

Cellular Tropism of HIV-1 Mediated and Constrained by Coreceptor Dependencies

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The ability of cells to migrate to specific sites is critical for the proper functioning of both innate and adaptive immune responses in primates. This selective responsiveness is largely orchestrated by small proteins known as chemokines, which direct immune cells expressing an array of chemokine receptors towards concentrations of intracellular and extracellular pathogens. Given the central role of chemokines and chemokine receptors in mediating immunity as well as homeostasis, it is perhaps not surprising that many pathogens, including HIV type 1 (HIV-1) and simian immunodeficiency virus (SIV), have targeted various aspects of chemokine biology. HIV-1 appears only able to use the chemokine receptors CCR5 and CXCR4 as coreceptors to CD4 to efficiently bind and infect cells *in vivo*. Interactions between HIV-1 and humans are thought to have evolved more recently than interactions between SIV and other primate species. Despite the ability of HIV-1 and SIV to readily mutate under immune pressure and the fact that CCR5 has undergone a range of adaptations in some primates that reduce the pathogenicity of HIV-1 infection, these viruses have maintained their dependency on the CCR5 coreceptor. This review discusses these issues of co-adaptation and the potential role and impact of coreceptor antagonists that are currently being developed for clinical use. *J Viral Entry* 2005;1(1):17–27.

One of the fundamental properties that characterizes living organisms is cellular motility – the ability to polarize and subsequently move towards or away from a stimulus. Cellular motility is of central importance for many biological processes in vertebrates, including embryological development, the migration of spermatozoa, and the movement of cell precursors from the bone marrow to tissues via the circulatory system. The ability of cells to migrate to specific sites is particularly relevant to the proper functioning of both innate and adaptive immune responses in primates. Selective responsiveness is largely orchestrated by the release and detection of extracellular proteinaceous ligands. Small proteins known as chemokines are involved in directing immune effector cells, which express an array of chemokine receptors, towards intracellular and extracellular pathogens. Due to the central role that chemokines play in

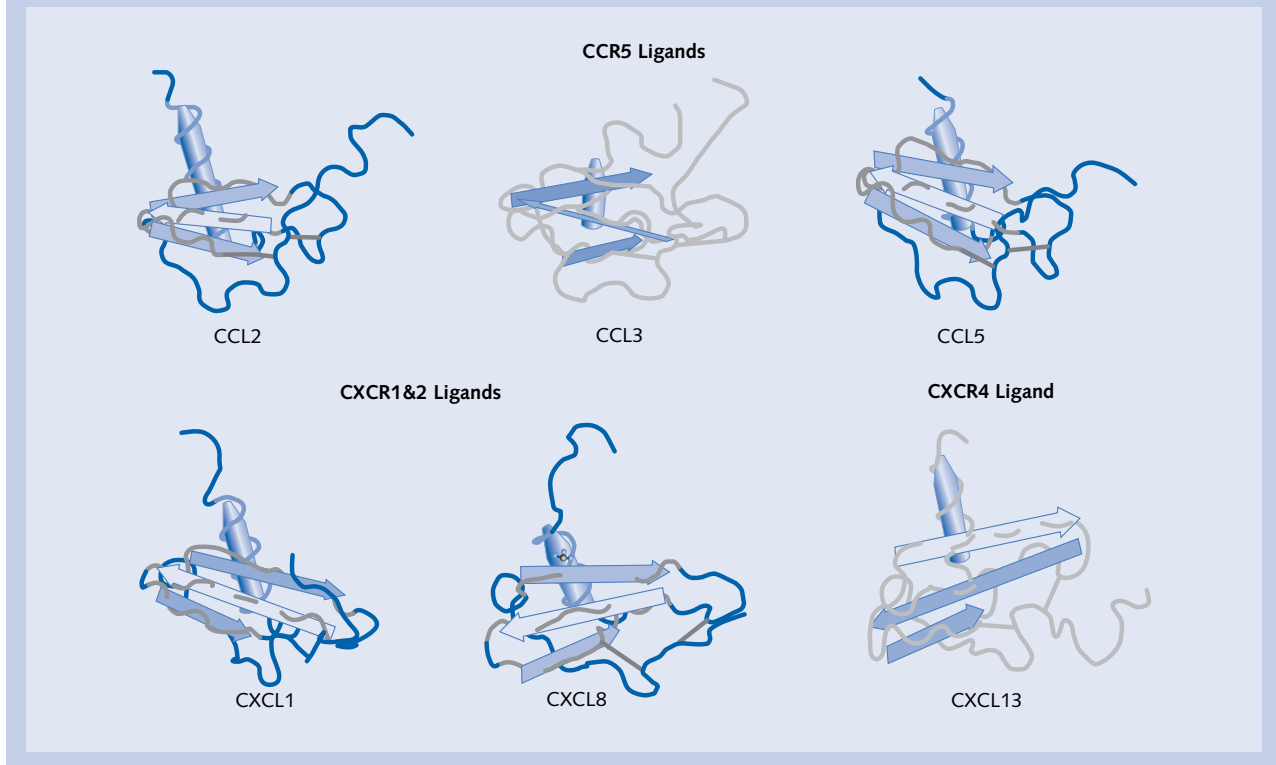
mediating both immunity and homeostasis, it is not surprising that many pathogens have targeted various aspects of chemokine biology, and, as with other targeted biological systems, there is evidence of a dynamic process of adaptation that affects both host and pathogen as these selective pressures shift over time. For a successful pathogen such as HIV type 1 (HIV-1), adaptation over generations may be measured in days and weeks, while for the human host it is possible that the shifting patterns of endemic and epidemic pathogens (of which HIV-1 is but one of the most recent) have guided the evolution of the chemokine system over centuries. This article reviews the interaction between chemokine receptors and their endogenous and pathogen-derived ligands with a specific focus on the role of chemokine–chemokine receptor systems in HIV-1 pathogenesis.

Chemokines and chemokine receptors: Divergent protein families with different and complementary functions

To understand the consequences for both the pathogen and the host that may result from targeting a particular

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Figure 1. Chemokines have a conserved tertiary structure despite substantial sequence diversity. All chemokines exhibit a highly similar tertiary fold, which consists of a flexible region located amino-terminally to the CC or CXC motif followed by a first loop region (N-loop), three anti-parallel β -strands and a carboxy-terminal α -helix. The 3D structures were created by Cn3D [75], using published data from the Molecular Modeling Database of the National Center for Biotechnology Information (National Library of Medicine, National Institutes of Health, Bethesda, MD, USA) [76,77].



chemokine or chemokine receptor for medical intervention, it is important to review the characteristics of this family of molecules. Chemokine receptors are members of the rhodopsin superfamily of receptors and have a characteristic structure that includes seven transmembrane-spanning α -helices. The conventional nomenclature for chemokine receptors reflects the conserved structural characteristics of their ligands; thus, CCR5 binds a selection of CC chemokines. Chemokines are bound via interactions with the amino terminus and one or more of the extracellular loops of the receptor. Signal transduction is dependent on the activation of G-proteins associated with the receptor, which phosphorylate intracellular targets that stimulate chemotaxis of the cell towards the source of the chemokine [1].

Chemokines can be broadly divided into two classes (Table 1):

- Those secreted in response to a proinflammatory stimulus.
- Constitutively expressed chemokines involved in homeostasis.

This functional classification system is distinct from the structural assignment of chemokines to one of four classes, which is related to the conserved cysteine residues present within their receptor-binding sites. In fact, all chemokines exhibit a highly similar tertiary structure, consisting of a flexible region located amino-terminally to the CC or CXC motif, followed by a first loop region (N-loop), three anti-parallel β -strands, and a carboxy-terminal α -helix (Fig. 1).

Phylogenetic analysis of the chemokine receptors reveals a highly divergent family of proteins, with the clustering of sets of receptor proteins reflecting physical genetic proximity and, in most cases, overlapping ligand interactions (e.g. CCR5, 2, 3, and 1; CXCR1 and 2) (Fig. 2). However, although the chemokine receptors exhibit low levels of amino acid similarity, they do retain a high degree of structural homology, which reflects their interaction with a restricted number of chemokine ligands.

Properties of chemokine receptors that affect ligand binding include sulphated tyrosine residues at the amino terminus that interact with the ligands and which convey an overall negative charge to this region on the receptor. This

Table 1A. Inflammatory/inducible chemokine receptors and ligands.

| Receptor | Ligand(s) | Receptor | Ligand(s) |
|----------|---|--------------|--|
| CCR1 | CCL3L1/MIP- α CCL5/RANTES CCL7/MCP-3 CCL9/MIP1- γ CCL14/HCC-1 CCL15/HCC-2 CCL16/HCC-4 | CCR11 | CCL2/MCP-1 CCL8/MCP-2 CCL13/MCP-4 |
| CCR2 | CCL2/MCP-1 CCL7/MCP-3 CCL12/mouse MCP-5 CCL13/MCP-4 | XCR1 | XCL1/lymphotactin α XCL1/lymphotactin β |
| CCR3 | CCL3L1/MIP-1 α CCL5/RANTES CCL7/MCP-3 CCL8/MCP-2 CCL11/eotaxin CCL13/MCP-4 CCL15/HCC-2 CCL24/eotaxin-2 CCL26/eotaxin-3 | CXCR1 | CXCL2/Gro- β CXCL3/Gro- γ CXCL5/ENA-78 CXCL6/GCP-2 CXCL7/NAP-2 CXCL8/IL-8 |
| CCR4 | CCL17/TARC CCL22/MDC | CXCR2 | CXCL1/Gro- α CXCL2/Gro- β CXCL3/Gro- γ CXCL5/ENA-78 CXCL6/GCP-2 CXCL7/NAP-2 CXCL8/IL-8 |
| CCR5 | CCL3/MIP-1 α CCL4/MIP-1 β CCL5/RANTES CCL8/MCP-2 | CXCR3 | CXCL9/Mig CXCL10/IP-10 CXCL11/I-TAC |
| CCR8 | CCL1/I309 CCL4/MIP-1 β | CXCR3B | CXCL4/PL4 |
| CCR10 | CCL27/CTACK CCL28 | CX3CR1 | CX3L1/Fractalkine |
| | | CCL6/C10 | Unidentified |
| | | CCL10 | Unidentified |
| | | CCL18/DC-CK1 | Unidentified |

Table 1B. Constitutive/homeostatic chemokine receptors and ligands.

| Receptor | Ligand(s) | Receptor | Ligand(s) |
|----------|---------------------------------|--------------|--------------------|
| CCR6 | CCL20/MIP3- α | CXCR7 | CXC12/SDF-1 |
| CCR7 | CCL19/MIP3- β CCL21/6kine | CX3CR1 | CX3CL1/Fractalkine |
| CCR9 | CCL25/TECK | Unidentified | CCL18/DC-CK1 |
| CCR10 | CCL27/CTACK | Unidentified | CCL23/MPIF-1 |
| CXCR4 | CXCL12/SDF-1 | Unidentified | CXCL14/BRAC |
| CXCR5 | CXCL13/BLC | Unidentified | CXCL15/Lungkine |
| CXCR6 | CXCL16 | | |

interaction has been demonstrated experimentally for two of the most promiscuous ligand-binding receptors: CCR5, which binds a range of CC chemokine ligands (including HIV-1) [2], and the human Duffy antigen, which uniquely has both CC and CXC ligand-binding affinities [3]. The same post-translational tyrosine sulphation is also present on the other central HIV coreceptor, CXCR4 [4]. However,

additional structural characteristics of the amino terminus seem to be more important than the interactions with sulphated tyrosine residues in determining the specificity of these receptors. The specificity of CCR2 for its ligands (CCL2, 7, 12, and 13), and of CCR3 for its ligands (CCL3, 5, 7, 8, 11, 13, 15, 24, and 26) appears to depend on intrinsic characteristics of the amino termini of the receptors, but also

requires interactions with extracellular loop domains for maximal signaling [5]. Interestingly, this is not true for CCR5 and its ligands (CCL3, 4, 5, and 8), where experimental swapping of its amino terminus with that of CCR2 does not change its ligand-binding selectivity [6].

The cellular components of the early pro-inflammatory response are governed in part through the selective expression of chemokine receptors by monocytes (chiefly CCR2–5), neutrophils (CXCR1 and 2) and eosinophils (CCR3) and by the temporal or tissue-specific secretion of chemokines [7,8]. This means that a given pathogenic stimulus in a specific tissue will generate a distinct local chemokine profile that influences the resulting cell infiltrate (eosinophils in the lung, neutrophils in the gut, and monocytes and neutrophils in the skin) [7,9–11]. Nearly all inflammatory chemokine receptors bind multiple high-affinity ligands (Table 1). Constitutive chemokine secretion exists in many tissues, and responsiveness to these gradients is regulated by the expression of suitable chemokine receptors by responsive cells. This is important for the homeostasis of many cell populations in peripheral tissues, such as the trafficking of dendritic cell (DC) precursors and activated T cells to the skin [12,13], the access and egress of naïve T cells to and from lymph nodes [14], the migration of maturing DC to lymph nodes [15], and the positioning of B cells within lymph nodes [16].

Taken together, the chemokine–chemokine receptor system shows both redundancy (ensuring the maintenance of vital processes in case of the loss of certain molecules), and specificity (allowing a targeted response to specific stimuli).

Viral subversion of the chemokine–chemokine receptor system

As mentioned above, the range of chemokines that interact with one or more chemokine receptors during an inflammatory response is broad; thus, the blockade of a single chemokine–chemokine receptor interaction might have little effect in limiting the inflammatory response. Indeed, this has been revealed in the generation of genetically modified mice with a single proinflammatory chemokine (CCL11, CCL3, or CCL5) “knocked out” [17,18]. In contrast, the targeted deletion of a chemokine receptor gene often has more profound effects on cell recruitment (CCR2, CCR5, or CCR7) [18–20], although only the deletion of CXCR4 has proved embryonically lethal in knockout mice [21]. The overlapping and frequently redundant nature of this signaling system is also reflected in the capacity of many viral proteins to bind host chemokines or chemokine receptors, such as the 35 kDa protein of the vaccinia virus and the MC148R protein of the Molluscum

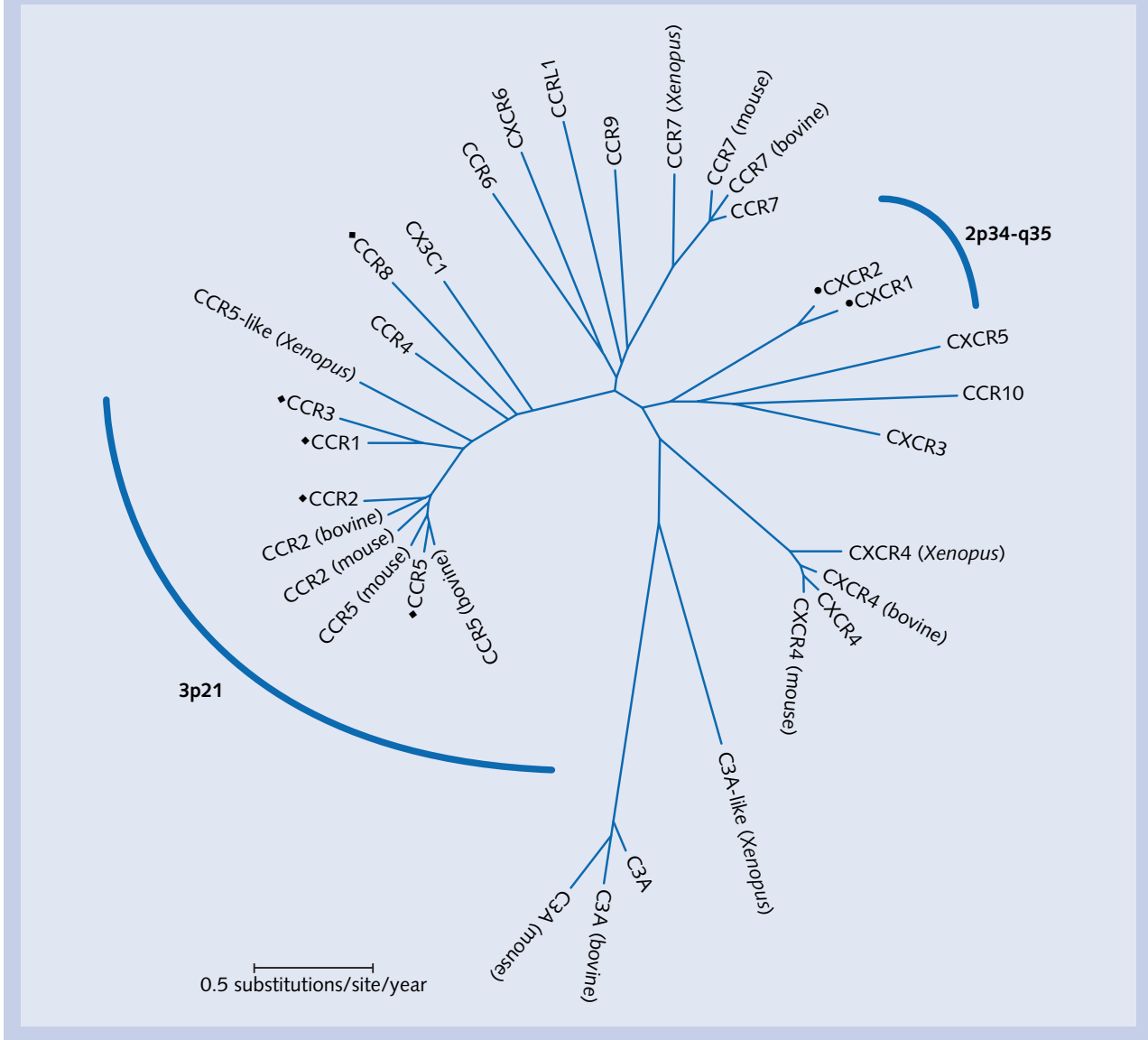
contagiosum virus [22,23]. The broad chemokine binding properties of these viral proteins are provoking much interest as potential anti-inflammatory agents at present [24]. While broadly directed chemokine–chemokine receptor binding represents a generic strategy to limit the host immune response, some viruses also employ alternative approaches to manipulate components of the immune response, including the production of chemokine agonists, such as vMIP2 by Kaposi’s sarcoma-associated herpes virus and the US28 protein by human cytomegalovirus [25,26], as well as viral chemokine receptor analogues [27]. Both of these approaches are thought to promote the recruitment of target cells to a site of active viral replication in order to aid the local spread of infection. For a more detailed discussion on the breadth of viral immune escape see recent reviews by Murphy and Lucas and colleagues [28,29].

A number of lentiviruses, including HIV-1 and the simian immunodeficiency virus (SIV), use a different strategy in their targeting of the chemokine–chemokine receptor system of their primate hosts. They produce molecular mimics of endogenous chemokines that are used to gain entry into the target cells through binding of a range of host cell surface receptors, including chemokine receptors. The following section aims to review evolutionary and current aspects of these adaptable host–pathogen interactions.

Entry via the front door: An ancient strategy

The targeting of CCR5 and CXCR4 by HIV-1 in humans is thought to be a recent pathogenic threat; thus, human population genetics may show little evidence of an adaptive response at present. By contrast, closely related viruses such as SIV appear to have affected primates for long enough to bring about significant changes in the genetic diversity of the targeted gene products [30]. The study of different primate populations has begun to provide insights into the possible array of host/lentiviral adaptations that have not yet occurred within the human and HIV-1 genomes. In this context, it is notable that chimpanzees appear to have lost significant genetic diversity within the CCR5 receptor locus [30]. This selective sweep of genetic diversity, which has also affected the major histocompatibility complex (MHC) region, suggests an evolutionary “bottleneck” generated during the chimpanzee encounter with SIV, in which variant CCR5 (and MHC) alleles conferred a critical survival advantage. Similarly, the CCR5 locus in the African green monkey (AGM) appears to show evidence of co-evolution with SIV isolates from the monkey’s natural habitats. SIV infection in AGMs is endemic but has a benign clinical course. Many of the allelic variants present within the AGM block SIV infection of susceptible cell lines *in vitro*, without

Figure 2. Phylogenetic analysis of the chemokine receptor family reveals a divergent family of proteins with clustering of receptors with overlapping ligands and close physical genetic proximity. Amino acid sequences of the human, mouse, bovine, and *Xenopus* chemokine receptors were aligned using ClustalW followed by manual editing [78]. The resultant alignment was used in the program MEGA v2.1 to create a Neighbor-Joining tree using the gamma model with pairwise deletion [79,80]. Receptors with overlapping ligands are indicated by closed circles and diamonds. Receptors in close genetic proximity on 2q34–q35 and 3p21 are indicated. CCR9, 4, and 8 are located on 3p22–23. Human chemokine receptors are indicated by name only.



interfering with chemokine–chemokine receptor binding [31]. Thus, despite encounters with a range of primate species that might have provided pressure to select for additional primary coreceptors, the use of CCR5 seems intrinsic to SIV/HIV-1 viral fitness, and is most probably linked to successful infection of the target. From the perspective of the host, it is important to note that CCR5

has not been lost from these primate populations. Instead, they remain susceptible to a SIV/HIV-1 infection that is effectively controlled by antiviral immunity, whose efficacy has been honed by the evolutionary history of the virus–host interactions described above. This illustrates the important point that blocking of infection is not the only route to reducing the pathogenicity of a virus.

In the case of humans, genetic diversity within the chemokine networks may serve to highlight those biological processes that are most critical (and least redundant) to allowing HIV-1 infection to proceed.

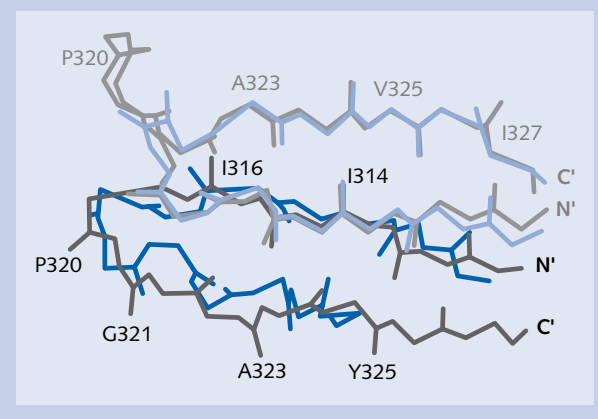
HIV-1 cellular tropism conveyed through structural mutability

It is well established that two main strains of HIV-1 are found within an infected human host: the macrophage-tropic HIV-1 (R5 virus), which preferentially uses CCR5 as a coreceptor during binding of the gp120 envelope protein to CD4 on a target cell [32,33] and the T cell-tropic HIV-1 (X4 virus), which requires CXCR4 as a coreceptor [34]. Before the identification of coreceptors, HIV-1 strains were categorized by their ability to induce the fusion of infected cells into a syncytia of cells [35]. Subsequently, it has been repeatedly demonstrated that syncytia-inducing (SI) forms of HIV-1 are most often X4 viruses, and non-syncytia inducing (NSI) forms, most often R5 viruses. However, there are reports of SI R5 viruses [36,37] and we therefore feel it is not safe to define coreceptor dependency on the basis of this biology.

Selectivity for either CCR5 or CXCR4 is thought to be conferred by structural changes in the V2 and V3 loops of the gp120 protein [38,39]. Sequence comparison approaches using sequences from the *env* sequence clade of B-subtype HIV-1 have identified susceptible positions within the V3 loop that are often mutated to positively charged amino acids – the so-called 11/25 rule [40]. The structural changes potentially induced by accumulated V3 mutations have been modelled by Sharon and colleagues, who examined a V3 peptide derived from the MN R5 tropic strain (V3MN) and one derived from the X4 tropic IIB laboratory-derived strain (V3IIB), which has a glutamine-arginine insertion near the tip of the V3 loop (present in <10% of HIV-1 isolates) [41]. Remarkably, the predicted β -hairpin structures of these two peptides almost completely overlaid the structural features of the β -sheets of the CCR5 ligand CCL3L1 and the CXCR4 ligand CXCL12, respectively (Fig. 3).

It has been shown *in vitro* that conversion between R5 and X4 strains is possible given the correct selective pressure. For example, the genotype and coreceptor selectivity of a cloned R5 HIV-1 virus changed to those of an R5X4 virus after prolonged culture in lymphoid cells [39]. The selective pressure appears to involve interactions of CXCR4 and its ligands, as specific blockade of CXCR4 using the bicyclam AMD3100 prevented emergence of CXCR4 use. Indeed, blockade of CXCR4 in cells infected with X4 viruses led to the emergence of R5X4 dual-tropic or R5-tropic variants [39]. The critical question of whether this

Figure 3. Analogy between the dual hairpin conformations formed by the HIV-1 gp120 V3 loops and the conformations of the homologous β hairpins in MIP-1 and SDF-1. Backbone superimposition of the homologous structures in CXCL12 (light blue), V3IIB (grey), CCL3L1 (blue), and V3MN (dark grey). Note that the loop of V3IIB is extended owing to the insertion of two amino acids.



conversion between strains occurs in the human host has not been conclusively determined, although there is growing evidence that there are X4 quasi-species present at baseline testing even in so-called R5-infected patients [42]. Jensen and van 't Wout also draw attention to the important point that R5 and X4 comprise two distributions of viruses with a range of mutational differences in the V3 loop [43]. Thus, the level of mutation required for switching from a particular R5 sequence to an X4 sequence varies.

Human diversity: The search for tools to disarm HIV-1 pathogenicity?

Despite its remarkable mutability, which provides a successful response to immune pressure during the course of an infection, HIV-1 is constrained by the necessity to maintain existing, or develop new, mechanisms that efficiently initiate an infection in a new host. Thus, if novel affinities for alternative coreceptors develop within the viral population of an infected host, suitable targets for these must also be present at the sites of primary infection for them to be retained within the population.

Given the results of the phylogenetic analysis of the chemokine receptor family (Fig. 2), it is somewhat surprising that CXCR4 is targeted, as it is not as closely related to CCR5 as other receptors; this suggests that phylogenetic analyses are not sufficiently sophisticated to examine structural or binding site potential at present. Furthermore, this switch in receptor tropism has been estimated to involve between one and six amino acid substitutions [43,44], which has reinforced the idea that R5 and X4 viruses are

not derived from each other *in vivo*. This reasoning forms the basis of strategies to select patients for treatment with specific receptor inhibitors, as further discussed below.

Immature mucosal dendritic cells (DCs) are likely to be among the first cells to become infected with HIV-1. In man, these are both CD4 and CCR5 positive [45]. Additionally, these DCs express the C-type lectin DC-Sign, which, although not an HIV-1 coreceptor, binds gp120 without internalizing the virus. This bound virus is then carried by the DCs when they migrate to secondary lymphoid organs, which facilitates viral access to T cells [46].

The use of additional coreceptors by HIV-1, which has been demonstrated using indicator cell lines *in vitro* has generated much interest [47]. However, it has not been possible to demonstrate efficient infection mediated by these receptors expressed on peripheral blood mononuclear cells when interactions with CCR5 are absent as a result of a homozygous $\Delta 32$ polymorphism, or when CXCR4 is specifically blocked [48,49]. The CX3CR1 receptor for CX3CL1 (fractalkine) has been identified as an HIV-1 coreceptor *in vitro*; a polymorphic variant of this receptor (T280M) has been shown to occur at a higher frequency in cohorts of rapid-progressor patients, which suggests that it acts as an alternative coreceptor for some HIV-1 viral strains [50].

Analyses of polymorphic variation in large patient cohorts provide information on the biologically limiting factors that shape HIV-1 infectability and progression. A recent study revealed an influence of gene dosage of the CCR5 high-affinity ligand CCL3L1: levels higher than the population average had protective effects on both infectability and disease progression in different human populations [51]. This finding seems consistent with the large body of experimental evidence showing that natural ligands for CCR5 reduce HIV-1 infectability in a concentration-dependent manner via competitive inhibition of gp120 binding [52]. Additionally, the binding of CCL3L1 to its receptors has been suggested to reduce the level of host cell cellular activity that is critical for efficient viral replication, by reducing intracellular cyclic-AMP levels through inhibitory G-proteins [53,54]. This is in contrast to the binding of gp120, which appears to induce a constitutive activation of host cell transcription factors, including AP-1 [54].

As CCL3L1 is a proinflammatory chemokine, its protective effect is only mediated during the course of an inflammatory event. Under normal circumstances, there is not a high concentration of this protein, either in the periphery or the circulation. It is therefore tempting to speculate that the induction of CCL3L1 and/or other inflammatory chemokines may play a role in conferring

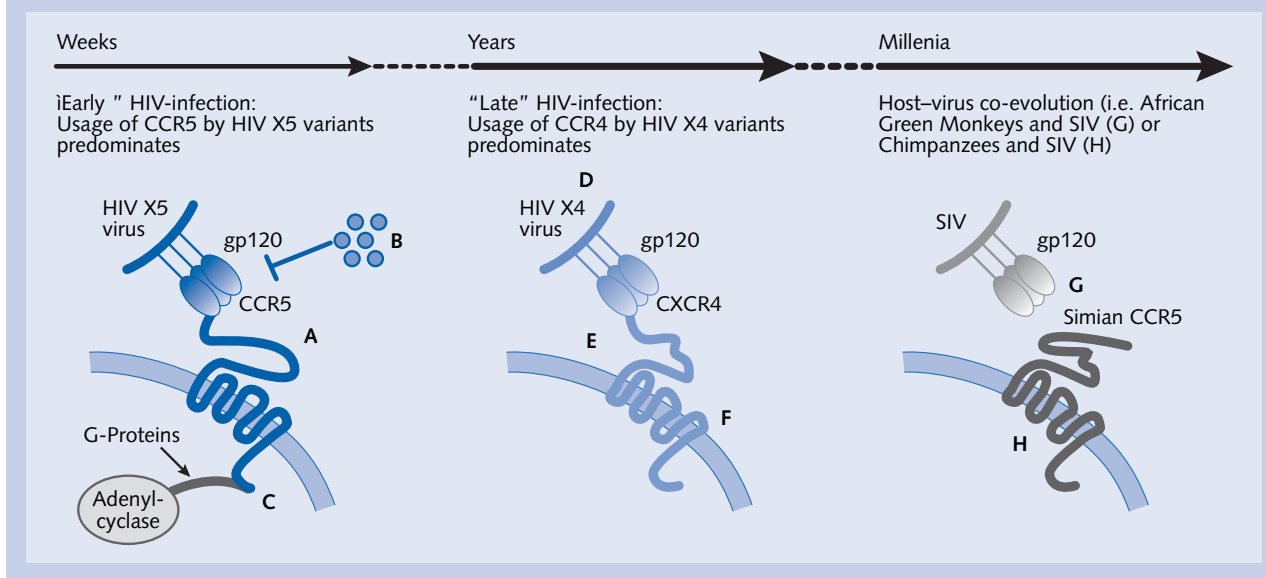
protection against HIV infection under selected circumstances. Such a mechanism may be implicated in cases of Kenyan sex workers repeatedly exposed to multiple strains of HIV-1 who have remained uninfected [55]. These individuals lack the protective effect of the CCR5 $\Delta 32$ mutation, but may have a chronic induction of CCL3L1 within the vaginal epithelium associated with local pro-inflammatory signaling, triggered by the damage associated with sexual intercourse and opportunistic sexually transmitted infections. The enhanced levels of CCL3L1 would be expected to fall in the absence of a continuing inflammatory stimulus, which is consistent with the loss of protection observed in this cohort once they retired from this profession [56].

Shift of HIV-1 from R5 to X4 variants and disease progression

It is well established that R5 viruses predominate in early HIV-1 infection and that disease progression is associated with a shift from R5 to X4 variants [57,58]. Furthermore, changes in coreceptor use, predominantly from CCR5 to CXCR4, have been associated with an accelerated loss of CD4⁺ cells and a faster progression to AIDS [57,59]. Schuitemaker and colleagues reported that this switch from R5 to X4 viruses occurred in around 50% of patients and that the incidence of appearance of X4 variants was increased in individuals with CD4⁺ T cell counts <500/ml [60] (summarized in Fig. 4). The presence of dual R5X4 or mixed populations of CXCR4-using viruses may occur at all CD4⁺ cell levels and viral loads, but is more common at lower CD4⁺ cell counts and high viral loads [61].

One possible explanation for the predominance of R5 forms of the virus early in the infection is that there are no suitable X4 target cells during this period. This is partly explained by the fact that CXCR4 is predominantly expressed on the naïve, unstimulated subset of T lymphocytes [62]. Naïve T cells appear to be poor targets for efficient viral replication [63]. It is possible that an increased stimulation of these T lymphocyte subsets in late HIV-infection might facilitate replication within these naïve T cells [64]. If these naïve cells are now targeted and killed this will impact on the CD4⁺ cell frequency markedly ultimately reducing the size of the memory CD4 pool. [64]. Although some studies have proposed that X4 variants are more cytopathic than R5 HIV-1 variants [44,65], others have suggested that CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T cell targets and that the accelerated loss of T cells is attributed simply to the different targets of the virus, as outlined above [66]. *In vitro* studies have highlighted that the binding of X4 viruses to CXCR4⁺ T cells induces apoptosis

Figure 4. Co-adaptation of HIV/SIV and chemokine receptors. **A:** CCR5 expression predominates in cells initially exposed to HIV-1 (macrophages, dendritic cells, mucosal T cells). **B:** Protective effect of high levels of certain chemokines (i.e. CCL3L1) on HIV infectability. **C:** gp120-dependent, G-protein-linked activation of adenylyclase leads to activation of CCR5⁺ cells. **D:** Possible immune-driven selection of HIV X4 viruses during HIV infection. **E:** Increased proliferation of resting and naïve T cells (with predominantly CXCR4 receptors) in “late” HIV-infection. **F:** Shift to CXCR4 use of predominant immune targets through co-infections (i.e. tuberculosis). **G:** Co-evolution drives selective mutations in simian CCR5 receptors that inhibit SIV gp120 binding, with only minor effects on chemokine binding and signaling. SIV survives without causing disease in the natural host. **H:** “Bottleneck-effect” - genes encoding detrimental CCR5 receptors are lost from the host genome after encounters with highly pathogenic viruses.



independently of successful infection of the bound cells and that this is not a property shared by R5 viruses [67].

Manning the roadblocks: The approaching receptor antagonist revolution?

The discovery that individuals who are homozygous for the CCR5 $\Delta 32$ allele are protected from HIV infection has clearly demonstrated that viral entry is a highly effective limiting step in the progression of HIV-1 infection [68]. Just as significant is the fact that these individuals have no known immunological defects related to their lack of CCR5 [68]. The corollary of these observations is the prediction that the pharmacological ablation of CCR5 responses would not produce harmful side effects, which has been borne out by good tolerance to a number of different inhibitory molecules in Phase I clinical trials [69].

Although it is not clear whether there is a direct switch of viral tropism from a pure R5 HIV-1 population to dual tropic or X4 HIV-1 variants *in vivo*, it is highly probable that the viral population infecting a new host contains viruses of mixed tropisms. This is clinically important, as the presence of detectable X4 strains at the onset of antiretroviral therapy is related to a worse clinical outcome [70].

Accumulating evidence obtained *in vitro* using specific inhibitors of coreceptor binding demonstrates the lability of coreceptor preference under focused selective pressure. Thus, the targeting of CCR5 in patients with a mixture of R5 and X4 viruses might provide a selective pressure that favors the X4 strain. Additionally, co-infections with other pathogens may alter the selective environment to favor X4 strains. For example, *Mycobacterium tuberculosis* co-infection has been associated with an upregulation of CXCR4 on alveolar macrophages [71]. For this reason, clinical trials have been careful to administer CCR5 antagonists to patients whose HIV-1 populations have been phenotyped as R5. The CCR5 antagonists that have been evaluated in Phase I and Phase II trials all tend to cause dramatic reductions in viral load [69]. However, there is evidence of an expansion of either a pre-existing R5X4 virus or a switching of coreceptor preference via *in vivo* adaptation in a small number of patients [72]. Interestingly, the high titers of X4 virus decreased as the CCR5 antagonist was lost from the patients' system, perhaps reflecting competition for CCR5⁺ cellular targets. New specific antagonists that block both CCR5 and CXCR4 are being developed to prevent X4 expansion [73].

The long-term consequences of CXCR4 blockade have not been evaluated, and initial trials of CXCR4 antagonists were stopped following reported side effects on the Q wave to T wave interval of the patients' heart rhythm. Although the next generation of CXCR4 antagonists has so far been well tolerated [69], the loss of signaling through this non-redundant chemokine-chemokine receptor system could have deleterious effects on a range of important biological processes, including immune responses. Thus, it may be necessary to use independent antagonists for CCR5 and CXCR4 so that the X4 antagonist can be withdrawn if complications arise. Although antagonists have been reported to reduce viral loads significantly [69], it will be important to use them as an adjunct to current antiretroviral therapy protocols to reduce the probability that resistant viruses emerge. This is not just a theoretical concern as the emergence of viral variants resistant to CCR5 antagonists has been described *in vitro* [74].

Conclusion

Comparative sequence analysis is beginning to provide important insights into the variety of responses that have been selected for across the primate and lentiviral families. These approaches are identifying susceptible and resistant genotypes within the human population, as well as revealing the remarkable dependency of HIV-1 on receptor binding for entry into host cells. Recently introduced specific antagonists to CCR5 and CXCR4 are an exciting new set of therapeutic tools for use in the war against HIV-1 pathogenicity. Examples of successful battles of primate species with similar viral pathogens are extremely informative. The AGM adaptation of CCR5 to inhibit viral envelope binding but preserve native ligand signaling should encourage further innovations in coreceptor antagonist design, whilst the reduction in both CCR5 and human leukocyte antigen diversity in the chimpanzee indicates that the adaptive immune response plays an important role in overcoming viral pathogenicity. Ongoing studies on the influence of genetic diversity on HIV-1 and -2 and SIV in man and primates will continue to identify critical processes involved in initial infection and early adaptive immune responses.

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