

Mini-Review

The CCR5 and CXCR4 Coreceptors—Central to Understanding the Transmission and Pathogenesis of Human Immunodeficiency Virus Type 1 Infection

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ABSTRACT

In this review, we will discuss what is known, what is suspected, and what still remains obscure about the central role played by coreceptor expression and usage in the transmission and pathogenic consequences of human immunodeficiency virus type 1 (HIV-1) infection. An emphasis will be on the HIV-1 phenotypic variants that are defined by their usage of the CCR5 or CXCR4 coreceptors, and how the different cellular tropism of these variants influences how and where HIV-1 replicates *in vivo*. We will also review what might happen when coreceptor antagonists are used clinically to treat HIV-1 infection.

INTRODUCTION

THE INDEPENDENT DISCOVERIES IN LATE 1995 by Lusso and Gallo that CC-chemokines could inhibit HIV-1 replication, and by Berger in early 1996 that the CXC-chemokine receptor CXCR4 was the long sought-after coreceptor for some strains of HIV-1, opened up an entirely new area of AIDS research.^{1,2} Soon it was shown that the counterpart to CXCR4 for the most commonly transmitted strains of HIV-1 was the CC-chemokine receptor CCR5, a receptor for the same chemokines that had been shown to be inhibitors of HIV-1 replication only a few months earlier.^{3–7} Moreover, the complete absence of CCR5 from some humans (those homozygous for the defective, $\Delta 32$ -CCR5 allele) was found to be strongly protective against HIV-1 infection *in vitro* and *in vivo*, while decreased CCR5 expression caused by heterozygosity for the $\Delta 32$ -CCR5 allele reduced the rate of disease progression in HIV-1-infected people.^{8–11} In the 7 years since these early, seminal observations, the central role that coreceptor expression and usage play in HIV-1 pathogenesis has become increasingly obvious.

In this article, we will focus on the role played in pathogenesis and transmission by the HIV-1 phenotypic variants that are defined by their usage of CCR5 or CXCR4. It is not our intent to rigorously review every aspect of the relevant literature

that already exists in abundance. For example, there are several, complex ways in which phenotypic variants can affect how the human immune system responds to HIV-1 infection that we will not discuss. The complexities of all the possible interactions between HIV-1 and the many cell types of the immune system are also beyond the scope of this article. Instead, a recent and thorough review by Douek *et al.*¹² should be consulted. HIV-1 infection of the brain will also be ignored, since this organ represents *terra incognita* for the senior authors. Instead, we will highlight some specific topics that we believe merit further discussion.

CORECEPTORS: THE BASICS

The two coreceptors that are the most relevant to HIV-1 replication *in vivo* are CCR5 and CXCR4. More than a dozen other G-protein-coupled receptors can mediate the entry of some HIV-1 strains when they are expressed in transfected cells *in vitro*.^{13,14} However, with very rare exceptions,^{15–19} these receptors are not used by HIV-1 to enter primary CD4⁺ cells *in vitro*, and probably not *in vivo*. Thus, HIV-1 replication in primary cells *in vitro* is usually completely blocked by inhibitors specific for CCR5 or CXCR4, and there is no correlation between disease progression and the use of coreceptors other than

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CCR5 or CXCR4.^{16–18,20–26} *In vivo*, the absence of CCR5 is strongly protective against HIV-1 infection, which is powerful evidence for the paramount importance of this coreceptor for viral replication.^{8,9,11} Moreover, so far as is known, the rare individuals that do acquire HIV-1 infection despite their genetic lack of CCR5 expression are infected by strains that use CXCR4 (and sometimes CCR5 *in vitro*), but not other potential coreceptors.^{27–30} Thus despite the opportunity to diversify their coreceptor usage, the CXCR4-using strains of HIV-1 do not do so under *in vivo* conditions. For these reasons, only CCR5 and CXCR4 are the foci of drug development aimed at inhibiting HIV-1 entry at the coreceptor binding stage.^{31–33} This is not to say that other coreceptors are not, or could not be, relevant to HIV-1 pathogenesis—only that there is at present little evidence in favor of their importance.

The simian immunodeficiency virus (SIV) strains that naturally or experimentally infect macaque monkeys almost never use CXCR4. In contrast, they all use CCR5 and one or more of a wide range of other G-protein-coupled receptors to enter receptor-transfected cells *in vitro*.^{13,14,34} There is some evidence that some SIV (and HIV-2) strains can use an unknown coreceptor(s) to enter primary cells *in vitro*, but whether this coreceptor(s) is relevant to viral replication in macaques is not yet clear.^{24,35} However, the paramount importance of CCR5 and CXCR4 for HIV-2 replication, and of CCR5 for SIV replication, seems apparent.^{24,35–38}

Years before the coreceptors were identified, it was recognized that two major phenotypic variants of HIV-1 existed, and that they had different impacts on the rate of disease progression in infected people.^{39,40} In those days, the phenotypic variants were differentiated by their ability to replicate, and form syncytia, in the MT-2 cell line. The viruses that were able to form syncytia in MT-2 cells were named “syncytium-inducing” (SI) viruses, while those that could not were, logically enough, designated “non-syncytium-inducing” (NSI) viruses.³⁹ The isolation of SI viruses from an infected person was a poor prognostic indicator; the overt appearance of these viruses was associated with an accelerated rate of loss of CD4⁺ T cells, and a relatively rapid progression to AIDS and death.^{40–43} Fortunately, it was also apparent that SI viruses were relatively rarely transmitted (or, more accurately, that they were relatively rarely found during primary and early stage infection).^{40,44} Many people who died of AIDS did so with only NSI viruses detectable in their blood. However, in perhaps 50% of infected individuals after about 5 years of infection, SI viruses became detectable.^{41,45} The appearance of these viruses became known as the “phenotypic switch” and, as noted above, it heralded a poor prognosis for the patient.

With the identification of the coreceptors, the SI/NSI nomenclature became archaic, once it was realized that SI viruses were able to use CXCR4 (and sometimes CCR5 as well) whereas the NSI viruses used CCR5 only.^{20,25} The MT-2 cell assay “worked” and was valuable because MT-2 cells, like most permanent CD4⁺ T cell lines, expressed CXCR4 but not CCR5. Hence the formation of syncytia in MT-2 cells heralded the presence of CXCR4-using viruses. A new nomenclature based on coreceptor usage was soon introduced; CCR5-using viruses were designated R5, CXCR4-using viruses, X4, and viruses able to use both receptors, R5X4.⁴⁶ Some clonal viruses have been proven to be able to use both CCR5 and CXCR4 and prop-

erly justify the designation R5X4.^{47,48} However, most isolates that replicate on both CCR5-positive and CXCR4-positive cells probably do so because they can contain a mixture of R5 viruses and X4 viruses. A better term for such isolates would be R5 + X4, but since it is technically very difficult to distinguish between a true dual-tropic virus and an isolate containing a mixture of phenotypic variants, we will continue to use the now-conventional term, R5X4.

Just as many humans die of AIDS without the overt acquisition of X4 viruses, so do macaques experimentally infected with SIVmac and related viruses. In this model, there is good evidence that R5 SIV strains can evolve to become more virulent, but without broadening their tropism to gain CXCR4 usage.⁴⁹ The same phenomenon has also been reported to occur in some humans.⁵⁰

ARE THERE BLOCKS TO X4 VIRUS TRANSMISSION, OR POSTINFECTION REPLICATION BLOCKS?

As noted above, X4 viruses rarely predominate in the early years of HIV-1 infection. Does this mean that there is a block to the transmission of X4 viruses that occurs at, for example, the genital or rectal mucosa, perhaps due to the lack of CXCR4 expression at or near the sites of virus deposition? It has been argued that R5 viruses are preferentially transmitted because of the patterns of expression of coreceptors and their ligands at mucosal sites after virus deposition during sexual intercourse.^{51,52}

There are many uncertainties about the mechanism(s) by which HIV-1 is transmitted sexually, and controversies surround the identity of the first infected cells.^{50,53} Genital and rectal subepithelia stromal tissues are densely populated with dendritic cells, macrophages, and T cells that express CD4, CCR5, and, to a lesser extent, CXCR4.^{54–56} Each of these cell types is, therefore, susceptible to HIV-1 infection. In the macaque model, infection of all three cell types can be detected within 1 hr of the addition of SIV to the macaque vagina, most commonly where the epithelium is abraded to allow the virus better access to the underlying, target-cell-enriched tissues.⁵⁴ Immature dendritic cells present in the epithelium express 10-fold more CCR5 than CXCR4,⁵⁷ and they selectively replicate R5 strains.⁵⁸ The Langerhans cell, a member of the dendritic cell family, has been proposed to play a particularly important role in the early stages of sexual transmission.⁵¹ CXCR4 is usually undetectable on human Langerhans cells, whereas CCR5 is present at low levels on a significant minority of these cells,⁵⁹ rendering a low percentage (<5%) susceptible to productive R5 HIV-1 infection *ex vivo*.⁶⁰ It has been suggested that the level of CCR5 on Langerhans cells is a major determinant of sexual transmission, and an important factor in the preferential transmission of R5 viruses.⁵¹ The expression of CCR5 but not CXCR4 on intestinal epithelial cells may also be relevant to the preferential transmission of R5 viruses via the rectal route.^{52,61} The high levels of the CXCR4 blocking ligand, SDF-1, that are present in the intestinal lumen could also be a factor in suppressing the transmission of X4 viruses.⁶²

One model of sexual transmission is, therefore, that CCR5 on target cells within or near the sexual mucosa acts as a local

“gatekeeper.” According to this model, the level of CCR5 expression has a major influence on the efficiency of HIV-1 transmission, and the more abundant local expression of CCR5 compared to CXCR4 explains the R5 phenotype of the most commonly transmitted viruses.

There may, however, be a different or additional explanation for the apparent block to the sexual transmission of X4 viruses. The dominance of R5 viruses early after infection is true in both adults and children, so any block to the transmission of X4 viruses must be relevant to not just sexual, but also vertical^{63–70} and intravenous^{71,72} infection routes. Moreover, epidemiological analyses do not suggest that the route of infection is a major influence on the rate of disease progression.^{72,73} This would not be the case if X4 viruses generally dominated the infections of those infected directly via the blood; in that scenario, rapid progression to AIDS and death would be more common in intravenous drug users (IVDU) and hemophilia cohorts than is, in fact, the case.^{71–73} Furthermore, X4 SHIVs are perfectly infectious for macaques after atraumatic vaginal deposition,⁷⁴ again arguing against the presence of an insuperable physical block to X4 virus transmission.

If X4 viruses can be successfully transmitted, why do they not dominate the overall pool of virus that becomes amplified during primary HIV-1 infection, a stage at which R5 viruses almost always dominate? After all, *in vitro*, X4 viruses have more cellular targets in lymphoid cell cultures and in lymphoid tissue blocks,⁷⁵ and there are many more CXCR4⁺ CD4⁺ T cells in the blood than there are CCR5⁺ CD4⁺ T cells.^{12,76} We will discuss this critical issue at more length later in the article. However, in the setting of acute infection, a key point may be that, *in vivo*, R5 viruses have a selective advantage over X4 strains due to their preferential tropism for the dendritic cell in the context of dendritic cell–T cell conjugates.^{58,60,77} Members of the dendritic cell family can bind HIV-1 particles efficiently via CCR5- and CXCR4-independent mechanisms, then internalize the virus into intracellular endocytic vacuoles without being productively infected themselves.^{79,80} Dendritic cells are natural sentinel cells that sample incoming pathogens or their antigens at, for example, the vaginal epithelium, transport them to regional lymph nodes, and there present them to T and B cells for the initiation of adaptive immune responses.⁸¹ Unfortunately, after internalization, HIV-1 can remain infectious within a dendritic cell for up to 5 days.^{79,80} When the dendritic cell interacts with, and activates, CD4⁺ T cells within T cell-rich regions of the lymph nodes, the virus is in a perfect environment for its rapid and efficient amplification, with R5 viruses dominating because of the expression of CCR5 on activated CD4⁺ T cells.^{80–82} According to this model, then, it is not the expression of CCR5 on the transporting dendritic cell that determines the preferential transmission of R5 viruses, it is the advantage provided to R5 virus replication by dendritic cell–T cell conjugates within lymphoid tissues. The selective amplification of R5 viruses within the lymphoid tissue would presumably operate irrespective of the route of transmission, be it rectal, vaginal, vertical, or intravenous, for it should not matter how the virus reaches the lymph node. The same factors would apply also to HIV-1 infection of tissue macrophages, because most R5 viruses replicate more efficiently than do most X4 strains in these cells.^{34,83}

Whether their advantage is gained during the early events of

transmission or later during the initial amplification stage within the first infected lymph node, R5 viruses then go on to dominate the generally homogeneous quasispecies pool that is present during primary infection. There are, perhaps, no specific, insurmountable obstacles to the transmission of X4 viruses in any setting; only a limitation to their relative replication capacity once viral dissemination to the most active sites of replication has occurred. Two anecdotal transmission cases, one by intramuscular inoculation, the other by needle-stick, are quite revealing.^{84,85} In both examples, X4 viruses were identifiable in the donor, and the first samplings of blood from the recipients suggested that X4 viruses had, in fact, been successfully transmitted. However, as primary infection progressed, R5 viruses came to dominate the pool of viruses in the blood of the recipients, while the X4 viruses became a minor, and eventually almost invisible species.^{84,85} Although several explanations of these phenomena are possible, the one that we favor, as outlined below, is that R5 viruses simply outcompete their X4 counterparts in the race to replicate *in vivo*, once they are successfully transmitted. It may even be that X4 viruses evolve to become R5 strains under the conditions of primary infection.

WHERE DOES HIV-1 REPLICATION OCCUR *IN VIVO*, AND WHY? THE ROLE OF GALT

There is an increasing appreciation of the central role that the gut-associated lymphoid tissue (GALT) plays in the replication of HIV-1 *in vivo*, and hence in the pathogenesis of HIV-1 infection overall. This important issue has been reviewed in detail by Veazey *et al.*⁸⁶ The GALT contains over half the human body’s total of T-lymphocytes, and these cells tend to be more activated than T cells of the peripheral blood—a reflection of the role played by GALT in combating intestinal and food-borne pathogens. Moreover, the T cells of the GALT are not in rapid equilibrium with their counterparts in the peripheral blood, so that events such as viral replication that affect one organ do not necessarily have the same effect on the other.⁸⁶ This is nicely revealed by the outcome of infection of macaques with SHIV-162P.⁸⁷ That virus caused rapid and extensive depletion of CD4⁺ T cells from GALT, but there was no significant loss of CD4⁺ T cells from the blood at the time the GALT was being destroyed; only later did peripheral CD4⁺ T cell counts decline. Thus an apparently minimally pathogenic viral infection, judged by what is happening in the blood, was actually an infection that was having a severe impact on a major lymphoid organ.⁸⁷

It is notable that SHIV-162P is an R5 virus. In contrast, an X4 strain, SHIV-SF33A.2, depleted circulating CD4 T cells, while sparing the T cells in the GALT.⁸⁷ A similar situation occurs in macaques infected with SIV, which also causes a rapid depletion of CD4⁺ T cells that, initially, is seen exclusively in the GALT.^{88,89} The use of CCR5 by SIV is highly relevant in this context, once it is appreciated that the majority of the GALT CD4⁺ T cells are CCR5⁺, compared with only 5% in peripheral blood.^{90–92} The high levels of expression of the chemokine ligands for CCR5 that are present in the GALT may direct the trafficking of memory CCR5⁺ T-lymphocytes to this tissue.⁹³ The CCR5⁺ subset of CD4⁺ T cells is known to be preferentially depleted soon after infection with HIV-1 or SIV, in par-

ticalar at mucosal sites.^{55,92} Furthermore, CXCR4 expression in the GALT is inherently low.^{87,90} The localized production of the SDF-1 ligand for CXCR4 may further occlude this coreceptor and prevent its use for HIV-1 entry, both in mucosal tissues, and elsewhere.^{62,94} One significant implication of this pattern of coreceptor expression in the GALT will be discussed below in the context of the R5 to X4 phenotypic switch and why it does and does not occur.

Overall, it can be argued that HIV-1 and SIV infection might be considered as predominantly an infection of the GALT, at least in the early years of infection when R5 strains are the most abundant.⁸⁶ This is not to say that R5 virus replication does not occur elsewhere in the body, such as the peripheral lymph nodes; instead, we argue (as have others⁸⁶) that virus production in the GALT may initially dwarf production in other lymphoid tissues. Measuring CCR5 expression levels in the blood may allow only an imprecise and indirect understanding of factors that influence the replication of R5 viruses in environments such as the GALT. On the one hand, the reduction in CCR5 expression in the blood caused by CCR5-Δ32 heterozygosity is likely to be predictive of reductions in CCR5 expression elsewhere, because the genetic lesion will have the same effect in all cells. But conversely, the pronounced effects that CCR5 promoter polymorphisms can have on HIV-1 transmission and disease progression rates may be mediated by influences on CCR5 mRNA transcription that are cell-type specific or otherwise affected by the local milieu within a lymphoid tissue.⁹⁵⁻¹⁰² In other words, measurements of CCR5 expression on CD4⁺ T cells derived from the peripheral blood may not reveal every influence that a promoter polymorphism can have on CCR5 protein expression in all tissues relevant to HIV-1 replication *in vivo*.

A similar explanation may account for the apparent paradox that HIV-1-infected infants have very high levels of plasma viremia, usually involving R5 viruses¹⁰³⁻¹⁰⁶ yet CCR5 expression is very low on CD4⁺ T cells derived from the blood of infants (cord blood).¹⁰⁷ Again, this speaks to the limitations of relying on measurements of CCR5 expression that involve only cells from the blood. Perhaps in infants, as in adults, the more relevant levels of CCR5 expression are those present in the GALT. For understandable reasons, measurements of CCR5 expression levels, and the extent of HIV-1 replication, in the GALT of young infants have not been performed. Perhaps such studies could be done in the macaque models.

A final point is that the abundance of activated, CD4⁺ CCR5⁺ T cells in GALT may be an important factor when considering why receptive anal intercourse is such a high-risk sexual practice for HIV-1 transmission,⁶¹ particularly when the high levels of the HIV-1 attachment factor, DC-SIGN, on the rectal mucosa are also taken into account.¹⁰⁸

WHERE DOES HIV-1 REPLICATION OCCUR *IN VIVO*, AND WHY? THE ROLE OF THE THYMUS

It has been apparent ever since AIDS was first identified as a clinical syndrome in the early 1980s that the loss of CD4⁺ T cells was its central feature.^{109,110} Furthermore, HIV-1 is a cytopathic virus *in vitro*, causing massive destruction of CD4⁺

T cells. This is particularly true of the X4 viruses that were the first HIV-1 isolates to be extensively studied. Of course, these viruses have the X4 phenotype, because early isolations tended to be from symptomatic AIDS patients, and because permanent T cell lines were used for the isolations.^{12,111,112} It is, therefore, not remotely controversial to state that HIV-1 kills T cells. However, while T cell killing may be necessary for AIDS to occur, it is highly unlikely to be sufficient.^{113,114} The immune system has abundant regenerative capacity that it can use to repair some of the damage caused by T cell destruction, or at least prolong the period before the damage becomes fatal. Extremely high levels of replication of SIV variants can occur in naturally infected monkeys (e.g., SIVagm infection of African green monkeys or SIVsm infection of sooty mangabeys) without the onset of immunodeficiency; lentivirus production per se need not be lethal to the host.¹¹⁴⁻¹¹⁷ For humans to develop AIDS, it is becoming ever-more accepted that an important contributory factor must be viral impairment of the regenerative capacity of the immune system.^{12,118-124} This capacity is finite and it is age dependent, in that older people develop the symptoms of AIDS much sooner than younger people do.^{121,125,126} It is hard to imagine that T cell destruction is a function of patient age, but both T cell replenishment capacity^{126,127} and immune function¹²⁴ certainly are. The impressive CD4⁺ T cell recovery in children treated with HAART, compared to the lesser response in adults, speaks for itself.¹²⁸

The major organ of naive T cell production is the thymus, long thought to be dormant in adults but now known not to be. The central role of the thymus in HIV-1 pathogenesis has been reviewed by McCune, and will not be discussed at length here.¹¹⁸ However, a highly relevant issue is the role that HIV-1 phenotypic variants play in impairing thymic function, and in particular the local damage that can be caused to the thymus by X4 viruses that target the majority of developing T cells in the thymus.¹²⁹⁻¹³⁴ R5 viruses also replicate in the thymus, but they do not appear to cause the same level of destruction of developing T cells as do X4 strains, as discussed further below. Regardless of the strain, destruction of immature thymocytes either by direct infection or by alteration of thymic stromal function will lead to decreased production of naive T cells. If very immature cells are affected, this will result in decreased production of both naive CD4⁺ and CD8⁺ lineages, and the body's fight against HIV-1 or other infection may be diminished.

The pattern of CXCR4 and CCR5 expression in the human thymus is an important influence on the destruction of immature T cells, and overall, on the pathogenesis of HIV-1 infection. CXCR4 and CCR5 are independently regulated during T cell development. The modulation of each coreceptor renders CD4⁺ cells at different stages of development differentially susceptible to HIV-1 infection. A summary of CD4 and coreceptor expression patterns during lymphopoiesis is provided in Figure 1. A low level of CXCR4 expression can be observed on a subset of the earliest T cell progenitor, the CD34⁺ bone marrow hematopoietic progenitor cell, prior to its entry into the thymus.^{129,135} Furthermore, some CD34⁺ cells express CD4, which renders them infectable, albeit poorly, by HIV-1, at least *in vitro*.¹³⁶⁻¹³⁸ However, it is unclear as to whether CD34⁺ cells are infected *in vivo* and whether any such infection might have direct immunological consequences. Although low levels of virus have been reported in the CD34⁺ progenitor cell popula-

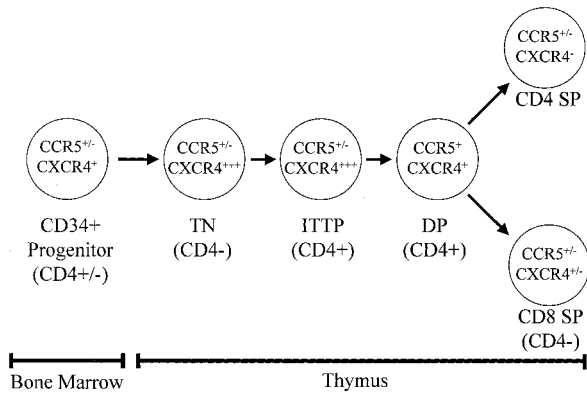


FIG. 1. CXCR4 and CCR5 expression during various stages of lymphoid development. The presence of CD4 on these cells is indicated below each subset. Maturation proceeds from left to right.

tion in a minority of studies, more often these cells have been found to not be infected^{139–141} (reviewed in ref. 142). In the fetal lymphoid system, CXCR4 expression is at its greatest in the thymus.¹⁴³ The receptor is expressed throughout thymopoiesis and appears to have a functional, SDF-1 activated signaling role in early T cell development.¹³⁵ This function of CXCR4 may not be absolutely required; T cells can develop normally in mice engineered to lack CXCR4 expression, although the overall CXCR4 null genotype was developmentally lethal.¹⁴⁴

CXCR4 is expressed on approximately 60–70% of primary human thymocytes.^{145,146} In the thymus, the highest amounts CXCR4 are found on early thymocytes, particularly the CD3⁻CD4⁻CD8⁻ [triple negative (TN)], the CD3⁻CD4^{lo}CD8⁻ intrathymic T cell progenitor (ITTP), and the CD3⁺CD4⁺CD8⁺ [CD4CD8 double positive (DP)] populations.^{129,146,147} CXCR4 is down-regulated during the DP stage of development, more specifically during the transition from the CD3^{lo}CD4^{lo}CD8^{lo} stage to CD3^{hi}CD4^{hi}CD8^{hi} stage.^{146,147} Further down-regulation occurs at the cells mature into the CD3⁺CD4⁺CD8⁻ and CD3⁺CD4⁻CD8⁺ [CD4 or CD8 single positive (SP)] populations.

CCR5 expression is relatively low during T cell development, the converse of what is seen with CXCR4.^{129,145,147,148} There is some evidence that CCR5 is expressed on the bone marrow-derived and thymic-resident CD34⁺ T cell progenitor populations.^{129,147,149} In the thymus, CCR5 is generally found on < 5% of total thymocytes.^{129,143,147,148,150} In general, CCR5 appears to be detectable at low levels on a few cells from every thymocyte subset. However, there is little consensus as to which particular subset(s) express(es) the highest levels of CCR5. In contrast to CXCR4, CCR5 is expressed poorly on CD34⁺ progenitors and ITTPs while it is present on a larger percentage of cells from the more mature populations. The use of different monoclonal antibodies that detect low levels of CCR5 with different efficiencies may explain some of the discrepant results from multiple laboratories. The anti-CCR5 monoclonal antibodies (mAbs) 3A9 and 5C7 appear to cross-react with CCR8, which is also expressed on thymocytes and where it can function as an HIV-1 coreceptor.¹⁵ The 2D7 mAb, however, is specific for CCR5.¹⁵⁰ Cross-reactivity could therefore help explain

discrepant results found in different studies. Some of the highest reported CCR5 levels (although still typically < 5%) are present on DP cells.^{129,143,147,148} CCR5 is also consistently expressed on a low percentage of thymic CD4 and CD8 SP cells. Within the thymus, while its expression is relatively low compared to CXCR4, CCR5 is present at approximately equal distributions on both the CXCR4⁺ and CXCR4⁻ populations.¹⁴³ Further, similar to CXCR4 expression, CCR5 expression in the thymus is clearly not obligatory, as humans homozygous for the CCR5-Δ32 mutation produce normal T cells.¹⁵¹ Furthermore, T cells develop normally in CCR5-deficient mice, although they appear to have some functional differences compared to T cells from wild-type mice.¹⁵² Thus, the differential distribution and expression of CXCR4 and CCR5 during T cell development suggest that different HIV-1 strains may target different cells at different stages of development; in some cases the virus may target the same subset of developing cells (i.e., CCR5⁺/CXCR4⁺/CD4⁺ cells). This pattern of tropism could dramatically influence viral pathogenesis within this primary lymphoid compartment.

The expression of both CXCR4 and CD4 at one of the earliest stages of T cell development in the thymus (i.e., ITTPs) renders these cells susceptible to infection and destruction by X4 HIV-1 strains. The different cellular transcriptional and replicative activity of the various thymocyte subsets also influences their susceptibility to productive infection. For example, the highly transcriptionally active DP^{Bright} subset is very vulnerable to infection with X4 strains whereas the less transcriptionally active CD4 SP population is less likely to be productively infected.^{153,154} Thus, multiple factors render thymocytes more or less permissive to HIV-1 infection. When thymocytes are infected with X4 strains either *in vitro* or in the SCID-hu mouse, the immature CD4 subset is depleted first, then the mature subsets.^{146,155–160} *In vitro* and in the SCID-hu mouse model, X4 HIV-1 isolates primarily deplete the immature CD4⁺ population that expresses the highest level of CXCR4.^{146,161} In the SCID-hu mouse, these CXCR4⁺ cells are regenerated following antiretroviral therapy.^{161,162} Hence X4 viruses do not permanently alter the ability of progenitor cells to express CXCR4 during development, even if the cells had previously been exposed to the virus.

R5 viruses are less cytopathic than X4 strains in cultured thymocytes, and they replicate to lower titers *in vivo* in the SCID-hu mouse.^{130,132,148,155,158,163,164} Children from whom X4 viruses can be isolated have a greater impairment of thymic function than those infected with R5 viruses; their ability to generate new CD4⁺ T cells is much diminished.¹³³ The relatively high levels of CXCR4 but low levels of CCR5 in the thymus condemn this organ to be particularly vulnerable to X4 strains. However, viruses with *env* gene sequences suggestive of the R5 phenotype have been identified in thymuses from HIV-1-infected individuals at autopsy.¹⁶⁵ Furthermore, R5 viruses can cause significant cellular alterations in infected thymocytes, and the outright destruction of these cells *in vivo* and *in vitro*.^{130,131,158,164,165}

Infection and involution of the thymus occurs as macaques infected with SIV (an R5 virus) progress to disease.^{166–168} The majority of SIV-infected cells in the thymus are found in the medullary region,^{167,168} where the mature thymocytes reside and where CCR5-expressing cells are present in the greatest

amount.¹⁶⁹ That CCR5 is expressed at lower levels than CXCR4 in the thymus may explain the decreased pathogenesis of R5 strains in thymocytes *in vivo*, as the extent of infection correlates with CCR5 expression level.¹⁴⁸ Although the mechanism for viral spread into new target cells is unclear, CCR5 may be transiently expressed by thymocytes during T cell development, which could then render these cells susceptible to R5 viruses. Indeed, it was shown recently that HIV-1 expands its tropism within the thymus by up-regulating CCR5.¹⁷⁰ This seems to occur through induction of interferon-alpha (IFN- α) by intrathymic predendritic cells that have responded to HIV-1 infection.¹⁷⁰ This induction of CCR5 was restricted to the TN and ITTP populations, which rendered these progenitors susceptible to HIV-1 infection and depletion. Hence this mechanism could explain the pathogenic effects of R5 strains in the human thymus, despite the low levels of CCR5 expressed by uninfected thymocytes. Recent studies have shown that the interleukin (IL)-7 cytokine alone¹⁷¹ or the combination of IL-4 plus IL-7¹⁷² increases the expression of CXCR4 on mature thymocytes cultured *in vitro*, so the cytokine microenvironment may influence coreceptor expression and therefore HIV replication in the thymus. Of further note is that two other components of the T cell arm of the immune response—immunoregulatory CD161⁺ natural killer (NK) T cells and T cell receptor (TCR) $\gamma\delta$ ⁺ T cells—are also present in the thymus. A significant fraction of the TCR $\gamma\delta$ ⁺ cells expresses CD4 in the thymus, but CD4 expression is lost in the periphery or gut. CD161⁺ T cells express CD4 both in the thymus and periphery.¹⁷³ In the thymus, these cell types both express CXCR4 and CCR5 and are susceptible to infection by multiple HIV-1 strains. Thus, HIV-1 can target diverse subsets of cells within the thymus. By doing so, the virus has a dramatic effect on the development of immature components of the immune system, which may contribute substantially to the immune impairment that is a hallmark of systemic HIV-1 infection.

THE R5 TO X4 PHENOTYPIC SWITCH: WHY DOES IT OCCUR (OR NOT)?

Experimentally, an R5 virus can be converted to an X4 virus by engineering a few (two or three) amino acid changes into the V3 loop of gp120.^{174–176} Given the extent of HIV-1 replication *in vivo*¹⁷⁷ and the associated error rate,¹⁷⁸ the conversion of an R5 virus into an X4 strain should occur rapidly, in every infected person. Moreover, *in vitro*, both in cell culture and in lymphoid tissue blocks, X4 viruses usually replicate more rapidly than R5 strains, and have more cellular targets available to them.^{12,75,76} Target cell availability is also not a driving force for the phenotypic switch *in vivo*, at least so far as can be determined from measurements of coreceptor expression on T cells of the peripheral blood.¹⁷⁹ Thus, measured after a year of infection, the percentage of CXCR4⁺ CD4⁺ T cells in the blood was *inversely* correlated with the later development of X4 viruses, whereas there was no relationship between the percentage of CCR5⁺ CD4⁺ T cells and the phenotypic switch.¹⁷⁹ X4 viruses are also more pathogenic *in vitro*, by a variety of mechanisms including direct cell killing and Env-induced apoptosis,^{180–183} yet the reduced levels of CXCR4⁺ CD4⁺ T cells found in cohort members with advanced disease and X4 vari-

ants is not simply due to virus-mediated killing of this T cell subset.¹⁷⁹ HIV-1 infection, of course, causes immune activation that leads to the up-regulation of CCR5 expression on CD4⁺ T cells.^{184,185} Overall, CCR5 and CXCR4 expression levels in the blood do not influence the rate of evolution of X4 variants; the availability of blood CXCR4⁺ CD4⁺ T cells does not cause X4 viruses to evolve more rapidly; and the ability to use CXCR4 is not an escape mechanism used by HIV-1 to overcome a limiting number of CCR5⁺ CD4⁺ target cells.¹⁷⁹ As noted earlier, however, different forces might influence coreceptor expression levels in other lymphoid organs that cannot readily be sampled in large cohorts.

So why do X4 viruses not dominate HIV-1 pathogenesis in every infected individual, causing AIDS to be a disease of rapid progression? The answer must presumably be that a selection pressure acts against the evolution and dominance of X4 viruses under *in vivo* conditions. It is still unclear whether this pressure has a virological or an immunological basis. Yet it is important to know which of these two possibilities applies, or indeed whether both do. The answer could help resolve a question that has been asked since SI (X4) viruses were first described^{89,40}: Is the emergence of X4 viruses the cause or consequence of severe immune system impairment?

As outlined earlier, arguments can be made that R5 viruses have a selective replication advantage under *in vivo* conditions. Hence, the early bias toward R5 viruses *in vivo* might be a fundamental property of the underlying virology of X4 and R5 virus replication. Moreover, the burst size (virions released per infected cell) has been reported to be about an order of magnitude greater for R5 viruses than for X4 viruses, as measured in lymphoid tissue blocks *in vitro*.¹⁸⁶ The CD4⁺ T cells infected by and releasing R5 viruses were activated memory cells and those producing X4 viruses were resting, naive cells. If the relative burst sizes are similar *in vivo*, the capacity of R5 viruses to outcompete their X4 counterparts over multiple replication cycles is obvious, particularly if much replication occurs in a tissue such as GALT in which CCR5 is abundant, CXCR4 sparse (see above).

There is also evidence that the selection pressure against X4 strains might have an immunological basis, which would imply that the emergence of X4 strains late in infection reflects the cumulative erosion of the suppressive capacity of the immune system. Several mechanisms have been proposed, mostly based on *in vitro* studies.^{187–190} Indirect evidence for an immunological component to the X4 suppressive mechanism is the observation that the development of X4 viruses is associated with low CD4⁺ T cell numbers, and hence with immunological impairment.¹⁷⁹ More direct, but still inferential, support is provided by a recent study in the SHIV-infected rhesus macaque model: an R5 virus (SHIV-162P3) replicates to dominance over a dual-infecting X4 strain (SHIV-33A).^{190a} The mechanism appears to involve preferential suppression of the X4 virus by the immune system. It is unlikely that there is any differential effect of neutralizing antibodies on the two SHIVs,^{190a} and earlier *in vitro* studies also argue against the involvement of this humoral response.^{191,192} Instead, there is evidence based on the use of a depleting anti-CD8 mAb that CD8⁺ cytotoxic T-lymphocyte (CTL) may be responsible for the suppression of X4 viruses.^{190a} One possibility is that the R5 SHIV has a greater propensity than its X4 counterpart for replication

in macrophages, and that these cells constitute a privileged site that is relatively resistant to CTL lysis. There is some independent evidence to support the latter concept.¹⁸⁸ The X4 SHIV, on the other hand, is argued as replicating predominantly in CD4⁺ T cells, which are efficiently suppressed by CTL activity, at least while the immune system remains intact, so fewer X4 viruses are produced. Determining how general these observations are and how broadly they relate to HIV-1 infection of humans will require more studies, perhaps with additional SHIV or SIV strains. Different primary X4 HIV-1 strains and X4 SHIVs vary greatly in their abilities to replicate efficiently in macrophages, compared to CD4⁺ T cells.^{83,193,194} Hence, even focusing on the above immunological model of preferential X4 virus suppression by CD8⁺ CTL, the likelihood of the R5 to X4 phenotypic switch might have a strong virological influence. And of course, any host genetic factors that affect the potency of the CTL response¹⁹⁵ would also have a significant impact on what happens, and when.

Adding to the complexity, environmental influences might also be important. Viruses with the X4 phenotype are infrequently found in individuals with infections with subtype C viruses, strains that are common in sub-Saharan Africa.^{196,197} The apparent preference for the R5 phenotype among subtype C strains might conceivably account for the relatively rapid heterosexual spread of HIV-1 in southern Africa.¹⁹⁷ One possibility is that the envelope glycoproteins of subtype C viruses are in some fundamental way different from those of the subtype B strains that are the most common in North America and Europe, disfavoring the R5 to X4 phenotypic switch on protein structure grounds. However, we doubt this is the explanation; subtype C X4 viruses certainly exist.^{196,198,199} Studies on cohorts that contain individuals infected with several different HIV-1 subtype yield little or no evidence that the subtype of the infecting virus is an important variable in disease progression rates.^{197,200–202} *In vitro*, subtype C R5 viruses are actually *less* fit than B R5 strains.^{202a} Instead, we favor the argument that the local environment is a more important influence on the spread of subtype C strains, and that this is influenced by coreceptor-expression patterns, not by any fundamental virological property of HIV-1 subtype C strains.

Supporting this hypothesis is a study that measured CCR5 protein levels on blood lymphocytes of Italians and Ugandans resident in Italy and Uganda.^{203,204} CCR5 expression varied significantly, but showed no correlation with the racial grouping of the blood donor; instead, the highest CCR5 levels were found in Ugandan residents, be they of Italian or Ugandan extraction; conversely, Italian residents had relatively low CCR5 expression.²⁰³ These observations were attributed to the relatively high levels of immune system activation found in Ugandan residents, particularly activation caused by parasitic infections.^{203,204} Immune activation is known to increase CCR5 expression on T cells of the peripheral blood.^{76,184,185} Sustained, high-level CCR5 expression, particularly if it also occurred in solid lymphoid tissues, could be an important selection pressure in favor of the retention of the R5 phenotype during HIV-1 infections in the African environment.

Intuitively, one might suppose that chronically elevated CCR5 levels might accelerate disease progression since a reduction in expression caused by $\Delta 32$ -CCR5 heterozygosity is

associated with a decreased rate of progression.⁹ However, if there is any selection against X4 strains caused by high CCR5 expression, this would work in the opposite direction, i.e., to slow the rate of progression. The complexities are nicely illustrated by the cohort-based studies of van Rij *et al.* summarized above.¹⁷⁹ Overall, the net effect on disease progression of elevating CCR5 expression is hard to predict without taking into account other variables, although higher CCR5 expression, local or systemic, is likely to act to increase the probability of a successful transmission and amplification of HIV-1 in the first place. It should also be noted that the elevated CCR5 expression in the blood of African residents may actually be a marker of what is happening elsewhere in the body. For example, intestinal parasitic infections may induce the up-regulation of CCR5 in the GALT, and this may be a more important influence on virus replication and disease progression than anything that is measured in the blood (see above). We are not aware of any comparative studies of CCR5 expression in the genital or rectal mucosa, or the GALT, of African and European/North American residents, but perhaps these experiments should be done to gain further insight into why HIV-1 spreads so rapidly in sub-Saharan Africa.

Might methodological considerations also be relevant? Although X4 subtype C infections appear to be rare, X4 viruses are much more common when subtype B viruses are the infecting strains, as is the case in North America and Europe.^{39–41} But how common are X4 viruses? Do most infected people have a low-level X4 HIV-1 infection or are some people completely “X4 free”? One issue is techniques used to detect and quantify X4 and R5 viruses. In the early years of AIDS research, HIV-1 isolations were performed using transformed CD4⁺ T cell lines, which are usually CCR5 negative but CXCR4 positive. Not surprisingly, the early isolates were X4 or, in some cases, R5X4 viruses, because of the obvious bias caused by the cell substrate. When peripheral blood mononuclear cells (PBMC) became the cell of choice for virus isolations, it was (and still is) standard procedure to activate them with phytohemagglutinin (PHA) plus IL-2, which up-regulates CCR5 expression.⁷⁶ Consequently, there is probably a bias in today’s standard isolation procedures toward finding R5 viruses. Other phenotypic methods using cell lines expressing CCR5 or CXCR4 tend to detect the presence of R5 or X4 viruses in plasma, without truly quantifying their relative abundance²⁰⁵; the same concern applies to the MT-2 cell assay for detecting X4 viruses. At best, all these cell-based assays are semiquantitative when it comes to estimating the relative amounts of R5 and X4 viruses in the plasma. Moreover it is entirely possible that X4 viruses will sometimes not be detected if they are present only at low levels relative to R5 viruses, or are present only in other anatomical sites, such as the thymus (see above).

An alternative method to quantify R5 and X4 viruses is a genetic procedure based on the detection of “signature” amino acids in the V3 loop of gp120 that are associated with the R5 or X4 phenotype.^{174,206} Although the genetic methods can be quite laborious and the correlation between the presence of signature residues and the X4 phenotype is probably not 100%, they may be more reliable for quantification purposes than their cell-based counterparts. Using the genetic method, Shankarappa *et al.* identified the presence of X4 viruses (or their genetic

signatures) in all HIV-1-infected individuals involved in the study.²⁰⁷ Of note is that a variable frequency of X4 viruses was observed throughout the course of infection, so an inability to detect X4 viruses in a patient at a single time point could be a false-negative finding, particularly if sampling were done following a period of viremia decline.

Chimpanzees experimentally infected with an X4 virus do have viral loads that are about 1–2 logs lower than those in animals challenged with an R5 strain.²⁰⁸ However, the small number of animals involved and the multiple differences between the challenge viruses other than coreceptor usage limit the robustness of any conclusions that could be drawn. Additional, although again still limited, evidence that X4 viruses are rarely produced in large quantities during HIV-1 infection of humans comes from the studies of the few individuals who are homozygous for the defective $\Delta 32$ -CCR5 allele, yet who become infected with HIV-1. Viral load data from most of the cases are missing, but where it is available, it does suggest that X4-specific viremia is not particularly high.^{27,28,209} Despite the moderate-to-low viral loads in these people, the rates of CD4⁺ T cell loss are high, again emphasizing the particularly destructive effect of the X4 virus on the immune system. CCR5 wild-type individuals infected with high levels of both R5 and X4 viruses, or genuine R5X4 viruses, most likely suffer the worst of both worlds—high levels of virus production and T cell loss caused by the R5 viruses, combined with a relatively modest level of additional viremia and the associated much greater immune system, particularly thymic, impairment caused by the X4 virus component. Hence their rapid progressor status.^{40,43,71}

WHAT WILL HAPPEN WHEN CORECEPTOR-SPECIFIC ENTRY INHIBITORS ARE USED IN THE CLINIC?

Specific inhibitors of HIV-1 entry via CCR5 or CXCR4 are now in human clinical trials.^{33,210,211,211a} Studies in the SCID mouse models suggest that they will have a beneficial effect on viral load,^{211,212} and emerging evidence from human studies is also encouraging.^{211a} Of course, a CCR5-specific inhibitor will block only the entry of R5 viruses, and conversely for a CXCR4-specific inhibitor. But will blocking one coreceptor drive the virus toward using the other? More specifically, will inhibiting HIV-1 entry via CCR5 drive the virus to use CXCR4, thereby increasing the prevalence of X4 viruses and exacerbating disease? Perhaps but not necessarily. In most, but not all,²¹³ studies a reduction in CCR5 expression caused by $\Delta 32$ -CCR5 heterozygosity does not lead to an increased rate of emergence of X4 variants *in vivo*.^{45,189,214,215} Moreover, a low number of CCR5⁺ CD4⁺ target cells in the blood does not drive the evolution of X4 variants from R5 viruses.¹⁷⁹ Again, however, the situation in solid lymphoid tissues may not be the same as in the blood.

To a large extent, the above questions can be resolved only by clinical trials under the most carefully monitored conditions, but some *in vitro* studies are relevant. Most studies of the escape pathways used by HIV-1 to avoid coreceptor antagonists have not been set up to permit coreceptor switching to occur (cell lines were used that expressed either CCR5 or CXCR4, not both). The outcome was predictable; the escape mutant still

used the same coreceptor, but in a way that ignored the presence of the inhibitor.^{216–220} In other words, the virus either evolved a different binding site for the coreceptor that was not affected by the inhibitor, and/or it developed a higher affinity for the coreceptor, so outcompeted the inhibitor. Three studies, however, were designed to permit coreceptor switching to occur. In two of these, CXCR4 antagonists were added to PBMC cultures containing either mixtures of R5 and X4 viruses, or an X4 virus alone.^{221,222} In both cases, the replication of X4 isolates was effectively inhibited, so that only R5 viruses replicated in the cultures. If the input inoculum contained a mixture of R5 and X4 viruses, only the former replicated; if the input was an X4 virus, it mutated to an R5 strain, which grew to dominance in the culture. Thus the intuitively predictable events occurred.^{221,222} However, a different outcome was observed when an R5 virus was cultured in the presence of a CCR5-specific antagonist, AD101.²²³ Although the input virus was derived from a patient in whom X4 viruses later grew to readily detectable levels, in the *in vitro* experiment no coreceptor switching to CXCR4 usage was seen; instead, the escape mutant still used CCR5 but in an inhibitor-insensitive manner. Similar results were observed with a second CCR5 antagonist, SCH-C, and a different R5 input virus.²²³ Thus, for whatever reason, CCR5 was the preferred coreceptor under the *in vitro* conditions that apply to a PBMC culture, at least in those experiments. Whether this is always the case will await the outcome of additional studies with other inhibitors and/or different HIV-1 strains.

What might happen *in vivo*? We can imagine two scenarios that depend very much on whether R5 and X4 viruses replicate in two independent pools of cells, or whether they fight for the same pool with the X4 viruses normally being outcompeted by their R5 cousin compound. In the former scenario, inhibiting the replication of R5 viruses by the use of a CCR5-specific compound would not in any way affect the X4 viruses, which would continue to replicate to the same extent in their own population of target cells (Fig. 2). For example, if R5 viruses are replicating predominantly in the GALT, inhibiting their replication would not necessarily cause X4 viruses to now replicate in GALT, because very little free CXCR4 is expressed in that tissue.⁹⁰ However, in the second scenario, the selective inhibition of R5 viruses would make available an evolutionary niche into which the X4 viruses could expand (Fig. 2). Time and experience will tell which of these scenarios is correct, or whether there will be an intermediate or alternative course of events.

OVERVIEW

We argue that HIV-1 infection of humans might almost be viewed as two separate diseases caused by two different lentiviruses that have different cellular tropisms: R5 HIV-1 strains and their close cousins, the X4 viruses. The most commonly transmitted viruses, the R5 strains, replicate preferentially in activated CD4⁺ CCR5⁺ lymphocytes and macrophages, cells found in abundance in the GALT. In that tissue, which is rarely sampled for analysis, localized virus production and T cell destruction occur for a prolonged period of time. Of course, R5 virus replication occurs also in other lymphoid organs, particularly the peripheral lymph nodes. In such solid tis-

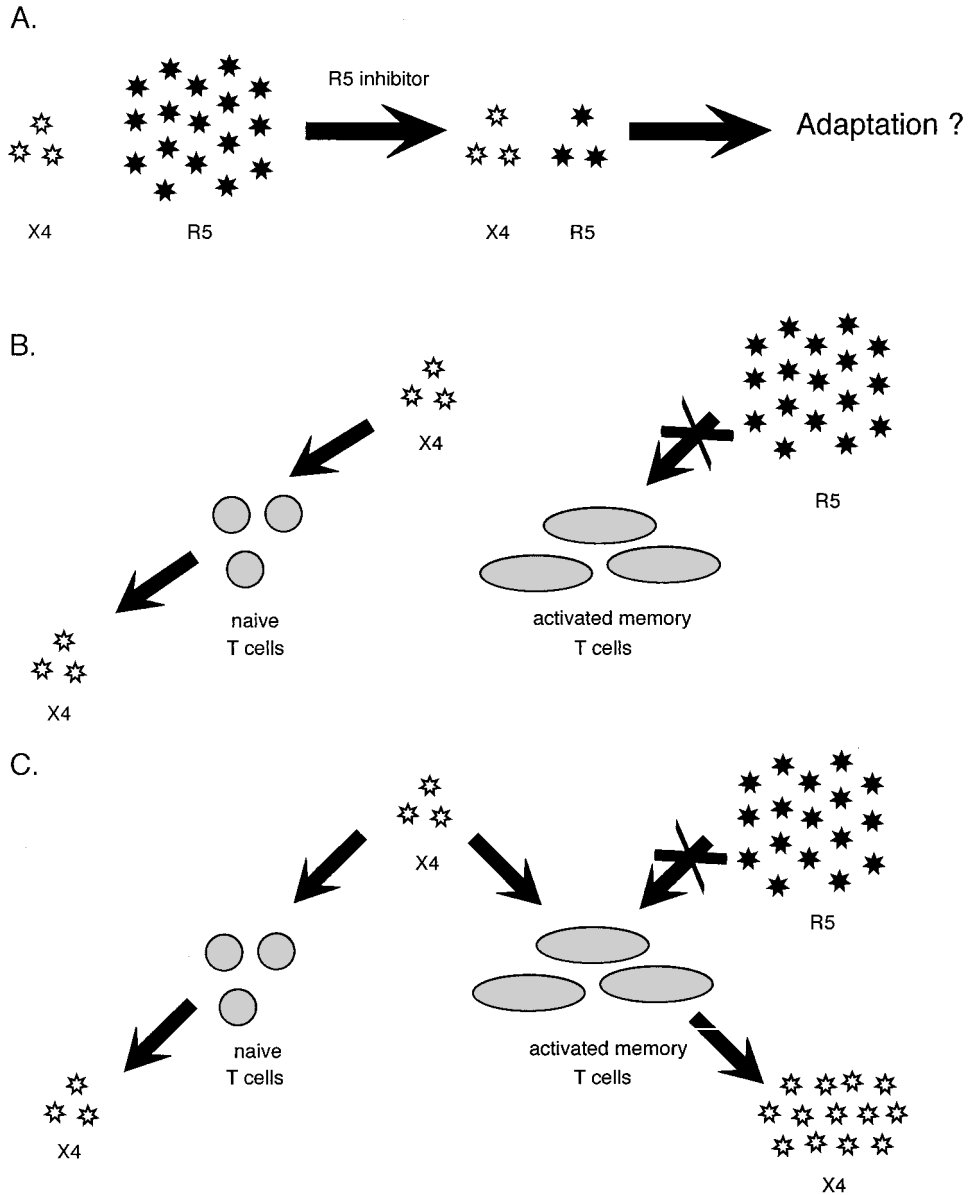


FIG. 2. Possible outcomes of suppressing R5 HIV-1 replication in an individual infected with R5 and X4 viruses. (A) Application of a CCR5 inhibitor initially suppresses the R5 population, followed by outgrowth of escape variants. In principle, the escape viruses could still use CCR5, or they could switch to use CXCR4 or even another coreceptor (not shown). (B) If X4 and R5 viruses normally replicate in independent pools of cells, suppression of the R5 population by a CCR5 inhibitor does not affect the X4 population. (C) If R5 viruses normally outcompete the X4 viruses for the pool of activated memory $CD4^+$ T cells, then suppression of the R5 population allows a niche into which the X4 population can expand, contributing to a greater amount of X4 virus.

sue environments, influences on the CCR5 promoter may be quite different from what applies in the blood where CCR5 expression is normally measured. Within the GALT, and perhaps within lymph nodes, there is little selection pressure for HIV-1 to evolve to CXCR4 usage, particularly if CXCR4 is occluded by SDF-1 or otherwise down-regulated. Moreover, if the relative burst sizes for R5 and X4 virus production are the same *in vivo* as they might be in lymphoid tissue blocks *in vitro*, and we suspect they might be, R5 viruses will simply outcompete their X4 counterparts. There has been a recent paradigm shift

in HIV-1 pathogenesis, with the rejection of the simplistic and incorrect "tap-and-drain" model of T cell turnover²²⁴ in favor of one based on chronic immune activation as the proximal cause of T cell loss.²²⁵ Immune activation will continue to generate $CCR5^+$ $CD4^+$ T cell targets for R5 viruses, because of the up-regulation of CCR5 on these activated T cells, so immune activation and HIV-1 replication go hand in hand.^{123,204,225} Eventually, however, the reservoir of $CCR5^+$ $CD4^+$ target cells in the GALT and elsewhere becomes exhausted, and other cells elsewhere in the body may become the

engine room of virus production. In macaques, there is good evidence that the macrophage can produce abundant quantities of virions in the later stages of an X4 SHIV infection, and presumably this applies earlier in infection as well.¹⁹³ Macrophages can be productively infected by both R5 and X4 viruses, the archaic "macrophage-tropic and T cell tropic" nomenclature notwithstanding.^{30,83,193,194,226} X4 viruses may be present in most people at low-to-intermediate levels for most of the time, and they may be responsible for a disproportionate amount of immune system damage, particularly via their capacity to infect and destroy naive CD4⁺ T cells, and especially in the thymus. The rare individuals in whom X4 or R5X4 viruses predominate early after infection can lose their immune systems with stunning rapidity,^{40,43,71} just as do macaques infected with acutely pathogenic, X4 SHIVs.^{193,227,228} In rapid progressors, the destruction of CD4⁺ T cell is often so swift and comprehensive that T cell help for B cells is insufficient even to permit seroconversion to the viral antigens.^{227,229,230} This could be considered as an entirely separate disease manifestation to that caused by the still lethal, but less rapidly destructive, R5 strains.²²⁸ And, as outlined above, the transition from the dominance of R5 viruses early in infection to the emergence of X4 strains in the later years is likely to be influenced by not just virological factors but also immunological and conceivably even environmental ones as well. Such an eclectic galilimaufry of interdependent variables defies simplistic analysis.

Indeed, when considering HIV-1 pathogenesis, it is important to fight the natural tendency for oversimplification by homogenization. HIV-1 can evolve complex phenotypes, humans are an outbred species (as too are macaques), and the efficiency with which the immune system responds to a viral infection varies between individuals.¹² These are complex, interlocking variables. To fully understand HIV-1 pathogenesis on a population basis is, therefore, no easy task; each individual's overall response to the infecting strain is usually subtly, and sometimes profoundly, distinctive. Hence different answers to questions of HIV-1 pathogenesis can be obtained that depend upon who is studied, when during the course of his or her infection, and what properties are possessed by the infecting virus, a parameter that can itself vary markedly over time. Until recently, there has been a tendency for immunologists to overlook the complexities caused by the HIV-1 phenotypic variants, for virologists to consider all CD4⁺ T cells as being much the same as one other, and for mathematical modelers to ignore most or all of the above variables as being beyond the scope of their equations. Perhaps the ever increasing knowledge of coreceptor expression and usage will enable HIV-1 infection to eventually be better understood than it is now.

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