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# The effect of different immune responses on the evolution of virulent CXCR4-tropic HIV

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We use mathematical models to determine possible mechanisms contributing to the evolution and rise of virulent CXCR4-tropic HIV *in vivo*. The models predict that the ability of the virus to specialize on a given target cell type depends on the exact fitness landscape of the viral mutants. Because this fitness landscape varies between people, this may explain why the evolution of fully CXCR4-tropic strains only occurs in about 50% of infected patients. Assuming that CXCR4-tropic HIV may evolve, we investigate the effect of different immune responses on the rise of such virulent strains. If we assume that CXCR4-tropic HIV is more cytopathic than CCR5-tropic virus, virulent CXCR4-tropic mutants remain suppressed at low levels both in the absence of an immune response, and in the presence of responses that act on the virus before integration into the host genome. On the other hand, this difference in cytopathogenicity is reduced by the presence of immune responses acting on infected cells, allowing CXCR4-tropic HIV to coexist with the CCR5-tropic virus. These results may help to interpret experimental data and are discussed with reference to the literature.

**Keywords:** cell tropism; coreceptors; cytopathogenicity; evolution; HIV; mathematical models

## 1. INTRODUCTION

Human immunodeficiency virus (HIV) is known to infect a variety of target cell types, mainly $CD4^+$ T helper cells and antigen-presenting cells such as dendritic cells and macrophages. More specifically, the tropism of HIV for macrophages compared with that for T cells is thought to be an important determinant influencing the course of disease progression (Crowe & Kornbluth 1994; Karlsson *et al*. 1994; Mosier & Sieburg 1994; Schuitemaker 1994; Crowe 1995; McKnight & Clapham 1995; Rudensey *et al*. 1995; Fauci 1996; Koot *et al*. 1996; Connor *et al*. 1997; Dittmar *et al*. 1997). At the beginning of the infection and during the asymptomatic phase, HIV is found to infect mainly macrophages and also primary T cells, and has a relatively slow rate of replication and a low degree of target-cell killing. These virus isolates tend to be of the non-syncytium-inducing (NSI) phenotype (Fenyo *et al*. 1988; Roos *et al*. 1992; Schuitemaker *et al*. 1992; Connor *et al*. 1993; Zhu *et al*. 1993; van't Wout *et al*. 1994; Rudensey *et al*. 1995). Since the use of the CCR5 chemokine receptor for cellular entry is a property of such virus strains, they have also been termed R5 viruses (Berger *et al*. 1998; Doms & Moore 1998). At a later stage in the disease process, at about 4–5 years after infection (Doms & Moore 1998), HIV evolves to show increased tropism for T cells as well as higher replication kinetics and virulence. This property is associated with the use of the CXCR4 receptor. In about 50% of the patients, the syncytium-inducing (SI) phenotype arises (Tersmette *et al*. 1988, 1989; Schuitemaker *et al*. 1992; Connor & Ho 1994; Rudensey *et al*. 1995; Fouchier *et al*. 1996). HIV may either use both the CCR5 and the CXCR4 receptor with similar efficiencies (called R5X4 virus) (Collman *et al*. 1992; Berger *et al*. 1998; Doms & Moore 1998), or use the CXCR4 receptor only, thus specializing on T cells and usually showing the SI phenotype (X4 viruses) (Berger *et al*. 1998; Doms & Moore 1998).

The reasons for the occurrence of SI strains in only about 50% of the patients and the selective forces contributing to the rise of virulent T-cell-tropic HIV are still not properly understood (Fauci 1996). In a previous paper (Wodarz & Nowak 1998*a*), we used mathematical models to identify factors necessary and sufficient for a virus population to specialize on two alternative target cell types. In terms of HIV, the full X4 phenotype may only evolve if these conditions are fulfilled. Assuming that this is the case, we expand on these studies to investigate how different kinds of immune responses influence the course of evolution *in vivo*. Assuming that CXCR4-tropic HIV is more cytopathic than CCR5-tropic virus, we find that CXCR4-tropic mutants remain suppressed at low levels both in the absence of an immune response, and in the presence of responses that act on the virus before integration into the host genome. On the other hand, immune responses acting on infected cells decrease this difference in cytopathogenicity, allowing X4 and R5 virus strains to coexist.

## 2. A MATHEMATICAL MODEL FOR THE EVOLUTION OF CELL TROPISM

Wodarz & Nowak (1998*a*) studied the interactions between $n$ virus strains and $m$ target cell types in an

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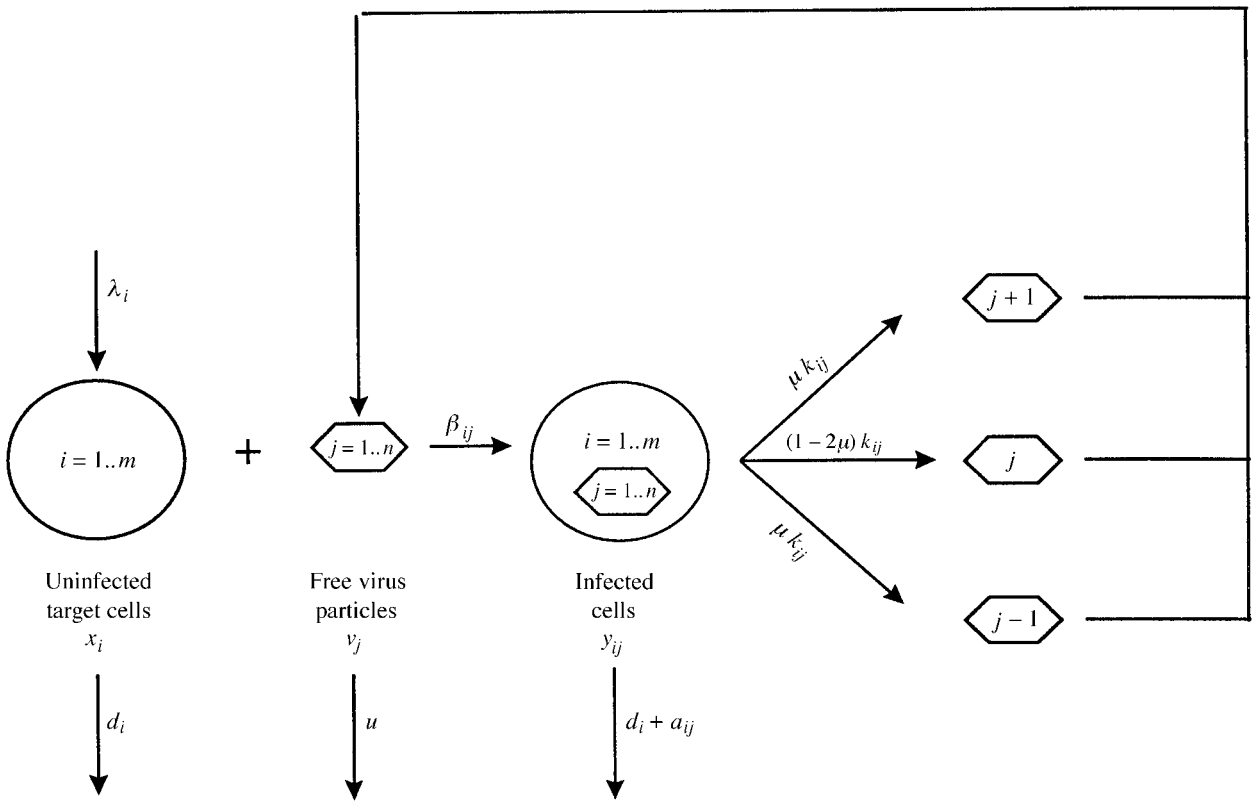


Figure 1. A mathematical model for the evolution of cell tropism. The model distinguishes between different types of target cells ($i=1 \ldots m$) and different virus strains ($j=1 \ldots n$). Uninfected target cells of type $i$ ($x_i$) are produced at a rate $\lambda_i$, die at a rate $d_i$, and become infected by a virus of strain $j$ ($v_j$) at a rate $\beta_{ij}$. Cells of type $i$ harbouring virus strain $j$ ($y_{ij}$), die at a rate $d_i + a_{ij}$, which is the sum of natural and virus-induced cell death. They produce free virus particles at a rate $k_{ij}$. During replication, virus of strain $j$ mutates to strains $j-1$ and $j+1$ at a rate $\mu$. Finally, free virus particles die at a rate $u$.

evolutionary context. Denoting uninfected target cells by $x_i$ ($i=1 \ldots m$), infected target cells by $y_{ij}$ ($j=1 \ldots n$), and free virus particles by $v_j$, the general model is given by

$$\dot{x}_i = \lambda_i - d_i x_i - x_i \sum_{j=1}^{n} \beta_{ij} v_j;$$

$$\dot{y}_{ij} = \beta_{ij} x_i v_j - (d_i + a_{ij}) y_{ij};$$

$$\dot{v}_j = (1-2\mu) \sum_{i=1}^{m} k_{ij} y_{ij} + \mu \sum_{i=1}^{m} k_{i,j-1}\, y_{i,j-1} + \mu \sum_{i=1}^{m} k_{i,j+1} y_{i,j+1} - u v_j.$$

The equations are explained graphically in figure 1. Uninfected target cells are produced at a rate $\lambda_i$, die at a rate $d_i$ and become infected by the different virus strains at a rate $\beta_{ij}$. Infected cells die at a rate $d_i + a_{ij}$ and produce new virus particles at a rate $k_{ij}$. During the process of virion production, a given virus strain $j$ mutates at a rate $\mu$ to strains $j-1$ and $j+1$. Finally, free virus particles decay at a rate $u$.

Assuming the existence of two target cell types, Wodarz & Nowak (1998*a*) analysed evolution in the rate of target-cell entry ($\beta_{ij}$). The values of this parameter for the different strains were assigned according to

$$\beta_{1j} = \beta_1 + f_1 (j-1)^r,$$

$$\beta_{2j} = \beta_2 + f_2 (n-j)^r,$$

where $\beta_1$ and $\beta_2$ denote the minimum rate of entry into the respective target cell types, $f_1$ and $f_2$ denote the rate of evolution in the two cell types, and $r$ denotes the pattern of change of $\beta_{ij}$ in the successive mutants. Thus, while the rate of target cell entry of a given virus strain increases in one cell type, it decreases in the alternative cell type (figure 2). A crucial parameter determining the behaviour of the model is $r$. In biological terms, this parameter represents the fitness landscape of the viral mutants based on their replication kinetics. If $r>1$, the change of the viral replication rate in the successive mutants is greater than linear (figure 2*b*). This may happen if, for example, the accumulation of mutations leads to an increasing effect on the viral replication kinetics. Under this condition, the virus population evolves to specialize on the two target cell types (figure 2*b*). That is, you observe the coexistence of two virus strains (strains 1 and $n$), one replicating mainly in the first cell type and the other replicating largely in the alternative cell type. On the other hand, $r<1$ (figure 2*a*) represents a less than linear change in the viral replication kinetics. This may occur if the initial mutations have a relatively large effect on viral replication, but subsequent mutations exert only a weaker effect. Under this condition, you observe the evolution of generalism, i.e. the coexistence of two virus strains that can grow in either of the two cell types with similar efficiencies. Therefore, whether the virus will evolve to

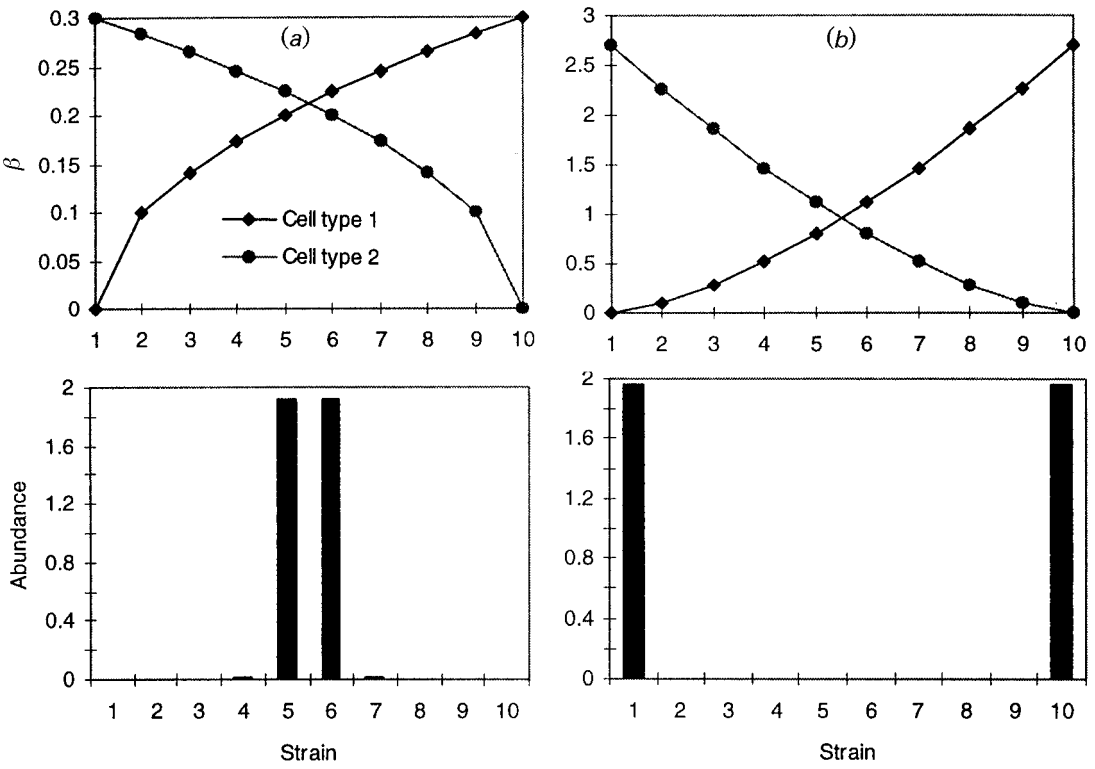


Figure 2. Evolution of generalism versus specialism assuming the existence of two target cell types. We also assume that the viral replication kinetics monotonically increase from strain 1 to $n$ in one cell type, while they monotonically decrease in the alternative cell type. The resulting fitness landscape 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 use both cell types with similar efficiency (generalism), whereas a greater than linear change leads to the evolution of specialism. Baseline parameters were chosen as follows: $\lambda_i=1$; $d_i=0.01$; $a_{ij}=0.5$; $k_{ij}=2$; $u=2$; $\mu=0.0001$; $\beta_1=0$; $\beta_2=0$; $f_1=0.1$; $f_2=0.1$; for (*a*) $r_1=r_2=0.5$; for (*b*) $r_1=r_2=1.5$.

specialize on a given target cell type depends on the exact fitness landscape of the viral mutants, determined by the value of $r$.

In the following section we will assume that the virus population is able to specialize on two target cell types ($r>1$). We will study this system in the context of CCR5 and CXCR4-tropic HIV and investigate how the course of evolution is influenced by various kinds of immune responses.

## 3. CCR5 VERSUS CXCR4 TROPISM IN HIV

The correlation between coreceptor usage and cell tropism is complex. Whereas macrophage infection is dependent on the CCR5 coreceptor, both the CCR5 and the CXCR4 coreceptor may promote T-cell infection. However, it has been reported that in T cells, expression of the CCR5 and CXCR4 receptors is subset-dependent and tends to be mutually exclusive (Bleul *et al.* 1997; Rowland-Jones & Tan 1997; Unutmaz & Littman 1997; Loetscher *et al.* 1998). Therefore, we can distinguish between two target cell types: those susceptible mainly to R5 strains and those susceptible mainly to X4 virus. In terms of the model described above, we denote cells susceptible to CXCR4-tropic strains as cell type 1 and cells susceptible to CCR5-tropic strains as cell type 2. We investigate the effect of two basic differences between R5 and X4 strains: they concern the replication rate of the virus and its virulence. CCR5-tropic HIV replicates relatively slowly and shows a low degree of cell killing whereas CXCR4-tropic mutants show faster replication kinetics and a higher degree of virulence. O'Brien (1994) reported that the difference in replication rates is partly due to macrophages slowing down the replicative process at the stage of reverse transcription. This may be due to the non-proliferative nature of this cell type, but other macrophage-dependent factors must also be involved, because addition of exogenous nucleotide precursors did not result in reverse transcription rates comparable to those observed in stimulated peripheral blood lymphocytes (O'Brien 1994). In our model, the rate of reverse transcription is included in the rate of target cell entry ($\beta_{ij}$) since it occurs before the production of new virions in the viral life cycle. Consequently, although we allow for evolution of this parameter in both virus strains, we assume that the rate of target cell entry may evolve to higher levels and at a faster rate for X4 compared with R5 variants ($f_1 \gg f_2$). The fitness landscape of HIV mutants assumed in our simulations is shown in figure 3.

We also take into account the observation that cells infected with R5 virus generally have a longer lifespan than cells infected with X4 virus (Rudensey *et al.* 1995; Fouchier *et al.* 1996). We make the simplest assumption that the half-life of infected cells ($a_{ij}$) is generally shorter for cells harbouring X4 rather than R5 strains (figure 3).

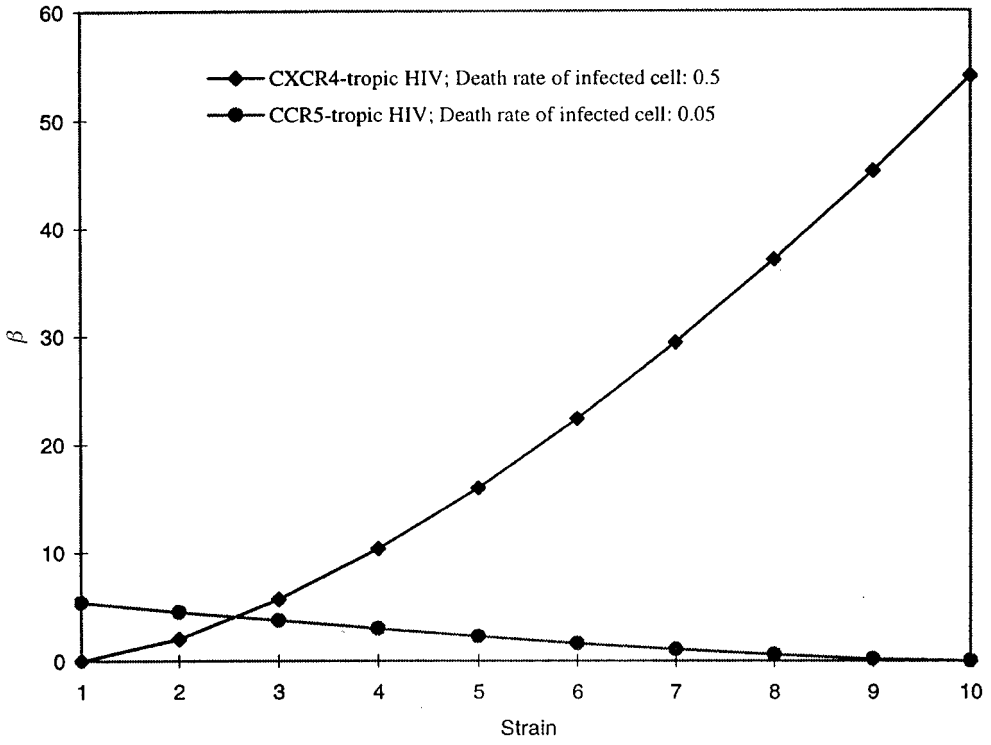


Figure 3. Fitness landscape assumed for CCR5- and CXCR4-tropic HIV. The change in the replication kinetics of the successive viral mutants is greater than linear. Therefore, the virus population may evolve towards specialism. According to empirical findings, we assume that X4 strains may evolve to faster 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. X4 strains are denoted by subscript 1 and R5 strains by subscript 2. The parameter values were chosen as follows: $\beta_1 = 0$; $\beta_2 = 0$; $f_1 = 2$; $f_2 = 0.2$; $r = 1.5$.

The simulation results of such a system are shown in figure 4*a*. Both CCR5- and CXCR4-tropic variants arise, but now R5 strains attain much higher abundances than X4 strains. Because our model includes only the dynamics between the virus population and target cells without the immune system being taken into consideration, this may correspond to the very early stage of HIV infection and could explain the fact that predominantly CCR5-tropic, slowly replicating and non-virulent virus strains are found at the beginning of the infectious process.

In the following, we incorporate four kinds of immune response into the model and explore how they might select for or against the rise of virulent and T-cell-tropic HIV variants. We model both CTL and antibody responses. With the CTL response, we distinguish between three general modes of action. First, CTLs may lyse infected cells. Alternatively, they may produce cytokines or chemokines inhibiting viral entry into infected cells. Finally, CTLs may release cytokines that inhibit the extent of virion production by infected cells. In a separate paper (Wodarz & Nowak 1998*b*), we explored the effect of these immune responses on viruses with different characteristics. The same functional responses that were used in these studies were incorporated into the basic tropism model and the equations for each immune response are set out in table 1. The results of simulations of each of the models are shown in figure 4*b*–*e*. It is clear that different kinds of immune response exert different selection pressures on virulent T-cell-tropic variants. Whereas an antibody response as well as CTL-mediated inhibition of virus entry selects against the rise of virulent X4 variants and leads to the dominance of CCR5-tropic strains, CTL-mediated lysis together with cytokines inhibiting virion production inside the host cell select for the rise of X4 variants. However, they do not simultaneously select against CCR5 tropism. R5-tropic variants will attain relatively high equilibrium levels with or without the presence of any immune response.

## 4. THE EFFECT OF IMMUNE RESPONSES ON VIRAL PHENOTYPE

The evolutionary dynamics of cell tropism studied in this paper offer insights into two basic questions concerning the pathogenesis of HIV infected patients: why virulent SI strains arise in only about 50% of the cases, and which factors might select in favour of these virulent strains if they arise.

We have shown that the ability of the virus to specialize on a given target cell type crucially depends on the exact fitness landscape of the viral mutants (Wodarz & Nowak 1998*a*). Specialization, and thus the evolution of full X4 strains, requires the replication kinetics of the virus to change in a greater than linear way in the successive mutants. On the other hand, if the change in the replication kinetics of the successive mutants is less than linear, the model predicts HIV to evolve towards an equally efficient use of both the CCR5 and the CXCR4 coreceptors. This corresponds to the R5X4 strains (Berger *et al.* 1998; Doms & Moore 1998). The fitness landscape of the virus

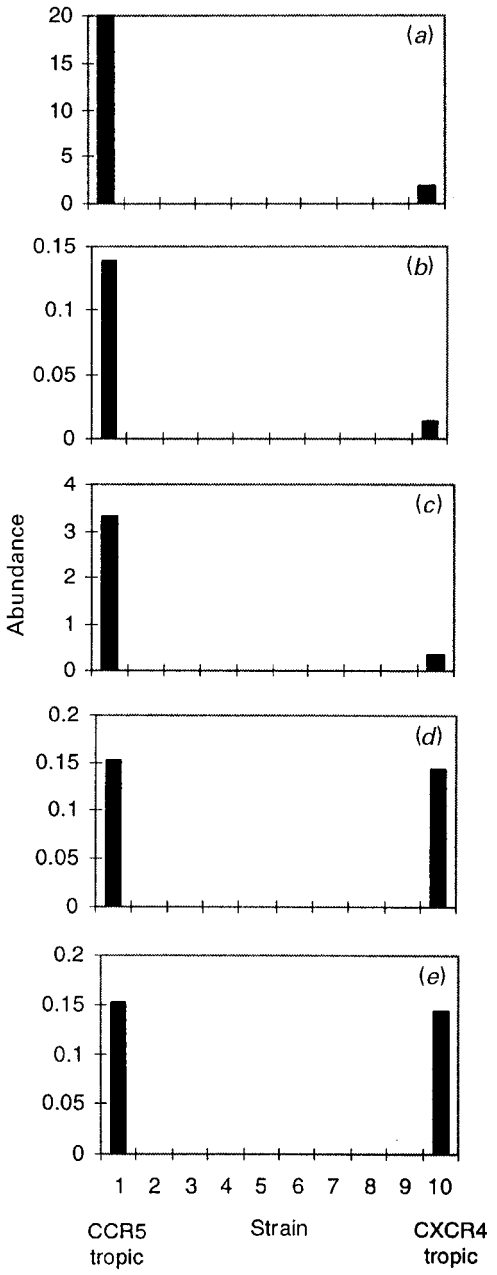


Figure 4. Effect of different immune responses on the rise of virulent CXCR4-tropic HIV. (*a*) No immune response; (*b*) antibody response; (*c*) CTL-mediated inhibition of virus entry; (*d*) CTL-mediated inhibition of virion production; (*e*) CTL-mediated lysis. The graphs show that no immune response as well as immune responses acting on the virus before integration into the host genome (*b*, *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 abundances similar to those of CCR5-tropic strains. These results are based on the models shown in table 1 and represent stable equilibria. Cells susceptible to X4 variants are denoted as cell type 1 and cells susceptible to R5 strains as cell type 2. The parameter values were chosen as follows: $\lambda_i = 1$; $d_i = 0.01$; $a_{ij} = 0.5$; $k_{ij} = 2$; $u = 2$; $\mu = 0.0001$; $\beta_1 = 0$; $\beta_2 = 0$; $f_1 = 2$; $f_2 = 0.2$; $r = 1.5$; $c_i = 2.5$; $b = 0.1$. For a lytic CTL response, the rate of virus inhibition $p = 1$. For soluble immune mediators, $p = 100$, because one immune cell can release many soluble mediators whereas it can only lyse one target cell at a time.

mutants may differ between HIV-infected patients. This may simply be due to chance in the mutations occurring and thus in the course of evolution taken by 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. Examples may be escape from interferon-mediated inhibition (Moskophidis *et al.* 1994; van den Broek *et al.* 1995*a*,*b*), antigenic variation (Nowak *et al.* 1991, 1995), TCR antagonism (Jameson *et al.* 1993; Bertoletti *et al.* 1994; Klenerman *et al.* 1994; Meier *et al.* 1994), or the occurrence of immune responses with inappropriate specificities (Jameson & Bevan 1995; Kalams & Walker 1995; Klenerman *et al.* 1995, 1996; McAdam *et al.* 1995; Borrow *et al.* 1997). A correlation between evolution of immune escape and tropism (McKnight & Clapham 1995) may further promote a greater than linear evolution of the viral replication rate. Such differences in the fitness landscape of HIV between patients may contribute to the fact that the emergence of X4 strains is only observed in about half of the patients, whereas in the other half the virus merely evolves towards the R5X4 phenotype (generalism). It would be interesting to design an experiment in which successive HIV isolates were tested *in vitro* for their ability to grow in $CXCR4^+$ and $CCR5^+$ cells and to see whether an increase in the replication rate in one cell type was accompanied by a decrease in the replication rate in the other cell type.

If the fitness landscape of the viral mutants is such that the evolution of specialization is possible, then the model suggests that different types of immune response may favour or act against the rise of virulent T-cell-tropic X4 viruses. Whereas no immune response and immune responses acting on free virus particles, such as antibody- and CTL-mediated inhibition of virus entry, may select against T-cell-tropic and virulent strains (X4 virus), immune pressures acting on infected cells, such as CTL-mediated lysis or inhibition of virion production, may favour the rise of virulent T-cell-tropic HIV. This follows directly from the finding that without an immune response or under the pressure of antibodies or CTL-mediated inhibition of virus entry, less cytopathic viruses attain significantly higher abundances than do cytopathic ones, whereas the fitness of cytopathic and non-cytopathic viruses is not significantly different under the pressure of CTL-mediated lysis of infected cells or inhibition of virion production inside infected cells (Wodarz & Nowak 1998*b*). Moreover, these studies have also demonstrated that although cytopathogenicity may be an important determinant of virus load, the replication kinetics of the virus do not significantly influence the equilibrium number of infected cells given that $R_0 \gg 1$. Thus, whereas a low degree of cytopathogenicity of R5 variants might give these virus strains a significant competitive advantage under certain immune responses, this is not cancelled out by the relatively slow replication kinetics of such HIV strains. Although this is a clear theoretical result, this issue has never been directly investigated by experiments. However, these ideas help to interpret some phenomena observed in HIV disease progression, and the literature also offers some support for this hypothesis.

Table 1. *Incorporation of different immune responses into the general model describing the evolution of cell tropism*

(The equations for the immune responses are based on Wodarz & Nowak (1998*b*). (*a*) Lytic CTL response. CTLs proliferate in response to a cell of type $i$ infected with virus strain $j$ at a rate $c_i$. Each virus strain is equally susceptible to the CTLs since we do not consider escape mutants. The CTLs lyse infected cells at a rate $p$ and die at a rate $b$. (*b*) CTL-mediated inhibition of virion production. We assume that CTLs secrete cytokines which inhibit virion production by infected cells at a rate $p$. Reduced levels of virion production result in reduced levels of antigen displayed on the surface of an infected cell. Therefore, the rate of CTL proliferation in response to antigen is also reduced by the cytokines at a rate $p$. Moreover, since production of new virus particles contributes to the cytopathic effect of the virus, the rate of virus-induced cell death is also reduced by cytokine action at a rate $p$. (*c*) CTL-mediated inhibition of virus entry. CTL proliferation is modelled in the same way as for the lytic CTL response. However, in this case, CTLs are assumed to secrete chemokines inhibiting the rate of virus entry into target cells at a rate $p$. (*d*) Antibody response. B cells are driven into proliferation by the interaction with T helper cells, which become activated by antigen-presenting cells (APCs). Because APCs take up free virus for display, we let expansion of the immune response be proportional to free virus rather than to infected cells. The antibody response kills free virus particles at a rate $p$.)

(*a*) CTL-mediated lysis

$$
\begin{aligned}
x_i &= \lambda_i - d_i x_i - x_i \sum_{j=1}^{n} \beta_{ij} v_j \\
y_{ij} &= \beta_{ij} x_i v_j - (d_i + a_{ij})\, y_{ij} - p y_{ij} z \\
v_j &= (1-2\mu) \sum_{i=1}^{m} k_{ij} y_{ij} + \mu \sum_{i=1}^{m} k_{i,\,j-1}\, y_{i,\,j-1} + \mu \sum_{i=1}^{m} k_{i,j+1}\, y_{i,\,j+1} - u v_j \\
z &= \frac{z}{\epsilon z + 1} \sum_{i=1}^{m} \left( c_i \sum_{j=1}^{n} y_{ij} \right) - bz
\end{aligned}
$$

(*b*) CTL-mediated inhibtion of virion production inside the cell via cytokines

$$
\begin{aligned}
x_i &= \lambda_i - d_i x_i - x_i \sum_{j=1}^{n} \beta_{ij} v_j \\
y_{ij} &= \beta_{ij} x_i v_j - \left( d_i + \frac{a_{ij}}{pz+1} \right) y_{ij} \\
v_j &= \frac{(1-2\mu) \sum_{i=1}^{m} k_{ij} y_{ij} + \mu \sum_{i=1}^{m} k_{i,j-1} y_{i,j-1} + \mu \sum_{i=1}^{m} k_{i,j+1} y_{i,j+1}}{pz+1} - u v_j \\
z &= \frac{z}{(\epsilon z + 1)(pz+1)} \sum_{i=1}^{m} \left( c_i \sum_{j=1}^{n} y_{ij} \right) - bz
\end{aligned}
$$

(*c*) CTL-mediated inhibition of virus entry into the cell via chemokines

$$
\begin{aligned}
x_i &= \lambda_i - d_i x_i - \frac{x_i \sum_{j=1}^{n} \beta_{ij} v_j}{pz+1} \\
y_{ij} &= \frac{\beta_{ij} x_i v_j}{pz+1} - (d_i + a_{ij})\, y_{ij} \\
v_j &= (1-2\mu) \sum_{i=1}^{m} k_{ij} y_{ij} + \mu \sum_{i=1}^{m} k_{i,\,j-1}\, y_{i,\,j-1} + \mu \sum_{i=1}^{m} k_{i,j+1}\, y_{i,\,j+1} - u v_j \\
z &= \frac{z}{\epsilon z + 1} \sum_{i=1}^{m} \left( c_i \sum_{j=1}^{n} y_{ij} \right) - bz
\end{aligned}
$$

(*d*) Antibody response

$$
\begin{aligned}
x_i &= \lambda_i - d_i x_i - x_i \sum_{j=1}^{n} \beta_{ij} v_j \\
y_{ij} &= \beta_{ij} x_i v_j - (d_i + a_{ij})\, y_{ij} \\
v_j &= (1-2\mu) \sum_{i=1}^{m} k_{ij}\, y_{ij} + \mu \sum_{i=1}^{m} k_{i,j-1}\, y_{i,j-1} + \mu \sum_{i=1}^{m} k_{i,j+1}\, y_{i,j+1} - u v_j - p v_j z \\
z &= \frac{cz \sum_{j=1}^{n} v_j}{\epsilon z + 1} - bz
\end{aligned}
$$

### (a) *Transmission*

At the beginning of HIV infection, after transmission, predominantly CCR5-tropic and NSI strains seem to be present in the body, with the sequence of the V3 region showing a strong degree of homology (Kuiken *et al.* 1992; McNearney *et al.* 1992; Zhang *et al.* 1993; Zhu *et al.* 1993; Connor & Ho 1994). Moreover, it has been reported that after sexual transmission of the virus, the most macrophage-tropic NSI variant transmitted emerged as the dominant strain in the recipient even if T-cell-tropic and SI strains were transmitted as well (Zhu *et al.* 1993; van't Wout *et al.* 1994). Various explanations have been put forward to account for these findings (Zhu *et al.* 1993; Schuitemaker 1994). The studies cited above argue against any mechanism of selection for macrophage-tropic variants in the donor. It has been suggested that in sexual or vertical transmission, macrophages are the first cells to be encountered by the virus in the mucosal or placental tissue, thus selecting for R5 strains. However, the same dominance of macrophage-tropic and NSI strains evolves if the virus is directly inoculated by injection of drugs or blood transfusion (van't Wout *et al.* 1994; Spijkerman *et al.* 1995). Along similar lines, Hirsch *et al.* (1999) demonstrated that even with intravenous infection of SIV into pig-tailed macaques, virus variants unable to infect macrophages (*vpx* mutants) were competitively inferior to the macrophage-tropic wild type.

The initial selection process seems to be independent of the pressure exerted by humoral or CTL responses: sequence uniformity for antibody as well as CTL epitopes at that time in the infectious process has been reported (Kuiken *et al.* 1992; Zhang *et al.* 1993; Zhu *et al.* 1993; Safrit *et al.* 1994). The mechanism underlying this selection has not yet been properly understood. However, our model offers a very simple explanation for the selective expansion of CCR5-tropic and NSI strains. As shown above, in the dynamic interactions between viruses and their target cells without an immune response being present, non-cytopathic viruses have a significant advantage over cytopathic ones. This advantage is due to the longer-lived target cell being able to produce many more virus particles during its lifespan than can the short-lived one. Thus, basic virus–immune system dynamics predict a selective expansion of CCR5-tropic NSI strains, although alternative mechanisms like the ones cited above almost certainly also play a role on top of these dynamics.

Along these lines, it also makes sense in terms of our theory that people who are homozygous for a deletion in CCR5 ($\Delta$CCR5) may only rarely allow HIV to establish a persistent infection (Dean *et al.* 1996; Dragic *et al.* 1996; Huang *et al.* 1996; Liu *et al.* 1996; Samson *et al.* 1996; Balotta *et al.* 1997; Biti *et al.* 1997; Rana *et al.* 1997; Theodorou *et al.* 1997). In these individuals, only T-cell-tropic phenotypes using the CXCR-4 receptor would be able to initiate an infection. As explained above, mathematical models predict that strongly cytopathic viruses will, at the very start of the infection, in the absence of any specific immune response, only attain relatively low abundances, thus facing a relatively high chance of going extinct or being eliminated by certain early unspecific immune responses. Moreover, in a previous paper (Wodarz & Nowak 1998*a*) we have shown that with the ability to infect fewer target cells, the basic reproductive ratio of the virus in the individual target cell types must be larger for the establishment of the infection to be successful.

### (b) *Immune responses*

Once the immune response rises, there is evidence that an efficient neutralizing antibody response contributes to the selection of CCR5-tropic and NSI strains rather than more virulent T-cell-tropic variants, supporting the results gained from our models. Thus, Tsang *et al.* (1994) followed the emergence of viral strains with different sensitivities to neutralizing antibodies in correlation with the viral phenotype. They found that at the early stage of the infectious process after seroconversion, viral variants were sensitive to neutralization by antibodies and were also macrophage-tropic and NSI. In contrast, viral variants later in the course of the infection were less sensitive to neutralizing antibodies, were less macrophage-tropic and tended to induce the formation of syncytia. Similar findings were obtained by Nkengasong *et al.* (1997), who followed disease progression in a married couple infected with HIV-1 group 0. A strong neutralizing antibody response corresponded with the suppression of HIV variants able to infect MT2 cells, whereas in the absence of an efficient neutralizing antibody response a switch from NSI to SI variants was observed.

It is now well established that, in the primary phase of HIV infection, the HIV-specific CTL response occurs at an earlier time point than does the rise of neutralizing antibodies (Koup *et al.* 1994; Connick *et al.* 1996). With this in mind it is interesting to consider findings by Cornelissen *et al.* (1995). They followed the sequential phenotypes of HIV variants as well as immunological measures in a patient who had received a deliberate intramuscular injection of a blood sample of an AIDS patient containing a mixture of NSI and SI strains. Before seroconversion, there was a temporary expansion of SI strains. At seroconversion, however, NSI strains took over as the dominant phenotype in the host. With our theory in mind, one could speculate that at the time of sampling when the SI phenotype expansion was observed, an HIV-specific CTL response had already arisen, which, through its lytic or cytokine action (CTL-secreted antiviral factor or CAF (Levy *et al.* 1996)), selected for a rise in SI variants before the emerging antibody response shifted the selection pressure against these strains. In addition, early unspecific immune responses acting on infected cells, such as IFN-$\gamma$ mediated inhibition of virion poduction or non-$CD8^+$ cell-mediated cytotoxicity (Connick *et al.* 1996) might have shifted the selective forces to a temporary advantage for SI variants. In a similar study, Lathey *et al.* (1997) analysed the role of neutralizing antibodies during primary infection in a patient infected by a needlestick injury. They found that the appearance of neutralizing antibodies resulted in the suppression of SI strains and the selection in favour of NSI strains, accompanied by reduced levels of virus load.

In addition to CTL-mediated lysis and secretion of cytokines inhibiting virion production by infected cells, it is interesting to consider CTL-mediated inhibition of viral entry into target cells. In HIV infection, this is mainly due to the $\beta$-chemokines macrophage inflammatory proteins MIP 1$\alpha$ and 1$\beta$ or RANTES (Cocchi *et al.* 1995; Zanussi *et al.* 1996; Gallo & Lusso 1997). They

inhibit only the less cytopathic R5 strains; the more virulent X4 mutants are not affected by these chemokines (Jansson *et al.* 1996). According to our models, more cytopathic virus strains may have a significant selective disadvantage when susceptible to CTL-mediated inhibition of virus entry, whereas this is not the case for less cytopathic strains. Therefore, the evolution of more cytopathic properties of the virus may require the evolution of resistance to the CTL-secreted chemokines, and therefore the use of the CXCR4 coreceptor. This may help to interpret the observation that the SI phenotype is usually associated with the use of the CXCR4 coreceptor even if the syncytium-inducing phenotype may also potentially arise in strains using the CCR5 receptor (Doms & Moore 1998).

To summarize, the overall selective advantage of HIV strains with different phenotypes will depend on a complex balance between the presence or absence of these different immune functions at any given point in time.

## 5. CONCLUSION

Past theoretical work on HIV has identified evolutionary dynamics of the virus as a key process responsible for the pattern of disease progression observed in HIV-infected patients (Nowak *et al.* 1991, 1995; DeBoer & Boerlijst 1994). The present paper extends this concept by showing that evolution of the viral replication kinetics may be a common thread in HIV disease progression, responsible for successively weakening the immune system and helping us to understand some of the key characteristics of the disease process from primary acute infection, through the asymptomatic period, to the development of full-blown AIDS. The overall replication kinetics of the virus is the product of several processes, the most important in the present context being the rate of virus entry into the target cell.

Directly after transmission, the simple dynamics between the virus strains and their target cells selects for CCR5-tropic variants that are relatively slowly replicating and not very cytopathic. Efficient immune responses inhibiting the virus before it has integrated into the genome of its host cells, such as a neutralizing antibody response, maintain this selection pressure in favour of less cytopathic R5 variants. However, evolution of resistance to the immune responses selecting in favour of R5 variants may shift the balance of selective forces towards a similar fitness of cytopathic and non-cytopathic virus strains, thus paving the way for the rise of virulent (SI) and fast-replicating T-cell-tropic mutants (X4 virus). The syncytium-inducing phenotype of the virus may then further suppress the overall function of the immune system, while viral evolution towards increased replication kinetics in T helper cells may specifically exhaust the CTL response (Wodarz *et al.* 1998), shifting the balance between HIV and the immune system further towards full-blown AIDS.

These theoretical studies also highlight the different roles of the various branches of the immune system in fighting progression to AIDS. The CTL response has often been considered to be the most important branch of the immune system specifically inhibiting HIV spread *in vivo* (Borrow *et al.* 1994; Koup *et al.* 1994; Klenerman *et al.* 1996). Although the CTL response is certainly of major importance for limiting virus load, we have shown that other branches of the immune system might be equally essential for delaying the onset of AIDS by various mechanisms. Thus, an efficient neutralizing antibody response may significantly contribute to the maintenance of non-virulent R5 strains, and immune functions limiting the overall replication kinetics of the virus in general may be vital for preventing the exhaustion of the HIV-specific CTL response (Wodarz *et al.* 1998).

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