Species and Tissue Tropisms of HIV-1: Molecular Basis and Phenotypic Consequences

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The tropism of a particular virus is defined by the species, and more particularly the tissues within a given species, that are capable of supporting a productive infection by that virus. Viruses rely extensively on host cell factors, including not only cell surface receptors but also a range of intracellular cofactors, for the successful completion of their life cycle. Therefore, they are unable to replicate effectively in differentiated cells that fail to express a particular cofactor or in species, distinct from their normal host(s), that express a variant form of an essential cofactor that is not capable of supporting a critical macromolecular interaction. The more cellular cofactors a particular virus is dependent on, the more constrained will be the pattern of permissive species and differentiated cell types. Human immunodeficiency virus type 1 (HIV-1) displays a remarkably narrow tropism in terms of not only the range of species, but also the range of tissues within a particular species, that are permissive for this pathogenic virus.

Understanding the molecular basis of HIV-1 tropism is important for several reasons. Firstly, the tissue tropism of primary HIV-1 isolates derived from individual patients has been shown to be predictive of both cytopathicity in culture and disease progression (Connor *et al*., 1997; Schuitemaker *et al*., 1992; Schramm *et al*., 2000). Understanding how tissue tropism is regulated, and how it evolves in infected individuals, therefore has the potential to improve the treatment of AIDS patients. Secondly, it would be extremely useful to have an animal model system, preferably a rodent model, that recreates the pattern of HIV-1 induced disease seen in infected humans. This is not feasible unless we can use transgenic approaches to repair the various molecular defects in the mouse, or other rodents, that currently render them non-permissive for HIV-1. Finally, the inability of a particular tissue or species to support HIV-1 replication provides a genetic tool to define cellular cofactors that are critical for specific steps in the viral life cycle. This has already facilitated the identification of cellular cofactors required for HIV-1 entry or transcription (Feng *et al*., 1996; Wei *et al*., 1998) and has the potential to identify other cellular cofactors involved in several other phases of the viral replication cycle. The identification and molecular characterization of these HIV-1 cofactors would in turn provide potential targets for the development of novel antiviral agents.

This article is primarily intended for scientists who have a good understanding of the various phases of the replication cycle of HIV-1. Other readers are directed to recent reviews describing the early phase of the viral replication cycle (Doms & Trono, 2000), the role of HIV-1 regulatory and auxiliary proteins (Cullen, 1998) and the steps in virion assembly and release (Freed, 1998).

Species Tropism of HIV-1

1. Infection of cells from non-human primates

All HIV-1 isolates recovered from humans appear to have evolved from a small number of zoonotic infections of humans by chimpanzee simian immunodeficiency viruses (SIVCPZ) (Gao *et al*., 1999). Therefore, it is not surprising that HIV-1 will replicate in both chimpanzee lymphoid cells and in chimpanzees themselves, although it does not generally cause disease when reintroduced into its species of origin. Surprisingly, however, HIV-1 replicates poorly in monkey cells, including in cells derived from rhesus macaques, the major primate experimental animal.

Attempts to infect macaque peripheral blood mononuclear cells (PBMC) with HIV-1 revealed that infection was blocked at a very early stage, prior to completion of reverse transcription (Shibata *et al*., 1991; Himathongkham & Luciw, 1996). Surprisingly, this defect was not, however, due to a problem in the HIV-1 envelope (Env) protein, as shown by two lines of evidence. Firstly, a macaque-derived SIV (SIVMAC) pseudotyped with the HIV-1 Env protein proved to be fully capable of infecting macaque PBMCs (Chackerian *et al*., 1997). Secondly, chimeric viruses constructed from SIVMAC and HIV-1, so-called SHIVs, that contained the HIV-1 *tat*, *vpu*, *env* and *rev* genes in an otherwise SIVMAC context, proved fully replication competent on macaque PBMCs (Shibata *et al*., 1995). Subsequent efforts to genetically define the gene product in HIV-1 that is non-functional in macaque PBMC have shown that this defect maps to HIV-1 *gag* and/or *pol* (Shibata *et al*., 1995). More recently, it has been demonstrated that substitution of a small fragment of *gag*, termed the capsid-p2 domain, from HIV-1 into SIVMAC confers a species tropism similar to that of HIV-1, *i.e*. this chimera can replicate in human but not macaque cells (Dorfman & Gottlinger, 1996). Unfortunately, the converse experiment has not been reported, i.e. rescue of HIV replication in simian PBMC by the SIV capsid-p2 sequence. Nevertheless, these data in sum argue that the Gag protein is likely to be the major determinant of HIV-1 primate species specificity.

While the host cell factor(s) that determines the primate species specificity of HIV-1 remains to be unequivocally established, one paper has suggested that it might be the co-receptor itself. Thus, Chackerian *et al*. (1997) reported that while several HIV-1 isolates were unable to infect macaque cells expressing human CD4, these same macaque cells became fully permissive for infection when they also expressed the relevant human co-receptor (see below for overview of co-receptor function). Therefore, these data imply that a primate species-specific block to infection that maps to the HIV-1 Gag protein can be overcome by expressing human CD4 and human co-receptor on macaque cells! While this result is at present difficult to understand, it may suggest a critical role for co-receptors during the early phase of the viral replication cycle but after virion entry.

While the Gag protein appears to be a key determinant of HIV-1 species tropism, other viral gene products appear likely to also constrain not only the primate species tropism of HIV-1 but also the ability of SIV variants to productively infect human cells. For example, Simon *et al*. (1998b) have demonstrated that the Vif proteins derived from an African green monkey SIV isolate (SIVAGM) or from Sykes' monkey SIV (SIVSYK) are not functional in human cells. Because Vif function is critical for both HIV-1 and SIV replication in primary cells (Gabuzda *et al*., 1992), the inability of the Vif proteins encoded by SIVAGM and SIVSYK to function in human cells would be predicted to be sufficient to preclude productive infection by these simian viruses. Similarly, Stivahtis *et al*. (1997) have shown that the Vpr proteins from SIVAGM and SIVSYK are incapable of blocking cell cycle progression in human cells, although both proteins, as well as HIV-1 Vpr, are active in simian cells. These data not only indicate that auxiliary proteins also play a role in controlling the primate species tropism of primate lentiviruses but also may explain why SIVAGM and SIVSYK appear to differ from SIVCPZ and sooty mangabey SIV (the progenitor of HIV-2; Gao *et al*., 1999) in not causing zoonotic infections in humans (Simon *et al*., 1998b). As neither the cofactor for Vif function nor for Vpr-mediated cell cycle arrest has as yet been clearly identified, the molecular basis for the primate species specificity of these viral proteins remains unexplained. However, the inability of, for example, SIVAGM Vif and Vpr to function in human cells may provide a simple way to validate candidate cellular cofactors for these two important HIV-1 auxiliary proteins.

2. HIV-1 Infection of rodent cells

Given that monkey cells are not permissive for HIV-1 replication, one might predict that more distantly related mammalian species, such as rodents, would be at least equivalently refractory. Indeed, it has been clear for some time that HIV-1 is unable to infect rodent cells, even when these have been engineered to express human CD4 (Ashorn *et al*., 1990; Clapham *et al*., 1991; Maddon *et al*., 1986). Further, it has also been known for several years that the HIV-1 Tat protein, an essential viral transcription factor, is not functional in either mouse or hamster cells, thus precluding significant levels of viral gene expression (Newstein *et al*., 1990; Alonso *et al*., 1992).

Although the inability of murine cells to be infected by HIV-1 or to support HIV-1 Tat-dependent transcription formed a major hurdle to the development of rodents as models for the study of HIV-1 pathogenesis, this defect also presented a potential way to identify and/or validate the human proteins that mediate these functions. Thus, the first HIV-1 co-receptor, now termed CXCR4, was initially cloned by Feng *et al*. (1996) by identifying a human cDNA that allowed fusion of murine NIH 3T3 cells expressing human CD4 with cells engineered to express a T-tropic HIV-1 Env protein. While the HIV-1 Tat cofactor, termed Cyclin T1, was in contrast identified by biochemical purification, it was only truly validated by the demonstration that expression of human cyclin T1 in rodent cells fully rescues HIV-1 Tat function (Wei *et al*., 1998). Interestingly, subsequent analysis has demonstrated that while murine CCR5 is indeed unable to support cell fusion mediated by CCR5 (R5)-tropic HIV-1 Env proteins, murine CXCR4 is actually fully competent to support CXCR4 (X4)-tropic Env dependent cell fusion (Bieniasz *et al*., 1997). However, murine CXCR4 is not expressed on most murine cell lines, including NIH 3T3. In contrast, murine cyclin T1 was shown to be unable to support HIV-1 Tat function due to the substitution of a critical cysteine residue, found in human cyclin T1, with tyrosine. Although murine cyclin T1 is able to bind to the HIV-1 Tat protein essentially normally, the lack of this critical cysteine prevents recruitment of the resultant Tat:cyclin T1 heterodimer to TAR, the cis-acting target for HIV-1 Tat function (Garber *et al*., 1998; Bieniasz *et al*., 1998)

Having identified the cellular receptor and co-receptor for the HIV-1 Env protein, and with the cellular Tat cofactor also known, it seemed possible that it might now be possible to render rodent cells permissive for HIV-1 by simply engineering rodent cells to express all three of these human proteins. However, this has not proven to be the case and it instead appears that rodent cells lack at least one, and possibly several additional factors required for the effective replication of HIV-1.

Two groups have extensively investigated the ability of rodent cells to support the replication of HIV-1 in culture. An initial report (Mariani *et al*., 2000), focused entirely on the murine cell line NIH 3T3, reported that mouse cells expressing human CD4 and co-receptor could be infected by HIV-1, albeit with reduced efficiency compared to human cells. The initial steps of the viral life cycle, i.e. penetration, reverse transcription, proviral integration and transcription, all proceeded efficiently in NIH 3T3 cells expressing human receptors and cyclin T1. However, the later stages of the viral replication cycle, particular including virion assembly and release, were very inefficient. A minor (~3 fold) drop in the relative abundance of the unspliced HIV-1 transcript was noted in HIV-1 infected NIH 3T3 cells, probably reflecting oversplicing of HIV-1 transcripts in rodent cells. However, the major problems were a drop in the efficiency of proteolytic processing of the HIV-1 Gag protein and a dramatic, and probably related, decrease in the release of HIV-1 virions. However, the small number of virions released did appear to be fully infectious. This study (Mariani *et al*., 2000) therefore suggested that murine cells were defective for HIV-1 replication primarily because they were unable to assemble HIV-1 virions effectively.

A second report (Bieniasz & Cullen, 2000) extended these earlier data to additional murine cell lines and also to rat and hamster cell lines. In contrast to Mariani *et al*. (2000), Bieniasz and Cullen (2000) reported that NIH 3T3 cells, as well as Rat 2 cells, were only poorly infectable by wild-type HIV-1 even when they expressed high levels of human CD4 and co-receptor. In contrast, hamster (CHO) cells supported HIV-1 infection as effectively as did human cells. This report did, however, confirm that the early steps of the HIV-1 life cycle, through proviral transcription, proceeded efficiently in rodent cells expressing a functional cyclin T1 protein if the infecting HIV-1 virions were pseudotyped with the G envelope protein from vesicular stomatitis virus.

In agreement with Mariani *et al*. (2000), Bieniasz and Cullen (2000) also saw modestly reduced levels of unspliced HIV-1 RNA and a significant drop in the efficiency of Gag polyprotein processing in most, but not all, rodent cell lines tested. Virion release was found to be very inefficient for murine and rat cells, although less affected in hamster cells. However, in disagreement with the earlier work of Mariani *et al*. (2000), these workers noted a significant drop in the infectivity of virions released from rat and mouse cells when compared to hamster and, particularly, human cells. Finally, Bieniasz and Cullen (2000) reported that HIV-1 replication in rodent cells could be rescued by fusion to human cells, thus

indicating that rodent cells lack one or more cofactors (in addition to receptors and cyclin T1) that are essential for critical aspects in the HIV-1 replication cycle, including particularly virion assembly and release.

More recently, Mariani *et al*., (2001) have extended their earlier work to other rodent cell lines with largely similar results. This more recent paper again reported only a small drop (~3 fold), relative to human cells, in the efficiency with which rodent cells bearing human CD4 and co-receptor can be infected. In addition, these workers extended and confirmed their earlier observation that HIV-1 virions released from a range of rodent cell lines were fully infectious. In agreement with Bieniasz and Cullen (2000), these workers also reported that HIV-1 can effectively replicate in human:mouse heterokaryons and that the hamster cell line CHO is significantly more permissive than either mouse or rat cells in supporting HIV-1 replication.

In conclusion, these three studies all indicate that rodent cells have a severe defect in the assembly and release of HIV-1 virions that is a recessive phenotype in mouse:human heterokaryons. In addition, there appears to be some deficiency in the ability of HIV-1 to utilize human receptors on the surface of rodent cells as well as a modest reduction in the level of Gag mRNA production when compared to human cells. The issue of whether the infectivity of HIV-1 virions released from rodent cells is attenuated remains to be resolved. However, if HIV-1 virions released from rodent cells are indeed fully infectious, then this would imply either that the HIV-1 Vif protein is fully active in rodent cells or that the cellular factor expressed in some, but not all, human cells that blocks the replication of Vif deficient HIV-1 (Simon *et al*., 1998a) is absent in rodent cells. In any case, it is clear that these defects, in total, are presently sufficient to preclude the spread of HIV-1 in cultures of rodent cells engineered to express human CD4, co-receptors and cyclin T1. The identification of the human cofactor(s) required for successful assembly and release of HIV-1 virions, that is apparently lacking in all rodent cell types, is clearly an essential prerequisite for the future establishment of a small animal model to study HIV-1 pathogenesis.

Tissue Tropism of HIV-1

1. Molecular basis for HIV-1 tissue tropism

The majority of research into the molecular biology of HIV-1 has used three related laboratory isolates termed IIIB, LAI (or BRU) and NL4-3. The IIIB and LAI isolates are very closely related while the 3' half of the chimeric NL4-3 provirus, including the critical *env* gene, is derived from LAI. As a result, these three laboratory isolates are very similar to one another in terms of their biological properties.

Both IIIB and LAI were derived after passage of virus derived from a single AIDS patient on CD4+ human T cell lines. It is now clear that passage of primary virus isolates on transformed T cells selects for several related phenotypes. These include a high affinity for CD4 and high sensitivity to neutralization by both soluble CD4 and by certain monoclonal antibodies. These properties correlate with the ability to grow on a range of CD4+ T cell lines, loss of the ability to grow on primary macrophages and induction of syncitia during replication on either T cell lines or primary T cells. This constellation of phenotypes was historically referred to as T cell or T-tropism. In contrast, most primary HIV-1 isolates, particularly at earlier stages of infection, are unable to grow on T cell lines, but do grow on primary macrophages and T cells. Primary viruses also generally do not form syncitia in culture, are resistant to neutralization by both soluble CD4 and antibodies and have a lower affinity for CD4 (Daar *et al*., 1990; Schuitemaker *et al*., 1991, 1992; Hwang *et al*., 1992; Moore & Ho, 1995; Kozak *et al*., 1997; Wrin *et al*., 1995; Platt *et al*., 2000). This constellation of phenotypes was originally referred to as macrophage or M-tropism, although M-tropic HIV-1 isolates do retain the ability to grow on primary T cells. It is important to emphasize that many viruses are actually intermediate in phenotype between the extreme forms of T-tropism and M-tropism defined here.

Efforts to identify the gene in HIV-1 that controlled these interrelated phenotypes rapidly led to the demonstration that these properties all mapped to the viral *env* gene (O'Brien *et al*., 1990; Shioda *et al*., 1991). More detailed mutational analyses subsequently revealed that the primary determinant of HIV-1 tissue tropism lay in the third variable, or V3, domain of the gp120 subunit of Env (Hwang *et al*., 1991,

1992; Fouchier *et al*., 1992; Chesebro *et al*., 1996) although regions in the first or second variable domain could also modulate tissue tropism to a lesser degree (Boyd *et al*., 1993; Koito *et al*., 1994).

The ability of T-tropic HIV-1 to replicate on T cell lines and primary T cells, while M-tropic HIV-1 could replicate only on primary T cells and macrophages, led to the hypothesis that there were two co-receptors required for HIV-1 infection in addition to CD4. In this hypothesis, T cell lines would only express co-receptor A, primary macrophages would express only co-receptor B and primary CD4+ T cells would express both co-receptors. The identification of CXCR4 as the co-receptor for T-tropic HIV-1 (Feng *et al*., 1996), while CCR5 was found to be the primary co-receptor for M-tropic HIV-1 (Alkhatib *et al*., 1996; Choe *et al*., 1996; Deng *et al*., 1996; Dragic *et al*., 1996), seemed initially to fully confirm this hypothesis. Indeed, CCR5 was found not to be expressed on transformed T cell lines, thus explaining why these could not support M-tropic virus replication.

It soon became apparent, however, that the molecular basis for HIV-1 tissue tropism could not be as simple as originally envisioned. Specifically, many primary M-tropic viruses were actually found to be able to use either CCR5 or CXCR4 as a co-receptor, or were even entirely CXCR4 (X4)-tropic. Nevertheless, many of these X4-tropic primary viruses were unable to grow effectively on CXCR4 expressing CD4+ T cell lines yet were able to grow on primary macrophages which, it now emerged, did indeed express a low level of CXCR4 (Simmons *et al*., 1998; Verani *et al*., 1998; Yi *et al*., 1998, 1999).

The question therefore became, why do X4-tropic primary HIV-1 isolates fail to replicate on T-cell lines that support replication by X4-tropic laboratory-adapted HIV-1 isolates and, conversely, why do the latter fail to replicate on primary macrophages that are permissive for primary HIV-1 X4 isolates? It now seems clear that the major, but perhaps not the only, determinant of these phenotypes is the relative affinity of viral Env proteins for the CD4 receptor and the CXCR4 co-receptor. As noted above, laboratory adaptation of primary viruses involves acquisition of a higher affinity for CD4 (Platt *et al*., 2000). As predicted, increased expression of CD4 on T cell lines was therefore able to at least partially rescue the ability of primary X4-tropic viruses to replicate on these transformed cells (Kozak *et al*., 1997; Platt *et al*., 2000). Similarly, it has recently been demonstrated that the inability of the T-tropic SIV isolate mac239 to replicate on primary simian macrophages can be rescued by increased expression of CD4 (Bannert *et al*., 2000).

Given that the inability of primary X-4 tropic HIV-1 to replicate on T cell lines largely reflects a low affinity for CD4, could the failure of laboratory adapted, X4-tropic viruses to replicate on primary macrophages be due to poor utilization of low levels of CXCR4? In fact, it has now been demonstrated that overexpression of CXCR4 on primary macrophages largely rescues both fusion with, and infection by, laboratory adapted X4-tropic HIV-1 (Dimitrov *et al*., 1999; Tokunaga *et al*., 2001).

Together, these data indicate that the relative affinity for CD4 and co-receptor may be a major rate limiting step in the efficiency of virus replication in culture. Interestingly, this also appears likely to be true in infected patients. For example, HIV-1 infected individuals that express lower levels of the co-receptor CCR5 have been shown to progress significantly more slowly than individuals that express high levels of CCR5 (Huang *et al*., 1996; Michael *et al*., 1997; Reynes *et al*., 2001). More generally, these observations suggest that the selective pressures acting on HIV-1 in the infected host may select not only for the ability to utilize other co-receptors but also for HIV-1 sequence variants able to infect target cell subsets that express suboptimal levels of CD4 or co-receptor. Such evolved viruses might be predicted to be potentially more fit than the initial infecting virus and, hence, more pathogenic (Quinones-Mateu *et al*., 2000). The appearance of an expanded cell tropism upon selection for ability to utilize very low levels of the co-receptor CCR5 has in fact been documented in vitro (Dejucq *et al*., 1999) and similar selection pressures may well exist in vivo, with similar results.

While the relative ability to use rate-limiting concentrations of CD4 or co-receptor therefore appears to be the primary determinant of HIV-1 tissue tropism, it is unlikely to be the only one (Gorry *et al*., 2001). Which other mechanism(s) could influence this process remains to be determined, although it has been suggested that the ability of the viral Env protein to signal via a co-receptor (Weissman *et al*., 1997; Liu *et al*., 2000) or to use antigenically distinct, tissue specific variants of CXCR4 (Lapham *et al*., 1999; Baribaud *et al*., 2001) may influence HIV-1 tissue tropism.

2. HIV-1 tissue tropism and disease progression

With few exceptions, viruses isolated during the initial phase of human infection are M-tropic viruses that exclusively utilize CCR5 as a co-receptor. However, during disease progression, highly cytopathic, T-tropic viruses that utilize CXCR4 as a co-receptor either in addition to, or instead of, CCR5 can evolve (Schuitemaker *et al*., 1991, 1992; Connor and Ho 1994; Connor *et al*., 1997; Van't Wout *et al*., 1994; Schramm *et al*., 2000). While CXCR4-tropic viruses eventually appear in up to 50% of patients infected with the subtype B viruses prevalent in the USA and western Europe, the incidence of CXCR4-tropism appears to be much lower in patients infected with subtype C, even though this widely distributed subtype appears equally pathogenic (Abebe *et al*., 1999; Ping *et al*., 1999). In any event, the observed increase in viral cytopathicity during disease progression appears to be encoded entirely within the *env* gene and correlates with changes in co-receptor usage (Kreisberg *et al*., 2001). An unresolved and important question is: why are the initial viruses generally M-tropic and why do T-tropic viruses appear over time?

One possible way to view this issue is that viral properties that enhance the likelihood of, particularly, sexual transmission may well be quite distinct from the properties that enhance viral replicative capacity in the infected host. Therefore, HIV-1 may evolve in the host in a way that actually selects against properties that enhance viral transmission frequency. Unfortunately, the factors that regulate this critical step remain largely unknown, although the simple hypothesis that M-tropic viruses are transmitted more effectively than T-tropic viruses because the first cells infected in the new host are macrophages appears to be incorrect (Schacker *et al*., 2001). In fact, even in individuals initially infected with T-tropic viruses that utilize CXCR4, selection for ability to replicate in macrophages occurs, even though this does not necessarily involve a change in co-receptor specificity (Beaumont *et al*., 2001). Therefore, it is clear that a major component of selection for M-tropism during the early phase of infection is actually subsequent to transmission. Presumably, M-tropic viruses predominate early because they replicate more effectively than T-tropic viruses in the available target cells and/or because the host is able to control T-tropic virus infection more readily at this early disease stage.

As noted above, many patients at later disease stages evolve HIV-1 variants that are T-tropic and use CXCR4 as a co-receptor. The appearance of T-tropic viruses clearly correlates with more rapid disease progression, although it is still not clear that this correlation implies causation. Nevertheless, a number of groups have reported that these late, T-tropic viruses are more cytopathic in vitro (Berkowitz *et al*., 1998; Grivel *et al*., 2000; Schramm *et al*., 2000; Kwa *et al*., 2001) although X4-tropic viruses are not necessarily more replication competent than R5-tropic viruses in culture (Quinones-Mateu *et al*., 2000). The appearance of X4-tropic HIV-1 therefore likely results from selection for an expanded target cell tropism, perhaps allowing HIV-1 to infect and kill additional T cell subsets that are not readily susceptible to infection with R5-tropic viruses, such as the naive CD4+ T cell subset that expresses low levels of CCR5 but readily detectable levels of CXCR4 (Bleul *et al*., 1997; Blaak *et al*., 2000; van Rij *et al*., 2000; Kwa *et al*., 2001). This change in tropism, by allowing HIV-1 to target novel, but potentially less optimal, T cell subpopulations that have not been depleted by the pre-existing R5 viruses, presumably supplies the selective pressure not only for the appearance, but also the maintenance, of X4-tropic viruses. A prediction of this hypothesis is that a reduction in viral load should reverse the selective pressures that favor the persistence of X4-tropic HIV-1 isolates by increasing the number of cells that are susceptible to R5-tropic viruses. In fact, it has now been documented that antiviral therapy indeed results in the preferential suppression of X4-tropic HIV-1 in infected patients (Equils *et al*., 2000; Philpott *et al*., 2001). In any event, although the appearance of T-tropic, CXCR4 utilizing viruses is clearly predictive of more rapid disease progression, it should be remembered that many patients progress to AIDS without evolving T-tropic viruses. Therefore, antivirals targeted exclusively at CXCR4-dependent viruses would appear likely to at best delay disease progression.

Conclusion

Our understanding of the mechanisms underlying HIV-1 species and tissue tropism, and the consequences of changes in the latter for disease progression, remains far from complete. In the case of

species tropism, the molecular basis for the inability of HIV-1 to replicate in simian cells remains obscure. On the other hand, resolution of two problems limiting replication in rodent cells, *i.e.*, the identification of HIV-1 co-receptors and of the cofactor for HIV-1 Tat, has merely facilitated the identification of other steps in the viral life cycle that are abortive in rodent cells. While the inability of HIV-1 to replicate in non-human cells offers the potential to identify additional human factors that are essential for HIV-1 replication, it appears that we are far from being able to construct a rodent model of HIV-1 pathogenesis. In the absence of a good animal model, progress in understanding how the evolution of HIV-1 tissue tropism affects viral pathogenicity in vivo will clearly be difficult, although the use of SHIVs containing different HIV-1 *env* genes may be helpful. Nevertheless, I would argue that intensified efforts to understand the tropisms displayed by HIV-1 will not only shed light on critical steps in the viral life cycle but also lead to the development of tools useful in the development of novel antiviral agents.

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