than linear, the virus population evolves to utilise both cell types with similar efficiency (generalism), while a greater than linear change leads to the evolution of specialism.

tion, but subsequent mutations exert only a weaker effect. Wodarz & Nowak (manuscript submitted) found that the virus population will only specialise on both target cell types if this change is greater than linear; otherwise, generalism will evolve (Fig. 7).

The correlation between co-receptor usage and tropism for macrophages and T cells is complex. While macrophage infection is dependent on the CCR5 receptor, both the CCR5 and the CXCR4 receptors may promote T-cell infection. However, it has been reported that, in T cells, expression of the CCR5 and the CXCR4 receptors is subset dependent and tends to be mutually exclusive (103–106). Therefore, we can distinguish between two 'target cell types': those that are susceptible mainly to R5 strains and those that are susceptible mainly to X4 virus. Consequently, our model for the evolution of cell tropism may be applied, and it predicts that whether HIV evolves to specialise on the CXCR4 co-receptor in a given patient depends on the exact fitness landscape of the virus. Full X4 strains will evolve only if the fitness landscape of the virus is greater than linear,

otherwise R5X4 strains will evolve, using both co-receptors with similar efficiencies. The fitness landscape may differ between patients. This may simply be due to chance in the mutations occurring in the course of evolution of the virus. Moreover, a greater than linear change in the replication kinetics of the successive mutants may also be promoted by escape from mechanisms limiting viral replication.

Assuming that the fitness landscape of the viral mutants allows the evolution of specialism, Wodarz & Nowak (107) adopted this model specifically for HIV and investigated the evolution of X4 strains in the absence and presence of various immune responses. The fitness landscape of HIV mutants assumed in these studies is shown in Fig. 8. In accordance with empirical findings (e.g. (33, 34, 108)), we assume that CXCR4 tropic HIV may evolve to higher replication kinetics and at a faster rate than CCR5 tropic strains, and that the half-life of cells infected with X4 virus is shorter than that of cells infected with R5 strains. This fitness landscape leads to the dominance of CCR5 tropic HIV with CXCR4 tropic strains being suppressed

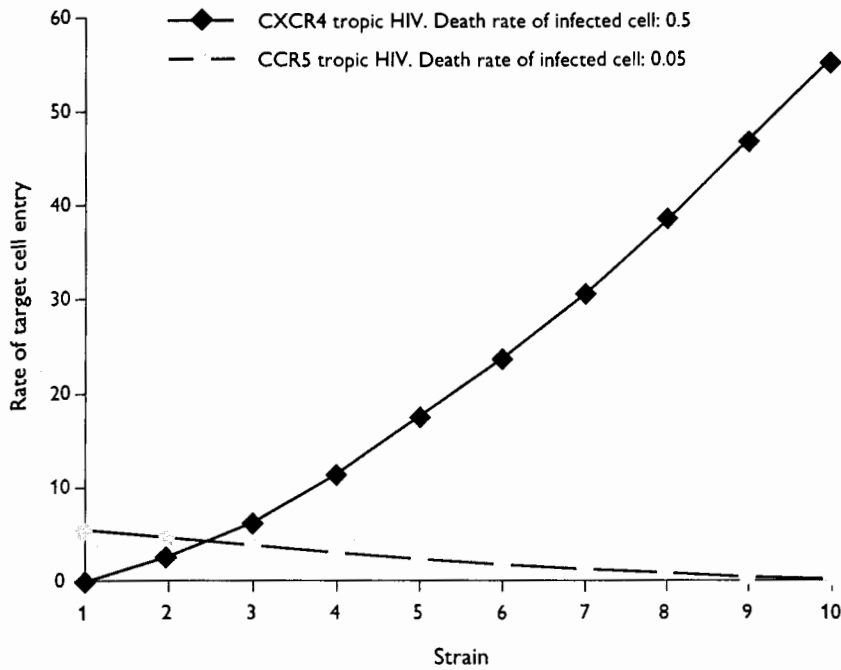


Fig. 8. Fitness landscape assumed for CCR5 tropic and CXCR4 tropic HIV. The change in the replication kinetics of the successive viral mutants is greater than linear. Therefore, the virus population may evolve towards specialism. According to empirical findings, we assume that X4 strains may evolve to faster replication kinetics and at a faster rate than R5 variants, and that cells infected with CXCR4 tropic mutants have a shorter half-life than those infected with CCR5 tropic virus.

to relatively low levels (Fig. 9A). We explored the effect of four types of immune responses on the course of evolution. These included an antibody response, CTL-mediated lysis, CTL-mediated inhibition of viral entry into target cells, and CTL-mediated inhibition of virion production. The evolutionary outcome of these models is shown in Figs 9B–9E. Clearly, different types of immune responses have different effects on the rise of CXCR4 tropic mutants. Immune responses acting on the virus before it has integrated into the host genome (antibodies and CTL-mediated inhibition of infection) select against the rise of CXCR4 tropic HIV, suppressing these strains to relatively low levels, while immune responses acting on infected cells (CTL-mediated lysis and inhibition of virion production) favour the rise of CXCR4 tropic strains, leading to the co-existence of R5 and X4 virus with similar abundances.

These results are the consequence of the finding that, without an immune response or under the pressure of immune responses acting on the virus before integration into the host genome, non-cytopathic viruses attain significantly higher lev-

els of virus load than cytopathic ones while this is not the case under immune responses acting on infected cells (109). Moreover, these studies have also shown that the viral replication kinetics do not significantly influence the equilibrium number of infected cells. Thus, while in certain circumstances non-cytopathic R5 variants may have a selective advantage over cytopathic X4 strains, this advantage is not cancelled out by the relatively slow replication kinetics of CCR5 tropic HIV.

Directly after infection, HIV can replicate freely before the rise of any immune responses. Since the models have demonstrated that such conditions select against the rise of virulent CXCR4 tropic mutants, these dynamics may also help us to understand why only non-virulent, slowly replicating R5 viruses are found at the beginning of HIV infection and why individuals carrying a deletion in the CCR5 receptor are unlikely to become successfully infected by HIV (94–102). Experimental studies investigating the rise of virulent X4 virus also support the result that different immune responses may exert different selection pressures on virulent CXCR4 tropic

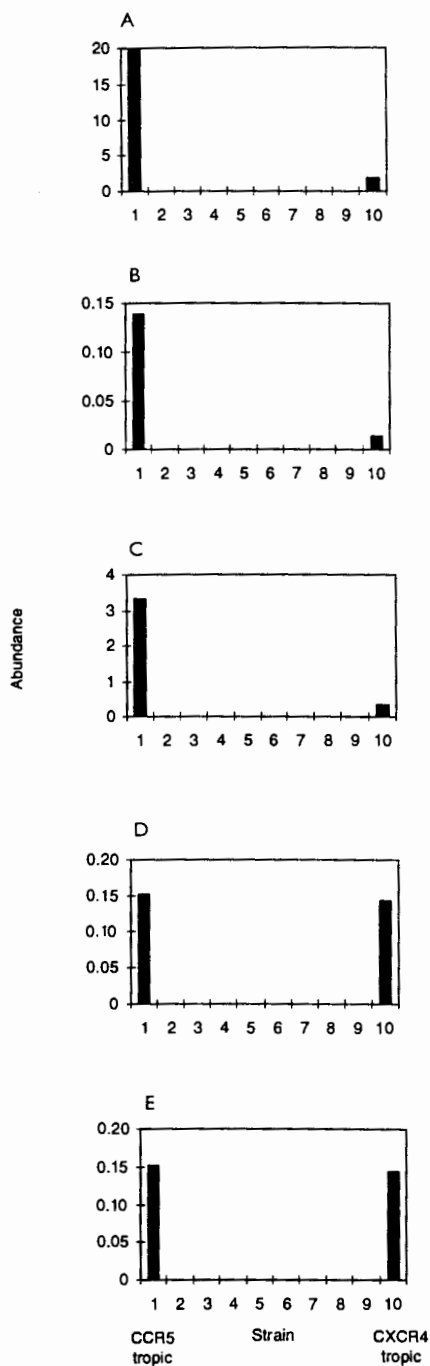


Fig. 9. Effect of different immune responses on the rise of virulent CXCR4 tropic HIV. **A.** No immune response. **B.** Antibody response. **C.** CTL-mediated inhibition of virus entry. **D.** CTL-mediated inhibition of virion production. **E.** CTL-mediated lysis. The graphs show that no immune response and immune responses acting on the virus before integration into the host genome (A & C) suppress virulent CXCR4 tropic strains to low levels. On the other hand, immune responses acting on infected cells (D & E) allow such strains to reach similar abundances compared to CCR5 tropic ones.

strains. Thus, it has been found that an efficient neutralising antibody response may lead to the suppression of X4 mutants, while evolution of viral resistance to neutralising antibodies correlates with the rise of virulent X4 strains (111–113).

It is interesting to consider CTL-mediated inhibition of viral entry in HIV infection. This is mainly due to the β -chemokines macrophage inflammatory proteins (MIP)1 α and 1 β or RANTES (114–116). They inhibit only the less cytopathic R5 strains while the more virulent X4 mutants are not affected by them (117). According to our models, more-cytopathic virus strains may have a significant selective disadvantage when susceptible to CTL-mediated inhibition of virus entry, while this is not the case for less-cytopathic strains. Therefore, the evolution of increased cytopathic properties of the virus may require the evolution of resistance to the CTL-secreted chemokines, and therefore the use of the CXCR4 co-receptor. This may help to interpret the observation that the syncytium-inducing phenotype is usually associated with the use of the CXCR4 co-receptor even if the syncytium-inducing phenotype may also potentially arise in strains using the CCR5 receptor (26).

Conclusion

This review has shown how mathematical models may help us understand the way in which viral evolution *in vivo* may contribute to the progression from the asymptomatic period of the infection to the development of AIDS. In particular, evolution towards increased viral diversity and replication may induce the destruction of the immune system, paving the way for the evolution of more pathogenic phenotypes that indicate progression to AIDS.

These findings also have implications for vaccination strategies to delay or prevent the onset of AIDS. Nowak & McLean (118) showed that the average number of escape mutants produced by a virus strain must be suppressed to below unity for the vaccine to be successful. This goal will only be achieved if the immune response against a sufficiently large number of strains is boosted, no matter how immunogenic the vaccine is. Therefore, the chances of successful vaccination will be maximised if a cross-reactive immune response is boosted (9, 118). In addition, because of the competition occurring between CTL against different epitopes, it would be advisable to boost the immune response against a single conserved epitope, even if this is not the immunodominant one, since this would lead to more stable and effective control of the virus population (47, 49).

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