Chemokines and chemokine receptors in HIV infection: Role in pathogenesis and therapeutics

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ABSTRACT

Chemokines are known to function as regulatory molecules in leukocyte maturation, traffic, homing of lymphocytes and in the development of lymphoid tissues. Besides these functions in the immune system, certain chemokines and their receptors are involved in HIV pathogenesis. In order to infect a target cell, the HIV envelope glycoprotein gp120 has to interact with the cellular receptor CD-4 and co-receptor, CC or CXC chemokine receptors. Genetic findings have yielded major insights into the *in vivo* roles of individual co-receptors and their ligands in providing resistance to HIV infection. Mutations in chemokine receptor genes are associated with protection against HIV infections and also involved in delayed progression to AIDS in infected individuals. Blocking of chemokine receptors interrupts HIV infection *in vitro* and this offers new options for therapeutic strategies. Approaches have been made to study the CCR-5 inhibitors as antiviral therapies and possibly as components of a topical microbicide to prevent HIV-1 sexual transmission. Immune strategies aimed at generating anti-CCR-5 antibodies at the level of the genital mucosa might be feasible and represent a strategy to induce mucosal HIV- protective immunity. It also remains to be seen how these types of agents will act in synergy with existing HIV-1 targeted anti viral, or those currently in developments. Beyond providing new perspectives in fundamental aspects of the HIV-1 transmission and pathogenesis, chemokines and their receptors suggest new areas for developing novel therapeutic and preventive strategies against HIV infections. Studies in this review were identified through a search for relevant literature in the pubmed database of the national library of medicine. In this review, some developments in chemokine research with particular focus on their roles in HIV pathogenesis, resistance and therapeutic applications have been discussed.

*C*hemokines are a superfamily of secreted proteins that function in leukocyte trafficking, recruiting and recirculation. They also play a critical role in many pathophysiological processes such as allergic responses, infectious and autoimmune diseases, angiogenesis, inflammation, tumor growth and hematopoietic development. They are secreted by a variety of cells in the immune system. All chemokines signal through seven transmembrane domain G-protein coupled receptors and many of these receptors exhibit promiscuous binding properties whereby several different chemokines can signal through the same receptor. To date, more than 40 distinct chemokines have been well characterized, some of which are listed in Table 1. Discoveries over the past few years have defined a close relationship between chemokines and HIV infection. Apart from their well established role in blocking viral entry by binding to their receptors, chemokines have additional roles in HIV pathogenesis. For several years, it has been known that CD 8+ T cells secrete factors that suppress HIV-1 replication in CD4 + T cells.[1,2] The nature of these factors remained unknown until Cocchi *et al* in 1995 showed that the β

chemokines MIP-lα (macrophage inflammatory protein 1α), MIP-Iβ (macrophage inflammatory protein 1β) and RANTES (regulated on activation, normal T expressed and secreted) contributed to the CD 8+ cell suppressive effect.[3] A breakthrough in our understanding of HIV pathogenesis was the identification of a chemokine receptor like molecule in 1996, called LESTR, subsequently designated as CXCR-4, as a necessary co-receptor for X 4 variants of HIV entry into cells.[4,5] In the absence of the second receptor, HIV-1 can bind to its target cells (via CD-4), but the fusion process is not initiated. The identification of the beta chemokine receptor (CC) CKR-5 (later renamed as CCR-5) as the primary co-receptor for macrophage-tropic, non-syncytium inducing (NSI) strains of HIV-1 was also reported in 1996, almost simultaneously by five independent groups of researchers.[6-10]

Chemokine receptors as co-receptors for HIV cell entry
HIV envelope proteins gp 120 and gp 41 mediate binding of virus to the host cell surface through high affinity interaction with CD-4, the primary virus receptor. Subsequent interaction with the appropriate chemokine receptor CCR-5 or CXCR-4

Table 1: Chemokines and their receptors

Chemokine family	Chemokines	Chemokine receptor	Cell type
CXC(α)	IL-8, GCP-2	CXCR 1	N, NK
Chemokines	IL-8, GCP-2, GRO-α/β/γ, ENA-78, NAP-2, LIX	CXCR 2	N, NK
	IP-10, MIG, I-TAC	CXCR 3	act T, NK
	SDF-1 α/β	CXCR 4	T, M
	BCL	CXCR 5	B
	CXCL 16 (Sex CKine)	CXCR 6	Th1 cells,
CC(β)	MIP-1α, MCP-4, MCP-3	CCR 1	M, act T, N, Ba, E, D
Chemokines	RANTES, MIP-5, MPIF-1, HCC-1 MCP-1, MCP-2, MCP-3, MCP-4, MCP-5	CCR 2	M, act T, Ba, D, NK
	Eotaxin, Eotaxin-2, Eotaxin-3, MCP-2, MCP-3, MCP-4, RANTES, MIP-5	CCR 3	act T, E, Ba, D
	TARC, MDC, RANTES, MIP-1α, MCP-1	CCR 4	act T, Ba, D
	RANTES, MIP-1α, MIP-1β, MCP-2	CCR 5	M, act T, D
	Exodus/LARC, MIP-3β	CCR 6	act T, B, imm D
	SLC/6Ckine, MIP-3β	CCR 7	T- and B-cell lines, D
	I-309, TARC, MIP-3β	CCR 8	Act T, NK
	TECK, MCP-1, MCP-2, MCP-4, MIP-1β, MIP-1α, RANTES, Eotaxin	CCR 9	D, thy
	C-TACK, MCP-1	CCR 10	Skin T cells
	MCP-1, ELC, SLC, TECK	CCR 11	Various organs
CX3C	Fracktalkine	CX3CR 1	M, act T, NK
C chemokine	Lymphotactin/SCM1α	XCR 1	act T, NK

Abbreviations: BLC, B lymphocyte chemokine; C-TACK, cutaneous T-cell-activating chemokine; ELC (Ebl-1), EBL-1-ligand chemokine; ENA-78, epithelial-cell-derived neutrophil-activating protein 78; GCP-2, granulocyte chemotactic protein 2; GRO, growth-related oncogene; I-309, (a nameless human chemokine), IL-8, interleukin 8; ITAC, interferon-inducible T-cell alpha chemoattractant; LIX, lipopolysaccharide-induced CXC chemokine; LARC, liver- and activation-regulated chemokine; MCP-1, monocyte chemoattractant protein 1, MDC, macrophage-derived chemokine, MIG, monokine induced by interferon g, MIP, macrophage inflammatory protein, MPIF-1, myeloid progenitor inhibitory factor 1; NAP-2, neutrophil-activating peptide 2; RANTES, 'regulated on activation, normal T-cell expressed and secreted'; SDF-1, stromal-cell-derived factor 1; SLC, secondary lymphoid tissue chemokine; TARC, thymus- and activation-regulated chemokine; TECK, thymus-expressed chemokine; act T, activated T cell; B, B cell; Ba, basophil; D, dendritic cell; E, eosinophil; imm D, immature dendritic cell; M, monocyte; N, neutrophil; NK, natural killer cell; T, T cell; Th1, T helper 1 cell; Thy, thymocyte

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triggers the final conformational changes in *env*, resulting in fusion between the viral and cellular membrane.[11-13] The observation that HIV-1 isolates differ in their ability to use two major co-receptors (CCR-5 and CXCR-4) has provided a key to understand the physiological basis of the biological variability of HIV-1. Different HIV-1 variants use either CCR-5 or CXCR-4 or both. According to the terminology based on co-receptor usage, CXCR-4 using viruses are termed X 4 and CCR5-using viruses, R 5. Viruses that were previously defined as SI which use both CXCR-4 and CCR-5 receptors are termed X 4 R 5 (R 3) viruses.[14,15] Using transfected cell lines other chemokine receptors such as CCR-3, CCR-2, CCR-8, CCR-9, STRL-33, Gpr 15, Gpr 1, APJ, Chem R 23 and CX 3 CR1 were identified and shown to be used by certain HIV strains for cell entry.[9,10,16-18] Recently a promiscuous CC chemokine receptor, D6, has been found that can function as a co-receptor for various primary dual-tropic isolates of HIV-1 and HIV-2.[19] Despite this broad spectrum of potentially available co-receptors, CCR-5 and CXCR-4 appear to be the most relevant co-receptors for HIV-1 *in vivo*. The host's natural ligands for these co-receptors are relevant because they might interfere with HIV entry into target cells by interfering with viral binding to the receptor or by down regulating the receptor. CCR-5 binds to RANTES, MIP-1α and MIP-1β, which are the members of the β chemokine family whereas CXCR4 binds to a member of α chemokine family, stromal cell derived factor 1 (SDF-1). CCR2 binds to monocyte chemotactic protein-1 (MCP-1) through MCP-5 and CCR-3 binds to MCP-3, MCP-4 and eotaxin1 and 2. CCR-5 using chemokine can block R 5 strains of HIV, whereas SDF-1 blocks X 4 strains. CCR 5 and CXCR-4 receptor has been expressed on a variety of cells and tissues. Chemokine receptor expression in a specific cellular type might be constitutive or inducible.[20]

Chemokines and HIV pathogenesis

Immunologic or genetic alterations that affect chemokine levels might have an impact on the susceptibility to HIV infection or the rate of progression once the infection is established. Inhibition of HIV entry by chemokines depends on two possible mechanisms: a steric effect that consists of the competitive blockade of viral entry by direct union of ligand to its receptor, or through the internalization of the receptor after chemokine binding. Alternatively, chemokine receptor dimerization mediated through binding of chemokine could account for inhibition of HIV entry and virus replication.[21-23] An *in vitro* study by the Multicenter AIDS cohort study (MACS) showed that AIDS free status and higher CD 4 counts correlate with higher CCR-5 ligands release from peripheral blood

mononuclear cells after activation *in vitro* by HIV antigen.[24] In addition, cells from individuals who are exposed but seronegative to HIV infection release significantly higher levels of chemokines than seronegative controls and HIV positive subjects, even in the absence of an *in vitro* stimulus.[24] These studies suggest that chemokine release is a very early response to the exposure to HIV. It is also reported that high levels of CCR-5 using chemokines are associated with slower disease progression.[25] An *in vitro* study which assessed the susceptibility to HIV infection of CD-4 + cells from HEPS (highly exposed persistently seronegative) individuals in a discordant cohort showed that CD-4 + lymphocytes from HEPS individuals were less susceptible to HIV infection by R 5 strains of HIV. In addition, CD-4 + lymphocytes of these HEPS subjects produced significantly higher levels of RANTES, MIP-1α and MIP-1β upon stimulation with phytohemagglutinin (PHA) and enhanced chemokine production was noticed against stimulation with HIV *gag* peptide.[26] Other studies have also reported increased β chemokine levels from serum samples in a group of Chinese HEPS individuals and enhanced production of MIP-1β by CD-8 + T cells from HEPS subjects in a discordant couple cohort.[27,28] Other studies have shown that CD-8 + T cells from asymptomatic individuals produce higher levels of MIP-1α and MIP-1β, but not RANTES, than CD-8 T cells from healthy donors or patients with rapid progression.[29-33]

Some other studies have found no association between chemokine production and HIV resistance in HEPS individuals and also between long term nonprogressors and rapid progressors.[34-37] Others have even suggested that RANTES, MIP-1α and MIP-1β may upregulate replication of HIV in macrophages and monocytes by recruiting activated target cells.[38-40] Other chemokines that affect HIV replication, although they are not involved in viral entry, are interferon-γ-inducible protein 10 (IP-10/CXCL-10) and monocyte chemotactic protein (MCP)-1.[41-43] These chemokines have been detected in the cerebrospinal fluid of HIV infected individuals. Another chemokine that may affect HIV infection is IL-8 (CXCL-8). Increased levels of circulating IL-8 have been detected in HIV infected individuals.[44] Upregulation of chemokine expression by cells of the immune system would have local and systemic effects that contribute significantly to the pathogenesis of HIV infection.[45,46] The exact role of chemokines in HIV-1 pathogenesis remains obscure, probably due to the fact that multiple chemokines may have different effects on viral replication and pathogenesis, or their effect might be compromised by viral factors.

CCR-5 gene mutations

The observation that chemokine receptors are used by HIV as co-receptors for cellular entry led to the discovery of genetic host factors that can affect susceptibility to infection with HIV or the rate of progression to disease once infection is established. In early HIV infection, a vast majority of HIV isolates use the CC chemokine receptor, referred to as CCR-5, which in blood is expressed on a variety of cells including CD-4, CD-8, memory and activated T cells. Individuals homozygous for a 32 base pair deletion in their CCR-5 gene (referred to as CCR5-Δ32), are almost completely resistant to HIV infection.[47] CCR5-Δ32 was the best characterized genetic trait, identified in 1996.[50,51] The mutation is a 32 base pair deletion corresponding to the second extracellular loop of the 7-transmembrane G-coupled protein receptor in the CCR-5 gene, which causes a frame shift, leading to a premature termination of translation and the resulted protein encoded by this mutant lacks three transmembrane segment of the receptor. Such a truncated protein is nonfunctional.[47] In epidemiological studies, the allelic frequency of the CCR-5 gene deletion was 10-20% among caucasians, particularly amongst those of Northern European descent with 1% homozygosity. This mutation is extremely rare in African and Asian population.[48,51] Individuals homozygous for the CCR5-Δ-32 allele do not express any of the CCR-5 receptor on their cell surfaces and in turn, they are largely resistant to infection by HIV-1. Different studies have shown that CCR-5-Δ-32 mutation is extremely protective against HIV-1 infection, although they can still be infected with X 4 strains of HIV, which use the CXCR-4 co-receptor for cell entry.[48-51] However, this protection is not complete, as a few individuals homozygous for this deletion were infected and the virus that was isolated from these individuals was X 4 type.[52,53] Some other studies have reported the presence of dual tropic R5X4 HIV strains in two individuals homozygous for CCR-5-Δ-32 allele.[54,55] Studies of CCR-5-Δ-32 mutation in exposed but uninfected individuals have revealed that a small proportion of them were homozygous for this mutation.[47,56,57,59] Some studies have found that CCR-5-Δ-32 heterozygosity was associated with delayed progression to AIDS in infected individuals and also reported that frequency of heterozygosity was significantly greater in long term non-progressors than in progressors and rapid progressors.[47-51,57-61] The mechanism of protection is not clear and it is believed that CCR-5 expression may be altered in these individuals. In our own cohort of such individuals, we were unable to detect any deletion.

A point mutation in the coding region of CCR-5 gene conferring *in vitro* and *in vivo* resistance to R 5 virus has been identified.[62] The mutation is characterized by an open reading frame single T to A base pair transversion at nucleotide 303 which indicates a cysteine to stop codon change in the first extracellular loop of the chemokine receptor protein at amino acid 101.[63,64] This mutation when found in the compound heterozygous state with CCR5-Δ-32 was associated with increased protection. However, this allele is very rare with an allelic frequency less than 1 percent.[64]

CCR-5 promoter and regulatory gene polymorphisms

Several genetic polymorphisms have been identified within the CCR5 regulatory or promoter region that might affect HIV transmission or disease progression, possibly through its effect upon levels of CCR5 expression.[58,64-67]

CCR-5 59029 G: This is an A/G polymorphism at base pair 59029 in the CCR-5 promoter. HIV infected persons who are homozygous for allele 59029 G within the CCR-5 promoter regulatory region progress to AIDS more slowly than those who are homozygous for allele 59029 A.[65] Another study shows an

association between *CCR-5* promoter polymorphisms and long-term asymptomatic HIV-1 infection, with individuals lacking the *CCR-5 59029A/CCR5 59353C* homozygous genotype likely to progress more slowly towards AIDS and/or death.[66] Yet another point mutation at position 59402 results in an A/G substitution and homozygosity of this allele is associated with reduced perinatal transmission. This mutation affects the CCR-5 expression on CD 4 + T cells.[67] Other promoter haplotypes may lead to more rapid disease progression.[58] Homozygosity of another polymorphism known as CCR-5 59356T has been strongly associated with increased rate of perinatal transmission and this polymorphism occurs more frequently in black persons than white.[67]

CCR-2b 64I mutation

The chemokine receptor CCR-2b is identified as a minor HIV-1 co-receptor.[59,62] The mutation within the CCR-2 gene is a conservative valine to isoleucine at position 64 in the first transmembrane domain of the CCR-2 receptor, which is present at an allelic frequency of 10-25% in different populations.[59] The presence of this mutation in either heterozygote or homozygote has been associated with delayed progression to AIDS and death in most, although not all, cohorts. In contrast with CCR-5-Δ-32 mutation, it provides protection against HIV disease progression in races other than the whites.[57,68,69-71] Increased frequency of CCR-2-64I homozygosity was observed in exposed uninfected individuals compared with HIV positive and HIV negative controls suggesting an association between CCR-2 64I homozygosity and resistance to heterosexual transmission.[18] However the mechanism of protection is not clear, since the CCR-2 is only rarely used as a co-receptor by HIV. It has been suggested that CCR-2 64I mutation tracks with another mutation through linkage disequilibrium, particularly in the regulatory or promoter region of CCR-5.[58,66,68] A polymorphism within the regulatory region of CCR-5 59653T is in linkage disequilibrium with the 64I mutation, but the functional significance of this finding is unclear.[68]

SDF-1 3' α mutation

Another genetic trait that might affect progression to AIDS involves stromal cell derived factor-1 (SDF-1), the chief ligand of CXCR4. SDF-1 blocks infection with X-4 variant of HIV-1.[72,73] The mutation SDF-1 3'α involves a G/A transition at position 801 of SDF-1 3' α untranslated region (UTR) and is associated with protection against HIV.[74] This mutation is common among all geographical regions of the world. Mutation may upregulate the synthesis of SDF-1, thus competitively inhibiting X 4 HIV from binding. Persons who are homozygous for this mutation have been shown to experience delayed progression to AIDS but do not exhibit decreased susceptibility to infection with HIV.[58,74,75] In contrast, other studies have shown that SDF-1 3' α homozygosity was associated with accelerated disease progression[66,76,77] and increased viral replication[78] or no effect on disease progression.[60]

Other mutations

Two single nucleotide polymorphism (SNP) sites, a cytosine to guanine transversion polymorphism at position - 28

(RANTES 28 C/G) and a guanine to adenine transition polymorphism at position - 403 (RANTES 403 G/A), in the promoter region of RANTES have been identified. RANTES 403 A and 28 G promoter polymorphism is associated with increased RANTES transcription and delayed progression to AIDS in HIV infected individuals.[79,80] No mutation has been described in the CXCR-4 region that might influence HIV infection.

Anti HIV-1 therapy based on chemokines and their receptors

The discovery of chemokine receptors as co-receptors for HIV-1 has opened the door for a number of novel antiviral approaches. The need for an improvement of current drug combination regimens, based on inhibitors of the viral reverse transcriptase and protease, is underscored by a series of important drawbacks, including the rapid rebound after withdrawal, the increasing emergence and transmission of multidrug resistance, side effects, difficulties in schedule compliance and, most importantly, the lack of virus eradication even after long term effective treatment.[81] While all the currently licensed antiviral drugs except peptide T 20 block HIV after its penetration into the target cell, co-receptor inhibitors target the early interactions between virus and cell membrane, thereby blocking HIV outside its target cells. However, major hurdles in the way towards developing safe and effective co-receptor inhibitors is the risk of interfering with the physiology of the chemokine system, causing potentially harmful side effects.[81] Therapeutic agents that interfere with chemokine co-receptors might also block membrane fusion and viral entry. Agents that block or prevent CCR-5 might prove useful and safe in the prevention of and treatment for HIV infection. The known inhibitory effects of CCR-5 ligands, RANTES, MIP-1a and MIP-1b, have led to the consideration of their use as potential therapeutic agents to limit HIV entry. But this approach has its own disadvantages as this might recruit HIV susceptible cells through chemotaxis, increase X 4 virus replication and even increase infectivity of R 5 viruses.[82,83]

Chemokine receptor antagonists

Another therapeutic approach is the usage of chemokine receptor antagonists. In this regard, a number of studies have demonstrated that N-terminal modification and truncation of chemokines can give rise to specific receptor antagonists. This approach has been used to create potential chemokine antagonists of HIV-1 co-receptors. The advantage of this approach is that these molecules can block HIV without activating chemotaxis or proinflammatory effect and without increasing the level of X 4 HIV.

CCR-5 inhibitors

Various CCR-5 ligands with antiviral properties have been described, including modified chemokines and monoclonal antibodies and more importantly small-molecule inhibitors with potential for oral administration Example for such modified or truncated CCR-5 antagonists are Aminooxypentane (AOP), N-nonanoyl (NNY)-RANTES, N (n-nonanoyl)-des-Ser (PSC) RANTES 9-68 RANTES and met RANTES. These compounds induce CCR-5 internalization.[84-

[87] Another study has generated a panel of recombinant RANTES analogues bearing natural amino acid substitution at the amino-terminus and two of them, L-RANTES and C1.C5-RANTES have anti HIV potency.[81] Another study reported a series of 1,3,4-trisubstituted pyrrolidine CCR-5 receptor antagonists containing a variety of fused heterocycles at the 4-position of the piperidine side chain with potent anti-HIV activity[89] A number of other small molecular weight compound like Tak 779,[90] Tak 220,[91] the spirodiketopeperazine derivative E-913,[92] monoclonal antibody PRO-140,[93] the small molecular weight compounds SHC-C and SHC-D[94] are under advanced clinical trials. Antiviral activity of anti CCR 5 monoclonal antibody PRO 140 was investigated.[93] In this study, PRO 140 is tested against a panel of primary HIV-1 isolates and the result showed that, low nanomolar concentrations of PRO 140 inhibited infection of primary peripheral blood mononuclear cells (PBMC) by all CCR-5 using (R 5) viruses tested.[93]

CXCR-4 inhibitors

In addition to modified chemokines, a peptide inhibitor of CXCR4 known as T 22 was identified. It is able to inhibit specifically the ability of T cell tropic HIV-1 which uses CXCR-4 co-receptor but not R5 HIV-1. In addition, T 22 also inhibits Ca^{2+} mobilization induced by SDF-1 stimulation through CXCR-4. Another report, describes an inhibitor of CXCR-4 known as Allelix (ALX)-40-4C. This compound is a highly cationic oligopeptide containing nine arginine residues. ALX 40-4C inhibits CXCR-4 dependent HIV-1 mediated membrane fusion and viral entry by T and dual tropic HIV-1 strains, but does not inhibit HIV-1 fusion mediated by CCR-5. In addition, ALX- 40-4C blocks SDF-1 mediated activation of CXCR-4 and binding of the CXCR-4 specific monoclonal antibodies12 G 5 to cells expressing CXCR-4.[95,96] Several other modified ligands of CXCR-4 receptor that are under investigation include peptoids CGP-64222, arginine conjugate such as R3G and NeoR, the bycyclams and the recently reported AMD070 and KRH 1636.[97-104] The bycyclam AMD-3100 blocks HIV-1 entry through CXCR-4 and inhibits binding of SDF-1 and 12G5 to CXCR-4 but does not itself trigger cell signaling.[101] AMD-3100 does not inhibit the binding of CC chemokine ligands to CCR-1, CCR-2b or CCR-5.

Another strategy to prevent HIV-1 infection is to reduce the surface expression of level of HIV-1 co-receptors. This principle has been used by some studies to develop a device to trap the HIV-1 co-receptors CCR-5 and CXCR-4 in the endoplasmic reticulum (ER), thus preventing their transport to cell surface.[105] Other areas of investigations are the administration of CD-4 cells with decreased CCR-5 expression, gene therapy to prevent receptor expression through antibodies or altered ligands and development of pseudo viruses or vectors that express CD-4 and chemokine receptors and thus could target HIV infected cells to deliver antiviral treatment or kill HIV infected cells. "Short interfering RNA" (siRNA) represent a new molecular tool that is able to selectively inactivate target genes.[106] Double stranded RNA is split by the enzyme dicer-1 into short pieces. This oligomer may complimentarily bind to longer RNA sequences that are subsequently degraded. The

use of siRNA against CCR-5 can prevent the expression of CCR-5 *in vitro*.[106-108] Another approach based on a hammerhead ribozyme and an RNA cleaving DNA enzyme was used against CCR-5 and shown that when these ribozyme under a strong eukaryotic promoter are introduced in to a mammalian cell, could interfere the co-receptor function.[108] The *in vivo* effectiveness of these molecular tools needs to be evaluated extensively before concluding its role in interfering co-receptor functions.

Chemokine as topical microbicides

Approaches using chemokine analog as a topical microbicide at the site of viral entry have been investigated in animal models. A study reported that topical application of high doses of PSC-RANTES, an amino terminus–modified analog of the chemokine RANTES, provided potent protection against intravaginal challenge in rhesus macaques without detectable toxicity or histological changes.[109,110]

Few studies using CMPD167, a small molecule that binds to CCR-5 to inhibit gp 120 association, were found to be protective against vaginal transmission of R 5 virus in rhesus macaque. These findings support the development of small molecule CCR-5 inhibitors as antiviral therapies and possibly as components of a topical microbicide to prevent HIV-1 sexual transmission.[111,112]

Issues associated with the usage of chemokine and its antagonists in HIV therapy

Blocking cellular receptors also presents challenges due to potential toxic effects from interference with their normal function in lymphocyte development and trafficking and inflammation. The following mechanisms of escape from co-receptor inhibitors are possible: (1) the escape mutant may continue to use the same co-receptor in an inhibitor-insensitive manner; (2) co-receptor switching may occur, so that R 5 viruses become able to use CXCR-4, or *vice versa*. CXCR-4 receptor and its ligand SDF-1 are involved in various physiologic processes like B cell lymphopoesis, cardiac and cerebellar development in embryogenesis, bone marrow myelopoisis and vascularization of the gastrointestinal tract.[113-115] Therefore, the clinical application of CXCR-4 blockers may be limited. A further challenge for agents targeting viral entry is the ability of HIV to become resistant to the selective pressure exerted by the drug. Even when given in the context of other antiviral agents, a low level of viral replication may suffice to select viral variants that use alternative co-receptors or entry mechanisms. This possibility of viral evolution has to be considered before using receptor antagonists in HIV therapy.[116] It is reported that acquisition of CXCR-4 use is not the dominant *in vitro* escape pathway for a small molecule, CCR-5 entry inhibitor. Instead, HIV-1 acquires the ability to use CCR-5 despite the inhibitor, first by requiring lower levels of CCR-5 for entry and then probably by using the drug-bound form of the receptor.[117] Another study has suggested that co-receptor expression levels also influenced sensitivity to fusion inhibitors and fusion kinetics. Thus, receptor expression levels and *env*/receptor affinity are cellular and viral determinants, respectively, that impact viral sensitivity to different classes of entry inhibitors.

Therefore, mutations that result in drug resistance may do so directly by altering inhibitor binding sites or indirectly by affecting the rate of membrane fusion. Individuals who express lower levels of CCR-5, such as CCR-5-32 heterozygotes, may consequently respond more favorably entry inhibitors and viruses that exhibit enhanced affinity for co-receptor may respond less well.[118] Moreover, entry inhibitors must overcome the obstacles such as their pharmacological toxic effects, bioavailability and affordability. In gene therapy, introduction of pseudoviruses or vectors into immunocompromised individuals might result in vector associated morbidity and mortality as these individuals might not be able to contain the replication and dissemination of vectors.

Conclusion

Research is progressing rapidly in the area of chemokines and their receptors in HIV pathogenesis. The coming together of the two major fields of research, HIV and chemokines, will produce significant new insights and new therapies that will enable us to combat HIV infection. Since the identification of the chemokine receptors as co-receptors for HIV entry, many of the mysteries of HIV pathogenesis have become clear. However, the mechanisms that allow interaction of HIV-1 with chemokine receptors and implications of virus –induced receptor signaling are yet to be answered. Moreover, the potential of this discovery for anti-HIV therapy and vaccine development remains to be explored further. The other areas like role of various chemokines in HIV pathogenesis *in vivo* and the mechanism of genetic host factors mediated protection need to be investigated further.

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