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Identification of interdependent variables that influence coreceptor switch in R5 SHIV<sub>SF162P3N</sub>-infected macaques

Ke Zhuang<sup>1</sup>, Andres Finzi<sup>2</sup>, Jonathan Toma<sup>3</sup>, Arne Frantzell<sup>3</sup>, Wei Huang<sup>3</sup>, Joseph Sodroski<sup>4</sup> and Cecilia Cheng-Mayer<sup>1*</sup>

Abstract

**Background:** We previously reported that adoption of an “open” envelope glycoprotein (Env) to expose the CD4 binding site for efficient receptor binding and infection of cell targets such as macrophages that express low levels of the receptor represents an early event in the process of coreceptor switch in two rapidly progressing (RP) R5 SHIV<sub>SF162P3N</sub>-infected rhesus macaques, releasing or reducing Env structural constraints that have been suggested to limit the pathways available for a change in coreceptor preference. Here we extended these studies to two additional RP monkeys with coreceptor switch and three without to confirm and identify additional factors that facilitated the process of phenotypic conversion.

**Results:** We found that regardless of coreceptor switching, R5 viruses in SHIV<sub>SF162P3N</sub>-infected RP macaques evolved over time to infect macrophages more efficiently; this was accompanied by increased sCD4 sensitivity, with structural changes in the CD4 binding site, the V3 loop and/or the fusion domain of their Envs that are suggestive of better CD4 contact, CCR5 usage and/or virus fusion. However, sCD4-sensitive variants with improved CD4 binding were observed only in RPs with coreceptor switch. Furthermore, cumulative viral load was higher in RPs with than in those without phenotypic switch, with the latter maintaining a longer period of seroconversion.

**Conclusions:** Our data suggest that the increased virus replication in the RPs with R5-to-X4 conversion increased the rate of virus evolution and reduction in the availability of target cells with optimal CD4 expression heightened the competition for binding to the receptor. In the absence of immunological restrictions, variants that adopt an “open” Env to expose the CD4 binding site for better CD4 use are selected, allowing structural changes that confer CXCR4-use to be manifested. Viral load, change in target cell population during the course of infection and host immune response therefore are interdependent variables that influence R5 virus evolution and coreceptor switch in SHIV<sub>SF162P3N</sub>-infected rhesus macaques. Because an “open” Env conformation also renders the virus more susceptible to antibody neutralization, our findings help to explain the infrequent and late appearance of X4 virus in HIV-1 infection when the immune system deteriorates.

**Keywords:** R5 SHIV, Coreceptor switch, CD4 binding, Macrophage infection

* Correspondence: cmayer@addcr.org

Aaron Diamond AIDS Research Center, New York, NY, USA

Full list of author information is available at the end of the article

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Background

Entry of the human immunodeficiency virus (HIV) is initiated by binding of the gp120 surface subunit of the viral envelope protein (Env) to the cellular receptor CD4 and either the CCR5 (R5 viruses) or CXCR4 (X4 viruses) coreceptor on the target cell [1]. Most HIV-1 transmissions result in a predominantly R5 virus infection [2-4]. With time, X4 variants arise and coexist with R5 variants in ~50% of subtype B infected individuals, and their emergence is associated with rapid CD4+ T cell loss and disease progression [5,6]. The determinants of phenotypic change from R5 to X4 map largely to the V3 region of the exterior envelope glycoprotein, gp120 [7], and can be inferred by analysis of the amino acid sequence of this region [8]. The underlying factors for virus coreceptor switch late in infection however remain uncertain, but several hypotheses that include high viral load and evolutionary rate, changes in target cell populations during the course of infection and/or differential immune recognition of X4 and R5 viruses have been proposed [9,10]. Because the presence of X4 virus is associated with poorer clinical prognosis and is a major limitation to the clinical use of CCR5 inhibitors [11-15], a better understanding of the pathway and selective pressures for the emergence of X4 virus should provide important insights into HIV-1 pathogenesis and treatment.

We recently reported coreceptor switch in macaques infected intravenously (iv), intrarectally (ir) or intravaginally (ivag) with the late R5 SHIVSF162P3N isolate [16-18]. The majority of infected macaques in which X4 virus emerged are rapid progressors (RP), with a clinical course that is characterized by persistent high levels of virus replication, early onset of clinical disease and undetectable or transient antiviral antibody titers that usually wane within 3-4 weeks of virus inoculation. Nevertheless, we showed that the genetic requirements for coreceptor switch in SHIVSF162P3N-infected RPs overlapped with those reported in humans that developed neutralizing antibodies [8,16,17,19,20], and transitioned similarly through dual-tropic intermediates with reduced replicative capacity and less efficient coreceptor use [21-24]. Furthermore, consistent with findings in HIV-1 infected patients [25-27], the appearance of X4 virus follows rather than precedes the initial decline of CD4+ T cells in the infected macaques. Since the newly emerging CXCR4-using viruses in both hosts are highly sensitive to neutralization with antibodies directed against the CD4 binding site [16,17,28,29], these observations suggest that immunological impairment provides a selective advantage for emergence and expansion of X4 virus. Thus, the mechanistic pathways and selection factors underlying phenotypic conversion are likely similar in some HIV-1 infected patients and in SHIVSF162P3N-infected RP monkeys.

For these reasons, we conducted a study to explore the mechanistic basis and blockade(s) for virus coreceptor switch in two R5 SHIVSF162P3N-infected RPs [30]. We found that R5 viruses evolved over time in these two macaques to become increasingly sensitive to sCD4, indicative of an “open” envelope conformation that exposes the CD4 binding site and improves receptor binding. Indeed, we observed that the increase in sCD4 sensitivity of the evolving R5 viruses correlates with the ability of their Envs to bind CD4 more efficiently as well as to mediate infection of cell targets that express low levels of the receptor. Furthermore, major antigenic changes in Env gp160 of the R5 viruses, including changes in the V3 loop that are important for coreceptor engagement, were seen near and at the time of coreceptor switch, consistent with global changes in Env conformation and structural plasticity that facilitate the remodeling needed to expand or switch to CXCR4 usage. These findings led us to propose that adoption of an “open” Env to expose the CD4 binding site for efficient CD4 binding and infection of CD4\textsubscript{low} cells represents an early event in the process of coreceptor switch, releasing or reducing Env structural constraints that have been suggested to limit the mutational pathways available for a change in coreceptor preference. Because these studies were limited with respect to the number of animals, and not all R5 SHIVSF162P3N-infected RP macaques exhibited coreceptor switch, the present work is conducted in two additional RPs with and three without tropism switch to verify and extend our earlier observations, and to identify additional factors that facilitated the process of R5-to-X4 conversion.

Results

R5 viruses evolve in RPs with coreceptor switch to infect primary macrophages more efficiently by improving their CD4 binding

To lend further support to our proposed mechanistic model that exposure of the CD4 binding site for improved CD4 binding and infection of CD4\textsubscript{low} cells is an early step in coreceptor switching, we generated viruses pseudotyped with CCR5-using Envs amplified over time from two additional SHIVSF162P3N-infected RPs with coreceptor switch (DE86, DG08). In addition to clinical indicators such as CD4 T cell loss in the periphery and lymph nodes, coreceptor switch in our studies is defined genotypically and phenotypically by envelope gp120 V3 sequence analysis in combination with an assessment of the ability of viruses recovered from blood and nodes of the infected animals at end-stage disease to utilize CXCR4. We determined the sensitivity of the evolving viruses to sCD4 and the CCR5 inhibitor PSC-RANTES as indirect measurements of their CD4 and CCR5 utilization efficiencies, respectively.
The ability of the viruses to infect primary macrophages that express low levels of the CD4 receptor and their gp120s to bind CD4-Ig was also examined. High and sustained levels of virus replication were seen in both rhesus monkeys, with progression to disease within 30 weeks post-infection (wpi) (Figure 1A). Two independent systemic R5-to-X4 switch events were identified in DG08 seven weeks prior to euthanasia at 20 wpi [17], with one in DE86 that is localized to the lymph node at the time of necropsy (12 wpi) (unpublished data; [33]). Blood CD4+ T cell count fluctuated in DE86, but declined in DG08, with precipitous loss towards end-stage disease. DG08 failed to mount a detectable anti-SHIV antibody response while the response in DE86 was transient, waning at 4 wpi (Table 1). Full-length gp160 Envs were derived by bulk PCR amplification from plasma collected at 2, 4, 5, 8 and 12 wpi for DE86, and at 2, 4, 8, 11, 12, 13, 14, 16 and 20 wpi for DG08. Three or more CCR5-using Env clones per time point were analyzed.

We found no significant difference in the entry efficiency (Figure 2A) or susceptibility to PSC-RANTES inhibition of R5 viruses evolving over time in DE86 (Figure 2B). In contrast, R5 viruses that evolved following the time of emergence of dual- and X4-tropic viruses in DG08 infected the CD4+hCCR5hi TZM-bl cells less efficiently (8.4- and 13-fold reduction in RLU for the w16 and w20 viruses as compared to the early w2-4 viruses respectively), with a 1.5- to 2-fold increase in susceptibility to PSC-RANTES inhibition of the w16 and w20 viruses that is suggestive of less efficient CCR5 usage. These findings of decreased replication and efficacy of CCR5 use with disease progression in DG08 are consistent with results in HIV-1 infected individuals with detectable CXCR4-using variants [6,34,35] and in the two SHIVSF162P3N-infected RP macaques with coreceptor switch studied earlier (BR24, CA28) [30]. Moreover, in agreement with our previous data, R5 viruses in both DE86 and DG08 evolved to become increasingly sensitive to inhibition with CD4-IgG2, a tetrameric soluble CD4 (sCD4) construct based on IgG. For both monkeys, the increase in sCD4 sensitivity that preceded the coreceptor switch occurred in the presence of adequate CD4+ T cell numbers (300-500 CD4+ T cells per ul blood; Figure 2C). Compared to the early w2 replicating virus, which required 1.45 μg/ml sCD4 to achieve 50% neutralization (IC50), a significant 3-4.5 fold increase in susceptibility was evident for viruses prior to (w8) and at the time of (w12) coreceptor switch in DE86. The w2 replicating virus in DG08 was as sensitive to sCD4 neutralization as the late DE86 viruses (IC50 0.42 μg/ml), but viruses replicating two weeks later were slightly more sCD4 resistant (IC50 0.6 μg/ml). Compared to the w4 viruses, R5 viruses present before (w11, w12) and after (w14, w16, w20) the time of switch in DG08 were also significantly more sCD4 sensitive, with a rebound in sCD4 resistance close to the baseline level seen at the time of switch (w13).

Importantly, and in support of our earlier findings [30], the increase in sCD4 sensitivity of the evolving R5 viruses in DE86 and DG08 was accompanied by a corresponding increase in the binding of the gp120s to CD4-
Ig and in infection of primary macrophages that express low amounts of the CD4 receptor (Figure 3A). The exceptions were R5 viruses present one week prior (w12) and at the time of coreceptor switch (w13) in DG08. Despite sCD4 sensitivity that was comparable to the w11 viruses, the w12 viruses in DG08 exhibited diminished ability to infect primary macrophages, with lower gp120/CD4-Ig binding as well. We did not observe any amino acid changes in the CD4 binding site, the Phe43 cavity or the inner domain layers of gp120 that could explain the loss in macrophage infection and CD4 binding of the w12 Envs. This dissociation between sCD4 sensitivity, better CD4 binding and infection of CD4low cells in DG08 prior to the time of switch had previously been observed in BR24 [30], suggesting that mechanism(s) other than exposure of the CD4 binding site (BS) for better CD4 use is conferring sCD4 sensitivity to the w12 viruses. Decreased infection of primary macrophages and receptor binding was also seen for the w13 viruses, but as noted above, there was a 2-fold increase in sCD4 resistance for R5 viruses at this time point.

Changes in envelope glycoprotein antigenic structure near and at the time of tropism switch

Marked changes in structure or accessibility of the CD4BS and V3 loop of gp120 and the pre-hairpin intermediate of gp41 were observed near the time of switch in our prior study of two RPs [30]. Accordingly, we probed the structure of the evolving R5 viruses in DE86 and DG08 by assessing their susceptibility to neutralization with the anti-CD4BS mAb b12, the anti-V3 loop mAb 447-52D and T20, which binds a triple-stranded coiled coil activated fusion intermediate composed of the N-terminal heptad repeat (HR-1) region of gp41. Results showed that the changes in sCD4 sensitivity and receptor use of the R5 viruses evolving around the time of coreceptor switch in the two macaques coincided with modulations in Env conformations. For DE86, there was a significant increase in b12 and 447-52D susceptibility for viruses close to (w8) and at the time of switch (w12) that may be indicative of changes in the structure and/or accessibility of the CD4 and coreceptor binding sites (Figure 3B). A significant increase in T20 sensitivity of the w12 viruses is suggestive of greater exposure or a longer half-life of the gp41 HR-1 groove on the Envs of these viruses. The changes in the antigenic structure of R5 Envs around the time of coreceptor switch in DG08, the macaque that harbored two independent R5-to-X4 evolutionary pathways, were more complex. Compared to the acute viruses, a statistically significant increase in b12 sensitivity was evident at 8 wpi, and remained so except for the w11 and w12 viruses. T20 sensitivity was also significantly increased, with the exception of viruses prior to (w12) and at the time of switch (w13). Furthermore, a dramatic decrease in 447-52D sensitivity was seen for the w11 viruses. Taken together, the data support Env structural changes near and following the time of switch in DE86 and DG08, the macaque that harbored two independent R5-to-X4 evolutionary pathways, were more complex. Compared to the acute viruses, a statistically significant increase in b12 sensitivity was evident at 8 wpi, and remained so except for the w11 and w12 viruses. T20 sensitivity was also significantly increased, with the exception of viruses prior to (w12) and at the time of switch (w13).

R5 viruses in RPs without coreceptor switch also evolved to infect macrophages more efficiently, but this was not accompanied by an increase in CD4 binding

To determine if acquisition of better CD4 binding is a unique characteristic of the R5 viruses in RPs with coreceptor switch, we characterized CCR5-using Envs over
time from three SHIVSF162P3N-infected RP macaques with no overt signs of coreceptor switch. BT78, CC39 and DN57 progressed to disease at 13, 12 and 30 wpi respectively, with preservation of peripheral and lymphoid CD4+ T cells at the time of euthanasia [17,33] (Figure 1B). Weak SHIV-specific binding antibody response could be detected throughout the course of infection in DN57, but was less persistent in BT78 (waned after 10 wpi) and absent in CC39 (Table 1). We derived full-length Envs by bulk PCR amplification of plasma collected at 1, 2, 5, 8 and 13 wpi for BT78, at 1, 4, 6, 9 and 12 wpi for CC39, and at 2, 4, 6, 10, 17, 25 and 30 wpi for DN57. At least three Env clones from each time point were assessed for their ability to mediate entry and PSC-RANTES susceptibility. Results showed that Envs amplified over time from BT78 and CC39 mediated comparable entry into TZM-bl cells, but the late viruses in DN57 (w17, 25 and 30) showed a 2- to 7-fold reduction in entry efficiency compared to the acute w2 viruses (Figures 4A). PSC-RANTES sensitivity fluctuated for the evolving R5 viruses in CC39 and DN57, but a trend towards resistance was seen in BT78 (Figure 4B). The difference in PSC-RANTES susceptibility between the early w2 and end-stage w13 viruses in BT78 approaches significance (p=0.06), consistent with reports that the late R5 viruses in HIV-1 infected individuals without detectable X4 viruses use the CCR5 coreceptor better [6,37-41].

sCD4 sensitivity, gp120/CD4-IgG binding and infection of primary macrophages of the evolving R5 viruses in the three RPs without evidence of coreceptor switch were then determined (Figure 5). Increase in sCD4 sensitivity was seen for R5 viruses evolving over time in BT78 and DN57, but was transient in CC39. The increase was ~3-fold for the end-stage (w13) viruses relative to the early (w2) viruses in BT78, but was significant
Figure 3 (A) Relationship between sCD4 sensitivity, CD4-Ig binding and infection of primary macrophages (mΦ) of DE86 and DG08 viruses. Values above the bars indicate fold increase in sCD4 sensitivity of evolving viruses compared to early viruses, and the vertical dashed line indicates the time of coreceptor switching. sgp120 binding to CD4-Ig was normalized to that of sgp120 binding to polyclonal serum from HIV-1 infected individuals. Infectivity in mΦ that express low levels of CD4 was expressed as a ratio of infectivity in autologous PBMCs that express high levels of CD4 and CCR5. The shaded area highlights the time prior and during coreceptor switch. For sgp120 CD4-Ig binding, data are the means and standard deviations from at least two independent experiments. For infection of macrophages, data are representative of at least 3 independent experiments (error bars, s.d.). * indicates statistically significant differences between the early and the evolving R5 viruses. (B) Changes in neutralization sensitivity of R5 viruses evolving over time in macaques DE86 and DG08. Susceptibility of R5 pseudoviruses to neutralization with IgG1b12, 447-52D and T20 was determined. The vertical dashed line indicates the time of coreceptor switching, and the shaded area designates the period of marked envelope conformational changes. Data are representative of at least two independent experiments (error bars, s.d.). * above the bars indicate IC50 values that are statistically different between the acute and the evolving R5 viruses, P<0.05 (Mann-Whitney U test).

Figure 4 Entry efficiency, PSC-RANTES and sCD4 sensitivity of R5 viruses evolving over time in BT78, CC39 and DN57. Entry of luciferase reporter viruses expressing CCR5-using Envs into TZM-bl cells expressed as relative light unit (RLU) (A), and susceptibility of the reporter viruses to neutralization with PSC-RANTES (B) and sCD4 (C) were determined. The numbers in the brackets indicate the number of clones analyzed at each time point and values above the bars indicate fold increase in sCD4 sensitivity relative to that of the w2 viruses. * P<0.05 (Mann-Whitney U test). Data are representative of 2-3 independent experiments (error bars, s.d.).
in DN57 (Figure 5A). Compared to the acute (w2) viruses, a 3-fold increase in sCD4 sensitivity was seen as early as 4 wpi, with further increases (8-13 fold) for the late viruses (w17, 25 and w30). Notably, an increase in the ability of the evolving viruses to infect macrophages was seen in all three RPs. The increase was significant for the w4 viruses in CC39 as compared to the w1 viruses (p<0.05), with differences that approach significance for the w9 and w12 viruses in this animal (p=0.07 and p=0.09, respectively). For BT78 and DN57, the difference in the ability to infect CD4low cells between the acute and late viruses was significant. Importantly, however, there was no corresponding increase in the ability of the gp120s of the evolving R5 viruses in these animals to bind CD4-Ig that would indicate increase accessibility of the CD4 binding site (Figure 5A). We recognize that CD4 binding was measured using monomeric gp120 and may differ from that of trimeric gp120. Nevertheless, because the comparison is between CD4 binding of monomeric gp120s evolving over time in macaques with and without coreceptor switch, the finding that increased gp120/CD4 binding is observed only in animals with tropism switch is still of interest. Thus, we conclude that while the selective pressure for infection of alternative target cells with lower receptor expression levels is present in RPs with and without coreceptor switch, R5 viruses in macaques that did not switch evolved to be more sCD4 sensitive and infected CD4low cells more efficiently via a mechanism other than adoption of an “open” Env conformation to expose their CD4BS for better receptor binding.

**R5 viruses in RPs without coreceptor switch employ a compensatory mechanism for poor CD4 binding in infection of macrophages and in sCD4 susceptibility**

Besides exposure of the CD4BS for efficient CD4 binding, increase sensitivity to sCD4 neutralization and infection of CD4low cells may mean that the conformational changes induced by CD4 binding are altered, that the virus has acquired additional contacts with CD4, or the interaction with CCR5 is improved [42-48]. The greater resistance to PSC-RANTES inhibition of the late viruses...
High-level replication is a strong predictor for R5-to-X4 virus evolution in RPs

The finding that not all SHIVSF162P3N–infected RP macaques adapt to use low levels of CD4 through adoption of an "open" Env to enhance CD4 binding, even though such an Env configuration would have relieved the structural constraints on change in coreceptor preference, suggests that there is no obligatory selection pressure for the virus to use CXCR4. It also implies that the absence or diminution of antibody-driven pressure by itself is insufficient to drive coreceptor switching, and is consistent with our report that depletion of B cells to abrogate or diminish antiviral antibody responses prior to infection of rhesus macaques with SHIVSF162P3N did not promote tropism switch [51]. In a number of cross-section studies of HIV-1-infected individuals, high viral load was shown to be the strongest predictor of R5-to-X4 evolution [37,52-59]. To determine if high viral load is also a predictor to R5-to-X4 evolution in RP macaques, we compared cumulative viral loads up to the time of euthanasia in R5 SHIVSF162P3N-infected RPs with (n=4) and without (n=3) coreceptor switch studied here and previously [30]. We found that cumulative viral load was significantly higher in monkeys with tropism switch than in those without, with a p value of 0.03 (Figure 6). Thus, similar to HIV-1 infection in humans, high virus replication is associated with the evolution and establishment of X4 variants in R5 SHIV-infected macaques.

Discussion

In this study, we confirmed adoption of an "open" Env to expose the CD4 binding site for efficient CD4 binding and infection of primary macrophages in two additional RPs with coreceptor switch. Moreover, changes in neutralizing antibody and T20 sensitivity around the time of X4 virus emergence are consistent with a more responsive quaternary configuration of the viral envelope spike. In agreement with reports in HIV-1-infected individuals, high virus replication is a strong predictor for R5-to-X4 conversion in SHIVSF162P3N–infected macaques. A certain level of viremia may provide the conditions optimal for coreceptor switching or, alternatively, the Env changes required for coreceptor switching may predispose to higher levels of virus replication. Adoption of an "open" Env conformation that confers structural flexibility to accommodate mutational changes, and high levels of virus replication to overcome the genetic hurdles for R5-to-X4 evolution therefore contribute to the coreceptor switch in nonhuman primates. Since an "open" Env conformation also renders the virus more susceptible to antibody neutralization, our findings help to explain the infrequent and late appearance of X4 virus in HIV-1 infection when the immune system deteriorates.

Figure 6 Comparison of cumulative viral load in R5 SHIVSF162P3N-infected RPs with (n=4) and without (n=3) coreceptor switch. Cumulative log_{10} RNA copies/ml plasma viremia up to the time of euthanasia were compared using unpaired t tests. Time to euthanasia for the four RPs with coreceptor switch is: w24 (BR24), w15 (CA28), w12 (DE86), w20 (DG08), and for the three RPs without coreceptor switch is: w13 (BT78), w12 (CC39) and w30 (DN57). P value of <0.05 was considered significant.
Diminished entry efficiency of the evolving R5 viruses following the time of switch, with increase in PSC-RANTES sensitivity that is suggestive of poorer CCR5 usage was seen in DG08 studied here (Figure 2) and in BR24 and CA28 reported earlier [30]. Interestingly, these are the three SHIV$_{SF162P3N}$-infected RPs that harbored pure X4 virus populations. Decrease in the efficiency of CCR5 usage had also been reported in HIV-1 infected individuals with detectable CXC4-using variants [6,34,35], suggestive of a decline in R5 fitness. HIV-1 and SHIV viruses with a pure X4 phenotype generally have higher replication kinetics in vitro [60-64] and in vivo [65-68]. Coupled with the broader cellular host range of X4 viruses in vivo [69,70], they are likely to out-compete R5 viruses once established. The decrease in the selective pressure for R5 viruses to maintain optimal replicative capacity and fitness with X4 emergence therefore, could be a reason for the decrease in entry efficiency and CCR5 utilization of R5 viruses in SHIV-infected macaques with pure X4 viruses. Nevertheless, R5 variants persist following the appearance of X4 viruses in these animals, perhaps because of their distinct target cell range [69,70].

Transmitted/founder viruses in acute and early HIV-1 infection have been reported to be sCD4 resistant and to replicate poorly in monocyte-derived macrophages that express low levels of the CD4 receptor [3,71,72]. Neutralization resistance in vitro is often accompanied by an increase in HIV-1 dependence on CD4 for entry [73-75]. Indeed, founder viruses were shown to require high levels of CD4 for entry [76], which may explain their deficiency in infecting CD4$^{low}$ cells. Consistent with these findings in humans, viruses that are sCD4 resistant and infected primary macrophages less efficiently predominated during acute infection in R5 SHIV$_{SF162P3N}$-infected RP macaques DE86, BT78, CC39 and DN57, and in the two RPs we previously studied [30], regardless of coreceptor switching. The exception was DG08, where the acute (w2) viruses were observed to be sCD4 sensitive but did not infect macrophages efficiently. This however, was rapidly replaced two weeks later by variants that are more resistant to sCD4, reinforcing the view that these viruses are selected for during primary infection. As this was observed despite intravenous inoculation (BR24, CA28, DE86, BT78, CC39) which circumvented mucosal barriers, and in macaques that failed to mount a strong humoral or cellular immune response, these factors are unlikely to be playing a role in the early expansion of sCD4-resistant viruses. We speculate that the abundance of target cells with high levels of cell surface CD4 expression in the new hosts maybe an underlying determinant for the expansion and propagation of viruses that are more dependent on CD4 for entry during acute SHIV$_{SF162P3N}$ infection in macaques and HIV-1 infection in humans. Further research on the importance of CD4 receptor density in HIV infection as well as the kinetics of virus replication in infected cells may help to understand the selective forces that govern the biological phenotype of HIV-1 transmitted founder viruses.

Enhanced infection of primary macrophages was seen in the RP macaques at end-stage disease irrespective of coreceptor switch, indicative of a strong selective pressure to replicate in cells with lower CD4 cell-surface expression levels over the infection course. Adaptation to macrophages has also been observed in SIV-infected RP macaques [77], and isolates from late in disease of HIV-1 infected individuals have been reported to be more macrophage-tropic compared to those from early in infection [78-80], suggestive of similar selection pressure in humans and rhesus monkeys. Presumably, as discussed above, ongoing virus replication in both hosts leads to loss in target cells (e.g., memory T cells) with high receptor expression levels, putting pressure on the virus to change so that it can use CD4 more efficiently to infect alternate CCR5+ cells such as macrophages that express low amounts of receptor. Infection-associated immune activation may also decrease receptor density, since CD4 is down-regulated with T-cell activation [81-83]. In RPs with coreceptor switch (BR24, CA28, DE86, DG08), infection of CD4$^{low}$ cells was achieved by adoption of a less constrained and more “open” Env conformation that exposes the CD4 binding site for better CD4 binding, allowing flexibility in accommodating the structural remodeling needed for the change in coreceptor preference. The V3 loop mutations that confer CXC4 usage in R5 SHIV$_{SF162P3N}$-infected RP monkeys and in HIV-1 infected individuals also render the virus sCD4 sensitive, consistent with the notion that improved CD4 binding is a necessary prerequisite for acquisition of CXC4 use [23,84,85]. In RP macaques that did not switch, R5 viruses appeared to have evolved to compensate for inadequate CD4 binding by improving CCR5 usage and/or the requisite post-binding conformational changes for virus entry in response to nonoptimal CD4. It is possible that given time, these latter macaques (e.g., BT78, CC39) could also adopt an “open” Env conformation to facilitate CD4 binding and infection of primary macrophages, setting the stage to tropism switch. Alternatively, the presence of host immune response may be a hindering factor. This is best illustrated in DN57, which, contrary to the other RP monkeys, mounted a persistent albeit weak anti-SHIV antibody response. R5 viruses evolved in this macaque to be highly sCD4 sensitive, with IC$_{50}$ values comparable to those seen in DE86, suggestive of a similar strong selective pressure for better CD4 usage. The
evolving R5 viruses in DN57 also infected macrophages efficiently, but this was not accompanied by a corresponding increase in CD4 binding that is indicative of exposure of the receptor binding site. Rather, the Env in DN57 evolved to adapt to less-than-optimal CD4 density by changes in receptor and/or coreceptor contact, or by acquiring a higher propensity for proceeding to fusion. Thus, although the pressure to infect cell targets that express low levels of CD4 is present in SHIVSF162P3N-infected RP macaques regardless of coreceptor switch, the mechanisms employed to overcome this selective pressure vary depending on other conditions in the host.

Conclusions

In summary, our studies revealed an ordered process of phenotypic switch that is now recapitulated in four R5 SHIVSF162P3N-infected RPs. The data reinforce the view that an interplay between positive (e.g., magnitude of persistent virus replication, evolutionary rate, change in target cell population) and negative (e.g., host immune response) selection forces that differ between individual hosts and at different stages of the infection course governs how R5 viruses respond to the changing environment and shape the frequency of expansion or switch to CXCR4 use. Greater viral burden translates into a faster reduction in the target cell population, higher mutation frequencies of the virus populations and increased chances of X4 evolution. In the absence of immunological constraints in the RPs, and with the conferred selective advantage of expanding cellular host range, X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread. Since an "open" Env conformation for X4-associated changes become fixed and these viruses rapidly spread.

Methods

Ethics statement

This work used blood from SHIV infected macaques housed at the Tulane National Primate Research Center (TNPRC) in accordance with the Animal Welfare Act and Guide for the Care and Use of Laboratory Animals. TNPRC is accredited by the Association and Assessment and Accreditation of Laboratory Animal Care (AAALAC #00594). The OLAW animal welfare assurance number for TNPRC is A4499-01 and the USDA registration number is 72-R-002. All procedures were performed on anesthetized animals and post-operative analgesics were administered as needed in accordance with the Tulane IACUC approval.

Cells

293T cells and Hela TZM-bl cells expressing CD4, CCR5 and CXCR4 and containing integrated reporter genes for firefly luciferase and β-galactosidase under control of the HIV-1 LTR [86] were maintained in DMEM supplemented with 10% fetal bovine serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. Human peripheral mononuclear cells (PBMCs) were prepared by Ficoll gradient centrifugation, stimulated with phytohemagglutinin (PHA, 3 μg/ml; Sigma, St. Louis, MO) in RPMI medium containing 10% FCS, penicillin, streptomycin, L-glutamine and 20 U/ml interleukin-2 (Novartis, Emeryville, CA). Monocytes were enriched by centrifugation of PBMCs through a 40% percoll cushion followed by plastic adherence, and cultured in RPMI 1640 medium supplemented with 10% FCS and 5% human AB serum for 5-7 days to allow for differentiation into macrophages [87].

Detection of antiviral humoral response

SHIV-specific antibodies in serum samples were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (GS HIV-1/ HIV-2 PLUS O EIA; Bio-Rad, Redmond, WA). This assay detects antibodies to HIV-1 gp160 and p24, and to the immunodominant region of the transmembrane glycoprotein gp36 of HIV-2. Optical density values at a 1:10 serum dilution that are three times above the cutoff value are considered positive.

Plasmid constructs and pseudotyped virus production

For expression of envelope glycoproteins (Env), viral RNA was prepared from 300-500 μl plasma using a commercially available RNA extraction kit (Qiagen, Valencia, CA) followed by reverse-transcription (RT) with Superscript III RT (Invitrogen, Carlsbad, CA) and random hexamer primers (Amersham Pharmacia, Piscataway, NJ). Full-length gp160 coding sequences were amplified from bulk RT products with primers SH43 (5’-AAAACAAGAATTGATGGAAGGAGATGGAAGAAG-3’) and SH44 (5’-AGAGAGGGATCTTATAGCAAGCCCTTTCAAGCCCT-3’), subcloned into the pCAGGS vector and sequenced for verification. To generate luciferase reporter viruses capable of only a single round of replication, envelope trans-complementation assay was used as previously described [88]. Briefly, Env expression plasmid and the NL4-3LucE-R+ vector were cotransfected with polyethylenimine (PEI, Polyscience, Warrington, PA) into 2.5 × 10⁶ 293T cells plated in a 100-mm plate. Cell culture supernatants were harvested 72 hours later, filtered through 0.45-μm filters, and stored at -70°C in 1-ml aliquots. Pseudotyped viruses were quantified for p24 Gag content (Beckman Coulter, Fullerton, CA).
Virus infectivity
For assessment of Env functionality and entry efficiency, 7 × 10^4 TZM-bl cells were seeded in 96-well plates 24 hours before use and infected, in triplicates, with 2 ng p24 Gag equivalent of the indicated pseudotyped viruses. Infected cells were cultured for 72 h at 37°C, at which time the cells were harvested, lysed and processed for luciferase activity according to the manufacturer’s instructions (Promega, Madison, WI). Entry, as quantified by luciferase activity, was measured with an MLX microtiter plate luminesimeter (Dynex Technologies, Chantilly, VA). For infection of primary cells, 10^5 and 10^6 cells of human PBMCs and macrophage respectively were infected in duplicates with 5 ng p24 Gag equivalent of the indicated pseudotyped viruses in each well of a 96-well plate. Infected cultures were harvested 72 hours later and processed for luciferase activity. To control for differences in Env entry efficiencies, infectivity in macrophages was expressed as a ratio of the infectivity for these cells compared to the infectivity in PBMCs from the same donor.

Receptor and coreceptor usage efficiency
For assessment of receptor usage efficiency, 2 ng p24 equivalent of the indicated pseudotyped viruses in 50 μl were incubated with equal 4-fold serial dilution volumes of CD4-IgG2 fusion protein (sCD4; PRO 542, Progenics Pharmaceuticals, Tarrytown, NY) for 1 h at 37°C and then added to cells, in duplicate wells, for an additional 2 hours at 37°C. 100 μl of medium was then added to each well and the virus–protein cultures maintained for 72 hours. Control cultures received virus in the absence of sCD4. At the end of the culture period, the cells were lysed and processed for β-galactosidase activity (Galanoclone Star System; Applied Biosystems, Bedford, MA). A neutralization curve was generated by plotting the percentage of neutralization vs sCD4 dilution, and 50% inhibitory concentrations (IC_{50}) were determined using the Prism 4 software (GraphPad, San Diego, CA). For assessment of coreceptor usage efficiency, 7 × 10^3 TZM-bl cells per well of a 96-well plate were inoculated, in duplicates, with 2 ng p24 Gag antigen equivalent of the indicated pseudotyped virus in the absence or presence of 4-fold dilutions of the CCR5 antagonist PSC-RANTES. The cells were lysed after 72 hours at 37°C, processed for β-galactosidase activity, and IC_{50} determined using the Prism 4 software.

Soluble gp120-CD4-Ig binding
To examine monomeric gp120-CD4 binding, gp120 glycoproteins from transfected 293T cells were metabolically radiolabeled for 48 hours with 100 μCi/mL [35S]-methionine/cysteine ([35S] protein labeling mix; Perkin-Elmer, Waltham, Mass) in Dulbecco’s modified Eagle’s medium lacking methionine and cysteine and supplemented with 5% dialyzed fetal bovine serum. Radiolabeled protein extracts were incubated with either a mixture of sera from HIV-1 infected individuals or CD4-Ig (a fusion protein in which the N-terminal two domains of CD4 are linked to the Fc component of immunoglobulin G [89]) in the presence of 70 μl of 10% Protein A-Sepharose (American BioSciences Inc, Boulder, CO) for 2 hr at 37°C. The precipitates were analyzed on NuPAGE Bis-Tris polyacrylamide gels (Invitrogen, Carlsbad, CA), followed by autoradiography and quantification with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Neutralization assay
Virus neutralization was assessed using TZM-bl cells in 96-well plates. Briefly, equal volumes (50 μl) of pseudotyped viruses (2-3 ng p24 Gag equivalent) and 4-fold serial dilutions of IgG 1b12, 443-52D and T20 were incubated for 1 h at 37°C and then added to cells, in duplicate wells, for an additional 2 hours at 37°C. 100 μl of medium was then added to each well and the virus–protein cultures maintained for 72 hours. Control cultures received virus in the absence of blocking agent. At the end of the culture period, the cells were lysed and processed for β-galactosidase activity. A neutralization curve was generated by plotting the percentage of neutralization vs agent dilution, and IC_{50} determined using the Prism 4 software.

Statistical analysis
The Mann-Whitney U or unpaired T test was used to evaluate differences in susceptibility to sCD4, 1b12, 447-52D and T20; binding of gp120 to CD4-Ig; and infection of macrophages between the early (w2-4) and the evolving R5 viruses. These tests were also used to examine differences in cumulative viral load between RPs with and without coreceptor switch. P-values <0.05 were considered statistically significant.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
KZ, AF, WH and CCM designed the study. KZ, AF, JT and A Frantzell carried out the experiments. KZ, AF, JS and CCM analyzed the results and drafted the manuscript. All authors read and approved the final manuscript.

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characteristics of coreceptor use and HIV type 1 V3 sequence in a large population of therapy-naive individuals. AIDS Res Hum Retroviruses 2008, 24:219–228.

35. Coetzee M, Nedellec R, Salkowitz J, McLaughlin S, Liu Y, Heath L, Mullins JL, Mosier DE: Evolution of CCR5 use before and during coreceptor switching. J Virol 2008, 82:21758–1766.

36. Huang W, Toma J, Franssen S, Stawski E, Reeves JD, Whitcomb JM, Parkin N, Metzger CL: Coreceptor tropism can be influenced by amino acid substitutions in the gp41 transmembrane subunit of human immunodeficiency virus type 1 envelope protein. J Virol 2008, 82:5584–5593.

37. Jansson M, Papovic M, Karlsson A, Cocchi F, Rossii P, Albert J, Wigzell H: Sensitivity to inhibition by beta-chemokines correlates with biological phenotypes of primary HIV-1 isolates. Proc Natl Acad Sci USA 1996, 93:15382–15387.

38. Koning FA, Kwa D, Boeser-Nunnink B, Dekker J, Vingerhoed J, Hiemstra H, Mosier DE: Evolution of CCR5 use before and during coreceptor switching. J Virol 2003, 188:8684–672.

39. Karlsson J, Antonssson L, Shi Y, Oberg M, Karlsson A, Albert J, Olde B, Oxman C, Jansson M, Fenyo EM: Coevolution of RANTES sensitivity and mode of CCR5 receptor use by human immunodeficiency virus type 1 of the RS phenotype. J Virol 2004, 78:11807–11815.

40. Rupits J, Oberg M, Edstrand P, Karlsson A, Albert J, Fenyo EM, Jansson M: Selection of human immunodeficiency virus type 1 RS variants with augmented replicative capacity and reduced sensitivity to entry inhibitors during severe immunodeficiency. J Gen Virol 2005, 86:2859–2869.

41. Etehad B, Fellows A, Kwambwa B, Karnat A, Feng Y, Lee S, Sagar M: Human immunodeficiency virus type 1 V1-to-V5 envelope variants from the chronic phase of infection use CCR5 and fuse more efficiently than those from early after infection. J Virol 2008, 83:9694–9708.

42. Orloff SL, Kennedy MS, Belperron AA, Maddon PJ, McDougal JS: Two mechanisms of soluble CD4 (sCD4)-mediated inhibition of human immunodeficiency virus type 1 (HIV-1) infectivity and their relation to primary HIV-1 isolates with reduced sensitivity to sCD4. J Virol 1993, 67:1461–1471.

43. Moore JP, McKeating JA, Huang YX, Ashkenazi A, Ho DD: Differences in CD4 dependence among primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. J Virol 1992, 66:235–243.

44. Thali M, Rehbein T, Helsen E, Repee H, Hodzic J: Lack of correlation between soluble CD4-induced shedding of the human immunodeficiency virus type 1 exterior envelope glycoprotein and subsequent membrane fusion events. J Virol 1992, 66:5516–5524.

Stamatatos L, Werner A, Cheng-Mayer C: Differential regulation of cellular tropism and sensitivity to soluble CD4 neutralization by the envelope gp120 of human immunodeficiency virus type 1. J Virol 1994, 68:4973–4979.

46. Haim H, Si Z, Madani N, Wang L, Couler JR, Princicato A, Kassa A, DeGrazia M, McGee-Estrada K, Melford M, et al: Soluble CD4 and CD4-mimetic compounds inhibit HIV-1 infection by induction of a short-lived activated state. PLoS Pathog 2009, 5:1003560.

47. Kabat D, Kozak SL, Wehrly K, Chesebro B: Differences in CD4 dependence for infectivity of laboratory-adapted and primary patient isolates of human immunodeficiency virus type 1. J Virol 1994, 68:2570–2577.

48. Platt EJ, Wehrly K, Kuhmann SE, Chesebro B, Kabat D: Effects of CCR5 and CD4 cell surface concentrations on infections by macrophage-tropic isolates of human immunodeficiency virus type 1. J Virol 1998, 72:2855–2864.

49. Moll R, Roosdams C, Demasi R, Heilek-Snyder G, Greenberg ML, Graham N: Lack of correlation between soluble CD4-induced shedding of the human immunodeficiency virus type 1 exterior envelope glycoprotein and subsequent membrane fusion events. J Virol 1992, 66:5516–5524.

50. Stamatatos L, Werner A, Cheng-Mayer C: Differential regulation of cellular tropism and sensitivity to soluble CD4 neutralization by the envelope gp120 of human immunodeficiency virus type 1. J Virol 1994, 68:4973–4979.

51. Tatsa S, Zhang K, Gettie A, Knight H, Blanchard J, Westmoreland S, Cheng-Mayer C: Effect of B-cell depletion on coreceptor switching in RS simian-human immunodeficiency virus infection of rhesus macaques. J Virol 2011, 85:3086–3094.

52. Bozzette SA, McCutchan JA, Spector SA, Wright B, Richman DD: A cross-sectional comparison of persons with syncytium- and non-syncytium-inducing human immunodeficiency virus. J Infect Dis 1993, 168:1374–1379.

53. Brumme ZL, Goodrich J, Mayer HB, Brumme CJ, Henrick BM, Wynbourn B, Asselin JJ, Cheung PK, Hogg RS, Montaner JS, Harrigan PR: Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. J Infect Dis 2005, 192:466–474.

54. Furrer H, Wendland T, Minder C, Christen A, von Overbeck J, Grunow R, Pichler W, Malinverni RP: Association of syncytium-inducing phenotype of HIV-1 with CD4 cell count, viral load and sociodemographic characteristics. AIDS 1998, 12:1341–1346.

55. Melby T, Despino M, Deras M, Heile-Snyder G, Greenberg ML, Graham N: HIV-1 coreceptor use in triple-class treatment-experienced patients: baseline prevalence, correlates, and relationship to enfuvirtide response. J Infect Dis 2006, 194:328–346.

56. Wilkin TJ, Su Z, Kurtzkes DR, Hughes M, Flemer C, Gross R, Coakley E, Greaves W, Godfrey C, Skolnik PR, et al: HIV-1 type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 2007, 44:591–595.

57. Moyle GJ, Warnke MD, van Benthem BH, Otto SA, Pins M, Miedema F, Schuitemaker H: Early viral load and CD4+ T cell count, but not percentage of CCR5+ or CXCR4+ CD4+ T cells, are associated with RS-to-X4 HIV type 1 virus evolution. AIDS Res Hum Retroviruses 2003, 19:389–398.

58. Kassaye S, Johnston E, McColgan B, Kantor R, Zijenah L, Katzenstein D: Envelope coreceptor tropism, drug resistance, and viral evolution among subtype C HIV-1-infected individuals receiving nonsuppressive antiretroviral therapy. J Acquir Immune Defic Syndr 2009, 50:5–18.

59. Woot AB Vt, Blaak H, Ran LJ, Brouwer M, Kuiken C, Schuitemaker H: Evolution of syncytium-inducing and non-syncytium-inducing biological virus clones in relation to replication kinetics during the course of human immunodeficiency virus type 1 infection. J Virol 1998, 72:5099–5107.

60. Sagi B, Morfeldt-Manson L, Albert J, Biberfeld G, Karlsson A, Lidman K, Fenyo EM: Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet 1986, 2864–2866.

61. Connor R, Mohri H, Cao Y, Ho DD: Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals. J Virol 1993, 67:1772–1777.

62. Cheng-Mayer C, Seto D, Tateno M, Levy JA: Biologic features of HIV-1 that correlate with virulence in the host. Science 1988, 240:80–82.

63. Harouse JM, Tan RC, Gettie A, Dailer P, Marx PA, Lucena PA, Cheng-Mayer C: In vitro infection of primate PBMC with simian/human immunodeficiency virus, SHIV(SF33A): correlation to in vivo outcome. J Med Primatol 1998, 9:7109–5107.

64. Igarshe T, Endo Y, Englund G, Sadjadpour R, Matano T, Buckler C, Buckler-White A, Plishka R, Theodore T, Shibata R, Martin M: Emergence of a highly pathogenic simian/human immunodeficiency virus in a rhesus macaque treated with anti-CD8 mAb during a primary infection with a nonpathogenic virus. Proc Natl Acad Sci USA 1999, 96:14049–14054.

65. Joag SV, Li Z, Firestone L, Stephen EB, Zhao LJ, Adany I, Pinson DM, McClure HW, Narayan O: Chimeric simian/human immunodeficiency virus that causes progressive loss of CD4+ T cells and AIDS in pig-tailed macaques. J Virol 1996, 70:3189–3197.

66. Reiman KA, Li J, Veeze R, Halloran M, Park NW, Karlsson GB, Sodroski J, Letvin NL: A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. J Virol 1996, 70:6922–6928.

67. Harouse JM, Buckner C, Gettie A, Fuller R, Bohn R, Blanchard J, Cheng-Mayer C: CD8+ T cell-mediated CXC chemokine receptor 4-simian/human immunodeficiency virus suppression in dually infected rhesus macaques. Proc Natl Acad Sci USA 2003, 100:1977–1982.
69. Ho SH, Stek L, Gettie A, Blanchard J, Cheng-Mayer C. V3 loop-determined coreceptor preference dictates the dynamics of CD4+ T-cell loss in simian-human immunodeficiency virus-infected macaques. J Virol 2005, 79:12296–12303.

70. Nishimura Y, Igarashi T, Donau OK, Buckler-White A, Buckler C, Lafont BA, Goeknet RM, Goldsag S, Hirsch VM, Martin MA. Highly pathogenic SHIVs and SIVs target different CD4+ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. Proc Natl Acad Sci USA 2004, 101:12324–12329.

71. Isaacman-Beck J, Herrmann EA, Yi Y, Ratcliffe SJ, Mulenga J, Allen S, Hunter E, Derdeyn CA, Collman RG. Heterosexual transmission of human immunodeficiency virus type 1 subtype C: Macrophage tropism, alternative coreceptor use, and the molecular anatomy of CCR5 utilization. J Virol 2009, 83:8208–8220.

72. Ochsengauer C, Edmonds TG, Ding H, Kelle BF, Decker J, Salazar MG, Salazar-Gonzalez JF, Shattock R, Haynes BF, Shaw GM, et al. Generation of transmitted/founder HIV-1 infectious molecular clones and monocyte-derived macrophages. J Virol 2012, 86:2715–2728.

73. Koaz SL, Platt EI, Madani N, Ferro FE Jr, Peden K, Kabat D. CD4, CCR-5, and CCR-5 dependencies for infections by primary patient and laboratory-adapted isolates of human immunodeficiency virus type 1. J Virol 1997, 71:873–882.

74. Platt EI, Madani N, Koaz SL, Kabat D. Infectious properties of human immunodeficiency virus type 1 mutants with distinct affinities for the CD4 receptor. J Virol 1997, 71:883–890.

75. Sullivan N, Sun Y, Sattentau Q, Thali M, Wu D, Denisova G, Geniseni J, Robinson J, Moore J, Sodroski J. CD4-induced conformational changes in the human immunodeficiency virus type 1 gp120 glycoprotein: consequences for virus entry and neutralization. J Virol 1998, 72:4694–4703.

76. Alexander M, Lynch R, Mulenga J, Allen S, Derdeyn CA, Hunter E. Donor and recipient envs from heterosexual human immunodeficiency virus subtype C transmission pairs require high receptor levels for entry. J Virol 2010, 84:4100–4104.

77. Brown CR, Zapiga M, Kabat J, Pang Q, Ourmanov L, Nishimura Y, Martin MA, Hirsch VM. Unique pathology in simian immunodeficiency virus-infected rapid progressor macaques is consistent with a pathogenesis distinct from that of classical AIDS. J Virol 2007, 81:5594–5606.

78. Gray L, Sterjovski J, Churchhill M, Ellery P, Nasr N, Lewin SR, Crowe SM, Wesselingh SL, Cunningham AL. SIVmac239 induces implications for coreceptor usage and diversity in SIVmac251. J Virol 2008, 82:12326–12332.

79. Li S, Juarez J, Alihi M, Owyer D, Collman R, Cunningham A, Naif HM. Persistent CCR5 utilization and enhanced macrophage tropism by primary blood human immunodeficiency virus type 1 isolates from advanced stages of disease and comparison to tissue-derived isolates. J Virol 1999, 73:9751–9755.

80. Tuttle DL, Anders CB, Aquino-De Jesus MJ, Poole PP, Lamers SL, Briggs DR, Pomeroy SM, Alexander L, Peden KW, Andiman WA, et al. Increased replication of non-syncytium-inducing HIV type 1 isolates in monocyte-derived macrophages is linked to advanced disease in infected children. AIDS Res Hum Retroviruses 2002, 18:353–362.

81. Ebert LM, McCall SR. Up-regulation of CCR5 and CCR6 on distinct subpopulations of antigen-activated CD4+ T lymphocytes. J Immunol 2002, 168:55–72.

82. Weyand CM, Goronyz J, Fatman CG. Modulation of CD4 by antigenic activation. J Immunol 1987, 138:1351–1354.

83. Paillard F, Sterkers G, Vaquerou C. Transcriptional and post-transcriptional regulation of TCR, CD4 and CDB gene expression during activation of normal human T lymphocytes. EMBO J 1990, 9:1867–1872.

84. Hwang SJ, Boyle TJ, Lyons HK, Cullen BR. Identification of envelope V3 loop as the major determinant of CD4 neutralization sensitivity of HIV-1. Science 1992, 257:535–537.

85. O’Brien WA, Chen IS, Ho DD, Darl Es. Mapping genetic determinants for human immunodeficiency virus type 1 resistance to soluble CD4. J Virol 1992, 66:3125–3130.

86. Wei X, Decker JM, Liu H, Zhang Z, Arabi RB, Kilby JM, Saag MS, Wu X, Shaw GM, Kappes JC. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob Agents Chemother 2002, 46:1896–1905.

87. Ho SH, Martin F, Higginbottom A, Pantridge LJ, Parthasaraty V, Moseley GW, Lopez P, Cheng-Mayer C, Monk PN. Recombinant extracellular domains of tetraspanin proteins are potent inhibitors of the infection of macrophages by human immunodeficiency virus type 1. J Virol 2006, 80:6487–6496.

88. Connor RI, Chen BK, Choe S, Landau NR. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. Virology 1995, 206:935–944.

89. Chowdhury IH, Koyanagi Y, Takamatsu K, Yoshida O, Kobayashi S, Yamamoto N. Evaluation of anti-human immunodeficiency virus effect of recombinant CD4-immunoglobin in vitro: a good candidate for AIDS treatment. Medical microbiology and immunology 1991, 180:183–192.