Biological and Molecular Aspects of HIV-1 Coreceptor Usage

Fransje A. Koning[1], Ronald P. van Rij*[1], and Hanneke Schuitemaker
Sanquin Research and Landsteiner Laboratory of the Academic Medical Centre, University of Amsterdam, Amsterdam The Netherlands
[1]both authors contributed equally to this review
**Present address:*
Dept Microbiology and Immunology, University of California, San Francisco, CA 94143-2280, USA

Correspondence to: Dr Hanneke Schuitemaker, Sanquin Research, Dept. Clinical Viro Immunology, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands, Tel: 31-20-5123317, Fax: 31-20-5123310, e-mail: h.schuitemaker@sanquin.nl

The identification of chemokine receptors as the coreceptors of HIV-1 has yielded many novel insights in the pathogenesis of HIV-1 infection with respect to the mechanism of viral entry, viral tropism, and differences in disease course among individual patients. Here we present a review on these insights and on the molecular aspects of HIV-1 coreceptor recognition.

HIV-1 coreceptor use

Fourteen chemokine receptors or structurally related molecules have been identified that can function as coreceptors for entry of HIV-1 *in vitro* (reviewed in [146, 37, 8]). Viral coreceptor use is determined on cell lines which lack endogenous expression of (co)receptors and which are transfected with CD4 and one of the putative HIV-1 coreceptors. Entry and subsequent replication in these cells indicates which coreceptors the viral isolate can use. Alternatively, cell fusion-based assays are used, in which fusion of a cell line expressing CD4 and a coreceptor with a cell line expressing HIV-1 gp120 is monitored [43, 104].

Coreceptor use is highly correlated with the ability to induce syncytia in the MT2 T cell line: non-syncytium-inducing (NSI) variants which in general establish new infections, predominate in the asymptomatic phase and [3, 123, 169 142], use CCR5 but not CXCR4. Syncytium-inducing (SI) variants, which emerge in about 50% of infected individuals preceding an accelerated CD4 cell decline and progressive clinical course of infection [77, 134], use CXCR4 and often additional coreceptors [13, 129]. Rapid/high (R/H) and slow/low (S/H) is another nomenclature for phenotypical different HIV populations [3] that partially overlap with SI/X4 and NSI/R5 virus populations, respectively.

A differential coreceptor usage of HIV variants has been demonstrated for all different clades [164, 166], although an underrepresentation of CXCR4 using HIV variants in clade C has frequently been reported [1, 102]. To date, there is no explanation for this phenomenon but as it has been observed among patients of different countries and different racial backgrounds, the involvement of viral genotypic differences rather than environmental differences seems likely.

Promiscuous coreceptor use on indicator cell lines was mainly observed among CXCR4-using variants while all described virus isolates most efficiently used either CCR5 and/or CXCR4, the main coreceptors for HIV [13, 24, 26, 28, 43-45, 66, 112, 113, 115, 160, 165]. A nomenclature based on coreceptor use has been proposed: R5 for CCR5-using variants, X4 for CXCR4 using variants and R5X4 for variants that use both CCR5 and CXCR4 [7].

Two approaches are available for the determination of coreceptor use in primary cells which have lower expression of CD4 and coreceptors than indicator cell lines. First, absent viral replication in peripheral blood mononuclear cells (PBMC) from healthy blood donors who are homozygous for the CCR5 Δ32 allele reflects the absolute dependence of the HIV variant on CCR5 for entry [91, 114]. Second, the availability of potent and specific coreceptor antagonists allows interference with entry via

the cognate chemokine receptor [4, 39, 40, 96, 119]. Using these approaches, it was shown that NSI HIV-1 variants depend on CCR5 for entry in primary cells, irrespective disease stage at the moment of virus isolation [160, 164, 166]. Disease progression thus can occur without an HIV expanded coreceptor use, which further implies that CCR5, is a promising target for the development of entry inhibitors.

CXCR4-using HIV-1 variants evolve from R5 viruses via a R5X4 phenotype towards an X4 phenotype as determined in indicator cell lines [72, 143]. Replication of CXCR4-using variants in the presence of saturating concentrations of potent CXCR4 antagonists would indeed indicate the capacity to use other coreceptors than CXCR4. However, replication of both R5X4 and X4 variants was completely inhibited by the CXCR4 antagonists AMD3100 and T22 in PBMC, despite the presence of sufficient levels of CCR5 for infection by R5 HIV-1 variants [53, 76, 122, 147, 167]. Thus, the vast majority of HIV-1 variants depend on either CCR5 or CXCR4 for replication in primary cells [164] and coreceptor use as determined on indicator cell lines does not necessarily reflect efficient use of these coreceptors in primary cells.

Cellular tropism

Co-expression of CD4 and an appropriate coreceptor have identified monocytes, macrophages, microglia, dendritic cells (DC), Langerhans cells and lymphoid cells such as thymocytes and CD4+ T helper cells as potential target cells for HIV-1. However, the mere co-expression of CD4 and an appropriate coreceptor does not warrant the capacity of a cell to support productive infection, illustrated by the relative resistance of CD4+CCR5+CXCR4+ macrophages to infection with X4 as compared to R5 HIV-1 variants [48, 124].

The relatively low CD4 levels on macrophages and a higher CD4 dependency of X4 variants than R5 variants [82, 103] may lead to a less efficient entry of X4 variants into macrophages. Over-expression of CD4 indeed rendered macrophages more permissive to infection with primary X4 variants [38, 138]. Alternatively, cell type specific differences in post-translational modifications of CXCR4 have been suggested to influence the susceptibility of macrophages to X4 HIV-1. A large proportion of the CXCR4 molecules on macrophages, but not monocytes, are present as high molecular weight species which can not associate with CD4 and may not mediate efficient viral entry [89]. Post entry restrictions at multiple levels of the replication cycle may also contribute to the resistance of macrophages to infection of X4 and R5 variants [48, 118].

The presence of macrophagetropic, R5 virus variants during early stages of HIV-1 infection, suggests that macrophages are a principal target for the establishment of infection in a new individual [142, 169]. DCs and Langerhans cells have also been implicated in transmission of HIV-1. These antigen-presenting cells capture antigen at peripheral tissues and transport this to lymphoid tissues for presentation to T cells. If DCs would get infected at the portal of entry, they could transfer the virus to CD4+ T cells upon migration to lymphoid tissues. The observed capacity of DCs to support extensive viral replication in T cells in DC-T cell cocultures may support this hypothesis [56, 108]. There has been some controversy whether HIV-1 can establish productive infection of DCs [18]. However, it has recently been shown that the immature DC specific C type lectin DC-SIGN allows intracellular capture and efficient presentation of virions to CD4+ T cells, without productive infection of the DC51. This capture is not coreceptor specific, and thus does not explain the predominance of R5 variants early in infection [51, 64].

The putative crucial role of DCs and macrophages in the establishment of infection has been challenged by in situ hybridisation studies of lymphoid tissues. Even a few days after onset of symptoms of acute infection, about 90% of the cells containing viral RNA were T cells, whereas a minority of infected cells were macrophages [116, 168]. The reported macrophagetropic phenotype of transmitted R5 or X4 variants [76, 142, 169] may thus merely reflect the ability of these virus variants to use a low CD4 density rather than the ability to infect macrophages *per se*.

The almost complete resistance from HIV-1 infection of individuals who are homozygous for CCR5 Δ32, underscores a major role of R5 variants in establishment of infection, irrespective of the route of transmission [91], although transmission of X4 HIV variants has been reported [5, 12, 76, 92,

97, 136, 142]. Several mechanisms have been suggested to play a role in the selective establishment of infection by macrophagetropic R5 HIV-1 variants. Escape from the immune system [125] and low immunogenicity of R5 variants [93] have been suggested to play a role, but this seems difficult to match with the abundant virus replication in activated CD4+ T cells [116, 168]. Selective capture of X4 HIV-1 variants to cell surface heparans would result in clearance of X4 variants due to binding to (but not infection of) CD4-negative cells [95]. Immature dendritic cells were shown to migrate towards R5 but not X4 HIV-1 variants, which would result in selective dissemination of R5 variants to lymphoid tissue [90]. Finally, high expression levels of SDF-1 and the virally encoded tat protein, which both interact with CXCR4, might limit transmission and evolution of X4 HIV-1 variants [52, 99, 158].

The differential tropism of R5 and X4 variants for CCR5+ and CXCR4+ T cell subsets [143] may offer an alternative explanation for the predominance of R5 variants early in infection. X4 variants mainly infect CXCR4+ cells, which generally have a naive or resting memory phenotype. The majority of CXCR4+ cells may therefore not provide the intracellular requirements for productive infection, resulting in viral entry but not productive infection [17, 161, 162]. Infected resting cells that lacked expression of the activation markers HLA-DR and Ki67 have been shown to contain lower numbers of viral transcripts than activated cells [168]. In agreement, a relatively low viral RNA load was observed in individuals who became infected despite a homozygous genotype for CCR5 [32 32, 97, 136]. This indicates a lower number of progeny X4 viruses may indeed be produced *in vivo*, which might be due to the resting phenotype of their target cells. Infection of activated CCR5+ cells may give R5 variants a replicative advantage over X4 HIV-1 variants, and hence an increased chance that R5 variants establish a productive infection in a new host.

A recent study has demonstrated the establishment of infection by X4 SHIV in macaques. However, when macaques were inoculated with a mixture of R5 and X4 SHIV, the X4 SHIV rapidly disappeared, suggesting a higher fitness of the R5 variant early in infection [60]. Clearance of X4 variants but not R5 variants has previously been reported in a parenterally infected human as well [31].

Throughout infection, CD4+ T cells are the major target cells for HIV-1. CCR5 and CXCR4 are differentially expressed during T cell development. CXCR4 is highly expressed on CD3- CD4+ CD8- intrathymic precursors, transiently downregulated during further development and again upregulated on naive CD4+ T cells when they leave the thymus. CCR5 is transiently upregulated on more mature CD3+ CD4+ CD8+ thymocyte subsets, and again downregulated on CD4 single positive thymocytes [10]. In the SCID hu-thy/liv mouse model, X4 variants replicated rapidly in thymocytes, resulting in significant depletion of these cells, whereas replication and depletion by R5 variants was much slower [9].

Naive and memory CD4+ T cells express high levels of CXCR4 and can be productively infected *in vivo* by CXCR4-using HIV-1 variants [14]. CCR5 is mainly expressed on activated memory CD4+ T cells [14, 15, 101] and consequently these cells are the target cells for CCR5-using HIV-1 variants [14].

Evolution of X4 HIV-1 variants

It is unclear why X4 variants only develop in approximately half of the infected individuals, and only when CD4+ T cell numbers decline below approximately 400 cells per µl blood [79]. Only a limited number of mutations are required for this phenotypic switch *in vitro* [16, 34, 67, 126, 149]. This suggests restraints on the ability to establish a productive infection or on the availability of susceptible target cells for X4 variants, rather than a difficulty to induce the switch *per se*. If so, the factors that limit the emergence of X4 variants in the course of infection may be similar to the factors that determine the predominance of R5 variants early in infection.

The differential tropism of R5 and X4 variants for CCR5+ and CXCR4+ T cell subsets may also provide an explanation for the limited evolution of X4 variants. Based on the low number of mutations required for a switch in phenotype, it could be expected that X4 variants evolve on multiple occasions throughout infection. These newly evolved X4 variants may not be able to establish productive infection due to the resting phenotype of their CXCR4+ target cells. The generalised immune activation in later stages of infection [33, 61] may result in proliferation of naive cells and/or a cytokine milieu that

may relieve the post-entry block in infection. This may allow productive infection of CXCR4+ cells or rescue of labile intermediates of HIV-1 replication [32]. At this stage newly emerging X4 HIV-1 variants may have a better chance of encountering appropriate target cells that efficiently support replication than in the earlier phases of infection and consequently, a X4 virus population can be established in the host. However, it remains unclear why this only occurs in 50% of progressing HIV-1 infected individuals.

It has been suggested that the number of available target cells, as defined by expression of the appropriate coreceptor plays a role in the development of X4 variants: a low number of CCR5 positive cells would select for X4 variants which can "escape" the limited number of available target cells. Additionally, availability of high numbers of CXCR4+ target cells would accelerate evolution of X4 variants. However, we have shown that a low number of CXCR4+ CD4+ cells was associated with an enhanced rate of evolution of X4 variants [145]. Furthermore, the mere number of CCR5+ cells nor a CCR5 Δ32 heterozygous genotype were associated with more rapid phenotypic switch [36, 144, 145].

Both R5 and X4 HIV variants continuously evolve as can be demonstrated in phylogenetic tree analysis on the basis of envelope sequences [147]. This evolution seems to be driven at least in part by increased efficiency of coreceptor usage. Indeed, as compared to the inhibition of R5 and X4 viruses early in infection, the inhibition of late stage obtained R5 or X4 HIV variants required higher concentrations of coreceptor ligands [147, 71, 75]. Moreover, pathogenic R5 HIV variants associated with neurodegenerative manifestations of AIDS showed increased CCR5 affinity as reflected by reduced sensitivity to CCR5 targeted inhibitors [55].

Cytopathicity and CD4+ T cell decline

The appearance of X4 HIV-1 variants has been associated with a subsequent more rapid decline of CD4+ T cells and more rapid disease progression [77]. Before the identification of chemokine receptors as the coreceptors for HIV-1, the differences in pathogenicity of R5 and X4 HIV-1 variants were thought to be due to differences in cytopathicity and replication rate. X4 variants in general replicate more rapidly and to higher levels than R5 variants and X4 infection results in a more massive depletion of cells *in vitro* [3, 27, 50, 141]. Now it is apparent that CCR5 and CXCR4 are not evenly distributed on the cells that have been used in these *in vitro* assays. Therefore, insights in cytopathicity based on these models need to be redefined in the context of the available target cells. Indeed, R5 and X4 HIV-1 variants were equally cytopathic for the target cells expressing the appropriate coreceptors, resulting in depletion of the cognate target cells [57, 83].

The enhanced CD4+ T cell decline associated with the presence of X4 variants may therefore not merely be due to a broader target cell range and more extensive replication of X4 HIV-1 variants, but rather to the infection and killing of naive T cells by which the exponential clonal expansion of a progeny memory T cell daughter population is prevented [14].

An additional mechanism may be based on the ability of X4 HIV-1 variants to infect and deplete thymocytes more extensively than R5 variants [9]. The finding that naive CD8+ T cells are somewhat reduced in patients with X4 variants as compared to patients with only R5 variants, may indeed suggest that thymocytes are infected and depleted by X4 variants [62]. Infection of thymocytes was suggested to further interfere with T cell renewal and contribute to the enhanced CD4+ T cell decline associated with X4 HIV-1 variants. However, thymectomy of macaques had absolutely no impact on the rate of CD4 cell decline after SIV infection [2].

Coexistence of R5 and X4 variants

After the evolution of X4 variants, R5 and X4 variants co-exist and both may even expand [78]. The coexistence of R5 and X4 variants can be explained by the availability of separate target cell niches within the pool of memory cells, defined by the differential expression of CCR5 or CXCR4 on subsets of memory CD4+ T cells. Indeed, R5 HIV could be isolated from the CCR5+ memory CD4+ T cell population whereas X4 variants were isolated from the CXCR4+ memory subset [14]. The existence of separate cellular niches was further supported by recent *in vitro* studies, which demonstrated that CD4+

cells that express the homing receptor for lymphoid tissue CD62L, also express CCR5 and CXCR4. These cells could only be infected with R5 HIV-1. In contrast, the CD4+CD62L+ cells, which express CXCR4 but not CCR5, were mainly infected by X4 HIV-1 variants [54, 54].

The separate phylogenetic clustering of R5 and X4 viruses on the basis of gp120 V3 sequences was not observed on the basis of p17gag sequences of the same viruses. Independent evolution of different genomic regions is considered evidence for recombination which implicates that R5 and X4 viruses in addition to their separate cellular niches must share a common target cell population [148].

Molecular determinants of HIV-1 Envelope coreceptor usage

The HIV-1 envelope protein mediates viral entry into the target cell. The HIV-1 160 kD (gp160) envelope precursor is cleaved by a cellular protease into two subunits: the membrane spanning gp41 and the soluble gp120 [41, 42, 157]. The gp41 and gp120 subunits are non-covalently linked and form trimers on the surface of the HIV particle.

Gp120 consists of five constant regions (C1–C5) and five variable regions (V1–V5), and the protein structure consists of three domains, an inner and an outer domain which are connected by a third domain, the so called bridging sheet. Only half of gp41 is exposed in the ectodomain, separated by a transmembrane region, the intracellular part of gp41 is thought to anchor the envelope complex to the underlying matrix [85]. The inner domain of gp120 contains the conserved N- and C-termini and is thought to interact with gp41 [19, 63, 81]. The outer domain is highly glycosylated and is relatively variable. The bridging sheet consists of four anti-parallel b-strands and is involved in the binding of gp120 to the chemokine receptor 84. The gp120 core consists of 25 b-strands, 5 a-helices, and 10 loop segments and is folded into a heart shaped globular structure [84].

Gp120 mediates attachment of the virion to the receptor complex on the cell surface. A highly conserved groove in gp120 associates with the most amino-terminal extracellular immunoglobulin-like domain of the CD4 receptor, consequently a conformational change of the envelope protein results in the exposure of the coreceptor-binding site on gp120. After interaction with the coreceptor, additional conformational changes within the gp41/gp120 trimer trigger the insertion of the gp41 fusion peptide into the plasma membrane. This eventually results in the fusion of the viral and cellular membrane and insertion of the viral core into the cellular plasma (reviewed in [22, 130, 157], see Figure 1).

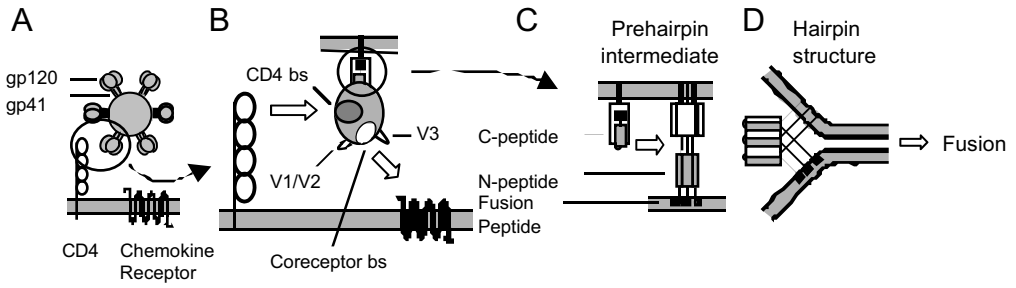

Figure 1. Schematic representation of the entry process of HIV-1. A. The viral envelope glycoproteins are expressed as a trimeric complex of gp120 noncovalently associated with gp41 on the surface of the virion. B. Binding of CD4 to the CD4 binding site (CD4 bs) of gp120 induces exposure of the coreceptor binding region (coreceptor bs) and the third variable region of gp120 (V3) which in succession bind to a chemokine receptor. C. After chemokine receptor binding, the fusion peptide of gp41 is inserted in the cell membrane via a prehairpin intermediate, and subsequently the viral and cellular membranes are drawn in close proximity in a hairpin structure (D), eventually resulting in fusion of the viral and cellular membranes and entry of the viral core into the cytoplasm.

While the mechanism of the phenotypic switch from CCR5 to CXCR4 specificity is still unclear, the interactions between gp120 and the two coreceptors CCR5 and CXCR4 are better understood. Much research has been done on gp120 coreceptor binding by mutagenesis, antibody and antagonist inhibition and X-ray crystallography. The predicted coreceptor binding site on gp120 has a hydrophobic core surrounded by a positively charged periphery and consists of both conserved and variable residues [84, 111, 156]. The effect of single amino acid substitutions on coreceptor binding differed among different viral isolates, indicating a certain level of plasticity in gp120-coreceptor binding. It is important to realize that a mutation at a given position in gp120 could directly influence the physical interaction between gp120 and the coreceptor but hypothetically could also modify the tertiary structure and stability of gp120 which could affect the coreceptor preference of affinity [65].

Below, we give an overview of what is known about the coreceptor binding site in gp120, in the light of coreceptor specificity and affinity. The information applies to subtype B HIV-1 variants, unless indicated otherwise. All gp120 amino acid numbering used here, is according to the HXB2 strain amino acid positioning.

Coreceptor usage and the variable regions in gp120

V3 loop

Even before it was recognized that HIV tropism was largely explained by a difference in coreceptor usage, it was found that the V3 loop was an important determinant for HIV-1 SI phenotype and tropism [23, 49, 69, 98, 127, 132, 140, 154]. Later it was recognized that even a single amino acid change in V3 could switch coreceptor usage [16, 34, 67, 126, 149]. The residues at positions 306 and 322 in the V3 region were specifically found to be of importance as one or both of these residues were positively charged amino acids in 98% of CXCR4 using SI HIV variants [49]. Furthermore, an increased positive charge of the V3 loop has often been associated with CXCR4 usage [20, 34, 49, 69, 128]. The involvement of the same fixed positions in the V3 domain in coreceptor usage was also demonstrated for clade A [133], clade C [163], clade E [73], and clade G [133] HIV-1 variants.

Moreover, also for these non-clade B viruses, an increased positive charge of the V3 loop has been associated with CXCR4 usage [25, 131]. Despite the similarities between clade B and non-B viruses, differences in CCR5 binding have been reported for these groups of viruses, which were determined by a cluster of residues in the second extracellular domain of CCR5 although the viral determinant has not yet been identified [137].

Recent studies have shown that CXCR4 usage is not a direct result of a high positive charge of the V3 loop [29, 68] but merely associated with the loss of an N-linked glycosylation site that accompanies a high positive charge of V3 sequences from primary isolates [105]. It has indeed been demonstrated that the loss of an N-linked glycosylation site due to mutation of V3 N301 enhanced infectivity of X4 variants and reduced infectivity of R5 variants [106] while the same mutation in X4R5 variants led to an inability to use CCR5 [100, 105, 107] Despite its enhanced CXCR4 binding, this mutant R5X4 virus showed an increased sensitivity to neutralizing antibodies and the natural CXCR4 ligand SDF-1 [59, 107, 120, 121]. N301 N-linked glycosylation thus seems to be directly involved in the interaction of R5 gp120 with CCR5 while it hinders CXCR4 usage and shields the V3 loop from binding to neutralizing antibodies.

Residue R298 in the V3 region is conserved among R5 and X4 HIV variants and is important for CCR5 and CXCR4 binding but not coreceptor specificity [6, 152, 153]. A 298R to K mutation had no effect on CCR5 binding, while other residues at this position gave a marked reduction in both CCR5 and CXCR4 binding.

V1/V2 loop

Glycosylation sites near the V1/V2 loop have been implicated in the efficiency of coreceptor usage, probably by maintenance of the conformation of the variable loops for interaction with the receptors [58, 100, 105]. However, the impact of changes in the glycosylation of V1/V2 was limited as

compared to importance of the V3 N-linked glycosylation at N301.

Delayed infection of a R5 chimeric clone with an HXB2 V3 loop was restored by a mutation in the V1 region which resulted in the loss of a potential glycosylation site (136N to D) [20]. This suggested a functional interaction between the V3 and the V1 region [86].

As discussed above, gp120 undergoes a conformational change after binding to CD4, which exposes the coreceptor binding site. HIV-1 strains that can replicate independent of CD4 have been generated. It is thought that in these viral isolates, the coreceptor binding site is already exposed. An HXB2 ΔV1/V2 mutant was found to bind to CXCR4 very efficiently in absence of soluble CD4 (sCD4) [6], which suggested that the V1/V2 loops are dispensable for coreceptor binding. A study on a CD4 independent HIV-1 IIIB env clone (IIIBx), showed that the determinants for CD4 independence of this clone map outside the V1/V2 and V3 regions [87]. By contrast, a single point mutation in the C4 region (G431E) could render the CD4 independent IIIB variant fully CD4 dependent. For the HIV-1 R5 clone Ada, elimination of a single N-linked glycosylation site at position D197 in the V1/V2 stem region was sufficient for CD4 independent infection [74].

Genetic variation occurs predominantly as point mutations, but deletions and insertions are also common, especially in the V2 and V4 regions. Length variation in V2 was found to be associated with an X4 phenotype in primary virus isolates [46, 58]. PCR fragment length analysis revealed that the V2 loop regions of X4 and R5 variants that were obtained just around the moment of X4 conversion were longer than those from R5 variants obtained from individuals who had never developed X4 variants. The increase in V2 length provided an additional potential glycosylation site. The association between CXCR4 usage and the length of the gp120 V2 domain was recently confirmed [70], although other studies have not found this correlation [30, 88, 150].

X4 and R5 variants isolated around the time of X4 conversion were also reported to have a significantly higher positively charged V2 loop than R5 variants [30, 58, 150]. Together with a higher V3 loop charge, this suggests that the envelope proteins of X4 variants have a higher net charge than R5 variants, which might be of influence on coreceptor specificity or affinity.

Conserved regions

CCR5 is used as a coreceptor by several primate immunodeficiency viruses, suggesting that highly conserved residues in gp120 might be involved in CCR5 binding. Indeed, the residues near the bridging sheet of gp120 that have been implicated in CCR5 coreceptor usage are highly conserved [11, 111, 151, 152]. This region is thought to undergo conformational changes after CD4 binding, resulting in an orientation towards the target cell, and facilitating binding to the coreceptor. HIV-1 neutralizing antibodies that target gp120 epitopes that are exposed after CD4 binding, the so-called CD4-induced (CD4i) epitopes, are able to block binding to CCR5 [139, 155]. Mutagenic analysis have suggested that these CD4i epitopes consist of elements of the conserved stem of the V1/V2 stem-loop and the C4 region. The concentration of basic residues in this region may facilitate the interaction of gp120 with the acidic extracellular regions of the chemokine receptor.

Mutagenesis of the 420-422 IKQ motif and G441 has revealed that these residues are important for CCR5 binding [110, 111]. Moreover, these mutations also had a negative effect on replication of a R5 chimeric virus with a HXB2 V3 loop in a CXCR4 expressing cell line. A single mutation in the C4 region (S440R) could restore the replication kinetics [21], indicating a functional interaction between V3 and C4 residues. A change in residue 440 exerted only modest effects on CCR5 binding [110]. These results suggest that a basic residue at position 440 is important for CXCR4 but not CCR5 usage.

Prediction of coreceptor usage on the basis of amino acid sequences?

The HIV-1 SI phenotype and coreceptor usage are strong predictors for disease progression [77, 117, 135], and much effort has been put into the prediction of these phenotypic traits of HIV-1. Sequence analysis might give an alternative to determination of the SI phenotype in the MT2 cell line, which is a very reliable, yet laborious, method to determine CXCR4 usage [80].

The V3 region is an important determinant for HIV-1 coreceptor usage and different models

based on the V3 region sequence have been proposed to predict HIV-1 coreceptor usage [35, 47, 49, 94, 159]. From these V3 sequence motif-based models, the presence of positively charged amino acids at positions 306 and/or 322 [35, 49] is the most reliable predictor of an SI phenotype [109]. However, prediction reliability for CXCR4 usage with this rule was still below 50%, which is probably due to the fact that a basic residue at position 322 alone can not discriminate between R5 and X4 sequences.

Others have tried to improve this reliability by generating a neural network to predict CXCR4 usage based on V3 sequences and found that the reliability of this method indeed was higher than the sequence motif-based method mentioned above [109]. Still, 69% reliability is too low for clinical use and for this purpose the MT2 assay remains the most reliable readout system of CXCR4 usage. However, the difficulty of predicting CXCR4 usage on V3 sequence alone underscores the importance of other determinants for coreceptor usage elsewhere in gp120. Indeed, a set of gp120 sequences available in the Los Alamos HIV Sequence Database has been analyzed for residues outside V3 that are associated to predicted R5 or X4 phenotype [65]. With this approach, which took the phylogenetic relationships among sequences into account, a strong linkage between predicted coreceptor usage and a number of residues throughout gp120 was found, of which residue 440 in C4 was most strong. As described above, residue 440 in C4 was also related to coreceptor usage in experimental settings. The R5 genotype was strongly associated with an arginine residue at this position, while glutamic acid at position 440 was over-represented in sequences with predicted X4 usage. In addition, amino acid positions 190 to 200 in the V1/V2 stem correlated with coreceptor affinity but not specificity while the X4 genotype was found to be correlated with an increased net positive charge in the V2 loop [65] while a correlation with V2 length was less pronounced.

These results show that neuronal networks and analysis of available sequences might well be useful to map patterns that are important for coreceptor usage apart from the more commonly used mutation and inhibition approach.

Concluding remarks

The identification of chemokine receptors as coreceptors for HIV-1 has provided many novel insights in AIDS pathogenesis. The major coreceptors for HIV-1 are CCR5 and CXCR4, which therefore represent promising targets for therapeutic interventions [4, 39, 40, 96, 119]. The current knowledge on HIV-coreceptor interactions may allow for the sophisticated design of new coreceptor antagonists that would be of great value in new therapy regimes. Alternatively, revealing the site of action of currently available coreceptor antagonists may improve our understanding on the viral molecular determinants for coreceptor usage.

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