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How HIV changes its tropism: evolution and adaptation?

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Abstract

Purpose of review—To present recent information on the evolution of coreceptor use from CCR5 alone to CCR5 and CXCR4, the impact CCR5 inhibitors have on this process, and new insights into HIV-1 binding to CD4 and CCR5.

Recent findings—The findings that are summarized include resistance to CCR5 inhibitors, genotypic predictors of coreceptor use, the link between coreceptor use and cell tropism, and new data on CCR5 structure and function.

Summary—Resistance to CCR5 inhibitors is uncommon, and frequently involves selection of minor populations of R5X4 virus. Genotypic predictors of coreceptor use need to take into account the entire envelope sequence, not just V3. Genetic polymorphisms in humans that affect CCR5 or chemokines that bind CCR5 affect not only virus entry but also immune reconstitution.

Keywords

CCR5; cell tropism; coreceptor; CXCR4

Introduction

The problem of coreceptor switching and cell tropism changes by HIV-1 is well recognized [1], but the explanation(s) remain elusive. HIV-1 infection of target cells is mediated by binding to the primary receptor CD4 and the chemokine coreceptors CCR5 (R5 viruses) alone, CCR5 and CXCR4 (R5X4 viruses), or, rarely, CXCR4 only (X4 viruses). Cell tropism is primarily determined by CCR5 or CXCR4 expression on target cells, although differences in CD4 and CCR5 expression levels can affect R5 virus infection of macrophages [2], so the translation from 'R5 virus' to 'macrophage-tropic' is imperfect. The two key questions of the coreceptor switch problem are: why do R5 HIV-1 isolates predominate in primary infection; and why do only approximately 50% of infected patients undergo the switch from R5 virus to R5X4 viruses? Insight into the second question should be aided by the introduction of potent CCR5 inhibitors (e.g. Maraviroc, Vicriviroc) into clinical use [3], and the widespread use of coreceptor testing prior to therapy with CCR5 inhibitors [4]. This brief review of recent literature will highlight insight gained from the use of CCR5 inhibitors in clinical trials and relate these results to studies of HIV-1 envelope evolution, CCR5 and CXCR4 function, and shifts in cell tropism.

CCR5 inhibitors and development of resistance

The finding that humans who lacked CCR5 expression because of the Δ32 mutation were resistant to HIV-1 infection [5] provided the impetus for development of small molecule

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inhibitors of CCR5, which culminated with the licensing of Maraviroc (Selzentry, Pfizer, New York, USA) for clinical use in 2007. Perhaps the biggest news of the year is that, although the introduction of CCR5 inhibitors into clinical practice has resulted in selection of R5X4 or X4 viruses in more than 50% of patients failing therapy, rapid declines in CD4 T cell count and disease progression were not observed [6]. Phenotypic typing of patient populations to define 'R5 only' HIV-1 strains [4] has helped to limit emergence of resistance, which remains relatively low (77/837; 11%) [6] as originally reported in the earlier phase II clinical trials [7]. Further insight into the success of CCR5 inhibitors has been provided by several recent studies. It is now clear that the transition from CCR5 to CXCR4 use begins with viruses that still prefer CCR5 as the functional coreceptor. Irlbeck *et al.* [8] demonstrated that 'R5X4' or 'dual-mixed' *env* clones from treatment-naive patients were dominated by clones capable of efficient CCR5 use, and R5X4 clones with close genetic relationship to R5 clones from the same patient were very poor at CXCR4 use in the Monogram Trofile assay. This result confirms the earlier work of Huang *et al.* [9] who proposed dividing R5X4 viruses into two categories: 'dual-R' (CCR5 preference) or 'dual-X' (CXCR4 preference), on the basis of their relative efficiency in mediating entry into target cells expressing CCR5 or CXCR4. A retrospective analysis of patients treated with the CXCR4 inhibitor AMD3100 [10] found that patients who responded to treatment had baseline R5X4 viruses with poor CXCR4 use ('dual-R'), whereas patients with poor responses had robust CXCR4 use ('dual-X').

Although there was one study [11] that resistance to CCR5 inhibitors could involve selection of CXCR4-using variants, this was based on in-vitro selection. Resistance to vicriviroc in one treated patient did not involve coreceptor switching, but was associated with V3 loop sequence changes and cross-resistance to TAK779 [12]. Importantly, the V3 sequence reverted to the pretreatment baseline when vicriviroc therapy was discontinued, implying a fitness loss associated with resistance [12]. Ogert *et al.* [13] found that resistance to vicriviroc selected by in-vitro virus passage mapped to determinants that included both V3 and other C2-V5 *env* mutations, so V3 mutations may be necessary but not sufficient for resistance.

The species selectivity of CCR5 inhibitors is an important consideration for their testing in primate models of infection, in which it has previously been noted that some compounds are much less effective at blocking rhesus CCR5 than human CCR5 [14]. This theme was extended by the work of Saita *et al.* [15] demonstrating that single amino acid differences between rhesus and human CCR5 determine the relative efficacy of different small-molecule CCR5 inhibitors. These observations are relevant for the preclinical development of CCR5 inhibitors as potential microbicides [16]. Ayouba *et al.* [17] reported a surprising finding in a model system relevant to microbicide development. They found that CXCR4 inhibitors in combination with the fusion inhibitors T20 or C34 not only failed to inhibit cell-mediated X4 virus transmission across a model trophoblast barrier, but actually enhanced transmission. This unexpected result was not seen with CCR5 inhibition and R5 virus challenge.

Genotypic predictors of coreceptor use

The introduction of CCR5 inhibitors into clinical use has increased the need for a rapid and reliable assay for coreceptor use by patient isolates [18]. Presently, the Monogram Trofile biologic assay [4] fills this need, but a number of groups have attempted to produce equally reliable prediction methods on the basis of the V3 *env* gene sequence. Garrido *et al.* [19] compared eight different genotypic predictors with a phenotypic assay for both subtype B and nonsubtype B HIV-1 isolates. The genotypic predictor success rate for R5X4 identification ranged from 71 to 84% for nonsubtype B viruses and as high as 91% for subtype B viruses. Lamers *et al.* [20] achieved a predictive accuracy of 75% for subtype B R5X4 viruses with evolved neural network computation. The addition of clinical data to the genetic sequence information improved the predictive power for R5X4 identification in a large patient cohort

infected with subtype B HIV-1 in work by Sing *et al.* [21]. However, almost all of the genotypic predictors rely on the V3 sequence alone, and it is abundantly clear that sequence changes in other regions of *env* are usually necessary for both coreceptor switching [22,23] and resistance to CCR5 inhibitors [13,24]. The future success of genotypic prediction may thus depend on including sequence information from the entire *env* gene. This conclusion is reinforced by an important study by Huang *et al.* [25•] that demonstrated that the gp41 sequence influences entry mediated by CCR5 or CXCR4 for *env* clones bearing identical V3 regions. A second study by Taylor *et al.* [26] also found impacts of the gp41 sequence on the efficiency of CCR5-mediated virus entry. It is not just about V3 anymore!

Envelope evolution leading to coreceptor switching/tropism shifts

Coreceptor switching occurs in approximately 50% of subtype B HIV-1-infected patients. What happens to CCR5 utilization in the remaining patients who progress to AIDS with only R5 virus detected? Four recent studies identified functional changes in R5 Env proteins from late-stage patients. Borggren *et al.* [27] demonstrated that a loss of an N-linked glycosylation site in V2 in late-stage isolates diminished the ability to utilize DC-SIGN for infection, and related studies by Repits *et al.* [28] found an increase in positive-charged residues in V1/V2 and V4/V5 that resulted in increased viral fitness and reduced sensitivity to entry inhibitors. Another change in late-stage R5 isolates that improved entry fitness was the addition of an N-linked glycosylation site (N362) near the CD4-binding site [29]. An important study by Low *et al.* [30•] found that entry efficiency via CCR5 continued to improve in *env* clones from patients that remained R5 to end-stage disease, whereas CCR5 entry declined in *env* clones from patients with R5X4 or dual/mixed viruses. This extends prior work showing that CCR5 use continues to improve with increasing duration of infection [31], unless coreceptor switching is eminent, in which case CCR5 use appears to decline [23]. Similar conclusions were reached by two studies of SHIV-SF162 *env* evolution during coreceptor switching in infected macaques [32,33]. R5X4 intermediates were more sensitive to both CCR5 and CXCR4 inhibitors than the parental R5 strain or the final X4 variant, in agreement with previous findings with HIV-1 coreceptor switch intermediates [22]. The emerging picture thus is that envelope evolution either continues to improve CCR5 use throughout the course of infection, or begins to show diminished entry activity via CCR5 as CXCR4 use is gained.

A study by Peters *et al.* [2] provided additional insight into the differences between coreceptor usage and cell tropism. Only a subset of R5 virus isolates was macrophage-tropic, and these showed an increased affinity for CD4 but no consistent changes in sensitivity to CCR5 inhibitors. The macrophage-tropic, R5 subset also showed increased sensitivity to b12-IgG, a CD4-binding, site-directed neutralizing antibody, reinforcing the idea that adaptation to use low CD4 levels on macrophages had altered the CD4 binding site. Xu *et al.* [34] isolated *env* clones directly from monocytes and described a unique phenotype: macrophage-tropic and specific R5 isolates that could not infect $CD4^+$ T cells, as well as R5X4, R5R3, and coreceptor promiscuous isolates that could infect both macrophages and T cells. These findings reinforce the concept that target cell selection helps shape the evolution of *env* during chronic infection.

Coreceptor switching has been reported to occur less frequently in subtype C HIV-1 infection, but one recent study [35] suggests that CXCR4-using viruses are increasing in frequency on the basis of sampling of 20 AIDS patients. This is a small sample, but the introduction of antiretroviral therapy may change the character of the subtype C epidemic [36], so continued attention to this possible shift is warranted. A study by Patel *et al.* [37] is relevant to this issue. They found evidence that the V3 conformation of subtype C isolates is different than subtype B isolates, and hypothesized that this difference might impair coreceptor switching.

More insight into the role of the V3 region in the use of CCR5 and CXCR4 was provided by two important related studies by Laakso *et al.* [38•] and Nolan *et al.* [39•]. Deletions in the V3 region of the R5X4 parental R3A isolate resulted in some mutants that could only use CCR5, but were resistant to inhibition by small-molecule CCR5 inhibitors. These results imply that the shortened V3 region can mediate interaction with the N-terminal domain of CCR5, but the conformation of the first and second extracellular loops (regions altered by most CCR5 inhibitors) is no longer relevant for viral entry. Structural information about the V3 loop (discussed below) is consistent with this interpretation.

Two studies presented evidence relevant to the concept that virus archived in specific tissues could contribute to changes in coreceptor use. Salemi *et al.* [40] analyzed sequence evolution and coreceptor use in four treatment-naive pediatric cases, and concluded that the thymus may play a role in the evolution of (R5)X4 variants. They also showed positive selection of V1/V2 and C2 domain mutations prior to V3 mutations conferring CXCR4 use, confirming prior in-vitro studies of coreceptor switching [22]. Madsen *et al.* [41] documented reappearance of an *env* sequence from 11 years prior to treatment interruption, suggesting that selective pressure can rescue archived sequence variants as well as selecting new mutants.

Another possible explanation for the predominance of R5 viruses during primary infection was raised by the important findings of Zhou *et al.* [42•]. They found that exposure of resting, memory $CD4^+$ T cells to X4 but not R5 virus induced rapid cytolysis of cells that required virus entry but not reverse transcription. These observations raise the possibility that primary transmission of X4 virus could lead to an abortive infection. This rapid cytolysis was not observed with activated $CD4^+$ T cells, the phenotype that is prevalent late in infection when (R5)X4 viruses appear.

Insights into CCR5/CXCR4 function

The most detailed structural information available on the interaction of CD4-bound gp120 with the CCR5 coreceptor was provided by a very important study by Huang *et al.* [43••]. They provided indirect evidence that the tyrosine-sulfated N-terminal domain of CCR5 interacts with the conserved arginine at the base of the V3 loop (CTRP—), and reinforce models in which the base of the V3 loop interacts with the N-terminus of CCR5 and the crown of the V3 loop interacts with other extracellular CCR5 domains [44]. This model was also supported by the V3 deletion mutant studies cited above [38•,39•]. Additional insight into the details of gp120:CD4:CCR5 interactions were provided by the single-molecule force spectroscopy study of Dobrowsky *et al.* [45•] that suggested that the initial gp120:CD4 binding event was unstable and rapidly reversible unless CCR5 binding also occurs.

Several recent studies deal with the cell biology of CD4 and CCR5 in the plasma membrane of the target cell. A photobleaching study performed by Baker *et al.* [46] showed association of CD4 and several CCR5 molecules both within and outside of microdomains consistent with lipid rafts. Platt *et al.* [47] selected *env* mutants capable of using human:mouse chimeric CCR5 molecules, and found that they could also mediate entry via an N-terminal domain-truncated CCR5. They propose an ‘allosteric machine’ hypothesis for the energetics of gp41-mediated fusion that is dependent on the number of coreceptors bound. The issue of CCR5 signaling versus internalization was studied by Cardaba *et al.* [48]. Internalization was not dependent on lipid raft association, but signaling via G_{alpha} G-proteins did require intact lipid rafts. Harmon and Ratner [49] studied the signaling pathways activated by virus binding to CD4 and coreceptors and found that activation of the actin cytoskeleton required for virus fusion was dependent on $G_{alpha}(q)$ but not other G_{alpha} proteins. Additional structural and functional studies of CCR5 will be aided by a technological advance in large-scale expression of the protein [50].

A number of human genetic studies shed light on the relationship between HIV-1 and other disease susceptibility and polymorphisms at loci influencing CCR5 or CCR5 ligand expression. Two very important studies by Ahuja *et al.* [51••] and Dolan *et al.* [52••] demonstrated that protective CCR5 and CCL3L1 genotypes influenced the extent of immune reconstitution during antiretroviral therapy and cell-mediated immunity independent of effects on viral entry, results reviewed by Moore and Klasse [53]. These articles imply that low CCR5 expression and high chemokine levels not only impair virus entry but also promote cellular immunity, providing at least two protective mechanisms that slow disease progression. A third study confirmed the impact of host genetic diversity on the response to antiretroviral therapy [54], including altering the kinetics of viral suppression and time to AIDS progression.

CCR5 deficiency, although protective against HIV-1, is now confirmed to increase the risk of symptomatic West Nile virus infection [55] in a study that extends the earlier, less definitive report [56]. There was also one publication [57] that suggested that polymorphisms in CCR5 and CCL5 might be associated with a rare case of systemic yellow fever infection following the administration of the usually well tolerated, attenuated vaccine. One additional case report of HIV-1 infection in a CCR5 Δ32 homozygote appeared [58], bringing the worldwide total cases reported to 12.

Conclusion

Figure 1 Figure 1 depicts the emerging consensus on coreceptor use during the course of chronic infection with subtype B HIV-1. Entry function via CCR5 either continues to improve during the entire course of infection or it declines as entry via CXCR4 begins to appear. This model has some interesting clinical implications for the use of CCR5 inhibitors; namely, that they might be useful against the ‘dual-R’ subset of R5X4 viruses that appear to be prevalent shortly after coreceptor switching. The answer to the question of why only 50% of patients undergo coreceptor switching remains elusive, but new insight into the continued evolution of HIV-1 envelope in patients who remain infected with R5 virus until end-stage disease suggest that options for improving CCR5 entry fitness may be limited. It has become increasingly clear this past year that the choice of coreceptor use is dictated by the entire gp120/gp41 sequence, and not simply a few charged residues in the V3 region. These findings pose major challenges for predicting coreceptor use from envelope sequence information.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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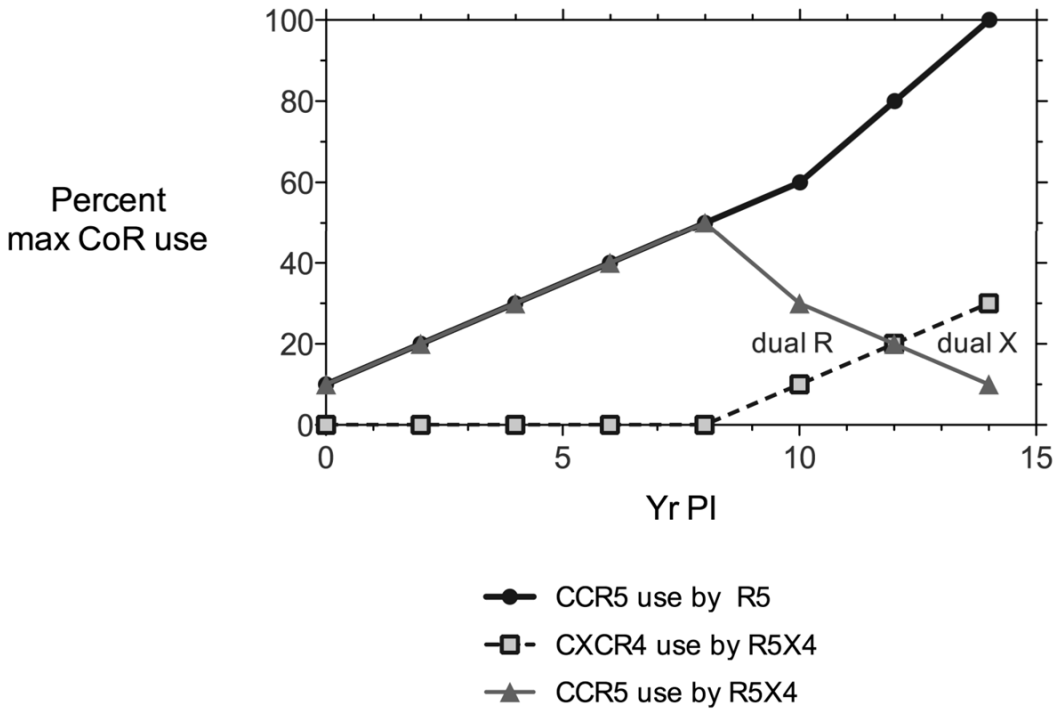

Figure 1.
Evolution of coreceptor use gr1
Schematic depiction of CCR5 and CXCR4 use during the course of HIV-1 subtype B infection. Yr PI, years postinfection; percentage max CoR use, percentage of maximal entry via CCR5 or CXCR4 during the course of infection; dual-R, R5X4 viruses with better entry via CCR5; dual-X, R5X4 viruses with better entry via CXCR4.