Dynamics of Macrophage and T Cell Infection by HIV

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We analyse mathematical models comparing the *in vivo* dynamics of macrophage- and T cell infection by HIV. Experiments suggest that HIV can only replicate in activated T cells whereas cell activation may not be required for successful replication in macrophages. These assumptions lead to fundamentally different conditions required to establish a persistent infection in the two cell types. While persistent replication in macrophages is achieved if the basic reproductive ratio of the virus, R_0, exceeds unity, the establishment of T cell infection may depend on a complex balance between host and viral parameters as well as initial conditions. More specifically, the replication rate of HIV needs to lie above a threshold level and the immune responsiveness of the host below a certain threshold for persistent T cell infection to be possible. In addition, initial virus load has to be intermediate and the initial abundance of CTLs low. Mathematical models predict that macrophage infection may be essential for the successful establishment of HIV in the primary phase of the infection. Acting as a reservoir, they allow the virus to evolve towards increased replication kinetics as well as away from immune recognition, thus paving the way for the rise of exclusively T cell tropic strains using the CXCR4-coreceptor.

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1. Introduction

During the course of the disease HIV may infect a variety of cell types. However, the tropism of HIV for macrophages and T cells is thought to be particularly important for disease progression (Schuitemaker, 1994; Berger *et al.*, 1998). Tropism of HIV is mainly determined at the level of cellular entry through membrane fusion with $CD4^+$ cells. However, successful entry also requires the presence of the appropriate coreceptors. Different HIV strains may use different coreceptors for cellular entry and this determines which target cell types the virus may enter. More specifically, two coreceptors are considered to be important factors governing the entry of HIV into macrophages and T cells. The CCR5 coreceptor is present both on macrophages and T cells, while the CXCR4 coreceptor is present on T cells only.

In the initial phases of HIV infection virus isolates tend to use the CCR5 coreceptor. Therefore, they can infect macrophages and primary T cells and have been termed R5 strains (Berger *et al.*, 1998; Doms & Moore, 1998). Such isolates are characterised by a slow rate of replication, are relatively acytopathic and tend to show the non-syncytium inducing (NSI) pheno-

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type (Schuitemaker *et al.*, 1992; van't Wout *et al.*, 1994; Rudensey *et al.*, 1995; Fouchier *et al.*, 1996; Berger *et al.*, 1998; Doms & Moore, 1998). Later in the course of the infection, HIV evolves to use the CXCR4 receptor. The virus may either use both the CCR5 and the CXCR4 coreceptors (R5X4 strains, Berger *et al.*, 1998; Doms & Moore, 1998), or in about 50% of the patients, HIV evolves to use the CXCR4 coreceptor only. Such specialist strains have been termed X4 viruses (Berger *et al.*, 1998; Doms & Moore, 1998). They are characterised by faster rates of replication, higher degrees of cell killing and tend to show the syncytium inducing (SI) phenotype, indicating progression to full-blown AIDS (Schuitemaker *et al.*, 1992; van't Wout *et al.*, 1994; Rudensey *et al.*, 1995; Fouchier *et al.*, 1996; Berger *et al.*, 1998; Doms & Moore, 1998).

The factors contributing to the eventual emergence of X4-tropic strains are not yet properly understood. Experimental studies (Zhu *et al.*, 1993; van't Wout *et al.*, 1994) have shown that macrophage tropic NSI strains initially evolve to dominate the virus population even if CXCR4-tropic SI strains are transmitted as well, thus arguing in favour of selection within the recipient rather than the donor or during transmission. The absence of T cells in the placental and mucosal tissues initially encountered by HIV may contribute to this selection, although macrophage tropic HIV also evolves to dominate the population if the virus is inoculated directly via drug injection or blood transfusion (Schuitemaker, 1994). Theoretical studies (Wodarz & Nowak, 1998) have shown how the presence or absence of various types of immune responses may select for or against the rise of CXCR4-tropic mutants, due to the difference in virulence of HIV in the two cell types.

In the present paper we focus on another difference between macrophage and T cell infection. While HIV can only replicate in activated T cells, activation and cell division is not required for macrophage infection (Stevenson & Gendelman, 1994; Stevenson, 1996). The reason for this difference rests in the mechanism underlying the transport of the HIV genome into the nucleus of the two cell types (Stevenson & Gendelman, 1994; Emerman *et al.*, 1994; Heinzinger *et al.*, 1994; Stevenson *et al.*, 1994,

1995; Bukrinskaya *et al.*, 1996; Stevenson, 1996). In contrast to onco-retroviruses such as murine leukaemia virus (Springett *et al.*, 1989; Miller *et al.*, 1990; Roe *et al.*, 1993; Lewis & Emerman, 1994), lentiviruses have evolved a mechanism circumventing the need of mitosis for the localisation of viral DNA into the nucleus of macrophages (Weinberg *et al.*, 1991). It has been shown that ATP is required for the HIV pre-integration complex to reach the nucleus, indicating facilitated active transport (Bukrinsky *et al.*, 1992; Lewis *et al.*, 1992). Among other HIV genes, *gag* and *vpr* seem to be required for successful infection (Stevenson, 1996). In contrast, this process does not work in quiescent T cells due to the presence of fewer nuclear pores and less efficient active transport (Bukrinsky *et al.*, 1991; Feldherr & Akin, 1993).

We show that this difference leads to the result that persistent macrophage infection is always possible given that the basic reproductive ratio of the virus in this cell type is greater than unity, while the rise of CXCR4-tropic mutants may require the replication rate of these strains to be above a certain threshold level as well as the effectiveness of the immune response to be below a certain threshold level.

2. Dynamics of Macrophage Tropic HIV

2.1. THE BASIC MODEL

As a first approximation, the dynamics between HIV and the macrophage population can be described by the simplest model of infection dynamics (Anderson & May, 1979, 1991; Nowak & Bangham, 1996; DeBoer & Perelson, 1998). Denoting uninfected macrophages by x and infected macrophages by y, and assuming that viruses are transmitted mainly by cell to cell contact, this model is given by:

$$x = \lambda - dx - \beta xy \qquad (1)$$
$$y = \beta xy - ay$$

Thus, uninfected macrophages are produced at a constant rate λ, die at a rate d and become infected at a rate β. Infected cells die at a rate a. The basic reproductive ratio of the virus is given by $R_0 = \lambda\beta/da$. If there is no infection or if

$R_0 < 1$, the trivial equilibrium will be attained given by (E_1) $x^{(1)} = \lambda/d$, $y^{(1)} = 0$. On the other hand, if $R_0 > 1$, the virus can establish an infection, and the system converges to the equilibrium (E_2) $x^{(2)} = a/\beta$, $y^{(2)} = \lambda/a - d/\beta$. Note that $R_0 > 1$ is equivalent to $y^{(2)} > 0$.

2.2. MODELLING A CTL RESPONSE

An equation for a CTL response can be added to the interaction between HIV and macrophages as proposed by Nowak & Bangham (1996). Denoting CTLs by z, the equations are given by

$$x = \lambda - dx - \beta xy$$

$$y = \beta xy - ay - pyz \qquad (2)$$

$$z = cyz - bz$$

Thus, CTLs proliferate in response to antigen at a rate c, die at a rate b and lyse infected macrophages at a rate p. If $R_0 < 1$, the trivial equilibrium (E_1) is attained. If $R_0 > 1$, an infection is established limited either by target cell availability alone or by a combination of target cell availability and the CTL response. If $c\ y^{(2)} < b$, levels of antigen are too low to stimulate a CTL response leading to equilibrium (E_2). On the other hand, if $c\ y^{(2)} > b$, the system approaches the equilibrium (E_3)

$$x^{(3)} = \frac{\lambda c}{dc + b\beta},$$

$$y^{(3)} = \frac{b}{c},$$

$$z^{(3)} = \frac{c(\beta\lambda - ad) - ab\beta}{p(dc + b\beta)}$$

3. Dynamics of T Cell Tropic HIV

3.1. THE BASIC MODEL

The dynamics between CD4$^+$ T cells and HIV are more complicated. We assume that HIV can only infect activated CD4$^+$ T cells. Thus, we devise a model including three variables: resting CD4$^+$ T cells (w), uninfected activated CD4$^+$ T cells (x) and infected CD4$^+$ T cells (y). Building on previous models (McLean & Kirkwood, 1990;

McLean, 1992; Essunger & Perelson, 1994; DeBoer & Perelson, 1998), it is described by the following set of differential equations.

$$w = \xi - fw - rwy - sw$$

$$x = sw + rwy - dx - \beta xy \qquad (3)$$

$$y = \beta xy - ay$$

Resting T helper cells are produced at a rate ξ, die at a rate f and become activated upon contact with antigen at a rate r. In addition, there is a constant rate of back-ground activation (s) due to e.g. antigen persistence at very low levels after clearance of another infection. Activated CD4$^+$ T cells die at a rate d and become infected upon contact with virus at a rate β. Finally, infected cells die at a rate a.

In this case, the basic reproductive ratio of HIV may be defined as $R_0 = \beta s\xi/[ad(f + s)]$. If $R_0 > 1$, the virus establishes a persistent infection. This state will be promoted by a relatively high rate of background activation of T cells (s), induced for example by other infectious agents being present in the host at the time of HIV infection. However, if $R_0 < 1$, initial conditions may determine whether the virus is successful in establishing a persistent infection. This is fundamentally different compared with macrophage infection and it is this case that we will focus on in further analysis of the model. Since the background rate of T cell activation is likely to be relatively low compared with T cell activation in response to HIV, we will demonstrate these dynamics for the limiting case $s = 0$. We will therefore concentrate on the following set of equations:

$$w = \xi - fw - rwy$$

$$x = rwy - dx - \beta xy \qquad (4)$$

$$y = \beta xy - ay$$

The trivial equilibrium is described by (E_1): $w^{(1)} = \xi/f$, $x^{(1)} = 0$, $y^{(1)} = 0$. On the other hand, the virus population may successfully establish an infection resulting in the coexistence of both activated and resting T helper cells. This is described by equilibrium (E_2)

$$x^{(2)} = \frac{a}{\beta}, \quad w^{(2)} = \frac{a(d + \beta y^{(2)})}{r\beta y^{(2)}},$$

where $y^{(2)}$ is given by the solution of a quadratic equation.

$$y^{(2)} = \frac{\beta(\xi r - fa) - ard + \sqrt{[\beta(\xi r - fa) - ard]^2 - 4a^2fdr\beta}}{2ar\beta}.$$

Even in this limiting case, where $R_0 = 0$, the virus may still establish a persistent infection, since permissive target cells are created through activation, which is induced by the virus itself.

The trivial equilibrium (E_1) is always stable. The internal equilibrium (E_2) is stable if

$$y^{(2)} > a \frac{a\beta d - f^2\beta + fdr}{r^2(\xi\beta - ad) + r\beta^2\xi + fra\beta - a^2d\beta}$$

Within the region of bistability, it depends on the initial conditions whether the virus population is able to establish an infection (Figs 1 and 2). If the initial dose of HIV is relatively low, not enough CD4$^+$ T cells become activated by the virus for persistent replication to be possible. In contrast, if the initial dose of HIV is relatively high, a sufficient number of CD4$^+$ T cells is activated so
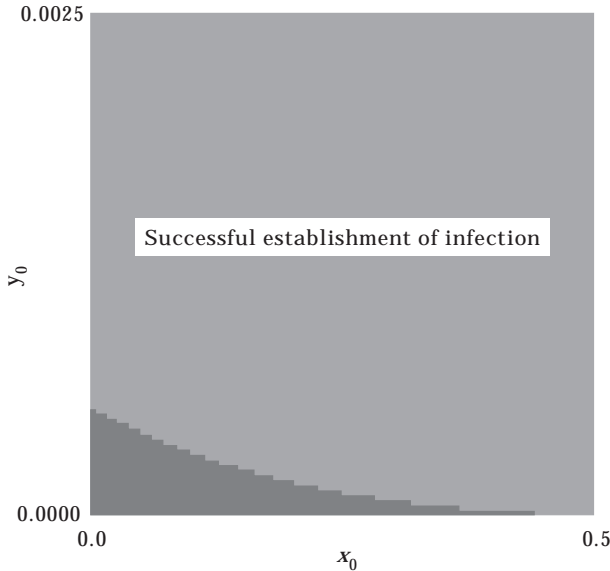

Fig. 1. Dependence of the outcome of T helper cell infection on the initial conditions without the presence of a CTL response [eqn (4)]. In the parameter space where both the virus extinction and persistence equilibria are stable, successful establishment of an infection requires the initial virus load (y_0) to be above a certain threshold in order to activate a sufficient number of target cells for persistent replication to be possible. The higher the initial number of activated target cells (x_0) the lower this threshold for initial virus load. Parameter values were chosen as follows: $\xi = 1$; $f = 0.01$; $r = 2$; $d = 0.1$; $\beta = 2$; $a = 0.5$.
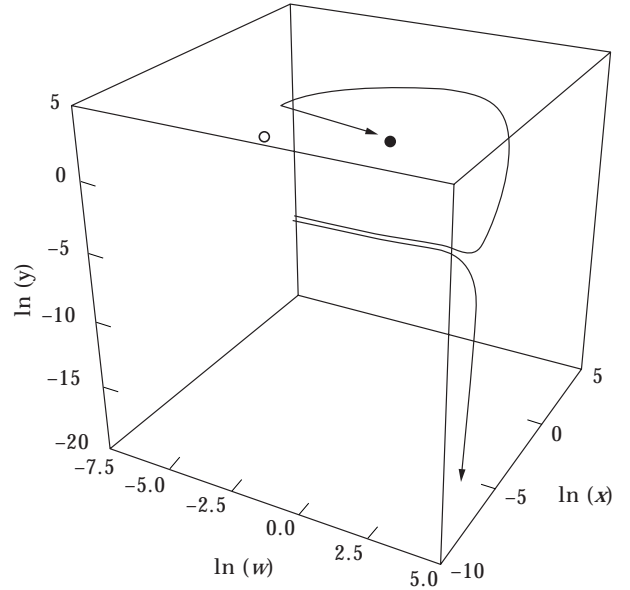

Fig. 2. Phase space plot showing how the dynamics of T cell infection depend on the initial conditions [eqn (4)]. ($\bullet$) represents a stable internal equilibrium while ($\circ$) indicates the position of an unstable internal equilibrium. The trajectories start close to the separatrix. The system moves to the virus persistence equilibrium if the initial virus load lies above a threshold, while an initial viral abundance below this threshold results in the extinction of the virus population. Parameters are the same as in Fig. 1.

that the virus may establish a persistent infection. More generally, the minimal initial virus load required to establish a persistent infection declines with increasing numbers of activated T helper cells initially present (Fig. 1).

3.2. THE EFFECT OF A CTL RESPONSE

The above model can be extended to include a lytic CTL response as in the previous case. It is given by

$$\begin{aligned} w &= \xi - fw - rwy \\ x &= rwy - dx - \beta xy \\ y &= \beta xy - ay - pyz \\ z &= cyz - bz \end{aligned} \qquad (5)$$

This system is characterised by three equilibria. In the trivial case, the virus is not able to establish an infection (E_1). If the virus does establish an infection, then virus growth is limited by target cell availability only (E_2), or virus growth is limited by a combination of

target cell availability and the CTL response. The latter outcome is described by the following equilibrium expressions (E_3).

$$w^{(3)} = \frac{\xi c}{fc + rb}, \; x^{(3)} = \frac{r\xi cb}{c[d(cf + rb) + \beta bf] + \beta b^2 r},$$

$$y^{(3)} = \frac{b}{c}$$

$$z^{(3)} = \frac{c[\beta b(r\xi - af) - ad(cf + rb)] - a\beta b^2 r}{p[dc(cf + rb) + \beta b(fc + rb)]}$$

Assuming that an infection may successfully be established without the presence of a CTL response, Fig. 3 shows how the stability property of equilibrium (E_3) depends on the immune responsiveness of the host (c). The graph plots the dependence of the real part of the dominant eigenvalue of the jacobian matrix of equilibrium (E_3) on c. The equilibrium is stable if the
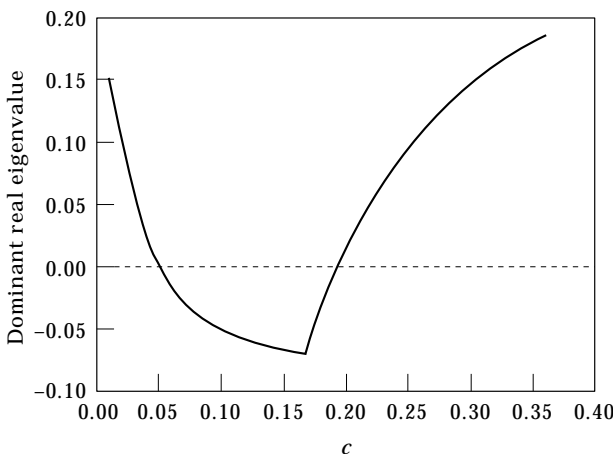

FIG. 3. Dependence of the stability of virus persistence controlled by CTLs on the immune responsiveness (c) of the host in the dynamics between HIV, T helper cells and a CTL response [eqn (5)]. The graph shows the dependence of the dominant real eigenvalue of the equilibrium describing virus persistence controlled by CTLs on the parameter c. The equilibrium is stable if the eigenvalue is negative, while a positive eigenvalue indicates instability. If the immune responsiveness of the host is too low, a CTL response cannot be established resulting in virus limitation by target cell availability only. If the immune responsiveness of the host lies above a threshold value, the CTL response efficiently reduces levels of virus load to very low levels. This in turn leads to a decrease in the number of activated target cells making it impossible for the virus to persist in the host. Successful establishment of persistent infection controlled by CTLs requires the immune responsiveness to be intermediate. Parameter values were chosen as follows: $\xi = 1$; $f = 0.01$; $r = 2$; $d = 0.1$; $\beta = 2$; $a = 0.5$; $b = 0.1$; $p = 1$.

eigenvalue is negative, whereas a positive eigenvalue indicates instability. If the immune responsiveness is too low, i.e. if $c \, y^{(2)} < b$, the population of CTLs will not be able to invade leading to virus infection limited by target cell availability only (E_2). On the other hand, if the immune responsiveness of the host is above a certain threshold level, equilibrium (E_3) is again unstable, but this time, the system moves to the disease-free equilibrium (E_1). The reason for this is that a relatively strong CTL response will depress levels of virus load to very low levels which are not sufficient to maintain the population of activated T helper cells. Since these are the targets of the virus, the infection will vanish. For intermediate values of c, equilibrium (E_3) is locally stable (Fig. 3). Now, it depends on the initial conditions whether an infection, controlled by a combination of target cell availability and the immune response, can be established (E_3), or whether the infection is cleared (E_1). Figure 4 shows the outcome of the model when starting with a wide range of different values for y_0 and z_0. Similarly to the model without a CTL response, virus load must lie above a 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 is very high, the CTL response can grow fast enough to quickly reduce the virus population 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 goes extinct. On the other hand, intermediate initial values of virus load allow virus replication to outrun the CTL response which can only grow at slower rate. Thus, persistent replication, controlled by the immune response, can be established. The higher the initial abundance of CTLs, the lower the threshold level of virus load required to achieve clearance of the infection. If initial levels of CTLs lie above a certain threshold level, the establishment of persistent infection becomes impossible. More generally, Fig. 4 shows that starting with different levels of activated target

(a) $x_0 = 0$

Virus extinction

Infection

$\log z_0$

-5

-20

-6 3

(b) $x_0 = 0.175$

Virus extinction
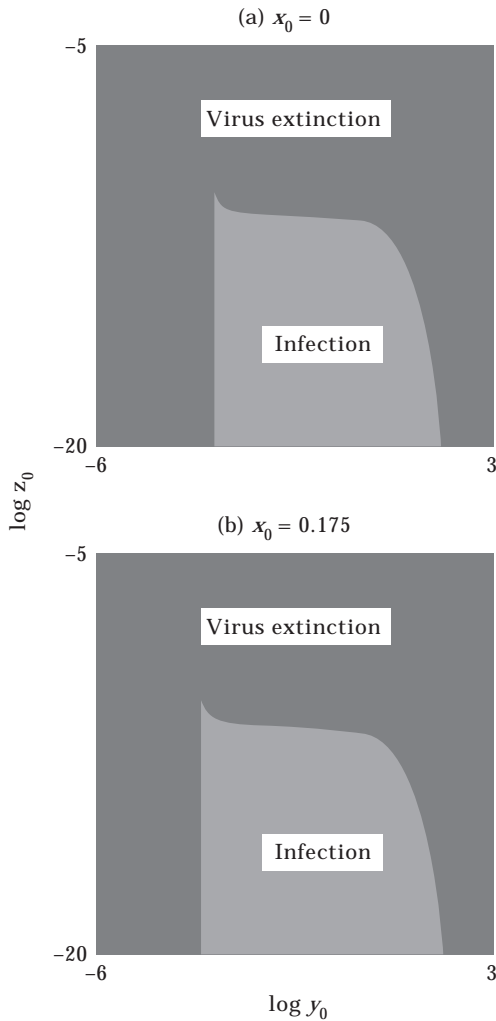
Infection

-5

-20

-6 3

$\log y_0$

FIG. 4. Dependence of the outcome of T helper cell infection on the initial conditions in the presence of a CTL response [eqn (5)]. As was the case without the presence of a CTL response, 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 also goes extinct if virus load lies above a certain threshold. This is because high initial virus load increases the initial growth rate of the CTL response, which in turn quickly suppresses virus load. This reduces the number of activated target cells below the levels required for persistent replication. Therefore, initial virus load needs to be intermediate for establishment of persistent infection to be successful. The initial number of CTLs (z_0) also plays an important role. The higher the initial levels of CTLs, the lower the upper virus load threshold leading to virus extinction. Above a certain initial abundance of CTLs, the establishment of persistent infection becomes impossible. If the initial numbers of activated target cells (x_0) is increased, the same general pattern holds. Only the exact parameter range over which persistent infection is possible becomes broader. Parameter values were chosen as follows: $\xi = 1$; $f = 0.01$; $r = 2$; $d = 0.1$; $\beta = 2$; $a = 0.5$; $c = 0.15$; $b = 0.1$; $p = 1$.

cells leads to similar results, the difference being in the initial loads required to maintain a persistent infection.

In addition, for the more general case $R_0 < 1$ and $s \neq 0$ the results remain qualitatively unchanged. Only the exact initial conditions allowing persistent infection will differ. The higher the basic reproductive ratio of the virus, the less stringent the initial conditions required for successful T cell infection.

4. Dynamics of Tropism of HIV for Macrophages and CD4$^+$ T cells

4.1. THE BASIC MODEL

The previous section has demonstrated that successful establishment of T cell infection may be difficult to achieve, depending on the fine balance between host and viral parameters as well as initial conditions. In this section we bring together the above models to investigate how the presence of strains able to infect macrophages may be vital for maintaining persistent HIV infection and how they may facilitate the rise of CXCR4-tropic mutants capable of infecting T cells only. Although CCR5-tropic HIV may infect both macrophages and primary T cells, we will consider a simplified model including two virus populations: one infecting macrophages, the other infecting T helper cells. This is justified since the basic difference between the two strains is that CCR5-tropic HIV may infect macrophages, whereas CXCR4-tropic HIV may not. The two virus populations are coupled through immune activation, i.e. CD4$^+$ T cells are activated in response to both macrophage tropic and CXCR4-tropic strains. As before, we assume $s = 0$. Denoting uninfected and infected macrophages by x_1 and y_1, resting T helper cells by w, and uninfected and infected activated T helper cells by x_2 and y_2, these assumptions lead to the following set of differential equations.

$$x_1 = \lambda - d_1 x_1 - \beta_1 x_1 y_1$$

$$y_1 = \beta_1 x_1 y_1 - a_1 y_1$$

$$w = \xi - fw - rw(y_1 + y_2) \qquad (6)$$

$$x_2 = rw(y_1 + y_2) - d_2 x_2 - \beta_2 x_2 y_2$$

$$y_2 = \beta_2 x_2 y_2 - a_2 y_2$$

This system is characterised by four equilibria. The disease-free equilibrium is given by (E_1) $x_1^{(1)} = \lambda/d_1$, $y_1^{(1)} = 0$, $w^{(1)} = \xi/f$, $x_2^{(1)} = 0$, $y_2^{(1)} = 0$.

Alternatively, only one of the virus strains may survive. If only macrophage tropic virus survives, the equilibrium is given by (E_2)

$$x_1^{(2)} = a_1/\beta_1, \quad y_1^{(2)} = \frac{\lambda\beta_1 - d_1 a_1}{a_1\beta_1},$$

$$w^{(2)} = \frac{\xi a_1\beta_1}{\beta_1(fa_1 + r\lambda) - rd_1 a_1}$$

$$x_2^{(2)} = \frac{r\xi(d_1 a_1 - \lambda\beta_1)}{d_2[rd_1 a_1 - \beta_1(r\lambda + fa_1)]}, \quad y_2^{(2)} = 0$$

The appropriate equilibrium expressions for the survival of CXCR4-tropic virus only are given by (E_3)

$$x_1^{(3)} = \lambda/d_1, \quad y_1^{(3)} = 0, \quad w^{(3)} = \frac{a_2(d_2 + \beta_2 y_2^{(3)})}{r\beta_2 y_2^{(3)}},$$

$$x_2^{(3)} = a_2/\beta_2$$

$$y_2^{(3)} = \frac{\beta_2(\xi r - a_2 f) - a_2 r d_2 + \sqrt{[\beta_2(\xi r - a_2 f) - a_2 r d_2]^2 - 4r\beta_2 f d_2 a_2^2}}{2r a_2\beta_2}$$

Finally, both virus strains may coexist and this equilibrium is described by (E_4)

$$x_1^{(4)} = a_1/\beta_1, \quad y_1^{(4)} = \frac{\lambda}{a_1} - \frac{d_1}{\beta_1}, \quad x_2^{(4)} = a_2/\beta_2$$

$$y_2^{(4)} = \frac{\xi a_1\beta_1 + w^{(4)}[rd_1 a_1 - \beta_1(fa_1 + r\lambda)]}{w^{(4)} r a_1\beta_1},$$

where $w^{(4)}$ is given by a solution of a quadratic equation.

Denoting

$$A = r f \beta_1 \beta_2 a_1$$

$$B = \beta_1 \beta_2 r (\xi a_1 + \lambda a_2)$$
$$- a_1 a_2 [d_2 \beta_1 r + \beta_2 (r d_1 + f \beta_1)]$$

$$C = \beta_1 \beta_2 a_1 a_2 \xi$$

$$w^{(4)} = \frac{B - \sqrt{B^2 - 4AC}}{2A}.$$

The question now arises under which circumstances the equilibrium describing the survival of

macrophage tropic virus only (E_2) becomes unstable, i.e. which factors favour the emergence of CXCR4-tropic strains. Equilibrium (E_2) becomes unstable if

$$\beta_2 x_2^{(2)} > a_2,$$

i.e. if

$$\frac{\beta_2 r\xi(\lambda\beta_1 - d_1 a_1)}{d_2[\beta_1(r\lambda + fa_1) - rd_1 a_1]} > a_2.$$

Assuming $\lambda \beta_1 \gg d_1 a_1$ and consequently $\beta_1 (r\lambda + f a_1) \gg r d_1 a_1$ this condition can be approximated by

$$\frac{\beta_2 r\xi\lambda}{d_2(r\lambda + fa_1)} > a_2.$$

In this case, the rate of background activation of T cells becomes irrelevant, since the basic reproductive ratio of T cell tropic HIV is pushed above unity by macrophage-tropic mutants. This is because macrophage-tropic strains induce immune-activation. Among the viral parameters, the most significant factor influencing the outcome of the above inequality is the replication rate of HIV in T cells (β_2). If the replication rate of CXCR4-tropic strains lies beyond a certain threshold, the equilibrium describing the persistence of macrophage tropic strains only (E_2) becomes unstable and CXCR4-tropic strains will certainly invade. On the other hand, if the above condition is not fulfilled, the establishment of persistent infection may generally depend on the initial conditions in the same way as for T cell infection alone. However, the initial conditions are irrelevant for the *in vivo* situation. This is because macrophage-tropic HIV will create CXCR4-tropic strains by mutation, thus ensuring relatively low initial abundances.

4.2. THE EFFECT OF A CTL RESPONSE

Similarly to the previous models, a CTL response can be incorporated in the tropism model. We assume that CTLs proliferate at different rates in response to infected macrophages and T cells (c_1 and c_2, respectively), and that the two cell types are killed by CTLs at different rates (p_1 and p_2, respectively). Denoting

the CTL population by z, this leads to the following set of differential equations.

$$x = \lambda - d_1 x_1 - \beta_1 x_1 y_1$$

$$y_1 = \beta_1 x_1 y_1 - a_1 y_1 - p_1 y_1 z$$

$$w = \xi - fw - rw(y_1 + y_2) \qquad (7)$$

$$x_2 = rw(y_1 + y_2) - d_2 x_2 - \beta_2 x_2 y_2$$

$$y_2 = \beta_2 x_2 y_2 - a_2 y_2 - p_2 y_2 z$$

$$z = (c_1 y_1 + c_2 y_2) z - bz$$

This system is again characterised by the trivial equilibrium (1) as well as by target cell limited virus growth (equilibria 2, 3, and 4). If levels of virus load are high enough to stimulate a CTL response, one of three alternative outcomes may again be seen. Only one of the virus strains may survive, or coexistence of both strains may be observed. The equilibrium expressions for the survival of macrophage tropic HIV only are given by (E_5)

$$x_1^{(5)} = \frac{\lambda c_1}{d_1 c_1 + b\beta_1}, \quad y_1^{(5)} = \frac{b}{c_1}, \quad w^{(5)} = \frac{\xi c_1}{fc_1 + rb},$$

$$x_2^{(5)} = \frac{r\xi b}{d_2(fc_1 + rb)}.$$

$$y_2^{(5)} = 0, \; z^{(5)} = \frac{c_1(\beta_1\lambda - a_1 d_1) - a_1\beta_1 b}{p_1(d_1 c_1 + b\beta_1)}$$

The survival of only CXCR4-tropic HIV is described by equilibrium (E_6)

$$x_1^{(6)} = \lambda/d_1, \quad y_1^{(6)} = 0, \quad w^{(6)} = \frac{\xi c_2}{fc_2 + rb}$$

$$x_2^{(6)} = \frac{r\xi c_2 b}{c_2[d_2(c_2 f + rb) + \beta_2 bf] + \beta_2 b^2 r},$$

$$y_2^{(6)} = b/c_2$$

$$z^{(6)} = \frac{c_2[\beta_2 b(r\xi - a_2 f) - a_2 d_2(c_2 f + rb)] - a_2\beta_2 b^2 r}{p_2[d_2 c_2(c_2 f + rb) + \beta_2 b(fc_2 + br)]}$$

The coexistence of both virus strains is given by a solution of a third degree polynomial and is not written out here.

Again, we are interested in the conditions leading to instability of the equilibrium describing the survival of macrophage tropic HIV only (E_5). This happens if

$$\beta_2 \, x_2^{(5)} - p_2 \, z_2^{(5)} > a_2,$$

i.e. if

$$\frac{\beta_2 r\xi b}{d_2(fc_1 + rb)} - \frac{p_2[c_1(\beta_1\lambda - a_1 d_1) - a_1\beta_1 b]}{p_1(d_1 c_1 + b\beta_1)} > a_2.$$

Assuming as before that $\beta_1 \, \lambda \gg a_1 \, d_1$ and that $c_1 \, \lambda \gg a_1 \, b$, the above inequality can be approximated by

$$\frac{\beta_2 r\xi b}{d_2(fc_1 + rb)} - \frac{p_2\beta_1 c_1\lambda}{p_1(d_1 c_1 + b\beta_1)} > a_2.$$

As was the case without a CTL response, the major viral parameter contributing to the instability of equilibrium (E_5) is a high replication rate of CXCR4-tropic virus strains (β_2). However, in addition, the effectiveness of the CTL response is also significant now. Concentrating on the immune response against infected macrophages first, a high rate of CTL proliferation in response to infected macrophages (c_1) counters the rise of CXCR4-tropic strains. This is because high values of c_1 significantly reduce levels of virus load and thus reduce the number of activated susceptible T helper cells. The pattern is different for the rate of CTL-mediated killing of infected macrophages (p_1). High values of p_1 reduce the equilibrium abundance of the CTLs and therefore facilitate invasion of CXCR4-tropic strains. However, as can be seen from the inequality given above, high values of p_1 do not offset the negative effect of a high rate of CTL proliferation (c_1) on the emergence of CXCR4-tropic strains.

As expected, the above inequality also shows that a high rate of CTL-mediated killing of infected T cells (p_2) counters the emergence of CXCR4-tropic HIV.

On the other hand, if the above condition is not fulfilled, invasion of CXCR4-tropic HIV may again depend on the initial conditions in the same way as for T cell infection alone. However, the initial conditions should again be irrelevant for the *in vivo* situation. T cell tropic strains are created by mutations and are thus present at initially low levels, and the initial number of CTLs at the time when mutation gives rise to CXCR4-tropic HIV is determined by the efficacy of the immune response against macrophage-tropic strains.

The invasion condition discussed above determines whether a CXCR4-tropic mutant has a positive growth rate when it is added to a macrophage-tropic HIV population at equilibrium. However, even if it does have a positive initial growth rate, this does not guarantee stable coexistence between CXCR4-tropic and macrophage-tropic strains. Broadly speaking, the dynamical behaviour of the coexistence equilibrium can be separated into two categories. Either the equilibrium is stable, or one observes oscillations which are characterised by relatively high amplitudes (Fig. 5). This behaviour is determined by the levels of immune responsiveness to both infected macrophages (c_1) and T

(a)

(b)

FIG. 5. Two types of dynamical behaviour can occur if CXCR4-tropic HIV has a positive initial growth rate [eqn (7)]. Either, (a) the equilibrium is stable reached by damped oscillations, or (b) the occurrence of stable limit cycles is observed. 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. Parameters were chosen as follows: $\lambda = 1$; $d_1 = 0.1$; $\beta_1 = 2$; $a_1 = 0.5$; $\xi = 1$; $f = 0.01$; $r = 2$; $d_2 = 0.1$; $\beta_2 = 2$; $a_2 = 0.5$; $p_1 = 1$; $p_2 = 1$; $b = 0.1$; for (a) $c_1 = 0.15$; $c_2 = 0.15$; for (b) $c_1 = 0.5$; $c_2 = 0.5$.
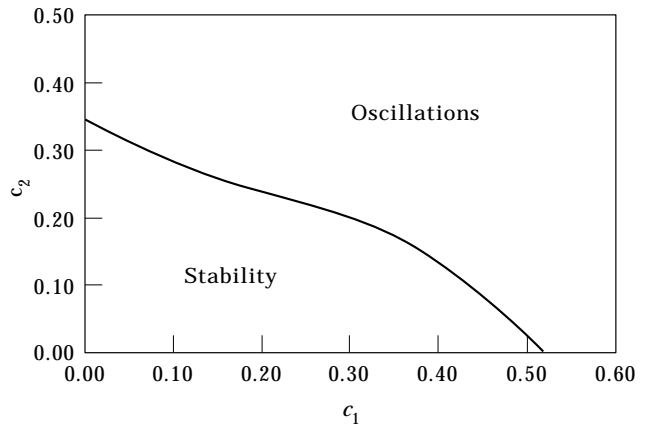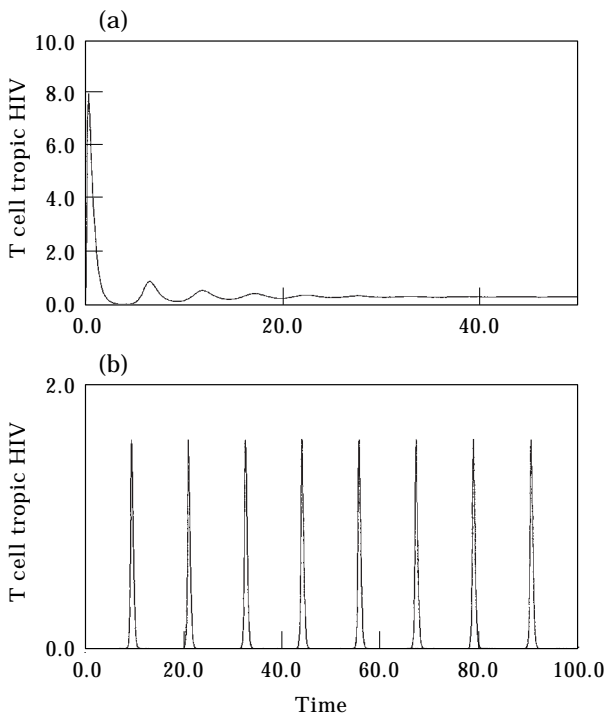

FIG. 6. Parameter space determining the dynamical behaviour of the model if CXCR4-tropic mutants can invade [eqn (7)]. The two important parameters are the growth rate of CTLs in response to infected macrophages (c_1) and T cells (c_2). If both of these parameters are still sufficiently high, the system is characterised by oscillations. Stable invasion of CXCR4-tropic HIV will only be observed if the efficacy of the immune system to both infected macrophages and T cells is sufficiently compromised. Parameters were chosen as follows: $\lambda = 1$; $d_1 = 0.1$; $\beta_1 = 2$; $a_1 = 0.5$; $\xi = 1$; $f = 0.01$; $r = 2$; $d_2 = 0.1$; $\beta_2 = 2$; $a_2 = 0.5$; $p_1 = 1$; $p_2 = 1$; $b = 0.1$.

cells (c_2) (Fig. 6). Thus, even if the value of c_1 is below the threshold level allowing invasion of CXCR4-tropic strains, this invasion may only be temporary if values of c_1 are still relatively high leading to oscillating dynamics. Similar considerations apply to the immune responsiveness to infected T cells (c_2). Although this parameter does not influence the invasion condition, a fast expansion of the CTL population in response to infected T cells (high values of c_2) induces only a temporary rise of CXCR4-tropic HIV with a subsequent suppression to very low levels. The permanent rise of CXCR4-tropic mutants will only be observed if the immune responsiveness to both types of infected cells is sufficiently small so as to allow stable dynamics. Therefore, the two types of dynamical behaviours have different biological interpretations. The stable equilibrium corresponds to stable coexistence of both macrophage-tropic and CXCR4-tropic virus. On the other hand, the occurrence of strong cycles indicates that if a CXCR4-tropic strain arises by mutation it has the ability to replicate to high levels, but is immediately reduced by the CTL response to such low levels that extinction is likely. If CXCR4-tropic mutants are generated

continuously, this will lead to the appearance of "blips" of T cell tropic HIV which rapidly become extinct again [Fig. 5(b)].

In summary, one can distinguish between three parameter regions. First, permanent extinction of the CXCR4-tropic strains may be observed. Alternatively, if the replication rate of HIV in T cells is sufficiently high and the effectiveness of the immune response lies below a threshold level, CXCR4-tropic strains may invade. In that case, if the immune responsiveness of the host is not reduced to sufficiently low levels, this invasion may only be temporary since the CTL response is still strong enough to suppress the rising HIV population. The permanent emergence of CXCR4-tropic mutants will only be observed if the efficacy of the immune system is reduced still further so that the CTLs are not capable of eliminating the virus.

5. Discussion and Conclusion

In this paper, we focussed on a basic difference in macrophages and T cells concerning the permissiveness of the cell for HIV infection. While macrophage infection does not require cell activation and division, only activated T cells allow HIV to complete its replication cycle (Stevenson & Gendelman, 1994; Stevenson, 1996). This difference leads to differences in the conditions required to establish a persistent infection. Strains able to replicate in macrophages can establish an infection if the basic reproductive ratio of the virus exceeds unity. On the other hand, the criteria for the establishment of HIV strains that can only replicate in T cells are more complicated given that the rate of background activation of T cells is not excessively large, e.g. due to other persistent infections being present in the host at the time of HIV infection. Considering T cell infection alone, we have shown that the replication rate of T cell tropic strains needs to be above a certain threshold and the immune responsiveness of the host below a threshold level for persistent infection to be possible. Moreover, establishment of infection requires the initial virus load to be intermediate and the initial number of CTLs to be low. If virus load lies below a certain threshold, not enough target cells become

activated in order to allow the virus to replicate at a sufficient rate. On the other hand, if the initial virus load or the initial number of CTLs lies above a certain threshold, the CTL population expands too quickly. This reduces virus load, and thus the number of activated target cells, to relatively low levels preventing the establishment of persistent replication.

Considering the model including both the dynamics of macrophage- and CXCR-4 tropic HIV, we have shown that a high rate of replication in T cells as well as a low immune responsiveness of the host contribute to the emergence of CXCR4-tropic mutants. Given that strains able to infect macrophages have established an infection, invasion of CXCR4-tropic strains simply requires the virus–immune system balance to have evolved in favour of fast replication and away from immune recognition, without the complex conditions required to infect T cells in the absence of macrophages. This indicates that macrophages are essential for the successful establishment of HIV and that they may act as a buffer or refuge contributing to the persistence of the virus during the disease process (Stevenson & Gendelman, 1994; Crowe, 1995).

Based on these results, our theory may help to interpret events occurring during the disease process. At the beginning of the infection the immune system is healthy and the virus is usually observed to be replicating at a relatively slow rate (Connor & Ho, 1994). Under these conditions, HIV will be able to infect macrophages, but any CXCR4-tropic mutants existing or arising would go extinct. During the course of infection, the virus evolves towards increased replication kinetics (Connnor & Ho, 1994; Nowak & May, 1991, 1992; DeBoer & Boerlijst, 1994; Schenzle, 1994; Wodarz *et al.*, 1998) and escape from the immune response. HIV may either escape recognition altogether (Nowak *et al.*, 1991; Phillips *et al.*, 1991; Nowak *et al.*, 1995; Borrow *et al.*, 1997; Goulder *et al.*, 1997) or alternatively bear a class of altered peptide ligands which partially activate T cells, act as TCR antagonists, (Jameson *et al.*, 1993; Bertoletti *et al.*, 1994; Klenerman *et al.*, 1994; Meier *et al.*, 1995), or promote responses with inappropriate specificities (Jameson & Bevan, 1995; Kalams & Walker, 1995; Klenerman *et al.*,
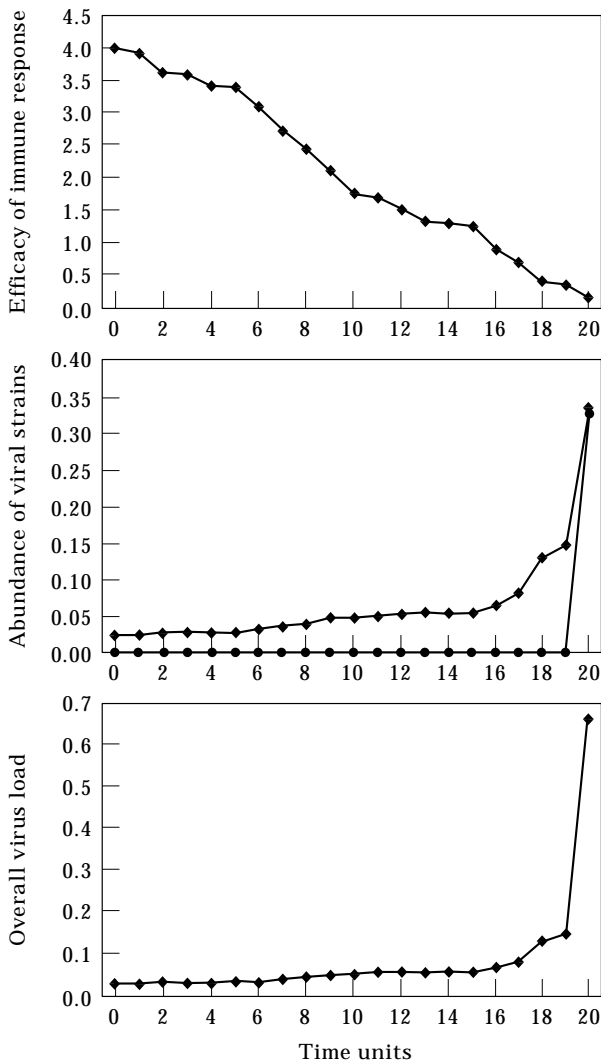
FIG. 7. Simulation of disease progression in HIV-infected patients based on viral evolution towards increased levels of immune escape. We assume that virus evolution leads to a reduction in the efficacy of the CTL response (c_1 and c_2) each time step by a random amount. For each time step the equilibrium values of virus load [eqn (7)] are calculated and plotted. If CXCR4-tropic mutants can invade but induce cycling dynamics, we set the equilibrium abundance of CXCR4-tropic strains to zero, since the limit cycles indicate rapid extinction. As can be seen from the graphs, a decrease in the efficacy of the CTL response leads to a slight increase in the levels of macrophage-tropic HIV while CXCR4-tropic mutants cannot establish a stable persistent infection as long as the rate of CTL proliferation in response to both cell types is still sufficiently large. Only a reduction in the efficacy of the immune response below a certain threshold leads to stable invasion of CXCR4-tropic HIV and therefore a sharp rise in the overall level of virus load, contributing to progression to full-blown AIDS. Parameters were chosen as follows: $\lambda = 1$; $d_1 = 0.1$; $\beta_1 = 2$; $a_1 = 0.5$; $\xi = 1$; $f = 0.01$; $r = 2$; $d_2 = 0.1$; $\beta_2 = 2$; $a_2 = 0.5$; $p_1 = 1$; $p_2 = 1$; $b = 0.1$. (◆) M-tropic; (●) T-tropic.

1995; McAdam et al., 1995; Klenerman et al., 1996; Borrow et al., 1997). Such evolutionary changes result in conditions favourable for the emergence of CXCR4-tropic HIV which in turn increases virus load and accelerates progression towards the final stage of the disease. A simulation of disease progression due to viral evolution leading to the rise of CXCR4-tropic strains is shown in Fig. 7.

Our model also sheds light on observations made in people lacking a functional CCR5 coreceptor required for viral entry into macrophages. It has been shown that successful establishment of HIV infection is a rare event in individuals bearing the ΔCCR5 deletion (Dean et al., 1996; Dragic et al., 1996; Huang et al., 1996; Liu et al., 1996; Samson et al., 1996; Biti et al., 1997; Rana et al., 1997). Along similar lines, Hirsch et al. (in prep.) 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. As discussed above, establishment of T cell infection alone is very difficult to achieve and depends on the complex balance between host and viral parameters as well as initial conditions.

We conclude that macrophages are essential for maintaining persistent HIV infection. Acting as a buffer they allow the virus to evolve towards conditions permissible for the emergence of CXCR4-tropic mutants which in turn drive progression to AIDS.

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