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Chemokine Receptors as Fusion Cofactors for Human Immunodeficiency Virus Type 1 (HIV-1)

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Abstract

CD4 is the primary cellular receptor for human immunodeficiency virus type 1 (HIV-1), but is not sufficient for entry of HIV-1 into cells. After a decade-long search, the cellular coreceptors that HIV-1 requires in conjunction with CD4 have been identified as members of the chemokine receptor family of seven-transmembrane G-protein coupled receptors. The discovery of distinct chemokine receptors that support entry of T-cell tropic (CXCR-4) and macrophage tropic HIV-1 strains (CCR-5) explains the differences in cell tropism between viral strains, the inability of HIV-1 to infect most nonprimate cells, and the resistance of a small percentage of the population to HIV-1 infection. Further understanding of the role of chemokine receptors in viral entry may also help explain the evolution of more pathogenic forms of the virus, viral transmission, and HIV-induced pathogenesis. These recent discoveries will aid the development of strategies for combating HIV-1 transmission and spread, the understanding of HIV-1 fusion mechanisms, and the possible development of small animal models for HIV-1 drug and vaccine testing.

Key Words

HIV
Chemokine receptor
CXCR-4
CCR-5
Entry
Fusion
Cofactor

Introduction

The primary receptor for human immunodeficiency virus type 1 (HIV-1) is CD4 . Although the gp120 subunit of the HIV-1 envelope protein (env) binds with high affinity to human CD4, binding of CD4 is not sufficient to elicit the conformational changes in env that are required for membrane fusion and virus entry *(1,2)*. Rather, a fusion coreceptor is required in conjunction with CD4 for fusion

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to occur. The coreceptor requirement is most clearly demonstrated by studies showing that expression of CD4 in most nonhuman cells fails to make them permissive for virus infection or env-mediated syncytia formation *(3–5)*. However, nonhuman cells expressing CD4 support HIV-1 fusion following the introduction of human cell membranes *(6)* or the formation of transient heterokaryons between human and nonhuman cells *(7,8)*. These studies suggest that one or more components ("accessory molecules," "cofactors," or "coreceptors") in human cells can render nonhuman cells susceptible to HIV-1 infection.

Coreceptors have also been thought to be responsible for variations in cellular tropism between strains of HIV-1. Macrophage-tropic (M-tropic) strains of HIV-1 infect macrophages and peripheral blood lymphocytes (PBLs), are typically nonsyncytia-inducing (NSI), are less pathogenic, and appear to be the type of HIV-1 that is preferentially transmitted from one infected individual to another via sexual contact, vertical transmission, and direct blood transmission *(9–12)*. T-tropic strains of HIV-1 can infect both transformed T-cell lines and PBLs, are typically syncytia-inducing (SI), and are associated with more aggressive forms of the virus *(10,13)*. Viral tropism has been mapped to the env protein, but must involve factors other than CD4, since all known HIV-1 env proteins bind CD4.

A number of molecules have been proposed to serve as coreceptors for HIV-1, including a leukocyte adhesion receptor (LFA) *(14)*, a monocytic serine protease *(15)*, CD26 *(16)*, CD7 *(17)*, and CD44 *(18)*. Although some of these molecules may enhance syncytia formation under certain circumstances, none has proven to be necessary or sufficient for viral entry and cell-to-cell fusion mediated by the HIV-1 env glycoprotein. Other molecules, such as galactosyl-ceramide, appear to be viable alternate receptors for HIV-1 entry, but mediate an inefficient, CD4-independent entry pathway *(19)*. Within the past year, the true coreceptors for HIV-1 entry and fusion have been identified as members of the chemokine receptor family of seven-transmembrane G-protein coupled receptors (GPCRs).

Although the marriage of the chemokine receptor field to the HIV field is still young, it has been remarkably productive and insightful. Recent findings can now explain the species restriction of HIV-1 entry, the cellular tropism of HIV-1 strains, the molecular basis of the $CD8^+$ cell-inhibitory factors, and the genetic basis for HIV-1 resistance. In addition, the molecular basis for the evolution of pathogenic forms of HIV-1 may soon be answered, and the understanding of the mechanism by which env-mediated fusion occurs has taken a remarkable leap forward.

Chemokine Inhibition of HIV

The chemokines are chemoattractant cytokines involved in the chemotactic immune response of phagocytic cells, such as macrophages, neutrophils, basophils, eosinophils, and lymphocytes, to areas of inflammation (for reviews, *see* Horuk *[20]* and Schall and Bacon *[21]*). The chemokines are generally divided into two groups, the CXC (α) and the CC (β) chemokines, based on the spacing of the first two cysteine residues of the chemokine molecule. The CXC chemokines primarily attract neutrophils, and members of this family include interleukin-8 (IL-8) and stromal cell-derived factor-1 (SDF-1). The CC chemokines attract macrophages, T-cells, eosinophils, and basophils, and members of this family include regulated on activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, eotaxin, monocyte chemoattractant protein-1 (MCP-1), MCP-2, and MCP-3. The chemokines have been shown to bind to G-protein-coupled seven-transmem-

brane domain receptors on the surface of target cells and to induce a Ca^{2+} flux that ultimately leads to cell chemotaxis *(20–22)*.

The HIV field was introduced to chemokines in December 1995 when RANTES, MIP-1α, and MIP-1β were identified as the major HIV-suppressive factors produced by $CD8^+$ T-cells *(23)*. Antiviral activity was found in several human T-cell leukemia virus (HTLV-1)-immortalized $CD8^+$ T-cell lines when the cells were stimulated with IL-2. Biochemical purification of media from these cells identified peaks of inhibitory activity, and sequencing of purified peptides from these peaks identified the antiviral agents as the chemokines RANTES, MIP-1α, and MIP-1β. These chemokines, but most notably RANTES and MIP-1β, were found to inhibit replication of M-tropic strains of HIV-1, such as Ba-L and MN, as well as some simian immunodeficiency virus (SIV) and HIV type 2 (HIV-2) viruses, but did not inhibit the HIV-1 T-tropic strain IIIB. Elimination of all three chemokines simultaneously was required to restore HIV-1 replication, indicating that all three of the chemokines possess antiviral activity. Although the antiviral activity was not traced to an inhibition of viral entry, this discovery was seen as an important first step toward developing alternative strategies for helping the natural immune system combat HIV-1 infection.

The CXCR-4 Coreceptor

The identification of CXCR-4 (also known as LESTR/fusin) as a T-tropic coreceptor was reported from the laboratory of Dr. Edward Berger (NIH) in May 1996 *(24)*. Using a vaccinia virus-based reporter gene assay to screen for fusion events, CXCR-4 was recovered from a HeLa cDNA library as a molecule that, in conjunction with CD4, was sufficient to allow fusion and viral entry of the T-tropic HIV-1 strain IIIB into otherwise nonpermissive murine 3T3 cells. The nature of the fusion event mediated by CXCR-4 is consistent with its identity as an HIV-1 coreceptor. CXCR-4 does not permit fusion in the absence of CD4, but is required in conjunction with CD4 for fusion and viral entry. Nonfunctional forms of env do not support fusion with CXCR-4, and the fusion mediated by CXCR-4 is cell-type-independent. CXCR-4 is expressed in cell lines and cell types consistent with the tropism of T-tropic strains of HIV-1, including a number of B-, T-, and monocyte-derived cell lines as well as primary T-cells. CXCR-4 permits fusion with envelopes from the T-cell tropic HIV-1 strains IIIB, LAV, and RF, but not from the M-tropic HIV-1 strains Ba-L, SF-162, or JR-FL *(24,25)*. Rabbit sera directed against the N-terminus of CXCR-4 inhibits fusion mediated by the T-tropic HIV-1 LAV, but not by the M-tropic HIV-1 Ba-L *(24)*. All of this evidence indicates that CXCR-4 is the T-tropic env coreceptor and does not simply mediate a general fusion event unrelated to HIV-1 env-mediated fusion.

Prior to its discovery as an HIV-1 coreceptor, CXCR-4 was cloned (and named) by six independent laboratories from human cDNA libraries derived from lung ("L5") *(26)*, fetal brain ("hFB22") *(27)*, fetal spleen ("pBE1.3") *(28)*, an HL60 cell line ("HUMSTSR") *(29)*, and monocytes ("HM89," "LESTR") *(30,31)*. Because of the molecule's ability to support HIV-1 fusion, it was also named "fusin" *(24)*. The recent discovery of its ligand, the CXC chemokine SDF-1, now permits its renaming as CXCR-4, in keeping with the standards of nomenclature agreed on by the chemokine receptor scientific community *(32,33)*. SDF-1 inhibits fusion and entry of T-tropic strains that use CXCR-4 as a coreceptor, but does not inhibit fusion and entry of M-tropic strains *(32,33)*. Thus far, CXCR-4 is the only known receptor for SDF-1, and SDF-1 is the only known ligand for CXCR-4. Transgenic mice that lack functional SDF-1 die perinatally with severely reduced numbers of B-cell progeni-

tors and myeloid progenitors, suggesting the importance of this chemokine in B-cell lymphopoiesis and bone-marrow myelopoiesis *(34)*. Although SDF-1 itself appears to be absolutely required for normal development, it will be important to determine whether CXCR-4 is also essential.

CXCR-4 is a 352 amino acid protein that is a member of the GPCR class of seven-transmembrane receptors. The receptor shares approximately 30% homology with the chemokine receptor family of GPCRs and was considered an orphan receptor at the time of its discovery as an HIV-1 coreceptor. Based on its homology with better characterized receptors and its utilization of N-linked glycosylation sites *(see below)*, CXCR-4 likely exhibits the topology depicted in Fig. 1A. Like other GPCRs, CXCR-4 contains conserved proline residues in its transmembrane domains, conserved motifs in its intracellular loops that are predicted to bind G-proteins, and serine/threonine residues in its C-terminus that may play an important role in the phosphorylation and downregulation of the receptor (for review of conserved structural motifs in GPCRs *see* Probst et al. *[35]*). The four cysteine residues in the ectodomain of CXCR-4 are highly conserved, and are predicted to form disulfide bonds between the first and second extracellular loops and between the N-terminus and third extracellular loop. Western blot of CXCR-4 protein separated in SDS-PAGE/urea gels reveals a protein of mol wt 50 kDa *(24,25)*. The protein is *N*-glycosylated and runs at its predicted mol wt of 40 kDa when treated with endoglycosidase F to remove all N-linked carbohydrates *(25)*. A higher-mol-wt band, consistent with a dimer form of CXCR-4, is consistently seen in such gels and shifts to its predicted mol wt when treated with endoglycosidase F, but the relevance of this dimer to the functional structure of CXCR-4 is not clear.

The CCR-5 Coreceptor

Although the identification of CXCR-4 as a T-tropic HIV-1 coreceptor marked a major advance in HIV research, the coreceptor for M-tropic strains of HIV-1 was still unknown. However, the ability of chemokines to block infection by M-tropic strains of HIV-1, and the homology of CXCR-4 to the chemokine receptor family did not go unnoticed. Initial screens of known chemokine receptors in early 1996, including CC chemokine receptors 1-4, did not demonstrate any that supported fusion by M-tropic strains of HIV-1. The publication in March 1996 of CCR-5 (also referred to as CKR-5) as a novel chemokine receptor capable of binding RANTES, MIP-1α, and MIP-1β provided the missing link needed for the discovery of the primary M-tropic coreceptor *(36)*.

CCR-5 was cloned from human genomic DNA and was characterized as a novel chemokine receptor strongly activated by MIP-1α, weakly activated by MIP-1β and RANTES, and not activated by MCP-1, MCP-2, MCP-3, or IL-8 *(36)*. CCR-5 is a 352 amino acid protein with a predicted mol wt of 40.6 kDa. Like other chemokine receptors, including CXCR-4, CCR-5 contains conserved G-protein coupling sequences in its intracellular loops, conserved proline residues in its transmembrane domains, and conserved cysteine residues in its extracellular loops. CCR-5 has one potential N-linked carbohydrate site in its third extracellular domain which is not utilized in the cell types examined *(37)* (*see* Fig. 1B).

Less than 2 mo after the identification of CXCR-4 as the T-tropic fusion accessory factor, five independent groups simultaneously reported that CCR-5 was a coreceptor for M-tropic strains of HIV-1 *(38–42)*. CCR-5 supports fusion and entry of M-tropic strains of HIV-1, including Ba-L, SF-162, JR-FL, and ADA, but does not support infection by T-tropic strains of HIV-1, such as LAV and

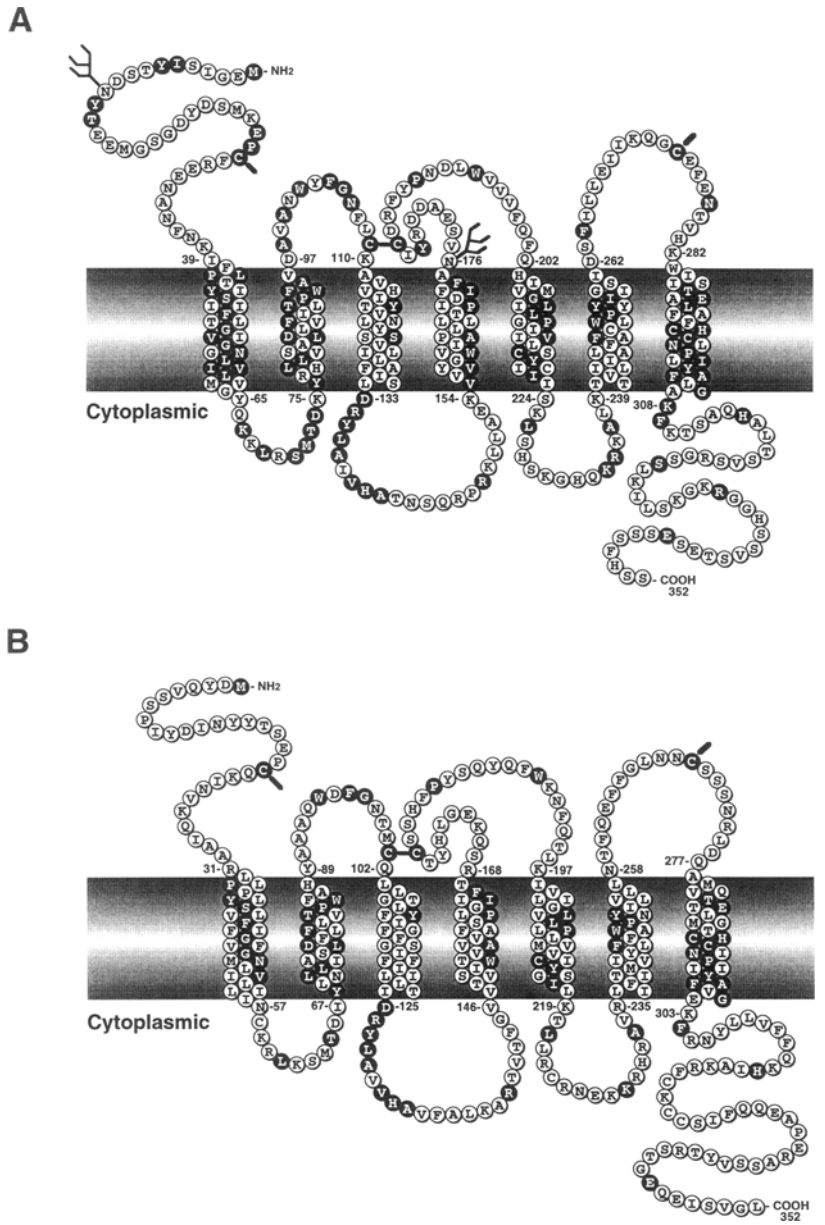

Fig. 1. (A) The predicted membrane topology of CXCR-4 (Lestr/fusin) with homology to CCR-5. CXCR-4 is known to be *N*-glycosylated *(25)*, but it is not known if both potential *N*-glycosylation sites are utilized. Shaded items indicate identical residues between CXCR-4 and CCR-5 (30% identity). **(B)** The predicted membrane topology of CCR-5 (CKR-5) with homology to CCR-3, CCR-2b, and CXCR-4. The potential N-linked glycosylation site in the third extracellular loop of CCR-5 is not utilized in the cells examined *(37)*. Shaded items indicate identical residues between CCR-5, CCR-3, CCR-2b, and CXCR-4 (20% identity), all of the fusion coreceptors identified to date.

HxB2. Diverse HIV-1 isolates from clades A, B, C, and E also utilize CCR-5 *(42)*, although a rigorous characterization of the coreceptor usage patterns of a diverse array of HIV-1 viruses must still be performed. The coreceptor usage patterns of HIV-2 and SIV viruses have yet to be fully characterized, but initial analysis indicated that these viruses can also use CXCR-4 and CCR-5 for fusion (our unpublished data). Primate homologs of CCR-5 appeared to function for HIV-1 entry, but rodent homologs of the chemokine receptors varied in their ability to support HIV-1 entry (our unpublished data). The pattern of CCR-5 expression has not yet been well characterized, but CCR-5 is clearly present in many cell types that are known to be susceptible to M-tropic strains of HIV-1, including macrophages, peripheral blood mononuclear cells (PBMCs), and the $CD4^+$ T-cell line PM1 *(38,39)*.

As predicted, the chemokines RANTES, MIP-1α, and MIP-1β prevent entry of M-tropic strains of HIV-1 by inhibiting their utilization of CCR-5 *(38–40)*. Other chemokines, such as MCP-1 and MCP-3, which are not natural ligands for CCR-5, have little or no effect on utilization of CCR-5 *(38)*. The relevance of chemokine blocking in vivo is still uncertain, since the concentration of chemokine that is required for inhibition in vitro appears to vary widely depending on cell type, mode of expression (e.g., transfection, stable expression, native expression), timing of blocking, and mode of fusion/infection. The nature of this inhibition (e.g., direct blocking or downregulation) is still being characterized.

The chemokine receptor family now consists of five CC chemokine receptors, four CXC chemokine receptors, the promiscuous Duffy receptor, several viral homologs of the chemokine receptors, and several new orphan receptors. Although many of these have already been tested for coreceptor function, the significance of many functional coreceptors remains to be determined. At least three primary isolates, 89.6, YU2, and ADA, have been reported to utilize CCR-3 in addition to CCR-5 *(41,42)*. The Duffy antigen receptor was a likely candidate for coreceptor function given previous results demonstrating that red blood cell ghosts, a primary source of Duffy, could, when fused to murine cells, render those cells susceptible to HIV-1 infection *(6)*. The Duffy antigen binds RANTES, IL-8, and MCP-1 *(43,44)* and, in a strange twist of evolution, also acts as a receptor for the malarial parasite *Plasmodium vivax (43)*. Nevertheless, the Duffy antigen does not function as a fusion coreceptor for any virus tested, including 89.6, Ba-L, ADA, JR-FL, YU2, and IIIB *(39,41,42)*.

The dual-tropic primary HIV-1 isolate 89.6 uses an impressive array of chemokine receptor cofactors, including CCR-2b, CCR-3, CCR-5, and CXCR-4 *(41,42)*. The ability of 89.6 to utilize multiple coreceptors suggests an evolutionary mechanism by which a virus of a defined tropism can switch coreceptor usage. The tropism of HIV-1 env proteins can be altered by relatively subtle differences in sequence *(45)*, and the nature of these differences implicates regions of env that may interact with the chemokine receptors and control coreceptor specificity. Initial analysis of coreceptor usage by chimeric envelope proteins indicates that the V3 loop is involved in coreceptor utilization *(42)*. Although the V3 loop of gp120 has clearly been implicated in cellular tropism *(45)*, it may not be the sole determinant of tropism *(46)*. With the isolation of CCR-5 and CXCR-4, the regions of env that define tropism can now be mapped in greater detail.

The ability of an env protein to utilize a variety of coreceptors may have a profound impact on the pathogenesis of HIV-induced diseases. One model for the emergence of increasingly pathogenic strains of HIV-1 over

the course of infection is that the virus transmitted to an individual uses CCR-5, but evolves its coreceptor usage to CXCR-4, perhaps due to selective pressures against env epitopes required for CCR-5 recognition. The 89.6 HIV-1 isolate may represent such a transitional isolate and, in the process of evolving, previously uninfected targets may become susceptible to these transitional strains. Eosinophils, which express CCR-3 and are known to be infectable by HIV-1 *(47,48)*, may be such a population of newly susceptible cells. The ability of CCR-3 to support fusion by three different primary HIV-1 isolates suggests that utilization of fusion coreceptors other than CCR-5 and CXCR-4 may have in vivo relevance for HIV-1 transmission, spread, and pathogenesis. Further examination of the expression patterns of the known HIV-1 coreceptors, CCR-2b, CCR-3, CCR-5, and CXCR-4, will be required to ascertain the true relevance of these receptors for HIV-1 pathogenesis.

It is important to note, however, that the expression pattern of the chemokine receptors can change with cell stimulation. For example, IL-2 can upregulate chemokine receptors CCR-1 and CCR-2 in some cells *(49)*, and such events may significantly influence the susceptibility of cell populations to HIV-1 infection. Thus, the natural chemokine receptor expression pattern of unstimulated cells may not reflect the true available targets of HIV-1 in vivo where populations of cells may have altered susceptibility depending on their state of activation.

CCR-5 Polymorphisms

The ability of individuals to resist HIV-1 infection and transmission has been suspected for several years, but has previously lacked a sufficient explanation *(50–52)*. The identification of chemokine receptors as HIV-1 coreceptors prompted the examination of the chemokine receptors and chemokine levels from such populations. $CD4^+$ T-cell clones from exposed-uninfected (EU) individuals appear to produce higher amounts (~10-fold) of the chemokines RANTES, MIP-1α, and MIP-1β than do clones from normal individuals *(40)*, and an *in trans* protective effect from these cells correlates loosely with chemokine inhibition *(50)*. However, the mechanism by which these cells resist HIV-1 infection is not due merely to increased chemokine production levels. HIV-1-resistant cells from EU patients do not render normal cocultured cells completely resistant to infection, and are not themselves rendered susceptible to infection by the addition of antichemokine antibodies *(40)*.

Screening of the coreceptors from these EU individuals revealed two identical copies of a defective CCR-5 gene *(53)*. Relatively normal amounts of CCR-5, CCR-1, and CXCR-4 mRNA were present in cells from EU individuals, but the CCR-5 gene contained a 32-bp deletion that caused a frameshift and introduced a premature stop codon in the second extracellular loop of CCR-5, a recombination event probably mediated by a 10 bp repeat on either side of the deletion *(53,54)*. The mutant form of CCR-5, ΔCCR-5, is produced by the cell, but does not reach the cell surface. As such, M-tropic viruses that require CCR-5 for entry, including JR-FL, ADA, SF-162, and Ba-L, cannot infect ΔCCR-5 homozygous T-cell clones, but T-tropic and dual-tropic strains of HIV-1 can infect the cells, presumably by using functional CXCR-4 *(53,54)*.

Cells heterozygous for the mutation are susceptible to infection by M-tropic and dual-tropic strains, but appear to have a small level of protection compared to cells with two copies of functional CCR-5. This protection is reflected in a lower level of replication in heterozygous cells *(53)*, a consistently reduced level of fusion mediated by cells expressing both the ΔCCR-5 mutation and the wild type

CCR-5, and by a decreased frequency of ΔCCR-5 heterozygotes in an HIV-seropositive population *(54)*. Disease progression in infected ΔCCR-5 heterozygotes also appears to occur more slowly than in infected individuals with normal CCR-5 alleles *(55)*. The CCR-5 mutation is genetically inherited and is surprisingly prevalent (allele frequency of about 0.10) in Caucasian populations of European descent *(54,55)*. No individuals who are homozygous for the mutation and who are seropositive for HIV-1 have yet been identified *(53–55)*.

The discovery of the ΔCCR-5 polymorphism identifies CCR-5 as the primary M-tropic coreceptor involved in HIV-1 transmission. The apparent resistance of ΔCCR-5 homozygotes to HIV-1 transmission implies that CCR-5 is absolutely required for HIV-1 transmission—no other chemokine receptor or coreceptor can compensate for the role of CCR-5 early in HIV-1 entry. Preliminary examination of other critical populations of high-risk individuals, such as intravenous drug users and hemophiliacs, suggests that ΔCCR-5 homozygous individuals in these populations may also be protected from HIV-1 transmission *(55)*. Thus, CCR-5 appears to be required for all routes of HIV-1 transmission. Further studies will be important for understanding what tissues HIV-1 infects early after transmission, what target cells are critical for maintaining viral reservoirs, and what target cells are responsible for the pathogenesis of HIV-1.

The facts that individuals who lack functional CCR-5 genes appear to be completely normal and that the allelic distribution of the ΔCCR-5 polymorphism obeys Hardy-Weinberg equilibrium suggest that CCR-5 plays a redundant role in normal chemokine-mediated signal transmission. Other receptors can also bind RANTES and MIP-1α (CCR-1, CCR-4), but their cellular distribution and functional overlap with CCR-5 are currently unknown. The apparent redundancy of the chemokine receptors, however, offers an optimistic outlook for the development of drugs that may target and inactivate CCR-5 without causing major side effects in these patients. On the other hand, the lethal effects of SDF-1 knockout mice *(34)* suggests that the chemokines themselves may be critical for normal development. Knockout mice for the CC-chemokines or for the fusion-active chemokine receptors remain to be characterized. Understanding these issues will be particularly important before drug strategies can be attempted, since alteration of viral tropism has the potential either to slow or to hasten the evolution of the virus to a potentially more aggressive form that could utilize other coreceptors.

The rapid identification of the ΔCCR-5 polymorphism was aided by its high prevalence in a single population. The same polymorphism was not present in other populations examined, including a Venezualian cohort *(53)*, a Japanese cohort, and a West- and Central-African cohort *(54)*. It is likely, however, that other polymorphisms in CCR-5, in CXCR-4, and in the transcription elements of these receptors remain to be identified. The natural variation in another chemokine receptor, the Duffy antigen, has previously been examined, and polymorphisms include an apparently normal open reading frame that is not expressed, as well as a rare deletion-frameshift allele, both of which are predicted to protect individuals from the spread of malaria *(56,57)*.

Several populations of individuals exist who may carry similar polymorphisms in HIV-1 coreceptors. The exposed-uninfected group initially examined by the laboratory of Richard Koup (Aaron Diamond AIDS Research Center) consisted of 25 individuals, only 3 of whom encoded the ΔCCR-5 polymorphism; only wild-type CCR-5 was detected in the remaining individuals *(50,53)*. T-cell

clones from the ΔCCR-5 homozygous individuals varied in their production of chemokines and in their response to infection by both M-tropic and T-tropic strains of HIV-1 *(40)*. Although such differences may be explained by alternative receptors, heterologous desensitization, or a lack of negative feedback for chemokine production, these individuals will certainly receive more attention for the identification of other protective factors.

Other populations that have previously been studied and may harbor coreceptor polymorphisms include uninfected prostitutes, uninfected spouses of infected individuals, exposed-uninfected hemophiliacs, and infected patients with nonprogressive disease (long-term nonprogressors). Although the causes of apparent HIV-1 resistance may include defective viruses *(58,59)*, a heightened immune response *(60,61)*, and delayed, but normal disease progression, at least some of the individuals in these populations may harbor coreceptor polymorphisms. Moreover, any polymorphisms present in these populations may offer unique insights into the pathogenesis of HIV. For example, a polymorphism in a population of long-term nonprogressors would not prevent the individual from becoming infected, but might slow or prevent disease progression, an exciting insight into understanding the causes of HIV-1 pathogenesis.

Mechanisms of Coreceptor Function

The mechanism by which HIV-1 utilizes the chemokine receptors as fusion accessory factors is currently unknown, but understanding this relationship will be a major goal in the near future. One obvious mechanism by which HIV-1 may use the chemokine receptors for fusion is by structural homology of env to the chemokine ligands. Although HIV-1 envelope proteins do not share any obvious sequence homology to the ligands of CCR-5, the high-resolution structures of both RANTES *(62)* and MIP-1β *(63)* may offer some valuable clues. In addition, the solution structure of an IL-8 chemokine dimer bound to an N-terminal fragment of IL-8R-A has been solved *(64)*, suggesting the possibility that structural regions involved in fusion may eventually be visualized.

The ligand binding sites for GPCRs have been predicted using receptor chimeras, site-directed mutants, antibody and peptide inhibition, crosslinking studies, photoaffininty labeling, fluorescence emission spectra, and computer modeling. The ligand binding sites for GPCRs can include the N-terminus, the extracellular loops, and the hydrophobic residues of the transmembrane-spanning domains, largely depending on the size of the ligand *(35)*. The N-terminal domain of chemokine receptors has proven to determine ligand specificity for Duffy *(44)*, IL-8R-B *(65–67)*, CCR-2 *(68)*, and the related C5a GPCR *(69,70)*. However, other regions of the receptors also appear to be important, and a second, independent contact site has been hypothesized for CCR-1, CCR-2, IL-8R, and C5a-R *(65,67–70)*. These independent contact sites have been modeled in a two-step mechanism of binding and activation of the receptor in which the N-terminus mediates initial binding of the ligand, while the loops or transmembrane domains mediate a subsequent binding event that leads to receptor activation.

Since the dual-tropic primary isolate 89.6 can use both CCR-5 and CCR-2b, but M-tropic viruses can use only CCR-5, chimeras of CCR-5 and CCR-2b (76% homology) were constructed to map the determinants of coreceptor function. Preliminary mapping studies were consistent with a model in which HIV-1 env interacts with CCR-5 at two independent locations, one of which was the N-terminus *(37)*. M-tropic strains of HIV-1 required either the N-terminus or the first extracellular domain of CCR-5, but both sites were not

required simultaneously, and the importance of other domains could not be excluded. The primary isolate 89.6 required different residues on the N-terminus than did JR-FL, and 89.6 was not capable of using an N-terminal truncated version of CCR-5 that JR-FL was capable of using. These results suggest that the use of CCR-5 by HIV-1 env may parallel, although probably not imitate, the use of CCR-5 by its ligands, and that different envelopes can utilize different regions of the same coreceptor *(37)*.

The mechanism by which the chemokine receptors, in conjunction with CD4, trigger the fusion conformation of HIV-1 envelope is completely unknown. The most obvious possibility is that, like other viral coreceptors, interaction with a chemokine receptor leads to conformational changes in the env protein that induce exposure of the env fusion peptide and subsequent membrane fusion. Evidence gathered before the identification of the fusion coreceptors suggested that CD4 triggers a conformational change in env that then mediates the formation of a trimeric complex between CD4, env, and the coreceptor. The phorbol ester myristate acetate (PMA) can downregulate CD4, but not a truncated version of CD4 that lacks its cytoplasmic domain *(71,72)*. However, PMA-induced downregulation of the tailless CD4 did occur when cells were incubated with soluble gp120 prior to addition of PMA. Importantly, this did not occur when tailless CD4 was expressed in nonhuman cell lines, indicating that the molecule mediating the downregulation of the tailless CD4 and gp120 might be the putative coreceptor. These findings suggest that gp120 binding to CD4 induces conformational changes in either gp120 or CD4 that lead to complex formation with the coreceptor, which itself is downregulated by PMA *(71,72)*. In support of this model, another study found that the HIV-1 env protein is sufficient to downregulate chemotactic GPCRs and to inhibit the chemotactic response of cells *(73)*. Although a direct interaction between the chemokine receptors and the HIV-1 env protein has been difficult to demonstrate, such an interaction is predicted and will prove invaluable for understanding the function of the chemokine receptors as fusion coreceptors.

As an alternative mechanism of coreceptor function, the G-protein signaling capabilities of the chemokine receptors may be involved in mediating viral entry. GPCR signals can involve ion fluxes within the cell, but GPCRs are also known to be capable of internalization into endocytic vesicles. Either activity has the potential to impact the location and cellular environment in which viral fusion occurs. However, preliminary evidence indicates that the chemokine receptors need not signal for fusion to occur. Mutants of CCR-5 that lack signaling capabilities were capable of supporting cell-to-cell fusion and viral entry (our unpublished data).

The role of the chemokine receptors and the chemokines in HIV-1 infection may extend beyond their ability to support HIV-1 entry. The ability of the HIV-1 env protein to alter chemokine and cytokine levels has been implicated in a number of pathogenic events, including neurotoxicity. HIV-1 infection can alter the expression of chemokines *(74,75)*, and HIV-1 env protein can downregulate chemotactic GPCRs and reduce cell chemotaxis *(73)*. Stimulation of cells with env can alter the production of several cytokines, including IL-10, which can regulate chemokine production and inhibit HIV-1 replication *(76–78)*. Despite their inhibitory activity in entry events, the chemokines may actually enhance HIV-1 infection and/or replication in some cell types *(79)*. Although some of these effects may be due to CD4-mediated signaling events or unrelated stimulatory events, the role of the chemokine receptors will have to be re-examined.

Perspectives

The identification of CXCR-4 and CCR-5 as the major coreceptors used by T- and M-tropic strains of HIV-1 represents a remarkable leap forward in the understanding of HIV-1 entry, tropism, pathogenesis, and epidemiology. Understanding the role of the chemokine receptors in entry has obvious implications for understanding the mechanism by which HIV-1 env mediates the mixing of two lipid bilayers, and may help identify conformational epitopes that must be conserved for fusion to occur and that may be exploited for vaccine development. The ability of chemokines to selectively block M- and T-tropic strains of HIV-1 offers an immediate first approximation for the development of drugs to inhibit the entry of HIV-1 into new cells, and possibly to inhibit the spread and evolution of HIV-1 in previously infected individuals. The selective pressures involved in inhibiting one strain of HIV-1 versus another, as may occur in the natural evolution of HIV-1 during disease progression, must be understood before such a strategy can be employed. The location, function, and redundancy of the chemokine receptors and chemokines will need to be described to predict viral targets and to devise strategies to inhibit entry of HIV-1 at this stage. Finally, the discovery of the components required for HIV-1 entry into nonprimate cells may allow the development of small animal models of HIV-1 infection, possibly one of the largest hurdles in HIV-1 research, but with a potentially enormous impact on the development of drugs and vaccines against HIV-1.

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References

1 Sattentau QJ, Moore JP: Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. J Exp Med 1991; 174:407–415.

2 Sattentau QJ, Moore JP, Vignaux F, Traincard F, Poignard P: Conformational changes induced in the envelope glycoproteins of the human and simian immunodeficiency viruses by soluble receptor binding. J Virol 1993;67:7383–7393.

3 Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, Axel R: The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell 1986;47:333–348.

4 Clapham PR, Blanc D, Weiss RA: Specific cell surface requirements for the infection of CD4–positive cells by human immunodeficiency virus types 1 and 2 and by simian immunodeficiency virus. Virology 1991;181:703–715.

5 Ashorn PA, Berger EA, Moss B: Human immunodeficiency virus envelope glycoprotein/CD4–mediated fusion of nonprimate cells with human cells. J Virol 1990;64: 2149–2156.

6 Dragic T, Picard L, Alizon M: Proteinase-resistant factors in human erythrocyte membranes mediate CD4–dependent fusion with cells expressing human immunodeficiency virus type 1 envelope glycoproteins. J Virol 1995;69:1013–1018.

7 Dragic T, Charneau P, Clavel F, Alizon M: Complementation of murine cells for human immunodeficiency virus envelope/CD4–mediated fusion in human/murine heterokaryons. J Virol 1992;66: 4794–4802.

8 Broder CC, Dimitrov DS, Blumenthal R, Berger EA: The block to HIV-1 envelope glycoprotein-mediated membrane fusion in animal cells expresing human CD4 can be overcome by a human cell component(s). Virology 1993;193: 483–491.

9 Veenstra J, Schuurman R, Cornelissen M, van't Wout AB, Boucher CAB, Schuitemaker H, Goudsmit J, Coutinho RA: Transmission of zidovudine-resistant human immunodeficiency virus type 1 variants following deliberate injection of blood from a patient with AIDS: characteristics and natural history of the virus. Clin Infect Dis 1995; 21:556–560.

10 Connor RI, Ho DD: Human immunodeficiency virus type 1 variants with increased replicative capacity develop during the asymptomatic stage before disease progression. J Virol 1994;68:4400–4408.
11 van't Wout AB, Kootstra NA, Mulder-Kampinga GA, Albrecht van Lent N, Scherpbier HJ, Veenstra J, Boer K, Coutinho RA, Miedema F, Schuitemaker H: Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission. J Clin Invest 1994;94:2060–2067.
12 Zhu T, Mo H, Wang N, Nam DS, Cao Y, Koup RA, Ho DD: Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science 1993; 261:1179–1181.
13 Koot M, Keet IP, Vos AH, de Goede RE, Roos MT, Coutinho RA, Miedema F, Schellekens PT, Tersmette M: Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann Intern Med 1993;118:681–688.
14 Hildreth JE, Orentas RJ: Involvement of a leukocyte adhesion receptor (LFA-1) in HIV-induced syncytium formation. Science 1989; 244:1075–1078.
15 Avril LE, DiMartino-Ferrer M, Barin F, Gauthier F: Interaction between a membrane-associated serine proteinase of U-937 monocytes and peptides from the V3 loop of the human immunodeficiency virus type 1 (HIV-1) gp120 envelope glycoprotein. FEBS Lett 1993;317:167–172.
16 Callebaut C, Krust B, Jacotot E, Hovanessian AG: T-cell activation antigen, CD26, as a cofactor for entry of HIV in CD4+ cells. Science 1993;262:2045–2050.
17 Sato AI, Balamuth FB, Ugen KE, Williams WV, Weiner DB: Identification of CD7 glycoprotein as an accessory molecule in HIV-1-mediated syncytium formation and cell-free infection. J Immunol 1994;152:5142–5152.
18 Dukes CS, Yu Y, Rivadeneira ED, Sauls DL, Liao HX, Haynes BF, Weinberg JB: Cellular CD44S as a determinant of human immunodeficiency virus type 1 infection and cellular tropism. J Virol 1995; 69:4000–4005.
19 Harouse JM, Bhat S, Spitalnik SL, Laughlin M, Stefano K, Silberberg DH, Gonzalez-Scarano F: Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. Science 1991;253:320–323.
20 Horuk R: Molecular properties of the chemokine receptor family. Trends Pharm Sci 1994;15:159–165.
21 Schall TJ, Bacon KB: Chemokines, leukocyte trafficking, and inflammation. Curr Opin Immunol 1994;6:865–873.
22 Kelvin DJ, Michiel DF, Johnston JA, Lloyd AR, Sprenger H, Oppenheim JJ, Wang J-M: Chemokines and sepentines: the molecular biology of chemokine receptors. J Leukocyte Biol 1993;54:604–612.
23 Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P: Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. Science 1995;270:1811–1815.
24 Feng Y, Broder CC, Kennedy PE, Berger EA: HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 1996; 272:872–877.
25 Berson JF, Long D, Doranz BJ, Rucker J, Jirik FR, Doms RW: A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. J Virol 1996;70:6288–6295.
26 Herzog H, Hort YJ, Shine J, Selbie LA: Molecular cloning, characterization, and localization of the human homolog to the reported bovine NPY Y3 receptor: lack of NPY binding and activation. DNA Cell Biol 1993;12:465–471.
27 Jazin EE, Yoo H, Blomqvist AG, Yee F, Weng G, Walker MW, Salon J, Larhammar D, Wahlestedt C: A proposed bovine neuropeptide Y (NPY) receptor cDNA clone, or its human homologue, confers neither NPY binding sites nor NPY responsiveness on transfected cells. Regul Pept 1993;47: 247–258.
28 Federsppiel B, Melhado IG, Duncan AMV, Delaney A, Schappert K, Clark-Lewis I, Jirik FR: Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. Genomics 1993;16:707–712.
29 Neote K, DiGregorio D, Mak JY, Horuk R, Schall TJ: Molecular cloning, functional expression, and signaling characteristics of a C-C chemokine receptor. Cell 1993;72: 415–425.
30 Nomura H, Nielsen BW, Matsushima K: Molecular cloning of cDNAs encoding a LD78 receptor and putative leukocyte chemotactic peptide receptors. Int Immunol 1993;5:1239–1249.
31 Loetscher M, Geiser T, O'Reilly T, Zwahlen R, Baggiolini M, Moser B: Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. J Biol Chem 1994;269:232–237.
32 Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA: The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature 1996;382:829–833.
33 Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, Schwartz O, Heard JM, Clark-Lewis I, Legler DF, Loetscher M, Baggiolini M, Moser B: The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 1996;382: 833–835.
34 Nagasaw T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T: Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 1996;382:635–638.
35 Probst WC, Snyder LA, Schuster DI, Brosius J, Sealfon SC: Sequence alignment of the G-protein coupled receptor superfamily. DNA Cell Biol 1992;11:1–20.
36 Samson M, Labbe O, Mollereau C, Vassart G, Parmentier M: Molecular cloning and functional expres-

sion of a new human CC-chemokine receptor gene. Biochemistry 1996; 35:3362–3367.
37 Rucker J, Samson M, Doranz BJ, Libert F, Berson JF, Yi Y, Smyth RJ, Collman RG, Broder CC, Vassart G, Doms RW, Parmentier M: Regions in β-chemokine receptors CCR5 and CCR2b that determine HIV-1 cofactor specificity. Cell 1996;87:437–446.
38 Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA: CC CKR5: A RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996; 272:1955–1958.
39 Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, DiMarzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR: Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996;381:661–666.
40 Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA: HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 1996;381:667–673.
41 Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW: A dual-tropic primary HIV-1 isolate that uses fusin and the β-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 1996;85:1149–1158.
42 Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, Wu L, Mackay CR, LaRosa G, Newman W, Gerard N, Gerard C, Sodroski J: The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 1996;85:1135–1148.
43 Horuk R, Chitnis CE, Darbonne WC, Colby TJ, Rybicki A, Hadley TJ, Miller LH: A receptor for the malarial parasite *Plasmodium vivax*: the erythrocyte chemokine receptor. Science 1993;261:1182–1184.
44 Zhao-hai L, Zi-xuan W, Horuk R, Hesselgesser J, Yan-chun L, Hadley TJ, Peiper SC: The promiscuous chemokine binding profile of the Duffy antigen/receptor for chemokines is primarily localized to sequences in the amino-terminal domain. J Biol Chem 1995;270: 26,239–26,245.
45 Hwang SS, Boyle TJ, Lyerly HK, Cullen BR: Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science 1991;253:71–74.
46 Kim FM, Kolson DL, Balliet JW, Srinivasan A, Collman RG: V3-independent determinants of macrophage tropism in a primary human immunodeficiency virus type 1 isolate. J Virol 1995;69:1755–1761.
47 Weller PF, Marshall WL, Lucey DR, Rand TH, Dvorak AM, Finberg RW: Infection, apoptosis, and killing of mature human eosinophils by human immunodeficiency virus-1. Am J Respir Cell Mol Biol 1995;13:610–620.
48 Freedman AR, Gibson FM, Fleming SC, Spry CJ, Griffin GE: Human immunodeficiency virus infection of eosinophils in human bone marrow cultures. J Exp Med 1991;174:1661–1664.
49 Loetscher P, Seitz M, Baggiolini M, Moser B: Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. J Exp Med 1996;184:569–577.
50 Paxton WA, Martin SR, Tse D, O'Brien TR, Skurnick J, VanDevanter NL, Padian N, Braun JF, Kotler DP, Wolinsky SM, Koup RA: Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures. Nature Med 1996;2:412–417.
51 Donegan E, Stuart M, Niland JC, Sacks HS, Azen SP, Dietrich SL, Faucett C, Fletcher MA, Kleinman SH, Operskalski EA, Perkins HA, Pindyck J, Schiff ER, Stites DP, Tomasulo PA, Mosley JW, Transfusion Safety Group: Infection with human immunodeficiency virus type 1 (HIV-1) among recipients of antibody-positive blood donations. Ann Intern Med 1990; 113: 733–739.
52 Detels R, Liu Z, Hennessey K, Kan J, Visscher BR, Taylor JMG, Hoover DR, Rinaldo CR, Phair JP, Saah AJ, Giorgi JV: Resistance to HIV-1 infection. J Acquired Immune Defic Syndrome 1994;7:1263–1269.
53 Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR: Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996;86: 367–377.
54 Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapouméroulie C, Cognaux J, Forceille C, Muyldermans G, Verhofstede C, Burtonboy G, Georges M, Imai T, Rana S, Yi Y, Smyth RJ, Collman RG, Doms RW, Vassart G, Parmentier M: Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996; 382:722–725.
55 Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, Donfield S, Vlahov D, Kaslow R, Saah A, Rinaldo C, Detels R, Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study, O'Brien SJ: Genetic resistance of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Science 1996;273: 1856–1862.
56 Chaudhuri A, Polyakova J, Zbrzezna V, Williams K, Gulati S, Pogo AO: Cloning of glycoprotein D cDNA, which encodes the major subunit of the Duffy blood group system and the receptor for the *Plasmodium vivax* malaria parasite. Proc Natl Acad Sci USA 1993;90:10,793–10,797.
57 Mallison G, Soo KS, Schall TJ, Pisacka M, Anstee DJ: Mutations in the erythrocyte chemokine receptor (Duffy) gene: the molecular basis of the Fya/Fyb antigens and identification of a deletion in the Duffy gene of an apparently healthy individual with the Fy(a-b-) phenotype. Br J Haematol 1995;90: 823–829.
58 Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL, Desrosiers RC: Absence of intact nef sequences

in a long-term survivor with non-progressive HIV-1 infection. N Engl J Med 1995;332:228–232.
59 Learmont J, Tindall B, Evans L, Cunningham A, Cunningham P, Wells J, Penny R, Kaldor J, Cooper DA: Long-term symptomless HIV-1 infection in recipients of blood products from a single donor. Lancet 1992;340:863–867.
60 Travers K, Mboup S, Marlink R, Gueye-Nidaye A, Siby T, Thior I, Traore I, Dieng-Sarr A, Sankale JL, Mullins C: Natural protection against HIV-1 infection provided by HIV-2. Science 1995;5217: 1612–1615.
61 Cao Y, Qin L, Zhang L, Safrit J, Ho DD: Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. N Engl J Med 1995;332:201–208.
62 Chung CW, Cooke RM, Proudfoot AE, Wells TN: The three-dimensional solution structure of RANTES. Biochemistry 1995;34:9307–9314.
63 Lodi PJ, Garrett DS, Kuszewski J, Tsang ML, Weatherbee JA, Leonard WJ, Gronenborn AM, Clore GM: High-resolution solution structure of the β chemokine hMILP-1β by multidimensional NMR. Science 1994;263:1762–1767.
64 Clubb RT, Omichinski JG, Clore GM, Gronenborn AM: Mapping the binding surface of interleukin-8 complexed with an N-terminal fragment of the type 1 human interleukin-8 receptor. FEBS Lett 1994; 338:93–97.
65 Ahuja SK, Lee JC, Murphy PM: CXC chemokines bind to unique sets of selectivity determinants that can function independently and are broadly distributed on multiple domains of human interleukin-8 receptor B. J Biol Chem 1996;271:225–232.
66 Gayle RB, Sleath PR, Srinivason S, Birks CW, Weerawarna KS, Cerretti DP, Kozlosky CJ, Nelson N, Bos TV, Beckmann MP: Importance of the amino terminus of the interleukin-8 receptor in ligand interactions. J Biol Chem 1993; 268:7283–7289.
67 Hebert CA, Chuntharapai A, Smith M, Colby T, Kim J, Horuk R: Partial functional mapping of the human interleukin-8 type A receptor. J Biol Chem 1993;268: 18,549–18,553.
68 Monteclaro FS, Charo IF: The amino-terminal extracellular domain of the MCP-1 receptor, but not the RANTES/MIP-1α receptor, confers chemokine selectivity. J Biol Chem 1996;271:19,084–19,092.
69 Siciliano SJ, Rollins TE, DeMartino J, Konteatis Z, Malkowitz L, VanRiper G, Bondy S, Rosen H, Springer MS: Two-site binding of C5a by its receptor: an alternative binding paradigm for G protein-coupled receptors. Proc Natl Acad Sci USA 1994;91: 1214–1218.
70 DeMartino JA, Riper GV, Siciliano SJ, Molineaux CJ, Konteatis ZD, Rosen H, Springer MS: The amino terminus of the human C5a receptor is required for high affinity C5a binding and for receptor activation by C5a but not C5a analogs. J Biol Chem 1994;269: 14,446–14,450.
71 Golding H, Dimitrov DS, Manischewitz J, Broder CC, Robinson J, Fabian S, Littman DR, Lapham CK: Phorbol ester-induced down modulation of tailless CD4 receptors requires prior binding of gp120 and suggests a role for accessory molecules. J Virol 1995; 69:6140–6148.
72 Golding H, Manischewitz J, Vujcic L, Blumenthal R, Dimitrov DS: The phorbol ester phorbol myristate acetate inhibits human immunodeficiency virus type 1 envelope-mediated fusion by modulating an accessory component(s) in CD4-expressing cells. J Virol 1994;68:1962–1969.
73 Wahl SM, Allen JB, Gartner S, Orenstein JM, Popovic M, Chenoweth DE, Arthur LO, Farrar WL, Wahl LM: HIV-1 and its envelope glycoprotein down-regulate chemotactic ligand receptors and chemotactic function of peripheral blood monocytes. J Immunol 1989; 142:3553–3559.
74 Schmidtmayerova H, Nottet HSLM, Nuovo G, Raabe T, Flanagan CR, Dubrovsky L, Gendelman HE, Cerami A, Bukrinsky M, Sherry B: Human immunodeficiency virus type 1 infection alters chemokine beta peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. Proc Natl Acad Sci USA 1996;93:700–704.
75 Canque B, Rosenzwajg M, Gey A, Tartour E, Fridman WH, Gluckman JC: Macrophage inflammatory protein-1–alpha is induced by human-immunodeficiency-virus infection of monocyte-derived macrophages. Blood 1996;87: 2011–2019.
76 Borghi P, Fantuzzi L, Varano B, Gessani S, Puddu P, Conti L, Capobianchi MR, Ameglio F, Belardelli F: Induction of interleukin-10 by human immunodeficiency virus type 1 and its gp120 protein in human monocytes/macrophages. J Virol 1995;69: 1284–1287.
77 Seitz M, Loetscher P, Dewald B, Towbin H, Gallati H, Baggiolini M: Interleukin-10 differentially regulates cytokine inhibitor and chemokine release from blood mononuclear cells and fibroblasts. Eur J Immunol 1995;25:1129–1132.
78 Schols D, DeClerco E: Human immunodeficiency virus type 1 gp120 induces anergy in human peripheral blood lymphocytes by inducing interleukin-10 production. J Virol 1996;70:4953–4960.
79 Schmidtmayerova H, Sherry B, Bukrinsky M: Chemokines and HIV replication. Nature 1996; 382:767.