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The biology of CCR5 and CXCR4

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Abstract

Purpose of review—We discuss the current knowledge concerning the biology of CXCR4 and CCR5 and their roles in HIV-1 infection.

Recent findings—Important research findings reported in the last 2 years have advanced our knowledge in the field of HIV coreceptors and pathogenesis. Novel methods have been used to crystallize two new members of the G-protein coupled receptors. It has been demonstrated that expression and stability of the naturally occurring truncated CCR5 protein is critical for resistance to HIV-1. The first stem cell transplantation of donor cells with the CCR5 mutation provided proof of principle. This is far from being a therapeutic option. The Food and Drug Administration approved the first CCR5-based entry inhibitor. New CXCL12 isoforms were discovered, one isoform is a potent X4 inhibitor with weak chemotaxis activity.

Summary—The coreceptor discoveries revealed new insights into host and viral factors influencing HIV transmission and disease. The HIV/coreceptor interaction has become a major target for the development of novel antiviral strategies to treat and prevent HIV infection. The first CCR5-based entry inhibitor has been recently approved. New drugs that promote CCR5 and CXCR4 internalization, independent of cellular signaling, might provide clinical benefits with minimum side effects.

Keywords

CCR5; CXCR4; G-protein coupled receptor; HIV/AIDS

Introduction

Despite the rapid identification of CD4 cell as the ‘primary receptor’ for the AIDS virus, it soon became clear that additional molecules might be involved [1•]. CXCR4 (Fig. 1) was identified as the coreceptor for X4 HIV-1 isolates [2]. Five different groups independently demonstrated that CCR5 is the coreceptor along with CD4 that allows entry of R5 HIV-1 [3–7]. The discovery of the HIV coreceptors provided a logical explanation for the previously observed tropism of HIV-1 on primary cells and T cell lines (Fig. 2). This review will focus on the recent findings on CCR5 and CXCR4 in terms of their biological functions, including their role in HIV-1 infection.

CCR5 and CXCR4 are G-protein coupled receptors

CCR5 and CXCR4 are structurally related chemokine receptors belonging to the superfamily of the seven-transmembrane G-protein coupled receptors (GPCRs) [8•,9,10•]. The GPCRs are activated to induce a signal by small ligands that are either promiscuous or specific for a given

receptor. Agonist-activated GPCRs are rapidly phosphorylated at serine and threonine residues within the C-tail and the third intracellular loop [11•]. There are 21 potential phosphorylation sites in CXCR4 (Fig. 1) and only seven in CCR5. Chemokines, small low-molecular weight proteins, are the ligands that activate and signal through CCR5 and CXCR4 to mediate several cellular functions including development, leukocyte trafficking, angiogenesis, and immune response [12•].

Ligand binding to GPCRs induces a change in conformation of the receptor that is transmitted to the cytoplasmic domains of the protein, enabling the protein to couple with an intracellular heterotrimeric G protein [13]. The intracellular G protein acts as an intracellular signal by activating or inhibiting intracellular enzymes. This model of cellular communication became so successful through evolution that GPCRs are used to enable the senses of taste, smell, and vision and control numerous intracellular signaling systems. Nearly, 1000 seven-transmembrane receptors are thought to be present in the human genome [10•]. Diseases such as some forms of blindness, obesity, inflammation, depression, and hypertension, among others, can be linked to malfunctions of GPCRs. It is not surprising that about half of the drug targets in the pharmaceutical industry are GPCRs [9].

The lack of crystallography on CCR5 and CXCR4 is mainly due to the fact that such proteins are highly hydrophobic and cannot be readily purified. Until recently, X-ray crystallography had provided information on only one GPCR, bovine rhodopsin. This is due to the fact that rhodopsin is easily purified in milligram quantities from bovine retinal extracts. Recently, the crystal structures of two other GPCRs were resolved [14•,15•]. The crystal structures of the human A_{2A} adenosine receptor [15•] and β_2 -adrenergic receptor (β_2 AR) [14•] were determined by using a T4L fusion strategy, in which most of the third cytoplasmic loop was replaced with lysozyme from T4 bacteriophage, and the carboxy-terminal tail was deleted to improve the likelihood of crystallization. The A_{2A} adenosine ligand bound structure suggested that there is no general, family-conserved receptor binding pocket in which selectivity is achieved through different amino acid side chains. Rather, the pocket itself can vary in position and orientation, yielding more opportunity for receptor diversity and ligand selectivity [15•].

Biology of CCR5

CCR5 was first isolated as a functional GPCR that is antagonized by three CC chemokines [16,17]. It was later discovered that several other CC chemokines can bind CCR5 with different affinities and efficiencies in receptor activation [18]. CCL3, CCL4, and CCL5 bind efficiently to CCR5 and are full agonists, whereas CCL7, CCL8, and CCL13 bind less efficiently and exhibit different abilities in receptor activation. Interestingly, MCP-3/CCL7 has been found to bind CCR5 without inducing a signal and has been suggested as a natural antagonist [18]. Among the several CC chemokines that have been demonstrated to bind CCR5, CCL3, CCL4, CCL5, and CCL8 show the most suppressive activities in HIV-1 infection assays [18]. CCL7 binds efficiently to CCR5, but does not induce internalization or block HIV-1 infection [18]. Interestingly, CCL2 binds to CCR5 and acts as a potential enhancer rather than a blocker of HIV-1 replication [19•].

CCR5-deficient (CCR5^{-/-}) mice develop normally, but show reduced efficiency in clearance of *Listeria* infection and exert a protective effect against lipopolysaccharide (LPS)-induced endotoxemia, reflecting a partial defect in macrophage function [20]. Additionally, CCR5-deficient mice had an enhanced delayed-type hypersensitivity reaction and increased humoral responses to T cell-dependent antigenic challenge, indicating a novel role of CCR5 in downmodulating T cell-dependent immune response [20]. In a murine transplant model with intensive conditioning, the overall effect of absent CCR5 expression on donor cells results in greater graft-versus-host disease (GVHD) and donor T cell expansion [21].

CCR5-deficient (CCR5^{-/-}) *Homo sapiens* are represented in 2–3% of whites [22]. The lack of CCR5 expression in these individuals is caused by a naturally occurring 32 base pair deletion in the *CCR5* gene. Individuals who are homozygous for the mutant CCR5 allele are highly resistant to HIV-1 infection. The mutant allele is not associated with any obvious phenotype. Although homozygosity for CCR5 Δ 32 mutation is clearly associated with disease resistance, HIV-1 infection has been reported in hemophiliac patients [23] and several CCR5^{-/-} homosexuals [24–30], indicating that the protective effect of the CCR5 Δ 32 mutation is not absolute. In some cases, exclusive use of CXCR4 by the infecting virus isolates or the presence of Env sequences typical of CXCR4-using (X4) viruses was observed. In other cases, dual-tropic (R5X4) HIV-1 isolates have also been identified in three different HIV+CCR5^{-/-} homosexual individuals [26,31].

Our studies suggested that HIV resistance in CCR5 Δ 32 homozygote might result from both genetic loss of CCR5 on the cell surface as well as active downregulation of CXCR4 expression by the mutant CCR5 Δ 32 protein [32]. We have recently demonstrated that expression and stability of the truncated CCR5 Δ 32 protein in CCR5^{-/-} individuals is critical for the resistance phenotype [33–35]. These studies support the hypothesis that the CCR5 Δ 32 protein acts as an HIV-suppressive factor by altering the stoichiometry of the molecules involved in HIV-1 entry and provide insight into the development of drugs that mimic the CCR5 Δ 32 protein interactions [33–35]. Recently, Hutter *et al.* [36] reported the first successful allogeneic stem cell transplantation in an HIV-positive patient with a donor selected to be homozygous for the CCR5 Δ 32 allele. The patient managed transplantation without any remarkable irregularities and developed a functional reconstitution of his T cell immunity. Although this case provided a proof of principle to the resistance phenotype, the long-term effects of stem cell transplantation remain unknown.

Biology of CXCR4

CXCR4 was originally identified as an orphan receptor called leukocyte-derived seven-transmembrane domain receptor (LESTR) [37–41], but did not receive much attention until its isolation as a coreceptor for HIV-1 [2] and the discovery of its natural ligand, SDF-1/CXCL12 [42,43]. The identification of CXCR4 as an HIV coreceptor [2] triggered a wide range of research activities to investigate the biological roles of the CXCL12/CXCR4 axis. CXCL12 is a highly conserved chemokine that has 99% homology between mouse and human, allowing CXCL12 to act across species barriers. Recently, six isoforms have been identified for the CXCL12 [44]. We found that CXCL12 γ is a very weak agonist for CXCR4, but is at least 5–6 times more potent than CXCL12 α in HIV-blocking assays [45]. The potent blocking activity of CXCL12 γ correlated well with its efficient CXCR4 internalization.

CXCR4 is functionally expressed on the cell surface of various cancer cells and plays a role in cell proliferation and migration of these cells [46]. CXCL12 and CXCR4 gene-deleted mice displayed an identical, lethal phenotype, indicating a monogamous relation between CXCL12 and CXCR4. Mice lacking CXCR4 die *in utero* and are defective in vascular development, hematopoiesis, and cardiogenesis [47]. Mice lacking CXCL12/SDF-1 are characterized by deficient B-lymphopoiesis and myelopoiesis and abnormal neuronal and cardiovascular development [48]. The CXCR4-CXCL12 axis is functional in evolutionarily distant organisms such as zebra fish and mice, in which CXCR4 expression is a prerequisite for germ cell migration to CXCL12-expressing gonads during development [49].

Role for CCR5 and CXCR4 in HIV-1 entry

The spikes projecting from the surface of HIV-1 particles are composed of the envelope glycoprotein (Env), whose function is to promote HIV entry by a process of direct fusion between the virion membrane and the plasma membrane of the target cell [50,51]. Env consists

of two noncovalently associated subunits derived by proteolytic cleavage of the gp160 biosynthetic precursor: the gp120 external subunit, which is responsible for binding to specific target cell receptors, and the gp41 transmembrane subunit, which catalyzes the fusion reaction and anchors Env to the viral host-derived membrane. Biochemical, genetic, immunochemical, and structural analyses have implicated the sulfated N-terminus and the second extracellular loop of CCR5; on the gp120 side, the bridging sheet and third variable (V3) loop are directly involved, with sequences in the latter domain determining the coreceptor usage phenotype (R5, X4, and R5X5) [52].

In their function as HIV coreceptors, CCR5 and CXCR4 physically associate with CD4-activated gp120, as shown by direct binding and coprecipitation studies [53–56] and functional assays [57]. The functional envelope glycoprotein on the surface of the HIV particle or infected cells is organized as a trimer of three gp120-gp41 heterodimers (Fig. 3a). The HIV fusion reaction is initiated by sequential receptor binding of gp120, first to CD4 and then to a specific chemokine receptor, generally CCR5 or CXCR4 [1•]. In the generally accepted model for HIV entry [50,58], gp120 binds to CD4 and is induced to undergo a major conformational change that either creates or facilitates exposure of the coreceptor-binding site; gp120 interaction with coreceptor then triggers the gp41 subunit to promote the fusion reaction via another series of complex conformational changes (Fig. 3). These receptor interactions then trigger gp41 to promote membrane fusion; this reaction is thought to involve extension of the gp41 subunit to allow insertion of its N-terminal ‘fusion peptide’ into the target cell membrane, followed by refolding the prefusion intermediated into an energetically favorable six-helix bundle that brings the two membranes together so that fusion can occur (Fig. 3e).

Despite extensive studies aimed at defining the precise molecular composition of the gp120-coreceptor complex, the details remain obscure [52]. The lack of the coreceptor crystal structure represents a major obstacle; indeed to date, a three-dimensional X-ray crystallographic structure has been obtained for only three GPCRs [14•,15•,59]. It is unfortunate that the coreceptor structure is still absent in high resolution X-ray structures solved for gp120 in its CD4-bound [60] and unbound [61] forms, the postfusion core of gp41 [62,63], and the gp120-binding region of CD4 cell [64,65].

It is generally accepted that R5 viruses appear early in infection and are responsible for virus transmission, whereas X4 strains appear late and have been associated to faster decline of CD4⁺ T cells [1•]. Coreceptor usage and switching has been analyzed most extensively for clade B isolates, which predominate in North America and Western Europe [66]. Nonclade B viruses cause the vast majority of new HIV-1 infections worldwide and should, therefore, be the major focus of vaccine efforts and drug development efforts. Nonclade B viruses are understudied, and their immunogenic and biological properties remain largely unknown. Rapid depletion of CD4⁺ T-lymphocytes has been associated with a switch in viral coreceptor usage from CCR5 to CXCR4 in approximately 40–50% of infected individuals [67]. However, the majority of infected individuals who progress to AIDS harbor predominantly CCR5-dependent viral strains [67]. Additionally, CCR5-deficient HIV+CCR5^{−/−} patients progress to AIDS harbouring either X4 or R5X4 isolates [68]. There is evidence to suggest that the late-emerging R5 strains have reduced sensitivity to entry inhibitors and increased ability to cause CD4⁺ T-lymphocyte loss [69]. Recent studies used a simian immunodeficiency virus model to show that disease progression can be induced by a mucosally transmissible, pathogenic R5 nonclade B simian/human immunodeficiency virus [70•].

Receptor internalization and recycling

The mechanism(s) by which chemokines block HIV-1 infection is not well understood. One model proposes that chemokines bind to a receptor domain that overlaps with gp120 receptor-

binding domain (i.e. steric blockade). The other model proposes that chemokine-induced internalization of the coreceptor molecules leads to the downmodulation of receptor sites at the cell surface, making the cells invisible for the virus envelope interaction. Several studies demonstrated that chemokine-induced internalization of CCR5 and CXCR4 could contribute to the chemokine-mediated inhibition of HIV-1 entry [71–73].

To avoid prolonged activation of the receptors, GPCR complexes are endocytosed and either recycled back to the plasma membrane or sorted into the degradative pathway [11•]. A ubiquitin-associated system is crucial in regulating these processes and involves the conjugation of ubiquitin onto target proteins destined for degradation, mediated by a family of proteins called E3 ubiquitin ligases. A well characterized example is the agonist-dependent degradation of CXCR4, in which ubiquitination mediated by the E3 ubiquitin ligase, AIP4, has been shown to be required at multiple steps in the sorting process. Ubiquitinated CXCR4 is concentrated on HRS-positive microdomains together with AIP4. AIP4 mediates ubiquitination of HRS following CXCR4 activation, which is critical for MVB sorting. CISK phosphorylates and inhibits AIP4 activity and, thereby, inhibits endosomal sorting of CXCR4 and favors the recycling pathway. VPS4 also regulates the ubiquitination status of CXCR4 and MVB sorting. Ubiquitination functions as an endosomal sorting signal and is not required for CXCR4 internalization. Figure 4 outlines CXCR4 trafficking within the endosomal–lysosomal system.

Recent studies on CCR5 intracellular trafficking of both progressive truncation and substitution mutants of the C-terminal domain demonstrated that the C-terminal tip of CCR5 possesses a sequence necessary for recycling, which acts as a postsynaptic density 95/discs-large/zona occludens (PDZ) interacting sequence [74]. PDZ domains are protein-protein recognition modules that bind to C-terminal short, linear PDZ ligand sequences. This sequence is present at the C-terminal tip of CCR5 (Fig. 2), but not CXCR4. The recent findings on CCR5 trafficking strengthened the key role played by PDZ ligands in the intracellular sorting and reinforced the emerging concept that GPCR recycling is a regulated process [11•]. Ligand-induced internalization of CCR5 is believed to involve a similar mechanism described for CXCR4; however, the subsequent fate of CCR5 is not well established. CCR5 shows a constitutive turnover in the absence of ligand with a half time of 6–9 h. Addition of RANTES has little effect on the rate of CCR5 turnover [75].

Conclusion

The HIV coreceptors have created entirely new paradigms for understanding basic mechanisms underlying the transmission and pathogenesis of HIV-1 infection [76]. In the coming years, we will likely witness the development of coreceptor-based antiviral strategies. Indeed, the orally bioavailable CCR5 blocking agent, maraviroc, [77] is the first such therapeutic to be approved by the U.S. Food and Drug Administration. CXCR4-based blocking agents are less attractive due to the crucial role of CXCR4 in many biological processes; however, agents that are aimed at downmodulating CXCR4 expression might provide some benefits for HIV-positive patients. Antagonism of CXCR4 significantly improved survival from lethal infection through enhanced intraparenchymal migration of West Nile Virus (WNV)-specific CD8⁺ T cells within the brain, leading to reduced viral loads and, surprisingly, decreased immunopathology at this site [78•].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest

•• of outstanding interest

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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antagonism of CXCR4 significantly improved survival from lethal infection suggesting that targeting CXCR4 may have therapeutic utility for the treatment of acute viral infections of the CNS

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Figure 1. Schematic ball diagram showing the amino acid sequence and membrane orientation of CXCR4 gr1

The shaded residues represent potential phosphorylation sites (total of 25). Potential N-linked glycosylation sites are marked. Asterisks indicate the cysteine residues involved in disulfide bonding.

HIV-1 Variants

Target Cell Types

Figure 2. Coreceptor usage and HIV-1 tropism gr2

X4-tropic strains are specific for CXCR4 and infect continuous CD4⁺ T cell lines and primary CD4⁺ T cells. R5-tropic strains are specific for CCR5 and can infect primary macrophages and CD4⁺ T cells. R5X4-tropic strains can utilize both CXCR4 and CCR5. Reproduced with permission from [1•].

Figure 3. Mechanism of HIV-1 entry gr3

(a) Diagram of the major players involved in HIV-1 entry. Upon binding of CD4 cell (b), gp120 undergoes a conformational change that exposes a hidden coreceptor-binding site (c). Binding of gp120 to the coreceptor brings the envelope into close proximity to the cell surface and induces gp120 to undergo a second conformational change (d) that allows the gp41 protein to penetrate the cell membrane and form a six helices bundle. Through processes that are unknown, fusion occurs between the cell and viral membranes allowing entry of the viral capsid and proteins.

Figure 4. CXCR4 trafficking within the endosomal-lysosomal system

CXCL12-activated CXCR4 is ubiquitinated at the cell membrane by the E3 ubiquitin ligase AIP4. CISK inhibits endosomal sorting of CXCR4 and favors receptor recycling. Under conditions of high PI-3-kinase signaling (i.e during the development or cancer progression), the degradation of CXCR4 is strongly inhibited. AIP4, atrophin-interacting protein 4; CISK, cytokine independent survival kinase; ESCRT, endosomal-sorting complex required for transport; MVB, multivesicular bodies; Ub, ubiquitin; Vps4, vacuolar protein sorting. Adapted with permission from [11••].