MATHEMATICAL BIOSCIENCES
AND ENGINEERING
Volume **8**, Number **2**, April **2011**

doi:10.3934/mbe.2011.8.605

pp. **605–626**

PERSISTENCE AND EMERGENCE OF X4 VIRUS IN HIV INFECTION

Ariel D. Weinberger

Graduate Group in Biophysics
University of California, Berkeley
Berkeley, CA 94720, USA

Alan S. Perelson

Theoretical Biology and Biophysics, MS-K710
Los Alamos National Laboratory
Los Alamos, NM 87545, USA

Abstract. Approximately 50% of late-stage HIV patients develop CXCR4-tropic (X4) virus in addition to CCR5-tropic (R5) virus. X4 emergence occurs with a sharp decline in CD4+ T cell counts and accelerated time to AIDS. Why this phenotypic switch to X4 occurs is not well understood. Previously, we used numerical simulations of a mathematical model to show that across much of parameter space a promising new class of antiretroviral treatments, CCR5 inhibitors, can accelerate X4 emergence and immunodeficiency. Here, we show that mathematical model to be a minimal activation-based HIV model that produces a spontaneous switch to X4 virus at a clinically-representative time point, while also matching in vivo data showing X4 and R5 coexisting and competing to infect memory CD4+ T cells. Our analysis shows that X4 avoids competitive exclusion from an initially fitter R5 virus due to X4s unique ability to productively infect nave CD4+ T cells. We further justify the generalized conditions under which this minimal model holds, implying that a phenotypic switch can even occur when the fraction of activated nave CD4+ T cells increases at a slower rate than the fraction of activated memory CD4+ T cells. We find that it is the ratio of the fractions of activated nave and memory CD4+ T cells that must increase above a threshold to produce a switch. This occurs as the concentration of CD4+ T cells drops beneath a threshold. Thus, highly active antiretroviral therapy (HAART), which increases CD4+ T cell counts and decreases cellular activation levels, inhibits X4 viral growth. However, we show here that even in the simplest dual-strain framework, competition between R5 and X4 viruses often results in accelerated X4 emergence in response to CCR5 inhibition, further highlighting the potential danger of anti-CCR5 monotherapy in multi-strain HIV infection.

1. **Introduction.** Without antiretroviral therapy, human immunodeficiency virus type-1 (HIV) generally depletes an infected individuals immunologically critical CD4+ T cell population, leading to AIDS onset and death after approximately 10-12 years [11, 25, 62]. HIV targets CD4+ T cells, as the virus binds and infects cells displaying the CD4 receptor. Yet, binding to CD4 alone is insufficient for HIV to enter a target cell: a coreceptor is required. HIV strains that utilize CCR5 as a

2000 *Mathematics Subject Classification.* Primary: 92C37, 92C45; Secondary: 92C50.
Key words and phrases. HIV; coreceptor; phenotypic switch; X4; R5.

coreceptor are called R5 viruses while those that bind CXCR4 are known as X4 viruses [8, 19, 37, 66].

X4 viruses are rarely seen during early infection, where R5 viruses predominate, whatever the route of infection [3, 7, 19, 28, 37]. Moreover, individuals homozygous for a 32 base pair deletion in the allele for CCR5 (CCR5Δ32/Δ32) are almost entirely immune to HIV infection [37], implying that X4 viruses are at a severe selection disadvantage during early infection. However, in approximately 40-50% of progressing patients, X4 viruses emerge late in infection, often becoming the dominant strain [37]. X4 emergence is correlated with a steep decline in CD4+ T cell counts, which explains earlier work noting a phenotypic switch to a more virulent viral phenotype in many late-stage HIV patients [1, 40, 47, 49, 54].

In vitro competition assays between R5 and X4 virus usually result in X4 dominance [37]. Since about fivefold more lymphocytes are CXCR4+ rather than CCR5+ [29], one wonders why X4 is unable to dominate in vivo. A compelling explanation for R5s in vivo dominance and the basis for our models is CCR5s disproportionate presence on activated and recently activated memory CD4+ T cells. Memory CD4+ T cells can often be distinguished from their nave precursor cells, because memory cells display the cell surface receptor CD45R0 [1]. Nave cells generally display the receptor CD45RA, which is modified to its isoform CD45RO after an antigen nave CD4 T cell encounters its cognate antigen, thereby activating it into an effector memory cell.

Using the distinct cell surface receptors of naive and memory cells as well as antibodies that specifically bind to CCR5 and CXCR4, respectively, Lee et al. estimated the per-cell concentrations of CCR5 and CXCR4 molecules on nave and memory T cells, respectively [29] (Table 1). The authors went further, dividing both nave and memory cell populations into activated and quiescent subsets, based on whether the cells also expressed the receptor CD62L, which is displayed by nave and memory cells in quiescent states [24]. Using quantitative fluorescence-activated cell sorting (QFACS), they found an average of 4741 R5 antibody-binding sites on CD62L+ CD45RO+ quiescent memory cells with only 1,013 X4 binding sites on this cell population. Among highly activated memory CD62L- CD45RO+ CD4+ T cells the difference is even more pronounced, with 9,576 R5 binding sites and only 505 X4 binding sites (Table 1). Conversely, the authors measured virtually no R5 antibody binding sites on nave CD45RA+ CD4+ T cells on which X4 binding sites dominate. In general, as Table 1 shows, CXCR4 is more common on nave and quiescent cells, while CCR5 dominates in the effector memory population.

As a result of CCR5s higher per-cell density among memory cells, which are more likely to be activated than naive cells [10, 35], R5 viruses may have an advantage on the whole over X4 viruses. Comparative snapshots of CD4+ T cells during SIV infection show approximately five times as many virions surround infected, activated CD4+ T cells as surround infected, phenotypically-quiescent CD4+ T cells [70]. Moreover, phenotypically-activated (Ki67+) CD4+ T cells produce over 90% of the virions during the chronic phase of SIV infection [30].

The relevant question is then: how do X4 viruses emerge late in infection if R5 viruses are simply better at infecting the all-important subset of memory CD4+ T cells? Previous mathematical models have analyzed several hypotheses for this emergence [2, 27, 44-46, 67, 68]. Specifically, Regoes and Bonhoeffer [44] pursued a model where antiretroviral treatment disproportionately inhibits R5 virus, precipitating a switch to X4. This cannot explain the documented emergence of X4 virus

in treatment-nave individuals [6]. Other models [2, 67, 68] analyzed the impact of differential immune responses on phenotypic switching, but these immune-based models utilize specific assumptions that current data argue against. Wodarz et al. [67] neglects the fact that over 90% of productive R5 infection occurs in CD4+ T cells, not macrophages [17]. The model by Wodarz and Nowak [68] cannot explain the disproportionate increase in X4 viral loads (VLs) after CD8 depletion [19]. Finally, the model by Callaway et al. [2] appears inconsistent with the fact that the greatest correlate of disease progression in HIV patients, and the only consistent difference between pathogenic and non-pathogenic lentiviral infections, is increased immune activation, including increased cytotoxic T-lymphocyte (CTL) activation [14, 51]. Since X4 onset is strongly correlated with disease progression, an active cytotoxic immune response is more likely a cause or consequence of X4 emergence than an inhibitor.

Here, the co-occurrence of X4 emergence and immune cell activation is explored in mathematical detail, using current data to derive conditions under which increased target-cell activation over the course of dual R5, X4 HIV infection drives a late-stage switch to X4 virus. As in the above studies, the switch to X4 in our models is the result of progressive HIV infection altering the fitness landscape in favor of X4. Yet, the mechanism altering the fitness landscape is different here, as we use in vivo derived data to justify conditions showing changing T cell activation rates directly changing the fitness landscape in favor of X4. Building upon previous studies arguing that target-cell activation drives the switch to X4 [46, 63], we derive a minimal target-cell activation-based model for understanding multi-tropism and its attendant immunodeficiency in HIV.

2. **Results.**

Generalized conditions for a phenotypic switch

Curve fitting a data set measured in vivo [23], which determined the fractions of activated T cells using the cell-cycle activation marker (Ki67), Ribeiro et al. [25] found that the fractions of nave cells that are activated (a_n) and the fractions of memory cells that are activated (a_m) obey the following inverse relationships with respect to the total CD4+ T cell count:

$$a_n(CD4) = 10/CD4 - .0095 \tag{1}$$
$$a_m(CD4) = 10/CD4 + .05$$

Here, CD4 denotes the total number of uninfected and infected CD4+ T cells per microliter of blood. During HIV infection, CD4+ T counts decline, causing both an and am to increase. The increase in an lets X4 virus benefit from CXCR4s strong presence on activated nave CD4+ T cells [29] (Table 1), allowing for a switch.

In a previous paper [63], we claimed that one can generalize (1) to show that a switch can occur even if the fraction of activated nave CD4+ T cells increases at a slower rate than the fraction of activated memory CD4+ T cells. We then chose a minimal model in this more general setting, and used simulations to show that CCR5 inhibitors, i.e. drugs that bind to CCR5 and prevent X5 viruses from entering cells, can accelerate X4 emergence, a prediction supported by recent data [65, 69].

Weinberger et al. [63] examined the possibility of a phenotypic switch when

$$\begin{gathered} a_n(CD4) < a_m(CD4) \\ a_n'(CD4) < 0, a_m'(CD4) < 0 \\ \frac{d}{d(CD4)}\left(\frac{a_n(CD4)}{a_m(CD4)}\right) > 0 \end{gathered} \tag{2}$$

To justify these conditions, we note that throughout infection a far greater fraction of CD4+ memory cells are activated than naive CD4+ lymphocytes [10, 35]. Thus, we set $a_n < a_m$. Furthermore, increased immune activation is strongly correlated with CD4+ T cell decline in HIV patients [21, 22, 38, 51] and this increased activation is manifested in both nave and memory CD4+ T cells [23, 35], so $a_n'(CD4) < 0$ and $a_m'(CD4) < 0$. To justify the final condition in Eq. (2), we note that

$$\frac{d}{d(CD4)}\left(\frac{a_n(CD4)}{a_m(CD4)}\right) = \frac{a_n'(CD4)a_m(CD4) - a_m'(CD4)a_n(CD4)}{a_m(CD4)^2}$$

This derivative is negative if and only if

$$a_n'(CD4)a_m(CD4) - a_m'(CD4)a_n(CD4) < 0$$

Because $a_m'(CD4) < 0$, this is true if and only if:

$$a_n'(CD4)/a_m'(CD4) > a_n(CD4) > /a_m(CD4) \tag{3}$$

Clearly, Eq. (1) is a particular system satisfying Eq. (2), because in Eq. (1) we have $a_n < a_m$ and a_n'$= a_m$'< 0. So in justifying Eq. (2) we are allowing for a larger class of models.

To justify Eq. (3) and thus the final condition of Eq. (2), we note that if a_n' is a larger fraction of a_m' than a_n is of a_m, Eq. (3) holds. We have already shown $a_n < a_m$, implying that:

$$1 - a_m < 1 - a_n \tag{4}$$

Thus, the fraction of nave cells that is quiescent is greater than the fraction of memory cells that is quiescent. We let n_t and m_t represent the total numbers (i.e., activated + non-activated) of nave and memory CD4+ T cells, respectively. Because nave and memory cell counts are initially similar and because R5 virus disproportionately depletes memory CD4+ T cells [15, 61], we assume that $n_t > m_t$ during R5 infection, implying:

$$m_t(1 - a_m) < n_t(1 - a_n) \tag{5}$$

Furthermore, many of the newly activated memory CD4+ T cells were previously quiescent nave CD4+ T cells activated by interaction with antigen. These additions to am also increase an by reducing the number of quiescent naive CD4+ T cells. Thus, given the large measured differences between the fractions of activated nave and memory CD4+ T cells, we argue that discrepancies between the rates of increase of activated nave and memory CD4+ T cells will often be relatively small. That is, we claim that in many cases $a_n'(CD4)/a_m'(CD4) > a_n(CD4)/a_m(CD4)$, which is equivalent to Eq. (3). Data sets such as the one from which Eq. (1) was derived, give us evidence that this is reasonable. We note that Eq. (3) clearly holds when $a_n'(CD4) \leq a_m'(CD4) < 0$, that is, when the fraction of activated nave CD4+ T cells increases at least as quickly as the corresponding fraction of memory cells, as

CD4+ T cells decline. Such a scenario is obviously to the increasing benefit of X4 virus in an activation-based model. In fact, this idea was used to explain the switch in Ribeiro et al. [46]. Yet, because $a_n < a_m$, Eq. (3) is even satisfied in certain cases in which $0 > a_n'(CD4) > a_m(CD4)$ (i.e., when an increases at a slower rate than am in response to CD4+ T cell decline). Such a broadened scenario would occur if $a_n << a_m$ and a_n' only slightly less negative than a_m' . Of course, in situations where a_n increases far slower than a_m in response to CD4+ T cell decline, Eq. (3) would likely not hold.

Model 1: One Target Cell Population Yields Competitively Exclusive Switches

In our preceding paper [63], we began by extending the basic model of viral dynamics [25, 62] to the simplest dual-strain framework, denoted Model 1 there and below. Through simulations, we showed that R5-to-X4 switches arise from this model, but claimed that such switches are beset by competitive exclusion, given the single-compartment nature of that model. Competitive exclusion is not consistent with in vivo data, which show X4 and R5 coexisting post-switch [69]. Here, we analytically show that competitive exclusion is the result of Model 1 and further show that accelerated emergence of X4 virus due to anti-CCR5 treatment is a basic result of strain competition for target-cells and is present in even the simplest of competitive models.

$$\begin{aligned}
\dot{T} &= \lambda - (k_4 V_4 + k_5 V_5)T - d_T T \\
\dot{I_4} &= k_4 V_4 T - \delta I_4 \\
\dot{I_5} &= k_5 V_5 T - \delta I_5 \\
\dot{V_4} &= p a_n I_4 - c V_4 \\
\dot{V_5} &= p a_m I_5 - c V_5
\end{aligned}$$

In this model, all variables (capitalized) are concentrations per microliter ($1/\mu$l), λ has the units cells/(μl$\times$day), k_4 and k_5 have the units μl/(virions$\times$day), and the remaining parameters have units 1/day. Specifically, T represents the concentration of uninfected CD4+ T cells, and (without loss of generality) is given an initial value of 1000 CD4+ T cells/μl. I_4 and I_5 reflect the concentrations of CD4+ T cells abortively, latently, and productively infected by X4 and R5 viruses, respectively; V_4 and V_5, describe X4 and R5 virus concentrations. λ is the rate of production of CD4+ T cells and k_4 and k_5 are the respective infection rate coefficients for X4 and R5 infection of CD4+ T cells. Also, d_T is the death rate of uninfected CD4+ T cells and is set equal to λ/T_0 to allow for steady-state pre-infection, δ is the death rate of infected CD4+ T cells, p is the rate of viral production by activated infected cells, and c is the viral clearance rate. a_n and a_m are required to satisfy Equation (2) and represent the fractions of activated nave and memory CD4+ T cells for a given value of CD4. Since CD4 represents the total number of uninfected and infected CD4+ T cells per microliter, $CD4 = T + I_4 + I_5$.

We assume that when activated cells become infected they produce virus at rate p per cell. In our model, it is only these activated infected cells that produce virus. We thus multiply $a_n I_4$ and $a_m I_5$ by p, to obtain the total concentrations of virions produced each day. In a more complex model, one could allow a small amount of viral production from infected resting cells. Importantly, the products $a_n I_4$ and

$a_m I_5$ assume that infected cells are no more likely to be activated than uninfected cells. This is because infection in our model is not necessarily productive, and in general most infections have been measured to be non-productive [17].

Given the per-cell concentrations of CCR5 and CXCR4 recorded shown in Table 1, we assume that X4 virus only productively infects nave CD4+ T cells and thus make X4s viral production dependent on a_n, but not a_m. Conversely, we use the same dataset to justify making R5s production dependent on a_m, but not a_n. Because CXCR4s median cell surface density is almost three times as high as that of CCR5 across all lymphocytes [29], we also assume $k_4 > k_5$. As above, this does not imply that X4 productively infects more target cells than R5 at the beginning of infection, since very few nave cells are activated early in infection [23].

Deriving a Switch Threshold for Model 1 In analyzing Model 1, we first determine how many productively infected cells each strain has at a given point in time. Let $R_{\text{eff4}}$and R_{eff5} be time-dependent functions for the average number of infected cells that an average X4 and R5 infected cell produces. $R_{\text{eff4}}$and R_{eff5} are thus effective reproductive ratios, in contrast to the basic reproductive ratios, R_{04} and R_{05}, which evaluate $R_{\text{eff4}}$and R_{eff5} at the initial time point. The equations for $R_{\text{eff4}}$and R_{eff5} are

$$R_{\text{eff4}}(t) = p \times a_n(CD4(t)) \times k_4 \times T(t)/(c \times \delta) \tag{6}$$

$$R_{\text{eff5}}(t) = p \times a_m(CD4(t)) \times k_5 \times T(t)/(c \times \delta)$$

$$\frac{R_{\text{eff4}}(t)}{R_{\text{eff5}}(t)} = \frac{k_4 \times a_n(CD4(t))}{k_5 \times a_m(CD4(t))}$$

We note that while $R_{\text{eff4}}$and R_{eff5} are functions of t (time), the explicit time-dependencies of $R_{\text{eff4}}$and R_{eff5} cancel in the quotient $R_{\text{eff4}}/R_{\text{eff5}}$. This allows us to explore and subsequently differentiate $R_e f f 4/R_{\text{eff5}}$ as a function of CD4 alone.

Initially, we assume that $R_{\text{eff4}} < R_{\text{eff5}}$, because at the large CD4+ T cell counts prevalent during early infection $a_m >> a_n$, implying that $a_m \times k_5 > a_n \times k_4$ despite the fact that $k_4 > k_5$ (i.e., we assume that initially the disparity between a_n and a_m is greater than the disparity between k_4 and k_5). With a higher effective reproductive ratio, R5 virus is more efficient and dominates early, consistent with observation. As infection progresses, Eq. (2) shows that the relative fraction of activated nave cells increases as CD4+ T cells decrease. This yields

$$\frac{d}{d(CD4)}\left(\frac{R_{\text{eff4}}(t)}{R_{\text{eff5}}(t)}\right) = \frac{k_4}{k_5} \times \left(\frac{a_n(CD4)}{a_m(CD4)}\right) \tag{7}$$

In other words, if Eq. (2) holds, lowering CD4+ T cell counts preferentially benefits X4 by increasing its fitness relative to that of R5 virus. This accounts for the possibility of a switch at low CD4+ T cell counts. Here we show that when CD4 counts decrease enough for $R_{\text{eff4}}/\ R_{\text{eff5}}$ to go above 1, a switch to X4 virus occurs at a future time point. Conversely, if $R_{\text{eff4}}/\ R_{\text{eff5}}$ never increases beyond 1, a switch to X4 cannot occur, potentially explaining why 50% of patients do not exhibit a switch to X4 Virus during HIV infection.

In order for X4 virus to overtake R5 virus at time t^*, the following conditions are necessary and sufficient: $d/dt(V_4(t^*)) > d/dt(V_5(t^*))$ and $V_4(t^*) = V_5(t^*)$. Solving these switch equations simultaneously yields a necessary and sufficient switch condition for this model:

$$\frac{a_n(CD4(t^*))}{a_m(CD4(t^*))} > \frac{I_5(t^*)}{I_4(t^*)} \tag{8}$$

Equation (8) describes the threshold at which the switch to X4 occurs. We can find an earlier necessary and sufficient threshold for an/am above which a *future* switch to X4 is guaranteed to occur. To do so, we note that because $a_n/a_m < 1$ for all time, the right-hand side of Eq. (8) must be less than one. Thus, $I_4(t^*) > I_5(t^*)$ is a necessary condition for a switch. In biological terms, when X4s advantage, manifested in a greater number of infected cells, outweighs R5s advantage, manifested in a higher target-cell activation level (i.e., a higher probability that its infected cells are productively infected), the switch occurs.

For I_4 to overtake I_5 at t^*, a necessary condition is that at some earlier time point, $t^{**} < t^*$, the rate of growth of X4-infected cells was higher than that of R5-infected cells. Hence, $R_{\text{eff4}}(t^{**}) > R_{\text{eff5}}(t^{**})$ is a necessary condition for the R5 to X4 switch to occur. In fact, $R_{\text{eff4}}(t^{**}) > R_{\text{eff5}}(t^{**})$ is also a sufficient condition for the R5 to X4 switch. Because Eq. (8) implies that R_{eff4} is always increasing relative to R_{eff5} as CD4+ T cells decline, $R_{\text{eff4}}(t^{**}) > R_{\text{eff5}}(t^{**})$ means that $R_{\text{eff4}}(t) > R_{\text{eff5}}(t)$ for all $t \geq t^{**}$. Thus, if $R_{\text{eff4}}(t^{**}) > R_{\text{eff5}}(t^{**})$, X4 will eventually overtake R5.

An R5 to X4 switch always results in the eventual extinction of R5 in Model 1. This is because coexistence at steady state means:

$$d/dt(T) = d/dt(I_4) = d/dt(I_5) = d/dt(V_4) = d/dt(V_5) = 0 \textbf{ and } I_4, I_5, V_4, V_5 \neq 0. \tag{9}$$

$d/dt(V4) = d/dt(V5) = 0$ means

$p = (c \times V_4)/(a_n \times I_4) = (c \times V_5)/(a_m \times I_5)$, so

$$V_4 = (V_5 \times a_n \times I_4)/(a_m \times I_5). \tag{10}$$

Moreover, since $d/dt(I4) = d/dt(I5) = 0$,

$$\delta = (k_5 \times V_5 \times T)/I_5 = (k_4 \times V_4 \times T)/I_4$$

implying that

$$I_4 = \frac{(k_4 \times V_4 \times I_5)}{(k_5 \times V_5)} \tag{11}$$

Plugging Eq. (10) into Eq. (11) tells us that a necessary and sufficient coexistence condition is $(k_4/k_5) \times (a_n/a_m) = 1$. By Eq. (6), this is equivalent to the coexistence iff $R_{\text{eff4}} = R_{\text{eff5}}$.

Thus, a necessary and sufficient condition for a switch to occur at some point $t^* > t^{**}$ and for R5 to approach extinction is:

$$R_{\text{eff4}}(t^{**}) > R_{\text{eff5}}(t^{**}) \tag{12}$$

$R_{\text{eff4}}(t^{**}) > R_{\text{eff5}}(t^{**})$ means that a_n/a_m increases beyond $k_5\ /k_4$. Because a_n/a_m increases as CD4+ T cells decline, it is the level of CD4+ T cell depletion engendered by HIV that is directly implicated in the models switch. To quantify this for the measured functions of an and am given in Eq. (1), we substitute Eq. (1) into Eqs. (6) and (12) to yield the following necessary and sufficient threshold

beyond which a switch is guaranteed to eventually occur:

$$CD4(t^{**}) < 200 \times \frac{k_4 - k_5}{(k_5 + .19k_4)} \tag{13}$$

Since $k_4 > k_5$, the quotient on the right hand side is positive. Hence, at a CD4 count below a threshold, the switch condition is satisfied, guaranteeing that X4 will eventually take over. In Figure 1a, we reproduce such an R5 to X4 switch at a late-infection time point similar to those observed in patients. The parameters used are $\lambda = 33$ cells/(μl×day), $c = 23$/day, $p = 5750$/day, $\delta = 0.7$/day, $k_4 = 510^{-4}\mu$l/(virions×day), and $k_5 = 10^{-4}\mu$l/(virions×day). With the exception of changes to k_4 and k_5, it is clear from Eq. (13) that all changes to the models parameters that accelerate CD4+ T cell depletion accelerate an R5 to X4 switch. Conversely, mitigating the level of infection and consequent CD4+ T cell depletion lengthens the time until the switch occurs. Because the partial derivative of the right side of Eq. (13) is positive with respect to k_4, increasing k_4 also accelerates the switch by increasing the right hand side while decreasing CD4 counts through heightened X4 infection (Fig. 1b-d). Yet, the partial derivative of Eq. (13) with respect to k_5 is negative, meaning that both right and left sides of the equation decrease in response to higher levels of k_5, making it initially unclear as to whether increasing k_5 promotes a switch to X4 virus.

CCR5 Inhibitors Can Promote Switches to X4 virus in a Single Compartment Model

In general, reducing k_5–as occurs in CCR5 inhibitor treatments–increases k_4/k_5, but it also decreases a_n/a_m by increasing CD4+ T cell counts through decreased R5 infection. By Eq. (6), $R_{\text{eff4}}/R_{\text{eff5}}$ is the product of k_4/k_5 and a_n/a_m, so the question is whether the increase to k_4/k_5 is greater than the decrease to a_n/a_m. If so, $R_{\text{eff4}}/R_{\text{eff5}}$ increases in response to lowering k_5, implying that anti-CCR5 treatments can accelerate switches to X4.

We examined how modulating k_5 affects the switch to X4 virus. When a_n and a_m are defined as in Eq. (1), increasing k_5 from $1 \times 10^{-4}\mu$l/(virions×day) to $1.5 \times 10^{-4}\mu$l/(virions×day) accelerates the time at which X4 emerges (i.e., it increases R4/R5) (Fig. 2a, upper panel). However, increasing k_5 even further to $3 \times 10^{-4}\mu$l/(virions×day) prevents a switch (Fig. 2a, lower panel). In fact, the model predicts a steady state with high X4 viral loads only at intermediate values of k_5: increasing k_5 beyond a threshold blocks X4 emergence (Fig. 2b). To understand why increasing k_5 beyond a threshold prevents a switch to X4 Virus, we note that large values of k_5 (e.g. $k_5 = 3 \times 10^{-4}$) allow R5 to infect the vast majority of CD4+ T cells, leaving few uninfected R5 target cells. This causes diminishing returns in the number of new CD4+T cells that can be infected through further increases to k_5. As a result, when k_5 is initially large and k_5 is further increased, the increase to a_n/a_m from further CD4 T cell declines is unlikely to outweigh the decrease to k_4/k_5, causing a decrease in R4/R5 and inhibiting X4 emergence. Thus, if k_5 is initially large and a CCR5 inhibitor only partially decreases k_5–keeping us in the high k_5 diminishing returns regime–the increase to k_4/k_5 from decreasing k_5 can outweigh the decrease to an /am from the small increase in CD4+ T cell counts, increasing $R_{\text{eff4}}/R_{\text{eff5}}$ and promoting a switch to X4 (Fig. 2c). Significantly, these switches to X4 are prevented by antiretroviral cocktail therapies such as HAART which target both R5 and X4 equally (Fig. 2d, left panel) or dual CCR5 and CXCR4 inhibition (Fig. 2d, right panel). Model 1 is thus a simplified model in which we can rigorously see that competition for target cells may make anti-CCR5

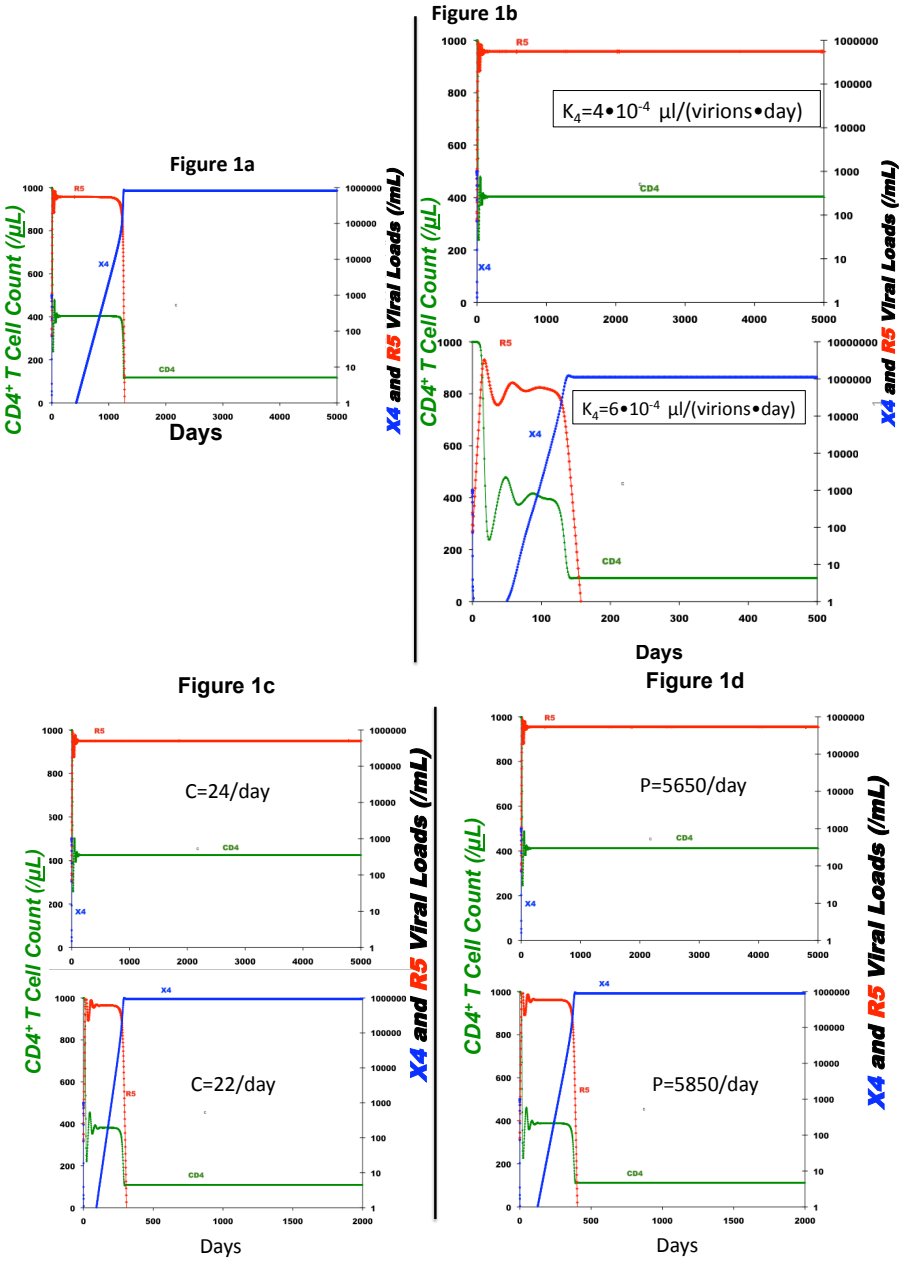


FIGURE 1. In Model 1, excluding changes to k_5, increasing virulence accelerates R5 to X4 switching. In (a) we simulate a common clinical outcome in which a phenotypic switch occurs after 3-4 years, with a drop in CD4+ T cell counts. Subsequently we modify: X4s infection rate coefficient k_4 in (b), the viral clearance rate c in (c), and the rate of viral production from infected cells p in (d). Parameter changes, except k_5, enhancing infection accelerate switches (bottom panels (b), (c), (d)), while those dampening infection hinder switches (top panels (b), (c), (d)). In each top panel, X4 stays below detection for all 5000 days of the simulation.

treatment a risky proposition. And while this simplified model seems to imply that CCR5 inhibitors do not do any damage insofar as the steady-state CD4+ T cell count is not lowered as a result of X4 emergence (Fig. 2c), the reality, as described more faithfully in Model 3 below, is that X4s emergence uniquely depletes the nave CD4+ T cell population, which serves as the pipeline for new memory CD4+ T cells.

Model 2: Coexistence, but no competition Having analyzed a simplified one-compartment switch-inducing model in detail, we are left with the problem of competitive exclusion. This all-or-nothing result is inconsistent with data that shows the possibility of coexistence after a phenotypic switch [42]. We previously claimed that maintaining distinct target-cell populations for R5 and X4 viruses is sufficient to produce coexistence [63]. Here we rigorously show this.

$$\begin{aligned}
\dot{N} &= \lambda + (1-2f)a_n N - k_4 V_4 N - d_N N \\
\dot{M} &= 2f a_n N + a_m M - k_5 V_5 M - d_M M \\
\dot{I}_4 &= k_4 V_4 N - \delta I_4 \\
\dot{I}_5 &= k_5 V_5 M - \delta I_5 \\
\dot{V}_4 &= p a_n I_4 - c V_4 \\
\dot{V}_5 &= p a_m I_5 - c V_5
\end{aligned}$$

The equations in this system are analogous to those in Model 1 but the uninfected CD4+ T cell population is now split into uninfected nave (N) and memory (M) subpopulations. The target cell death rates, d_N and d_M, are defined analogously to d_T in Model I, ensuring that both subsets of the uninfected CD4+ T cell population are in equilibrium pre-infection. Additionally, f is defined to be the fraction of nave cells activated by antigen, which then divide and differentiate into CD45RO+ memory cells. The rest of the activated cells are assumed to have been upregulated via cytokines or other antigen-T cell receptor independent processes and thus remain phenotypically nave (CD45RA+) [55, 59, 60]. Again, for simplicity it is assumed that X4 virus solely infects naive cells and that R5 virus only infects memory cells. Since the target cell population is now split, the effective reproductive ratios of R5 and X4 become functions of distinct target cell populations:

$$\begin{aligned}
R_{\text{eff4}}(t) &= p \times a_n(CD4(t)) \times k_4 \times N(t)/(c \times \delta) \qquad (14) \\
R_{\text{eff5}}(t) &= p \times a_m(CD4(t)) \times k_5 \times M(t)/(c \times \delta) \\
\frac{R_{\text{eff4}}(t)}{R_{\text{eff5}}(t)} &= \frac{k_4 \times a_n(CD4(t)) \times N(t)}{k_5 \times a_m(CD4(t)) \times M(t)}
\end{aligned}$$

An immediate result is that $k_4 > k_5$ is no longer required for a switch to occur. In fact, if R5 depletes most of its target cells, X4 virus will have an advantage even when X4 has a lower infection rate coefficient. That is, $an \times k4 \times N > am \times k5 \times$ M is possible even when $an \times k4 < am \times k5$.

Because of the differential target-cell compartments, after a phenotypic switch R_{eff5} can rebound and increase relative to R_{eff4}, a fact that could not occur in the above single-compartment model. This occurs because when X4 viral loads burgeon during a switch, X4 encounters an untapped nave target cell pool, while most memory target cells have already been depleted by R5 infection. This means that the nave CD4+ T cell population will decrease more rapidly than the corresponding

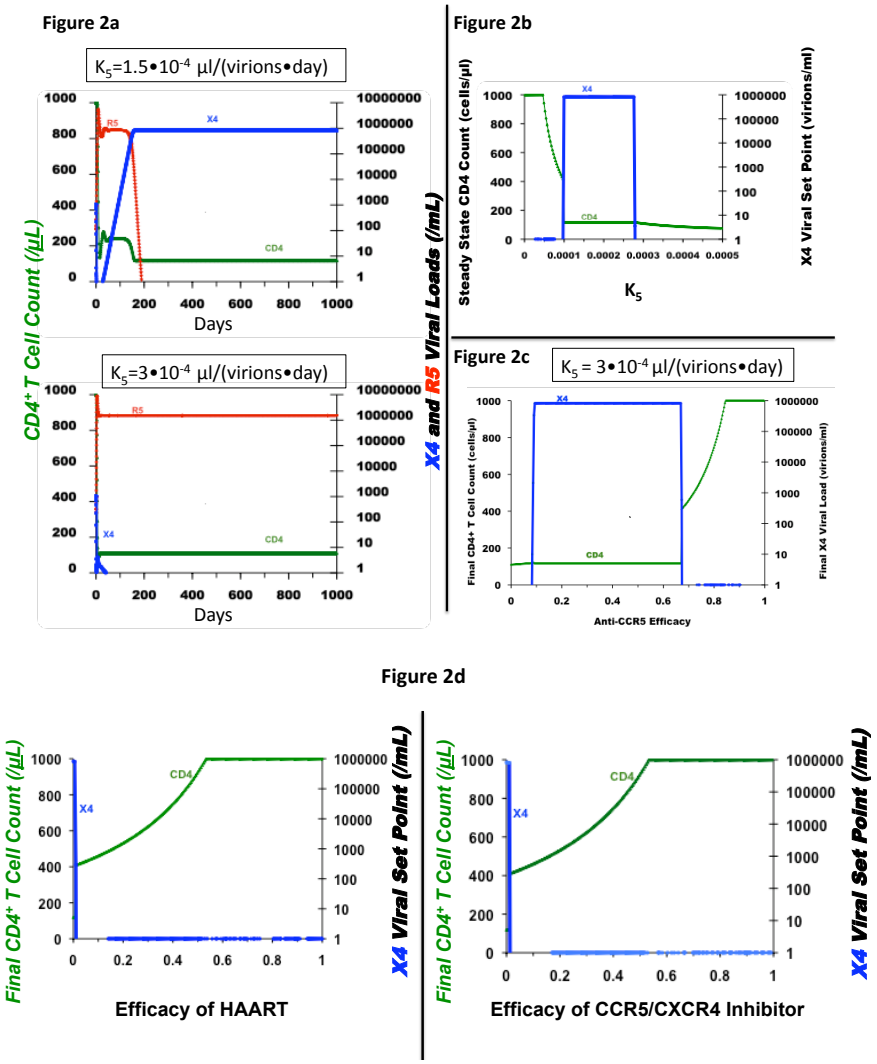


FIGURE 2. Even in the simplest target-cell competition model (Model 1), diminishing returns can cause CCR5 blockers to promote X4 Emergence. Unless noted, parameters values are from 1a. While Fig. 1 implies that increasing the level of infection accelerates switches, changes to k_5 have more complicated kinetics. In (a) Model 1 is simulated at two increased k_5 levels. As expected from Fig. 1, an initial increase of the R5 infection rate coefficient to 1.5×10^{-4} accelerates X4 emergence (top panel). However, increasing k_5 more significantly to 3×10^{-4}, prevents X4s onset (bottom panel). In (b), X4 is shown to go extinct as k_5 crosses a threshold. In (c), where $k_5 = 3$, anti-CCR5 therapy with efficacy below 70% actually promotes X4 emergence. In (d) combining anti-HIV therapies (i.e., HAART) to target R5 and X4 strains equally (left panel) or combining anti-CCR5 and anti-CXCR4 therapy (right panel) prevents X4 emergence and increases CD4 counts.

memory cell population after an R5-to-X4 switch. If the resulting decrease in N/M is greater than the increase in an/am that results from the lowered CD4+ T cell counts post-switch, then $R_{\text{eff4}}/R_{\text{eff5}}$ decreases by Eq. (14). Since $R_{\text{eff4}}/R_{\text{eff5}}$ can decrease after a switch in a two-compartment model, coexistence is now possible (Supporting Figure 1). The equations for V4 and V5 in the two compartment model are identical to those in the single-compartment model, so the same switch condition persists (found by setting V4=V5 and $d/dt(V4) > d/dt(V5)$).

Thus, as in Model 1, a switch occurs if and only if an/am goes above the threshold in (8), or, equivalently, if and only if there is sufficient CD4+ T cell depletion. Thus, modulating parameters to increase CD4+ T cell decline accelerates an R5 to X4 switch, while down-regulating infection, for example via drug intervention, inhibits X4 incidence. This result clearly extends to changes in k5, as X4 and R5 are independent viruses here so that X4 receives no advantage from a weakened R5 virus. Moreover, having a CD4+ T cell threshold for an R5 to X4 switch means that despite R5s ability to increase after X4 depletes the nave CD4 population post-switch, R5 is not likely to overtake X4 post-switch, because doing so requires an increase in CD4+ T cell counts.

While this two-compartment model can produce switching and coexistence, it is oversimplified in assuming that X4 cannot infect any memory CD4+ T cells. In fact, despite being outcompeted by R5 for CCR5+, CXCR4+ CD4+ T cells, X4 productively infects certain memory CD4+ T cells, predominantly those that are resting, CD62L+ [16, 29].

Model 3: Two Compartments with Competition

In order to account for the observed competition of X4 and R5 viruses for the infection of memory CD4+ T cells, which allows X4 to increase in response to CCR5 inhibition as seen in in vivo experiments [69], and in order to prevent competitive exclusion of the less fit viral strain, we combine Models 1 and 2, allowing X4s infection of both nave and memory CD4+ T cells:

$$\begin{aligned}
\dot{N} &= \lambda + (1-2f)a_n N - k_{N4} V_4 N - d_N N \\
\dot{M} &= 2f a_n N + a_m M - k_{M5} V_5 M - k_{M4} V_4 M - d_M M \\
\dot{N}_4 &= k_{N4} V_4 N - \delta I_4 \\
\dot{M}_4 &= k_{M4} V_4 M - \delta I_4 \\
\dot{M}_5 &= k_{M5} V_5 M - \delta I_5 \\
\dot{V}_4 &= p(a_n N_4 + a_m M_4) - c V_4 \\
\dot{V}_5 &= p a_m M_5 - c V_5
\end{aligned}$$

In this model, k_{N4}, k_{M4}, and k_{M5}, are the infection rate coefficients of X4 and R5 for naive (N) and memory (M) CD4+ T cells, while N4, M4, and M5 are the infected cell concentrations corresponding to the originating target cell and infecting viral strain. All other parameters, variables, and initial conditions have been previously defined. Because CCR5 is far more strongly expressed on memory CD4+ T cells than is CXCR4 [29] (Table 1), we set $k_{M5} > k_{M4}$. Conversely, CXCR4 is more highly expressed on naive CD4+ T cells than on memory CD4+ T cells [29], making $k_{N4} >> k_{M4}$. Given that X4 and R5 were already shown to

coexist in Model 2 due to decreases in N/M post-switch, coexistence results in this extended dual compartment model as well [63]. To show this graphically, we note that the relative fitness of X4 to R5 is given by:

$$R_{\text{eff4}}/R_{\text{eff5}}(t) = (k_{N4}/k_{M5}) * a_n(CD4(t))/a_m(CD4(t)) * (N(t)/M(t)) + k_{M4}/k_{M5} \tag{15a}$$

Simulations show $R_{\text{eff4}}/R_{\text{eff5}}$ rising to a local maximum before the R5 to X4 switch only to decrease post-switch due to an X4-driven decrease in N/M. $R_{\text{eff4}}/R_{\text{eff5}}$ eventually fixates at the value 1, allowing for coexistence of R5 and X4 strains post-switch (Supporting Figure 2).

In our previous paper [63], we derived the following switch conditions for Model 3:

$$(a_n(CD4(t^*))/a_m(CD4(t^*))) > (M_5(t^*) - M_4(t^*))/N_4(t^*) \tag{15b}$$

$$CD4(t^*) < 200 \times \frac{N_4(t^*) + M_4(t^*) - M_5(t^*)}{M_5(t^*) - M_4(t^*) + .19N_4(t^*)} \tag{16}$$

As in the preceding models, Equation (16) implies that, with the exception of changes to k_{M5}, modulating parameters to accelerate CD4+ T cell decline hastens an R5 to X4 switch while changing parameters to mitigate CD4+ T cell decline hinders a phenotypic switch. Thus, successful antiretroviral therapy will generally inhibit X4 emergence. However, as one might predict from our results in Model 1, because R5 and X4 are in competition, CCR5 inhibitors can generate more complicated kinetics. In fact, the utility of CCR5 inhibitors depends on the strength of the competition between X4 and R5 virus, which is modulated by X4s infection rate coefficient for memory CD4+ T cells, k_{M4}. To see how k_{M4} modulates the efficacy of anti-CCR5 treatments, we simulated Model 3 in two representative k_{M4} regimes, labeled non-competitive and competitive, respectively. In the non-competitive regime, the parameter values were: $\lambda = 33 cells/(\mu\text{l} \times \text{day})$, c=23/day, p=2100/day, f=0.8, δ=0.5/day, k_{N4}, k_{M4}, k_{M5} = 0.00108, 4×10^{-5}, and 0.0068 μl/(virions$\times$day), respectively. In the competitive regime, the only two changes are k_{M4} is increased to 5×10^{-4} and k_{N4} is correspondingly decreased to 0.001 to keep X4 in check. In the non-competitive regime, we found X4s viral set-point to be a monotonically decreasing function of the CCR5 inhibitors efficacy (Fig. 3a, left panel). This is because, due to the low value of k_{M4}, X4 is unable to infect the majority of target cells blocked from R5 infection, allowing CD4 counts to rise and causing a drop in activation levels. Conversely, in the competitive regime where k_{M4} is closer in value to k_{M5} but still less than k_{M5} to remain consistent with FACS data [44], we found X4s viral set-point to be a monotonically increasing function of the CCR5 inhibitors efficacy (Fig. 3a, right panel).

In the competitive regime the steady-state CD4+ T cell count is not decreased by CCR5 inhibition (Fig. 3a, right panel), which might lead one to suspect that these treatments are safe in this regime as well. Critically, however, the CD4+ T cell count crashes far sooner in the competitive regime when anti-CCR5 treatment is employed (Fig. 3b). Thus, CCR5 inhibitors may accelerate immunodeficiency in patients with a competitive X4 virus.

Why is this the case? CCR5 inhibitors decrease k_{M5}, causing R5s viral load to decline, and memory CD4+ T cell counts to increase. X4 is now able to infect some of these newly generated memory CD4+ T cells, but X4s ability to do so

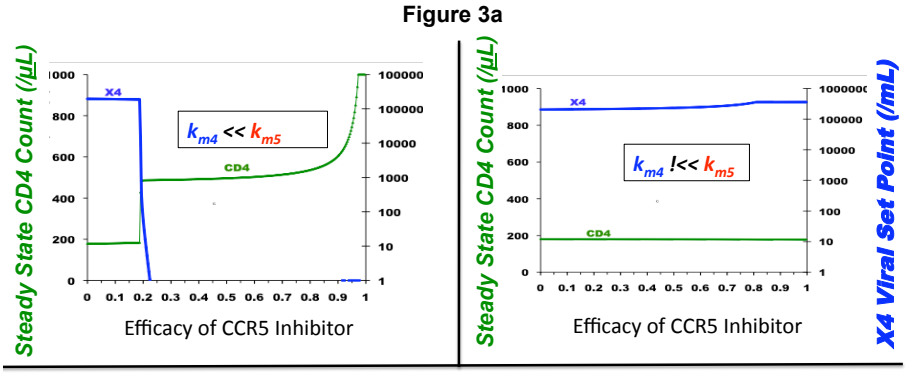
depends on k_{M4}. With k_{M4} sufficiently large (the competitive regime), X4 infects a non-negligible fraction of newly generated memory CD4+ cells, increasing X4s viral load. But with a larger viral load, X4 is now better able to infect its main target cell pool: nave CD4+ T cells, the untapped target cell reserve where CXCR4 is highly present. The slight increase in memory CD4+ T cell counts due to CCR5 inhibition thus causes severe and accelerated depletion of the nave CD4+ T cell population in this competitive regime (Fig. 3c). Thus, a single parameter, k_{M4}, controls the efficacy of anti-CCR5 therapy in dually infected HIV patients, highlighting the need for circumspection in prescribing these treatments.

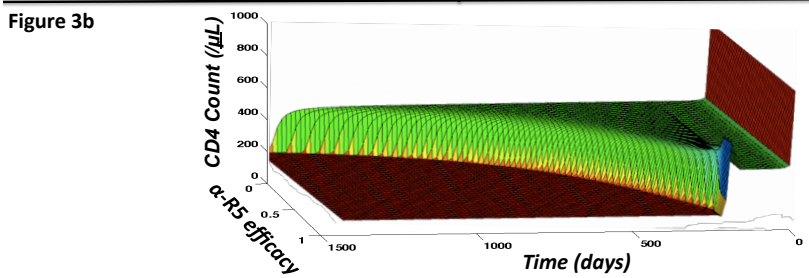
3. **Discussion.** In this paper a set of mathematical models for dual R5, X4 infection in HIV has been rigorously derived from a multi-strain version of the basic model of viral dynamics. The models were analyzed to show how an increase in the ratio of the fractions of activated nave and memory CD4+ T cells (an/am) can trigger an R5 to X4 phenotypic switch in dually infected individuals. Importantly, this allows for phenotypic switching even when the fraction of activated nave CD4+ T cells increases at a slower rate than the corresponding fraction of memory CD4+ T cells (as long as the ratio of the two fractions increases beyond a threshold). Our models also help explain why 50% of patients do not manifest a noticeable switch to X4 virus: their relevant parameter regimes may simply keep a_n/a_m below the threshold. Finally, anti-CCR5 treatment is shown to promote X4 virus even in the simplest competitive framework (Model 1).

While we predict that R5 blockers can promote X4 emergence in dual infection, we find that non-CCR5 specific, antiretroviral therapies such as HAART have the opposite effect. This prediction is in fact supported by a recent clinical trial on 15 women with X4 virus prior to undergoing HAART [42]. During HAART, the patients showed marked increases in CD4+ T cell counts as well as a correlated reversion in viral tropism toward CCR5. Other groups have also found that antiretroviral treatment inhibits X4 virus [12, 34, 53]. However, one group claims that HAART promotes R5 to X4 switching [9]. Delobel and colleagues conclusion arises from an analysis of the genotypes of peripheral blood mononuclear cells of patients on HAART for more than three years with viral loads below detection. Notwithstanding the overall utility of testing coreceptor usage when viremia levels are below detection, the genotypic algorithms used by Delobel et al. [9] do not always correctly predict the actual coreceptor usage [31]. To avoid such errors, the Philpott study, which found preferential suppression of X4 strains in patients on HAART, used a phenotypic MT-2 cell line characterization and a direct HOS-CD4 coreceptor binding assay in addition to a genotypic V3 analysis [42].

An activation-induced switch and its deleterious clinical effects are also consistent with the proposed new paradigm of lentiviral pathogenicity, which argues that immune overactivation is the distinguishing characteristic of symptomatic lentiviral infection in new hosts as opposed to asymptomatic lentiviral infection in natural hosts [4, 22, 23, 36, 38, 48, 50-52]. Evidence of a correlation between immune activation and disease progression is also supported by the fact that T cell activation levels are lowered almost immediately following successful HAART [23].

One might argue that phenotypic switching in HIV has little to do with target-cell activation levels and is instead the result of cumulative mutations that occur over the course of HIV. One would have two reasons for such an argument. First, given its status as a retrovirus, HIV is extremely prone to mutation [32]. Second, it





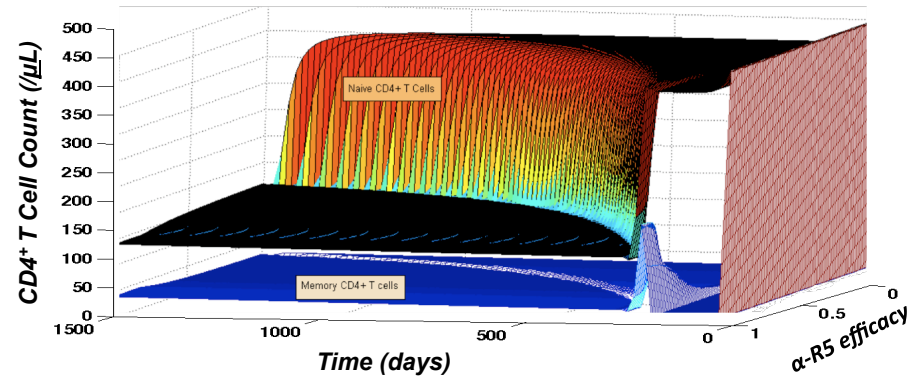


FIGURE 3. In the more realistic Model 3, CCR5 inhibitors can also fail to suppress X4 emergence. In the non-competitive regime of Model 3 (a, left panel), increasing the efficacy of a permanent CCR5 inhibitor first given at t=180 days, increases steady-state CD4 counts and decreases steady-state X4 levels. However, in the competitive regime (a, right panel), anti-CCR5 treatment does not depress steady-state X4 levels and it does not increase steady-state CD4 counts. While steady-state X4 levels and CD4 counts remain unchanged under this treatment schedule, in (b) the time at which this steady-state occurs is shown to be earlier. Thus, CCR5 inhibitors can accelerate CD4 depletion. In (c), this accelerated outcome is shown to occur via temporal gains in memory CD4 counts triggered by an initially effective anti-CCR5 treatment. The competitive X4 virus then increases by infecting newly generated memory cells, after which X4 can severely deplete nave CD4+ T cells, counteracting initial gains in memory CD4s.

takes very few mutations to go from R5 to X4 virus. For example, Ho et al. showed that in rhesus macaques ten amino acid changes in the V3 loop of an X4-tropic virus are sufficient to modify viral coreceptor usage to CCR5 [26]. That said, many V3 mutations yield viruses with lower fitness, implying that fitness troughs exist between R5 and X4 variants [39]. Perhaps as a result, mutation between R5 and X4 strains does not seem to be common in vivo [13, 57]. When drugs are employed to selectively block CCR5 in cases of R5-only infection, HIVs method of escape is not to evolve tropism for CXCR4 but to find a novel way of binding CCR5 despite the blockage [56, 57]. Finally, X4 is simply outcompeted by R5 during early dual-infection, arguing in favor of an early exogenous selection pressure toward R5, which is mitigated over the course of infection in switching patients. Current data are insufficient to test our conclusion that the efficacy of CCR5 blockers in dual infection depends on k_{M4}, because k_{M4} has an unknown value. The importance of testing whether CCR5 inhibitors have only partial regimes of utility stems from the fact that these treatments, in contrast to HAART and traditional antiretroviral drugs, are mainly non-toxic [58]. In fact, CCR5Δ32/Δ32, a natural deletion mutation that prevents CCR5 expression, is found in 1% of humans with no known side-effects. The question associated with CCR5 blockers is whether they promote X4 in R5s stead. This is because X4 virus quickly depletes R5-immune nave CD4+ T cells, compounding the earlier immunodeficiency that R5 engendered in the memory compartment. Naive CD4+ T cells are the source for new memory cells and a prime defense against unseen infections: hence, the victim of a phenotypic switch gets the worst of both worlds, memory CD4+ T cell loss by R5 followed by nave CD4+ T cell loss by X4, greatly lessening the chance of survival.

Methods

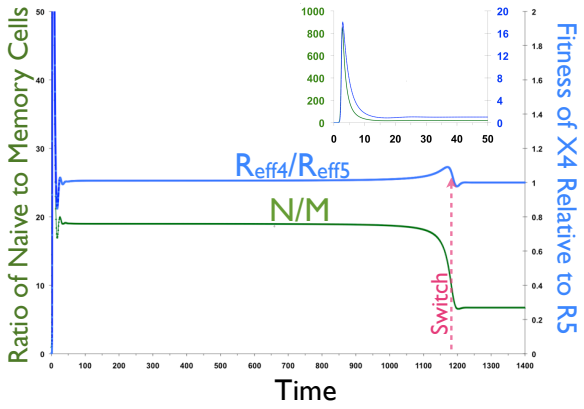
Models 1-3 were first solved numerically using the program Berkeley Madonna. We used the Rosenbruck algorithm for solving stiff ODEs with the parameters given in the Figures. Other than k_{N4}, k_{M4}, k_{M5} and f, these parameters have been estimated from in vivo measurements. Further, V4 and V5 were each given initial values of 1000 virions/ml, as in [46, 63], which reflects experiments in macaques in which high viral doses are given to ensure infection [69].

Since the purpose of the first two models is to motivate the added complexity in Model 3 and since our main conclusions are taken from Model 3, we offer a justification of the parameter values for Model 3. In particular, λ, the rate at which nave CD4+ T cells emigrate from the thymus, has been shown to remain relatively constant during HIV infection [20]. Following a recent theoretical analysis [64], we set λ to the constant value of 33 cells/(μl$\times$day). The viral clearance rate, c, has been directly measured to have an average value of 23/day [43]. The rate of virion production by productively infected cells, p, was set to 2100/day, which is line with the in vivo measure in [18] but smaller than the value reported in [5]. Finally, we set the infected cell death rate δ to 0.5/day, following the measurements in [41], although values as high as 1.0/day are also feasible [33]. The final four parameters k_{N4}, k_{M4}, k_{M5} and f have unknown values, but can nonetheless be substantially restricted. The fraction of nave CD4+ T cells that are activated by antigen, f, is between 0 and 1. Furthermore, the FACS data summarized in Table 1 leads us to restrict the infection rate coefficients as follows: $k_{N4} >> k_{M4}$, $k_{M5} >> k_{M4}$. We chose exact values for these 4 parameters, subject to the above constraints,

by repeated simulations of our Models so as to produce the general dynamics of long-term HIV infection, including the common phenotypic switch.

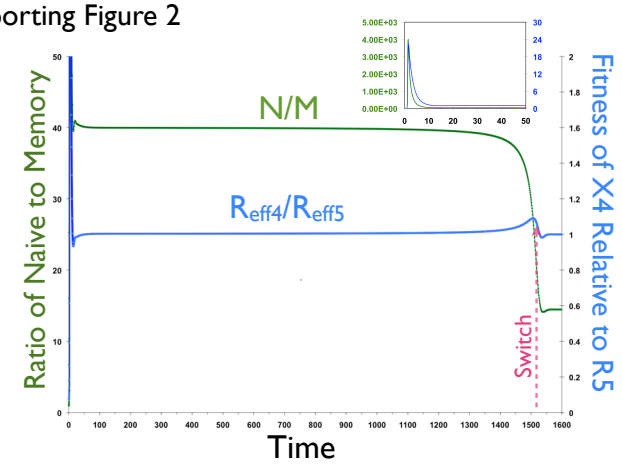
We also note that because simulations require an exact form for a_n and a_m, we used the particular form fit in (1). Of course, the analysis throughout this paper shows that we can apply any equations which satisfy (2), with obvious parameter adjustments. Subsequent to these simulations, we reproduced our work in MATLAB (with the stiff solver, ode23s) so that we could generate three-dimensional plots and show that the switch is accelerated when CCR5 is blocked in competitive regimes (i.e., those situations in which k_{M4} is relatively large). All code is available upon request.

Supporting Figure 1



Evolution of N/M and R_{eff4}/R_{eff5} in Model 2. A representative simulation of Model 2 illustrates how the fraction of naïve to memory CD4+ T cells (N/M) increases rapidly during the initial phase of HIV infection (inset shows a close-up of the first 50 days), decreasing to a quasi-set point that slowly drops throughout the pre-switch period. With N/M relatively constant in the period before the switch, R_{eff4}/R_{eff5} slowly increases due to a_n/a_m rising as CD4 counts decrease. N/M decreases post-switch when X4 depletes naïve CD4+ T cells, allowing for coexistence in Model 2 as R_{eff4}/R_{eff5} drops to 1.

Supporting Figure 2



Evolution of N/M and R_{eff4}/R_{eff5} in Model 3. A representative simulation of Model 3 shows the same pattern as observed above in Model 2. The fraction of naïve to memory CD4+ T cells (N/M) increases rapidly during the initial phase of HIV infection (inset shows a close-up of first 50 days), decreasing to a quasi-set point that slowly decreases throughout the pre-switch period. With N/M relatively constant in the period preceding the switch, R_{eff4}/R_{eff5} slowly increases as a_n/a_m rises from HIV-driven reductions in the CD4 count. Again N/M decreases post-switch as X4 depletes naïve CD4+ T cells, allowing for coexistence in Model 3 when $R_{eff4}/R_{eff5} = 1$.

Acknowledgments. We thank two anonymous reviewers for feedback that greatly improved this manuscript and U. Andrews, J. Bowman, H. Dahari, P. Hraber, A. Nourmohammad and L. Rong for helpful discussions. ADW also wishes to acknowledge H. Lecar, R. Ribeiro, and L. Weinberger for extensive and informative suggestions.

Portions of this work were done under the auspices of the U. S. Department of Energy under contract DE-AC52-06NA25396 and supported by NIH grants AI28433, RR06555, and P20-RR18754 (ASP). ADW gratefully acknowledges the support of the California HIV/AIDS Research Program (Dissertation Award).

REFERENCES

[1] H. Blaak, A. B. van't Wout, M. Brouwer, B. Hooibrink, E. Hovenkamp and H. Schuitemaker, *In vivo HIV-1 infection of CD45RA(+)CD4(+) T cells is established primarily by syncytium-inducing variants and correlates with the rate of CD4(+) T cell decline*, Proc. Natl. Acad. Sci. U.S.A., **97** (2000), 1269–1274.

[2] D. S. Callaway, R. M. Ribeiro and M. A. Nowak, *Virus phenotype switching and disease progression in HIV-1 infection*, Proc. Roy. Sci. B, **266** (1999), 2523–2530.

[3] C. H. Casper, P. Clevestig, E. Carlenor, T. Leitner, B. Anzén, K. Lidman, E. Belfrage, J. Albert, A. B. Bohlin, L. Navér, S. Lindgren, E. M. Fenyö and A. C. Ehrnst, *Link between the X4 phenotype in human immunodeficiency virus type 1-infected mothers and their children, despite the early presence of R5 in the child*, J. Infect. Dis., **186** (2002), 914–921.

[4] L. A. Chakrabarti, S. R. Lewin, L. Zhang, A. Gettie, A. Luckay, L. N. Martin, E. Skulsky, D. D. Ho, C. Cheng-Mayer and P. A. Marx, *Normal T-cell turnover in sooty mangabeys harboring active simian immunodeficiency virus infection*, J. Virol., **74** (2000), 1209–1223.

[5] H. Y. Chen, M. Di Mascio, A. S. Perelson, D. D. Ho and L. Zhang, *Determination of virus burst size in vivo using a single-cycle SIV in rhesus macaques*, Proc. Natl. Acad. Sci. U.S.A., **104** (2007), 19079–19084.

[6] E. Coakley, C. J. Petropoulos and J. M. Whitcomb, *Assessing chemokine co-receptor usage in HIV*, Curr. Opin. Infect. Dis., **18** (2005), 9–15.

[7] M. Cornelissen, G. Mulder-Kampinga, J. Veenstra, F. Zorgdrager, C. Kuiken, S. Hartman, J. Dekker, L. van der Hoek, C. Sol and R. Coutinho, *Syncytium-inducing (SI) phenotype suppression at seroconversion after intramuscular inoculation of a non-syncytium-inducing/SI phenotypically mixed human immunodeficiency virus population*, J. Virol., **69** (1995), 1810–1818.

[8] M. P. Davenporta, J. J. Zaundersb, M. D. Hazenbergc, H. Schuitemakerc and R. P. van Rijc, *Cell turnover and cell tropism in HIV-1 infection*, Trends Microbiol., **10** (2002), 275–278.

[9] P. Delobel, K. Sandres-Sauné, M. Cazabat, C. Pasquier, B. Marchou, P. Massip and J. Izopet, *R5 to X4 switch of the predominant HIV-1 population in cellular reservoirs during effective highly active antiretroviral therapy*, J. Acquir. Immune. Defic. Syndr., **38** (2005), 382–392.

[10] D. C. Douek, *Disrupting T-cell homeostasis: How HIV-1 infection causes disease*, AIDS Rev., **5** (2003), 172–177.

[11] D. C. Douek, L. J. Picker and R. A. Koup, *T cell dynamics in HIV-1 infection*, Annu. Rev. Immunol., **21** (2003), 265–304.

[12] O. Equils, E. Garratty, L. S. Wei, S. Plaeger, M. Tapia, J. Deville, P. Krogstad, S. Myung-Shin, K. Nielsen and Y. J. Bryson, *Recovery of replication-competent virus from CD4 T cell reservoirs and change in coreceptor use in human immunodeficiency virus type 1-infected children responding to highly active antiretroviral therapy*, J. Infect. Dis., **182** (2000), 751–757.

[13] J. M. Farber and E. A. Berger, *HIV's response to a CCR5 inhibitor: I'd rather tighten than switch!*, Proc. Natl. Acad. Sci. U.S.A., **99** (2002), 1749–1751.

[14] J. V. Giorgi, L. E. Hultin, J. A. McKeating, T. D. Johnson, B. Owens, L. P. Jacobson, R. Shih, J. Lewis, D. J. Wiley, J. P. Phair, S. M. Wolinsky and R. Detels, *Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage*, J.

Infect. Dis., **179** (1999), 859–870.

[15] F. Gondois-Rey, A. Biancotto, M. A. Fernandez, L. Bettendroffer, J. Blazkova, K. Trejbalova, M. Pion and I. Hirsch, *R5 variants of human immunodeficiency virus type 1 preferentially infect CD62L- CD4+ T cells and are potentially resistant to nucleoside reverse transcriptase inhibitors*, J. Virol., **80** (2006), 854–865.

[16] F. Gondois-Rey, J. C. Grivel, A. Biancotto, M. Pion, R. Vigne, L. B. Margolis and I. Hirsch, *Segregation of R5 and X4 HIV-1 variants to memory T cell subsets differentially expressing CD62L in ex vivo infected human lymphoid tissue*, AIDS, **16** (2002), 1245–1249.

[17] A. T. Haase, *Population biology of HIV-1 infection: Viral and CD4+ T cell demographics and dynamics in lymphatic tissues*, Annu. Rev. Immunol., **17** (1999), 625–656.

[18] A. T. Haase, K. Henry, M. Zupancic, G. Sedgewick, R. A. Faust, H. Melroe, W. Cavert, K. Gebhard, K. Staskus, Z. Q. Zhang, P. J. Dailey, H. H. Balfour Jr, A. Erice and A. S. Perelson, *Quantitative image analysis of HIV-1 infection in lymphoid tissue*, Science, **274** (1996), 985–989.

[19] J. M. Harouse, C. Buckner, A. Gettie, R. Fuller, R. Bohm, J. Blanchard and C. Cheng-Mayer, *CD8+ T cell-mediated CXC chemokine receptor 4-simian/human immunodeficiency virus suppression in dually infected rhesus macaques*, Proc. Natl. Acad. Sci. U.S.A., **100** (2003), 10977–10982.

[20] M. D. Hazenberg, S. A. Otto, J. W. Cohen Stuart, M. C. Verschuren, J. C. Borleffs, C. A. Boucher, R. A. Coutinho, J. M. Lange, T. F. Rinke de Wit, A. Tsegaye, J. J. van Dongen, D. Hamann, R. J. de Boer and F. Miedema, *Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naive T cell population in HIV-1 infection*, Nat. Med., **6** (2000), 1036–1042.

[21] M. D. Hazenberg, S. A. Otto, D. Hamann, M. T. Roos, H. Schuitemaker, R. J. de Boer and F. Miedema, *Depletion of naive CD4 T cells by CXCR4-using HIV-1 variants occurs mainly through increased T-cell death and activation*, AIDS, **17** (2003), 1419–1424.

[22] M. D. Hazenberg, S. A. Otto, B. H. van Benthem, M. T. Roos, R. A. Coutinho, J. M. Lange, D. Hamann, M. Prins and F. Miedema, *Persistent immune activation in HIV-1 infection is associated with progression to AIDS*, AIDS, **17** (2003), 1881–1888.

[23] M. D. Hazenberg, J. W. Stuart, S. A. Otto, J. C. Borleffs, C. A. Boucher, R. J. de Boer, F. Miedema and D. Hamann, *T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: A longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART)*, Blood, **95** (2000), 249–255.

[24] R. L. Hengel, V. Thaker, M. V. Pavlick, J. A. Metcalf, G. Dennis Jr, J. Yang, R. A. Lempicki, I. Sereti and H. C. Lane, *Cutting edge: L-selectin (CD62L) expression distinguishes small resting memory CD4+ T cells that preferentially respond to recall antigen*, J. Immunol., **170** (2003), 28–32.

[25] D. D. Ho, A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard and M. Markowitz, *Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection*, Nature, **373** (1995), 123–126.

[26] S. H. Ho, L. Shek, A. Gettie, J. Blanchard and C. Cheng-Mayer, *V3 loop-determined coreceptor preference dictates the dynamics of CD4+-T-cell loss in simian-human immunodeficiency virus-infected macaques*, J. Virol., **79** (2005), 12296–12303.

[27] M. Joly and J. M. Pinto, *CXCR4 and CCR5 regulation and expression patterns on T- and monocyte-macrophage cell lineages: Implications for susceptibility to infection by HIV-1*, Math. Biosci., **195** (2005), 92–126.

[28] E. E. Giorgi, J. F. Salazar-Gonzalez, J. M. Decker, K. T. Pham, M. G. Salazar, C. Sun, T. Grayson, S. Wang, H. Li, X. Wei, C. Jiang, J. L. Kirchherr, F. Gao, J. A. Anderson, L. H. Ping, R. Swanstrom, G. D. Tomaras, W. A. Blattner, P. A. Goepfert, J. M. Kilby, M. S. Saag, E. L. Delwart, M. P. Busch, M. S. Cohen, D. C. Montefiori, B. F. Haynes, B. Gaschen, G. S. Athreya, H. Y. Lee, N. Wood, C. Seoighe, A. S. Perelson, T. Bhattacharya, B. T. Korber, Hahn B. H. and G. M. Shaw, *Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection*, Proc. Natl. Acad. Sci. U.S.A., **105** (2008), 7552–7557.

[29] B. Lee M. Sharron, L. J. Montaner, D. Weissman and R. W. Doms, *Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages*, Proc. Natl. Acad. Sci. U.S.A., **96** (1999), 5215–5220.

[30] Q. Li, L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller and A. T. Haase, *Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells*, Nature, **434** (2005), 1148–1152.

[31] A. J. Low, W. Dong, D. Chan, T. Sing, R. Swanstrom, M. Jensen, S. Pillai, B. Good and P. R. Harrigan, *Current V3 genotyping algorithms are inadequate for predicting X4 co-receptor usage in clinical isolates*, Aids, **21** (2007), F17–24.

[32] L. M. Mansky and H. M. Temin, *Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase*, J. Virol., **69** (1995), 5087–5094.

[33] M. Markowitz, M. Louie, A. Hurley, E. Sun, M. Di Mascio, A. S. Perelson and D. D. Ho, *A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo*, J. Virol., **77** (2003), 5037–5038.

[34] T. Melby, M. Despirito, R. Demasi, G. Heilek-Snyder, M. L. Greenberg and N. Graham, *HIV-1 coreceptor use in triple-class treatment-experienced patients: Baseline prevalence, correlates, and relationship to enfuvirtide response*, J. Infect. Dis., **194** (2006), 238–246.

[35] H. Mohri, S. Bonhoeffer, S. Monard, A. S. Perelson and D. D. Ho, *Rapid turnover of T lymphocytes in SIV-infected rhesus macaques*, Science, **279** (1998), 1223–1227.

[36] H. Mohri, A. S. Perelson, K. Tung, R. M. Ribeiro, B. Ramratnam, M. Markowitz, R. Kost, A. Hurley, L. Weinberger, D. Cesar, M. K. Hellerstein and D. D. Ho, *Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy*, J. Exp. Med., **194** (2001), 1277–1287.

[37] J. P. Moore, S. G. Kitchen, P. Pugach and J. A. Zack, *The CCR5 and CXCR4 coreceptors–central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection*, AIDS Res. Hum. Retroviruses., **20** (2004), 111–126.

[38] M. Paiardini, I. Frank, I. Pandrea, C. Apetrei and G.Silvestri, *Mucosal immune dysfunction in AIDS pathogenesis*, AIDS Rev., **10** (2008), 36–46.

[39] C. Pastore, R. Nedellec, A. Ramos, S. Pontow, L. Ratner and D. E. Mosier, *Human immunodeficiency virus type 1 coreceptor switching: V1/V2 gain-of-fitness mutations compensate for V3 loss-of-fitness mutations*, J. Virol., **80** (2006), 750–758.

[40] M. L. Penn, J. C. Grivel, B. Schramm, M. A. Goldsmith and L. Margolis, *CXCR4 utilization is sufficient to trigger CD4+ T cell depletion in HIV-1-infected human lymphoid tissue*, Proc. Natl. Acad. Sci. U.S.A., **96** (1999), 663–668.

[41] A. S. Perelson, A. U. Neumann, M. Markowitz, J. M. Leonard and D. D. Ho, *HIV-1 dynamics in vivo: Virion clearance rate, infected cell life-span, and viral generation time*, Science, **271** (1996), 1582–1586.

[42] S. Philpott, B. Weiser, K. Anastos, C. M. Kitchen, E. Robison, W. A. Meyer, H. S. Sacks, U. Mathur-Wagh, C. Brunner and H. Burger *Preferential suppression of CXCR4-specific strains of HIV-1 by antiviral therapy*, J. Clin. Invest., **107** (2001), 431–438.

[43] B. Ramratnam, S. Bonhoeffer, J. Binley, A. Hurley, L. Zhang, J. E. Mittler, M. Markowitz, J. P. Moore, A. S. Perelson and D. D. Ho, *Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis*, Lancet, **354** (1999), 1782–1785.

[44] R. Regoes and S. Bonhoeffer, *HIV coreceptor usage and drug treatment*, J. Theor. Biol., **217** (2002), 443–457.

[45] R. R. Regoes and S. Bonhoeffer, *The HIV coreceptor switch: A population dynamical perspective*, Trends Microbiol., **13** (2005), 269–277.

[46] R. M. Ribeiro, M. D. Hazenberg, A. S. Perelson and M. P. Davenport, *Naive and memory cell turnover as drivers of CCR5-to-CXCR4 tropism switch in human immunodeficiency virus type 1: Implications for therapy*, J. Virol., **80** (2006), 802–809.

[47] D. D. Richman and S. A. Bozzette, *The impact of the syncytium-inducing phenotype of human immunodeficiency virus on disease progression*, J. Infect. Dis., **169** (1994), 968–974.

[48] N. Sachsenberg, A. S. Perelson, S. Yerly, G. A. Schockmel, D. Leduc, B. Hirschel and L. Perrin, *Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by*

Ki-67 antigen, J. Exp. Med., **187** (1998), 1295–1303.

[49] H. Schuitemaker, M. Koot, N. A. Kootstra, M. W. Dercksen, R. E. de Goede, R. P. van Steenwijk, J. M. Lange, J. K. Schattenkerk, F. Miedema and M. Tersmette, *Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: Progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population*, J. Virol., **66** (1992), 1354–1360.

[50] G. Silvestri, *Naturally SIV-infected sooty mangabeys: Are we closer to understanding why they do not develop AIDS?*, J. Med. Primatol., **34** (2005), 243–252.

[51] G. Silvestri, M. Paiardini, I. Pandrea, M. M. Lederman and D. L. Sodora, *Understanding the benign nature of SIV infection in natural hosts*, J. Clin. Invest., **117** (2007), 3148–3154.

[52] G. Silvestri, D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M. McClure, S. I. Staprans and M. B. Feinberg, *Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia*, Immunity, **18** (2003), 441–452.

[53] K. Skrabal, V. Trouplin, B. Labrosse, V. Obry, F. Damond, A. J. Hance, F. Clavel and F. Mammano, *Impact of antiretroviral treatment on the tropism of HIV-1 plasma virus populations*, Aids, **17** (2003), 809–814.

[54] I. J. Spijkerman, M. Koot, M. Prins, I. P. Keet, A. J. van den Hoek, F. Miedema and R. A. Coutinho, *Lower prevalence and incidence of HIV-1 syncytium-inducing phenotype among injecting drug users compared with homosexual men*, Aids, **9** (1995), 1085–1092.

[55] A. Suarez, L. Mozo and C. Gutierrez, *Generation of CD4(+)CD45RA(+) effector T cells by stimulation in the presence of cyclic adenosine 5'-monophosphate-elevating agents*, J. Immunol., **169** (2002), 1159–1167.

[56] J. C. Tilton, H. Amrine-Madsen, J. L. Miamidian, K. M. Kitrinos, J. Pfaff, J. F. Demarest, N. Ray, J. L. Jeffrey, C. C. Labranche and R. W. Doms, *HIV type 1 from a patient with baseline resistance to CCR5 antagonists uses drug-bound receptor for entry*, AIDS Res. Hum. Retroviruses, **26**, 13–24.

[57] A. Trkola, S. E. Kuhmann, J. M. Strizki, E. Maxwell, T. Ketas, T. Morgan, P. Pugach, S. Xu, L. Wojcik, J. Tagat, A. Palani, S. Shapiro, J. W. Clader, S. McCombie, G. R. Reyes, B. M. Baroudy and J. P. Moore, *HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use*, Proc. Natl. Acad. Sci. U.S.A., **99** (2002), 395–400.

[58] A. M. Tsibris and D. R. Kuritzkes, *Chemokine antagonists as therapeutics: Focus on HIV-1*, Annu. Rev. Med., **58** (2007), 445–459.

[59] D. Unutmaz, F. Baldoni and S. Abrignani, *Human naive T cells activated by cytokines differentiate into a split phenotype with functional features intermediate between naive and memory T cells*, Int. Immunol., **7** (1995), 1417–1424.

[60] D. Unutmaz, P. Pileri and S. Abrignani, *Antigen-independent activation of naive and memory resting T cells by a cytokine combination*, J. Exp. Med., **180** (1994), 1159–1164.

[61] R. S. Veazey, K. G. Mansfield, I. C. Tham, A. C. Carville, D. E. Shvetz, A. E. Forand and A. A. Lackner, *Dynamics of CCR5 expression by CD4(+) T cells in lymphoid tissues during simian immunodeficiency virus infection*, J. Virol., **74** (2000), 11001–11007.

[62] X. Wei, S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowak and B. H. Hahn, *Viral dynamics in human immunodeficiency virus type 1 infection*, Nature, **373** (1995), 117–122.

[63] A. D. Weinberger, A. S. Perelson, R. M. Ribeiro and L. S. Weinberger, *Accelerated immunodeficiency by anti-CCR5 treatment in HIV infection*, PLoS Comput. Biol., **5** (2009), e1000467.

[64] L. S. Weinberger, D. V. Schaffer and A. P. Arkin, *Theoretical design of a gene therapy to prevent AIDS but not human immunodeficiency virus type 1 infection*, J. Virol., **77** (2003), 10028–10036.

[65] M. Westby, M. Lewis, J. Whitcomb, M. Youle, A. L. Pozniak, I. T. James, T. M. Jenkins, M. Perros and E. van der Ryst, *Emergence of CXCR4-using human immunodeficiency virus type 1 (HIV-1) variants in a minority of HIV-1-infected patients following treatment with the CCR5 antagonist maraviroc is from a pretreatment CXCR4-using virus reservoir*, J. Virol., **80** (2006), 4909–4920.

[66] M. Westby and E. van der Ryst, *CCR5 antagonists: Host-targeted antivirals for the treatment of HIV infection*, Antivir. Chem. Chemother., **16** (2005), 339–354.
[67] D. Wodarz, A. L. Lloyd, V. A. Jansen and M. A.Nowak, *Dynamics of macrophage and T cell infection by HIV*, J. Theor. Biol., **196** (1999), 101–113.
[68] D. Wodarz and M. A. Nowak, *The effect of different immune responses on the evolution of virulent CXCR4-tropic HIV*, Proc. Roy. Sci. B, **265** (1998), 2149–2158.
[69] S. M. Wolinsky, R. S. Veazey, K. J. Kunstman, P. J. Klasse, J. Dufour, A. J. Marozsan, M. S. Springer and J. P. Moore, *Effect of a CCR5 inhibitor on viral loads in macaques dual-infected with R5 and X4 primate immunodeficiency viruses*, Virology, **328** (2004), 19–29.
[70] Z. Q. Zhang, S. W. Wietgrefe, Q. Li, M. D. Shore, L. Duan, C. Reilly, J. D. Lifson and A. T. Haase, *Roles of substrate availability and infection of resting and activated CD4+ T cells in transmission and acute simian immunodeficiency virus infection*, Proc. Natl. Acad. Sci. USA, **101** (2004), 5640–5645.

Received March 2, 2010; Accepted November 2, 2010.

E-mail address: asp@lanl.gov;arielw@berkeley.edu