Annu. Rev. Immunol. 1999. 17:657–700

CHEMOKINE RECEPTORS AS HIV-1 CORECEPTORS: Roles in Viral Entry, Tropism, and Disease

Edward A. Berger[1], *Philip M. Murphy*[2] *and Joshua M. Farber*[3]
[1]Laboratory of Viral Diseases, [2]Laboratory of Host Defenses and [3]Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; e-mail: Edward_Berger@nih.gov

KEY WORDS: AIDS, genetics, G protein-coupled receptors, pathogenesis, therapeutics

ABSTRACT

In addition to CD4, the human immunodeficiency virus (HIV) requires a coreceptor for entry into target cells. The chemokine receptors CXCR4 and CCR5, members of the G protein-coupled receptor superfamily, have been identified as the principal coreceptors for T cell line-tropic and macrophage-tropic HIV-1 isolates, respectively. The updated coreceptor repertoire includes numerous members, mostly chemokine receptors and related orphans. These discoveries provide a new framework for understanding critical features of the basic biology of HIV-1, including the selective tropism of individual viral variants for different $CD4^+$ target cells and the membrane fusion mechanism governing virus entry. The coreceptors also provide molecular perspectives on central puzzles of HIV-1 disease, including the selective transmission of macrophage-tropic variants, the appearance of T cell line-tropic variants in many infected persons during progression to AIDS, and differing susceptibilities of individuals to infection and disease progression. Genetic findings have yielded major insights into the in vivo roles of individual coreceptors and their ligands; of particular importance is the discovery of an inactivating mutation in the CCR5 gene which, in homozygous form, confers strong resistance to HIV-1 infection. Beyond providing new perspectives on fundamental aspects of HIV-1 transmission and pathogenesis, the coreceptors suggest new avenues for developing novel therapeutic and preventative strategies to combat the AIDS epidemic.

0732-0582/99/0410-0657$08.00

INTRODUCTION

The discovery that specific chemokine receptors function together with CD4 as coreceptors for the human immunodeficiency virus (HIV) emerged from two seemingly distinct puzzles in HIV-1 research: the specificity of HIV-1 entry into different target cell types and the control of HIV-1 infection by soluble suppressor factors. The molecular solutions to these problems led to a convergence of research on a viral pathogen that devastates the human immune system and on the chemokine regulatory network that orchestrates the immune system's response to pathogen invasion. Within a remarkably short period the coreceptor discoveries have engendered new perspectives on the major problems of HIV-1 disease, including the mechanisms of HIV-1 transmission and disease progression, and possibly new modes of therapeutic intervention.

This review focuses on coreceptors used by HIV type 1 (HIV-1), the major cause of AIDS worldwide and the subject of the research that led to the original coreceptor discoveries. HIV-2 and simian immunodeficiency virus have also been shown to use chemokine receptors and related orphans as coreceptors; for these subjects the reader is referred to recent review articles (1, 2).

IDENTIFICATION OF CHEMOKINE RECEPTORS AS HIV-1 CORECEPTORS

Historical Perspective: HIV-1 Tropism Suggests Existence of Coreceptors

HIV-1 enters target cells by direct fusion of the viral and target cell membranes. The fusion reaction is mediated by the viral envelope glycoprotein (Env), which binds with high affinity to CD4, the primary receptor on the target cell surface (reviewed in 3). By a similar or identical mechanism, cells expressing Env can fuse with $CD4^+$ target cells, sometimes leading to the formation of giant cells (syncytia).

The notion that a coreceptor is required for HIV-1 entry stemmed from the awareness that CD4 expression is not sufficient to explain HIV-1 tropism for different target cells in vitro (see 4 for review and citations). Two related phenomena led to this conclusion. The first series of findings, initially reported in the mid-1980s and extended through the early 1990s, was based on curious results with recombinant human CD4. The receptor was found to render cells permissive for Env-mediated fusion/entry/infection, but only when expressed on a human cell type. Experiments with cell hybrids supported the conclusion that this restriction was due to the requirement for a cofactor (coreceptor) of unknown identity that is specific to human cells, rather than to the presence of a fusion inhibitor on the nonhuman cells.

The second phenomenon concerned the distinct tropisms of different HIV-1 isolates for various $CD4^+$ human target cell types in vitro. All HIV-1 strains infect and replicate in activated primary $CD4^+$ T lymphocytes; the tropism distinctions emerge when other target cells are examined. Some isolates show efficient infectivity for continuous $CD4^+$ T cell lines, but poor infectivity for primary macrophages; this phenotype was originally observed with isolates that had been adapted in the laboratory to replicate in T cell lines and was subsequently observed with some clinical isolates. Such viruses are designated T cell line–tropic (TCL-tropic); they are generally syncytium-inducing in assays using a highly permissive T cell line. Other HIV-1 strains show the opposite preference, infecting primary macrophages much more efficiently than continuous T cell lines; these are designated macrophage-tropic (M-tropic) or nonsyncytium-inducing. Isolates that replicate efficiently in both target cell types are designated dual-tropic.

These in vitro tropism phenotypes were first revealed in the late 1980s and were soon demonstrated to have profound implications for HIV-1 transmission and pathogenesis (see 5 for original citations). The viral isolates obtained from peripheral blood of individuals shortly after infection and during the asymptomatic phase are predominantly M-tropic; as the infection progresses to AIDS, TCL-tropic viruses can be isolated from many (but not all) patients. TCL-tropic strains typically display higher cytopathic effects in vitro, suggesting that they may have a particularly important role in the decline of $CD4^+$ T cells in vivo, which is the hallmark of AIDS. In the early to mid 1990s, TCL- versus M-tropism was shown to result primarily from the fusion specificities of the corresponding Envs; again, experiments with cell hybrids revealed that these specificities derived from the requirement for an additional factor (coreceptor) in the permissive cell type rather than from an inhibitor in the nonpermissive type.

In the resulting model, TCL- versus M-tropism of different HIV-1 isolates was postulated to reflect the preferential fusogenic activity of the corresponding Envs for distinct coreceptors that are differentially expressed on these target cell types. Thus, by the mid-1990s, it was clear that the key to the HIV-1 entry/tropism problem rested on identification of these coreceptors. This was finally achieved in 1996.

Identification of CXCR4 and CCR5 as HIV-1 Coreceptors

CORECEPTOR FOR TCL-TROPIC HIV-1 The first HIV-1 coreceptor was identified using an unbiased functional cDNA cloning strategy based on the ability of a cDNA library to render a CD4-expressing murine cell permissive for fusion with cells expressing Env from a TCL-adapted strain (6). A single cDNA was isolated, and sequence analysis indicated that the protein product is a member of

the superfamily of the seven transmembrane domain G protein–coupled receptors, the largest receptor superfamily in the human genome. G protein–coupled receptor superfamily. The cDNA previously had been isolated and sequenced by several laboratories investigating G protein–coupled receptors, but no ligands or functional activities had been found; the protein was thus considered an "orphan" receptor. Because of its new-found activity in HIV-1 Env-mediated fusion, the protein was named "fusin" (6). Its role as a coreceptor was based on both gain-of-function experiments demonstrating that coexpression of fusin along with CD4 rendered nonhuman cells permissive for Env-mediated cell fusion and infection, and loss-of-function experiments showing that anti-fusin antibodies potently inhibited fusion and infection of primary human $CD4^+$ T lymphocytes. Most importantly, both types of analyses indicated that fusin functioned for TCL-tropic, but not M-tropic, HIV-1 strains. Fusin thus fit the criteria for the TCL-tropic HIV-1 coreceptor.

Among known members of the G protein–coupled receptor superfamily, fusin has the strongest sequence homology with peptidergic receptors, including chemokine receptors. Chemokines are small proteins ($\sim$70–90 amino acid residues) with chemotactic activity for leukocytes; they play prominent roles in leukocyte activation and trafficking to sites of inflammation (7). Receptors for chemokines (8) comprise a subfamily within the G protein–coupled receptors superfamily. All known human chemokines fit within four classes based on the cysteine motifs near the N-terminus. The two major classes are the CXC chemokines, in which the first two cysteines are separated by a single residue, and the CC chemokines, in which the first two cysteines are adjacent. Two minor classes have also been defined, each containing one known example: the C class with only a single cysteine residue in the motif and the CX_3C class with three residues separating the first two cysteines. Nearly all the receptors are selective for one class of chemokines; however, there is redundancy in the system, as individual receptors can bind multiple chemokines within a class, and many chemokines can function with more than one receptor.

CORECEPTOR FOR M-TROPIC HIV-1 The discovery of fusin, a putative chemokine receptor, as the coreceptor for TCL-tropic HIV-1 strains provided an impetus and direction for identifying the coreceptor for M-tropic isolates. The focus was narrowed to CC chemokine receptors by a link with an earlier study directed at a seemingly unrelated problem, namely the identity of soluble HIV-1 suppressor factor(s) released by $CD8^+$ T lymphocytes. This phenomenon was first described in the late 1980s (reviewed in 9). Biochemical identification of the molecule(s) in question promised to shed light on the natural mechanisms controlling HIV-1 infection in vivo, and could potentially lead to new modes of prevention and treatment. The first success was achieved in 1995 with the demonstration that the CC chemokines RANTES, MIP-1α, and MIP-1β are

major suppressive factors released by $CD8^+$ T lymphocytes (10) (although additional factors may contribute to the entire CD8 soluble suppressor phenomenon; see 9). Particularly intriguing was the fact that these CC chemokines potently suppressed infection by M-tropic HIV-1 strains but had little effect on a TCL-tropic strain.

Since fusin, the coreceptor for TCL tropic strains, was a putative chemokine receptor, an obvious mechanism for infection inhibition by CC chemokines was suggested: They bind to and block a chemokine receptor that functions as a coreceptor for M-tropic HIV-1. Fortuitously, at approximately the same time, a chemokine receptor was identified with precisely the corresponding specificity for RANTES, MIP-1α, and MIP-1β (11–13); it was designated CCR5, in keeping with the standard nomenclature system for chemokine receptors (fifth receptor for CC chemokines). Within the span of one week, five independent reports demonstrated that CCR5 is a major coreceptor for M-tropic HIV-1 strains (14–18); the evidence was based on both gain-of-function studies with recombinant CCR5, and loss-of-function studies using CCR5 chemokine ligands as blocking agents.

FUSIN AS A CHEMOKINE RECEPTOR (CXCR4) The coreceptor discoveries were soon followed by the demonstration that fusin is indeed a chemokine receptor, specific for the functionally equivalent CXC chemokines SDF-1α and SDF-1β (19, 20), which are formed by alternative splicing. Fusin was therefore renamed CXCR4 (fourth receptor for CXC chemokines). SDF-1 was shown to be a selective inhibitor of TCL-tropic HIV-1 strains.

PRIMARY HIV-1 ISOLATES AND GENETIC DIVERSITY The initial descriptions of coreceptor activity for CXCR4 and CCR5 were made with well-characterized prototypic HIV-1 strains. In subsequent studies, virtually all primary HIV-1 isolates were found to use one or both coreceptors (21–25). Isolates from different geographic regions display little relationship between genetic subtype and coreceptor usage (21, 23, 25, 26), although some distinctions have been reported (27). Thus, viruses from all clades can use CCR5 and/or CXCR4, and a marked correlation is observed between biological phenotype (including tropism) and coreceptor usage.

A SIMPLE MECHANISTIC MODEL FOR HIV-1 TROPISM The essential pieces of the tropism puzzle thus appeared to be in place. In a minimal model, the tropism of different HIV-1 strains can be explained by two considerations: the abilities of the corresponding Envs to use CXCR4 and/or CCR5, and the expression patterns of these coreceptors on different $CD4^+$ target cells (Figure 1). Thus, TCL-tropic strains preferentially use CXCR4, M-tropic strains prefer CCR5, and dual-tropic strains can use both; continuous T cell lines abundantly express CXCR4, primary macrophages express CCR5, and primary T cells express both.

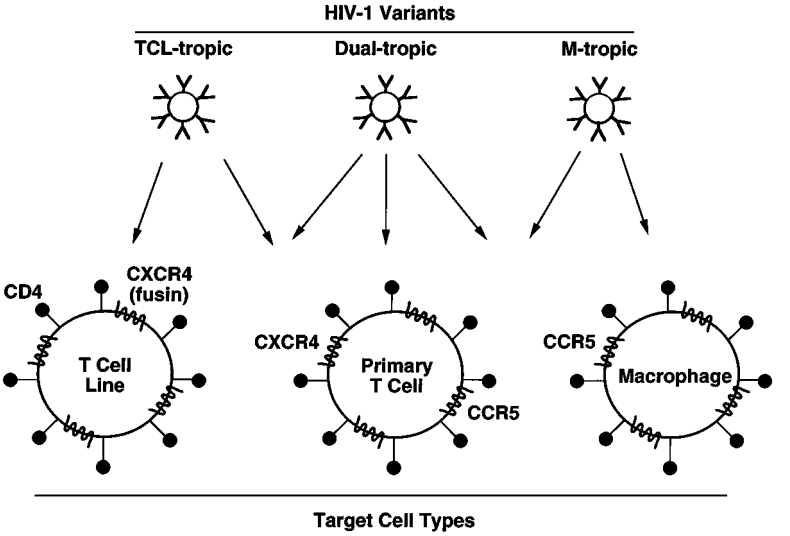

Figure 1 Model for coreceptor usage and HIV-1 tropism. TCL-tropic strains are specific for CXCR4 and can infect continuous $CD4^+$ T cell lines and primary $CD4^+$ T cells. M-tropic strains are specific for CCR5 and can infect primary macrophages and primary $CD4^+$ T cells. Dual-tropic strains can use both CXCR4 and CCR5, and can infect continuous $CD4^+$ T cell lines, macrophages, and primary T cells.

A NEW HIV-1 NOMENCLATURE SYSTEM In keeping with this model, it has been suggested that the designation of HIV-1 phenotype be revised to indicate coreceptor usage, rather than the less biochemically defined characteristics of target cell tropism or syncytium-inducing properties (28). Accordingly, throughout this review, HIV-1 variants are designated as either X4 (CXCR4-specific, generally corresponding to TCL-tropic and syncytium-inducing), R5 (CCR5-specific, generally corresponding to M-tropic and nonsyncytium-inducing), or R5X4 (using both CCR5 and CXCR4, generally corresponding to dual-tropic). While this designation provides a useful framework, not all features of HIV-1 tropism are fully explained by differential usage of CXCR4 and CCR5 (see below).

The Expanding Coreceptor Repertoire

OTHER CHEMOKINE RECEPTORS AND RELATED ORPHANS Additional complexity results from the findings that HIV-1 coreceptor activity is not limited to CXCR4 and CCR5. Studies with recombinant proteins have demonstrated coreceptor activity for several other human chemokine receptors and related orphans. Table 1 summarizes our current knowledge of the HIV-1 coreceptor repertoire and includes the chemokine receptors CCR2b (18), CCR3 (17, 18),

Table 1 HIV-1 coreceptor repertoire[a]

		Expression patterns			Evidence for coreceptor function		
					In vitro		*In vivo*
Coreceptors	Ligands	PBL[b]	Mo/MDM	Other CD4$^+$ cells	Recombinant protein	Primary cells	Genetic
Human chemokine receptors							
CCR2B	MCP-1, -2, -3, -4	+[c]	+	+	+	?	?
CCR3	Eotaxin-1, -2, RANTES MCP-2, -3, -4	+	+	+	+	+	?
CCR5	RANTES, MIP-1α, MIP-1β, MCP-2	+	+	+	+	+	+
CCR8	I-309	?	+	+	+	?	?
CCR9	CC chemokines	?	?	?	+	?	?
CXCR4	SDF-1α, -1β	+	+	+	+	+	?
CX_3CR1	Fractalkine	+	+	+	+	?	?
Human orphan receptors							
APJ	?	?	?	?	+	?	?
ChemR23	?	+	+	+	+	?	?
GPR15/BOB	?	+	+	?	+	?	?
STRL33/Bonzo	?	+	?	?	+	?	?
Other human receptors							
BLTR	Leukotriene B_4	+	?	?	+	?	?
Viral chemokine receptors							
US28	MIP-1α, MIP-1β, RANTES, MCP-1	+[d]	+[d]	?	+	?	?

[a]See text for references.
[b]PBL, peripheral blood lymphocytes; Mo/MDM, monocytes/monocyte-derived macrophages.
[c]+, expressed in cells or direct evidence obtained; ?, unknown, sometimes due to conflicting data.
[d]Expressed only in CMV-infected cells.

CCR8 (29–31), CCR9 (32), and CX_3CR1 (formerly designated CMKBRL1 or V28) (29, 33); the chemokine receptor–like orphans STRL33/Bonzo (34, 35), GPR15/BOB (35, 36), and Apj (32, 37). Not all members of the human chemokine receptor family can function as HIV-1 coreceptors; absence of activity has been noted in most studies for CCR1, CCR4, and CCR6, for CXC chemokine receptors other than CXCR4, and for several other chemokine receptor–like orphans.

In addition to these human proteins, HIV-1 coreceptor activity has been detected for US28, a CC chemokine receptor encoded by human cytomegalovirus (29, 38). Also interesting in this regard are the HIV-1 inhibitory effects of vMIP-I and vMIP-II, chemokine-like proteins encoded by Kaposi's sarcoma–associated herpesvirus (39, 40). These findings highlight the potential interplay of concurrent viral infections during HIV-1 pathogenesis.

DEVIATIONS FROM THE SIMPLE CHEMOKINE RECEPTOR/CORECEPTOR PARADIGM

The notion that coreceptor activity is restricted to chemokine receptors and related orphans has been challenged by the recent reports of coreceptor function for BLTR, the leukotriene B_4 receptor (41), and for Chem R23 (42), an orphan receptor more closely related to complement and formyl peptide receptors than to chemokine receptors.

While in most cases the HIV-1 inhibitory activity of chemokines can be attributed to their specific blocking of the corresponding coreceptors (10, 14–17, 19, 20, 30, 33), at least one puzzling exception has been noted. The CC chemokine designated MDC (macrophage-derived chemokine) has been purified based on its ability to block both R5 and X4 HIV-1 replication in PBMCs (43, 44). This activity is controversial, since it has been reproduced by one group using synthetic MDC (45), but not by others using synthetic and recombinant forms of the protein (46, 47). The mechanism of MDC inhibition is undefined, since its only known receptor is CCR4 (48) for which coreceptor activity has not been detected; moreover the HIV-blocking activity of MDC is observed in PBMCs, which R5 and X4 viruses clearly infect via CCR5 and CXCR4, respectively. These results raise the possibility that the infection-blocking activity of MDC occurs by a mechanism other than simple blocking of an HIV-1 coreceptor.

Major Questions About Tropism and the Coreceptor Repertoire

The breadth of the HIV-1 coreceptor repertoire poses important interrelated questions:

1. How well does CXCR4 versus CCR5 usage account for the selectivity of HIV-1 for different $CD4^+$ target cell types?

2. What are the relative efficiencies of the various coreceptors?

3. Is the activity of one coreceptor influenced by the presence of others on the same target cell?

4. How is the coreceptor repertoire used by diverse HIV-1 isolates?

5. Which coreceptors are significant on relevant human target cells?

6. Which coreceptors are significant for HIV-1 transmission and disease progression?

The failure of CD4-expressing nonhuman cell lines to allow HIV-1 entry/fusion/infection is clearly explained by the coreceptors. Murine CXCR4 can function for X4 strains, but it is not expressed on most murine cell lines (6, 49–51). Murine CCR5 does not display coreceptor activity (52–55).

However, ambiguities arise regarding the tropism of individual HIV-1 isolates for different $CD4^+$ human target cells. The situation is relatively clear with infection of continuous T cell lines. These targets generally express abundant levels of CXCR4 and only rarely CCR5; they are susceptible to infection by X4 and R5X4 strains. The macrophage problem is much more complex. Consistent with the model, CCR5 expression can account for the susceptibility of these cells to most R5 isolates (16, 56–60). However, CCR5 usage does not appear to be sufficient for macrophage infection, based on the isolation of HIV-1 strains that can use CCR5 yet fail to infect macrophages (61). Conflicting results have also been obtained regarding the effects of CCR5 chemokine ligands in macrophages, with some groups reporting the expected inhibition of fusion/entry/infection by R5 viruses (16, 62, 63), and others not observing this effect (15, 64, 65). One proposed explanation is that HIV-1 inhibition by CC chemokines is facilitated by chemokine interaction with cell surface heparan sulfate, and that this interaction occurs minimally with macrophages (66). To further complicate the matter, the effect of CC chemokines on HIV-1 replication in macrophages is reportedly highly dependent on the time of addition relative to virus, with stimulation occurring when they are added before infection, and inhibition occurring when they are added during or after infection (67). Perhaps even more puzzling is the resistance of macrophages to infection by some CXCR4-using strains, since several groups have demonstrated CXCR4 expression on macrophages and its functionality as a coreceptor for some HIV-1 isolates (25, 65, 68–71). As yet there is no simple resolution of these dilemmas, but several confounding issues have been noted, including variations in macrophage isolation and culture methods, donor-dependent macrophage differences, temporal modulation of CXCR4 levels during cell culture, possible

differences in coreceptor display on different cell types, varying sensitivities of different assay systems for coreceptor function, chemokine effects at multiple stages in the HIV-1 replication cycle, possible coreceptor-dependent post-entry effects on viral replication, and the fact that the resistance of macrophages to T cell line–adapted isolates is not absolute.

A major in vitro criterion for identifying members of the HIV-1 coreceptor repertoire is the ability of the recombinant proteins to confer HIV-1 permissiveness to a CD4-expressing target cell. However, numerous experimental variables have confounded efforts to assess the relative activities of each coreceptor. For one, the recombinant expression efficiencies can vary widely between different coreceptors. Thus, in several studies CCR3 has been reported as a minor coreceptor, based on the relatively inefficient activity observed with recombinant CCR3 compared to CCR5, and the limited number of HIV-1 isolates that could function with CCR3 (14, 15, 17, 18, 21–23). However, these findings were made under conditions in which recombinant CCR3 expression either was not monitored or was found to be very low compared to recombinant CCR5; in subsequent studies where CCR3 was expressed more efficiently, this coreceptor demonstrated activity comparable to CCR5 and/or CXCR4 and functioned with a broad range of isolates (25, 29). A related problem is the diversity of assay systems used to evaluate coreceptor activity (virus entry, Env-mediated cell fusion, productive infection); it is questionable whether the quantitative readout is proportional to the number of available coreceptor molecules in any of these assays, and whether the limiting molecular determinants are the same in each. Complexities associated with the varying dependencies of different HIV-1 strains on levels of both CD4 and coreceptor have been noted (72). These findings raise the issue of the "relevant" levels of coreceptor, which would seem to be the amounts that are endogenously expressed on natural human $CD4^+$ target cells. The reagents necessary to obtain such quantitative information (e.g. monoclonal antibodies, labeled chemokine ligands) have been used for CXCR4, CCR5, and CCR3, but less so for the other coreceptors, particularly the orphans.

Another important in vitro criterion in demonstrating the coreceptor activity of CXCR4 and CCR5 is based on the ability of coreceptor-specific blocking reagents to inhibit HIV-1 in natural human $CD4^+$ target cells (10, 14–16, 19, 20). In only a few instances has this been achieved for other coreceptors, e.g. for CCR3 in microglia (73) and in monocyte-derived dendritic cells (74). Again these approaches have been hampered by the limited availability of specific coreceptor-blocking agents. Moreover, the possible presence of multiple endogenous coreceptors on a natural target cell type complicates attempts to determine the relative importance of each.

Also critical for assessing the potential significance of an individual coreceptor is its expression pattern on diverse CD4-positive cells. It is possible

that virus replication in a specialized tissue compartment might select for viral variants, with enhanced usage of a coreceptor that is preferentially enriched in that compartment. Focus must therefore be extended to HIV-1 isolates from various specialized tissue compartments (e.g. thymus, genital tract, placenta).

The most powerful evidence for the significance of a given coreceptor is based on correlation of genetic variation in coreceptors with HIV-1 disease. Such analyses have provided convincing evidence of a central role for CCR5 in vivo. These findings are detailed in later sections.

CORECEPTORS IN THE HIV-1 FUSION/ENTRY MECHANISM

Model for Coreceptor Function in Fusion/Entry

ENV STRUCTURE The HIV-1 Env consists of two noncovalently associated subunits generated by cleavage of the gp160 precursor: (*a*) the heavily glycosylated external gp120 subunit, derived from the N-terminal portion of gp160 and containing the CD4 binding site, and (*b*) the membrane-spanning gp41 subunit, derived from the C-terminal portion of the precursor and containing at its N-terminus a hydrophobic fusion peptide, which is directly involved in membrane fusion (3). Native Env expressed on the surface of the virion or the infected cell is a trimeric structure containing three gp120/gp41 complexes associated via noncovalent interactions within gp41.

SEQUENTIAL CONFORMATIONAL CHANGES Env can be envisioned as a fusogenic machine, catalyzing direct pH-independent fusion between the virion membrane displaying Env and the target membrane displaying CD4 plus coreceptor. It seems logical that Env is activated only at the right time and place, i.e. when the virion encounters the target cell. This suggests that Env might be activated by receptor binding; indeed, numerous studies have demonstrated CD4-induced changes in Env conformation (3). The coreceptors represent new players in the fusion process, leading to more complex models involving multiple, and probably sequential, protein-protein interactions and conformational changes.

In the most favored model (Figure 2), CD4 binding induces conformational change(s) in gp120 that exposes, creates, or stabilizes the coreceptor-binding determinants; the gp120 interaction with the seven transmembrane domain coreceptor then induces a further conformational change(s) in Env that results in activation of gp41, presumably by exposing and extending its fusion peptide so that it can insert into the plasma membrane of the target cell. Several lines of experimental evidence support this model of receptor-induced conformational changes: (*a*) Env-mediated fusion generally requires the presence of

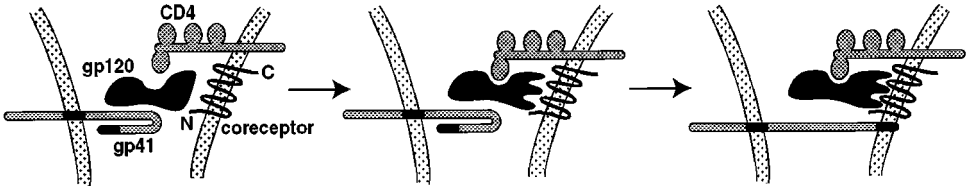

Figure 2 Model for coreceptor usage in HIV-1 entry. Upon binding to CD4, gp120 undergoes a conformational change that exposes, creates, or stabilizes the coreceptor-binding determinants. The interaction of gp120 with coreceptor triggers Env to undergo another conformational change, leading to extension of gp41 and insertion of the fusion peptide into the target cell membrane. Only a monomer of gp120/gp41 is shown for simplicity, though the native Env is a trimer.

CD4 as well as coreceptor on the target cell (6, 14–18); (*b*) complexes between CD4, gp120, and coreceptor have been isolated (75); associations between coreceptor and CD4 are enhanced in the presence of gp120, though they apparently occur to some extent in its absence (75–78); (*c*) soluble gp120 binds weakly to coreceptor expressed on cells, but the affinity is greatly increased upon CD4 binding (79–82); (*d*) treatment of Env-expressing cells with soluble CD4 activates them to fuse with coreceptor-positive CD4-negative target cells (K Salzwedel, ED Smith, EA Berger, unpublished); (*e*) soluble CD4 treatment renders fusion/entry/infection more susceptible to inhibition by monoclonal antibodies directed against the coreceptor-binding determinants of gp120 (83; also K Salzwedel, ED Smith, EA Berger, unpublished); (*f*) X-ray crystallographic structure analysis of a ternary complex containing gp120 bound to CD4 and a monoclonal antibody against the coreceptor-binding region (84), combined with mutational (85) and antigenic (86) analyses, suggests that the gp120 captured in the bound state has undergone dramatic conformational changes and that both the CD4 binding site and the coreceptor-interacting regions probably exist in very different conformations prior to CD4 binding.

FUSION INDEPENDENCE FROM CORECEPTOR SIGNALING AND INTERNALIZATION

The effector functions of chemokines are mediated by coupling of their receptors to complex intracellular signaling pathways mediated by G proteins. However, Env-mediated fusion can occur without such signaling or coreceptor internalization (52, 53, 87–91). Moreover, inhibition of fusion/entry/infection by chemokine ligands of the coreceptors does not require receptor activation/signaling and occurs by at least two mechanisms: downmodulation of the coreceptor and direct blocking of the Env/coreceptor interaction (26, 89, 92–96).

CD4-INDEPENDENT CORECEPTOR USAGE

The model described above accounts for the major features of the CD4-dependent mechanism, which is undoubtedly

the major route of HIV-1 fusion/entry/infection. However, in certain cases, Env/coreceptor interactions have been reported in the absence of CD4. These phenomena, which have been observed in assays of both Env-mediated fusion/entry/infection and gp120/coreceptor binding, were first described with HIV-2 (97–100) and then with SIV (99, 101, 102) and HIV-1 (82, 103, 104). It has been suggested that the gp120 molecules displaying CD4-independent interaction with coreceptor have already undergone (at least partially) the conformational changes normally induced by CD4.

These CD4-independent entry pathways are relatively inefficient; moreover, Envs capable of mediating CD4-independent interaction with coreceptor retain the ability to bind CD4, and CD4 binding still markedly enhances functional interaction between gp120 and coreceptor. The significance of CD4-independent Env/coreceptor interactions in vivo is thus questionable. However, an intriguing hypothesis deduced from these findings is that the evolutionary predecessor of HIV-1 strictly used the chemokine receptors for entry, and that the CD4 requirement evolved later as a means of conferring greater target cell specificity as well as protecting the coreceptor-binding region from the humoral immune system. According to this notion, the designation of CD4 as the primary receptor and the chemokine receptor as the coreceptor is a reflection of both the historical sequence of their discoveries and the kinetic sequence by which they function. These designations do not imply that the chemokine receptor plays only a secondary role; to the contrary, the coreceptor is believed to be essential for triggering the fusogenic activity of Env, and may represent the primordial receptor for primate immunodeficiency retroviruses.

Structural Correlates of the Env-Coreceptor Interaction

DETERMINANTS ON gp120 The gp120/coreceptor interaction is extremely intricate, involving multiple discontinuous regions on each protein. The gp120 molecule contains five variable loops designated V1-V5, interspersed with five relatively conserved regions designated C1-C5 (3). This framework has provided a basis for defining coreceptor interaction sites on gp120 (17, 24, 84–86, 105–114). In keeping with the long-appreciated role of the V3 loop as a critical determinant of Env fusogenicity and tropism, this region has a major role in gp120's activity and specificity for coreceptor binding. In particular, basic residues at fixed positions on either side of the conserved tip determine usage of CXCR4. V3 is critical for gp120 binding to coreceptor; in the fusion process, V3 functions not alone but rather in concert with other gp120 regions including V1, V2, and C4. Recent X-ray crystallographic structural determinations (84) coupled with mutagenic (85) and antigenic (86) analyses have led to a model in which coreceptor interacts with the V3 loop and a conserved "bridging sheet" composed of the V1/V2 stem and an antiparallel,

four-stranded structure including sequences in the C4 region. As noted above, the coreceptor binding site appears to be exposed, created, or stabilized upon CD4 binding.

DETERMINANTS ON CORECEPTORS The coreceptor is topologically arranged with seven transmembrane segments, the N-terminus and three loops extracellular, and the C-terminus and three loops intracellular (Figure 2). Regions of coreceptor involved in Env interaction have been studied by analyzing chemokine receptor chimeras and site-directed mutants, comparing chemokine receptor homologues from different species, and assessing the blocking activities of chemokine ligands and anti-coreceptor antibodies (51–55, 68, 99, 106, 115–131). The results are exceedingly complex and, in some cases, contradictory.

The extracellular regions of the coreceptors have been the focus of most studies, with the assumption that Env probably makes initial direct contacts with these regions. However, the transmembrane and/or cytoplasmic regions of the coreceptor also critically influence activity, perhaps by affecting display of the extracellular regions. Each extracellular region has been implicated in coreceptor function, with several studies suggesting a particularly important role for the N-terminal segment. Interestingly, the effects of a particular coreceptor modification or anti-coreceptor agent can vary markedly for different Envs; in many cases the activities cluster with the class of Env (R5, X4, or R5X4). It has also been suggested that evolution of the viral quasispecies within the infected host (from R5 to R5X4 or X4, see below) is associated with changes in those extracellular regions of coreceptor that are most critical.

Major Questions About Coreceptors in the HIV-1 Fusion/Entry Mechanism

The coreceptors provide a new focus for mechanistic questions about Env-mediated fusion:

1. What are the precise determinants of gp120 and coreceptor involved in intermolecular contacts?
2. How do the interacting determinants vary with different isolates using the same receptor, and a given isolate using different coreceptors?
3. What are the precise conformational changes in Env induced by interaction with CD4 and coreceptor?
4. Does the coreceptor undergo essential conformational changes upon interaction with Env?
5. Do molecular interactions between CD4 and coreceptor have significance for fusion/entry?

6. How does Env interaction with CD4 and coreceptor function in the context of the trimeric Env structure?

7. Are there additional target cell molecular components involved in fusion/entry?

A critical focus for future work will involve detailed characterization of the intricate conformational changes associated with the mechanism described above. The recent success in determining the X-ray crystallographic structure of a core fragment of gp120 complexed to CD4 and a Fab against the coreceptor binding region (84), coupled with the X-ray (132, 133) and NMR (134) structural determinations of gp41, provide a framework for future analyses. Particularly critical are the structures of gp120 prior to CD4 binding, and complexed to coreceptor; the latter problem will undoubtedly be extremely difficult in view of the challenges associated with crystallization of proteins containing multiple membrane-spanning regions.

In considering the regions on gp120 and coreceptor involved in fusion, it is particularly puzzling that many Envs can function with a wide variety of coreceptors that have minimal sequence homology. For example, the dual-tropic 89.6 strain can use CCR5, CCR2, CCR3, CCR8, CXCR4, CX_3CR1, and STRL33/Bonzo (18, 29, 33, 34); several R5 strains (e.g. Ba-L, JR-FL), though they cannot use CXCR4, can function quite efficiently with other coreceptors such as CCR3 (25, 29). Thus, in spite of the findings that individual amino acid substitutions in both Env and coreceptor can dramatically affect activity, the interactions may involve structural features not revealed by simple considerations of amino acid sequence. Also of interest is the possibility that gp120 binding might induce conformational changes in the coreceptor, which in turn trigger Env to initiate the final step in the fusion mechanism.

Finally, little is known about how the CD4 and coreceptor interactions occur in the context of the trimeric (and possibly higher ordered) Env structure. Preliminary information indicates that an individual gp120 subunit must interact with both CD4 and coreceptor; these interactions can then promote activation of gp41 subunits which can be on other members of the oligomeric complex (K Salzwedel, EA Berger, unpublished).

CORECEPTORS IN HIV-1 DISEASE

Each member of the HIV-1 coreceptor repertoire was discovered using in vitro model systems; thus far there is limited information on which ones actually play a role in vivo in HIV-1 pathogenesis. Coreceptor parameters relevant to this question include the range of viral isolates recognized, the pattern of expression on target cells and tissues, the ability to mediate infection of primary cells,

and allelic polymorphisms associated with altered clinical outcome. By these criteria, the strongest evidence for a role in HIV-1 disease is for CCR5.

Evolution of Viral Tropism in Disease

The coreceptors put a molecular face on one of the most puzzling phenomena of HIV-1 disease, namely the evolution of viral tropism in vivo in a specific pattern (reviewed in 5). M-tropic variants are found early after infection and throughout all stages of disease; TCL-tropic variants are detected in many infected persons, but only at late disease stages (Figure 3). The failure to find TCL-tropic variants in the newly infected individual occurs even when such variants are present in the transmitting source; moreover this pattern is observed whether transmission is by mucosal or parenteral routes. Following the coreceptor discoveries, it was determined that R5 viruses predominate at early stages, with X4 and R5X4 variants appearing at late stages. In a longitudinal study of vertical HIV-1 transmission, disease progression in infants was associated with loss of viral sensitivity to CC chemokines and emergence of CXCR4-using variants (24). While there is as yet no precise understanding of the selective factors underlying early R5 restriction and the late R5 → X4 evolution in

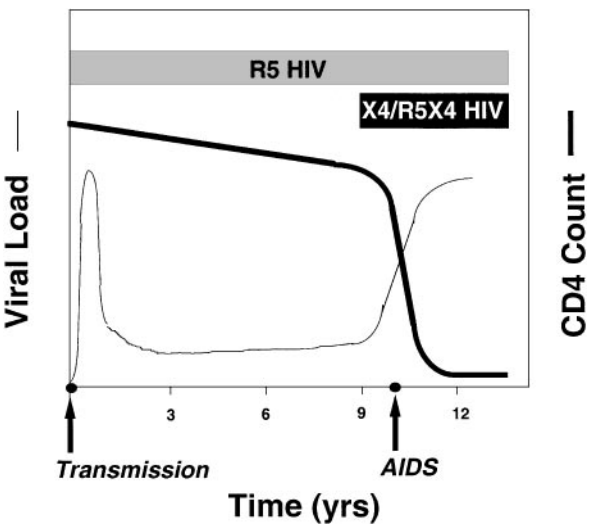

Figure 3 Temporal evolution of HIV-1 tropism during HIV-1 pathogenesis. HIV-1 transmission is restricted to R5 HIV-1 variants, which persist throughout the asymptomatic period as well as after the onset of AIDS. In many but not all individuals, viral tropism broadens to include X4 and R5X4 variants near the time when AIDS-defining symptoms are first observed.

many individuals, and the contributions of these phenomena to pathogenesis, the results suggest an important role for CCR5 during initial viral transmission, and for CXCR4 and/or possibly other coreceptors in late stages of disease progression.

CCR5 and CXCR4 are both expressed on known cell and tissue targets of HIV-1, consistent with roles in disease transmission and progression. In particular, CCR5 expression has been documented on cell and tissue types that may be important targets in the establishment of initial infection, including $CD4^+$ T cells (13, 58, 135), monocyte/macrophages (12, 136, 137), dendritic cells (74, 138, 139), Langerhans cells (140, 141), and the mucosa of rectum and colon (137) as well as vagina and cervix (137, 141a). CXCR4 is expressed in many of the same cells and tissues as CCR5 (135, 137–141a).

Role of Coreceptors in HIV-1 Transmission

THE CCR5 Δ32 MUTATION: PROOF OF A CRITICAL ROLE FOR CCR5 IN HIV-1 TRANSMISSION The realization that CCR5 is the molecular factor mediating entry of the preferentially transmitted M-tropic HIV-1 variants led to a focus on this coreceptor as a possible determinant of transmission. Definitive evidence came from the discovery of a mutant CCR5 allele designated CCR5 Δ32 and the association of this allele with resistance to HIV-1 infection. CCR5 Δ32 was discovered independently by several groups using different methods, including direct sequencing (142, 143), single stranded conformational polymorphism analysis (144), and heteroduplex mobility shift analysis (145) of CCR5 alleles. CCR5 Δ32 has a 32 base pair deletion in the region of the open reading frame encoding the second extracellular loop, causing a frame shift and premature stop codon in transmembrane domain 5. The truncated protein product is not expressed on the cell surface (57, 142, 146).

Homozygous CCR5 Δ32 and HIV-1 resistance The first report of CCR5 Δ32 described the mutation in homozygous form in two homosexual men who remained uninfected with HIV-1 despite repeated high-risk exposure (the so-called exposed-uninfected or EU phenotype) (142). Molecular epidemiological studies revealed highly statistically significant and reciprocol distortions in expected genotypic frequencies in HIV-1-infected versus ELL populations (143–145, 147, 148). Thus, the Δ32/Δ32 genotype was found to be significantly enriched in several EU cohorts; conversely, Δ32/Δ32 homozygotes were not found among several thousand HIV-1-infected individuals tested. Most importantly, these genotypic distortions were noted in cohorts of individuals exposed via mucosal or parenteral routes, including homosexual men, intravenous drug users, and hemophiliacs. In vitro experiments provided a

dramatic correlation to the population data: PBMCs and PBL from EU Δ32/Δ32 homozygotes were shown to be susceptible to infection with X4 viruses, but completely resistant to R5 viruses (56, 57, 142, 143, 149).

Heterozygous CCR5 Δ32 and HIV-1 resistance Despite the unequivocal protective effect of CCR5 Δ32 in homozygotes, no significant effect of the heterozygous genotype (+/Δ32) on transmission has been found in most studies of homosexual, heterosexual, vertical, blood, and blood product transmission of HIV-1 (144, 145, 147, 150–157). Exceptions, all of which suggest a modest protective effect, include (*a*) a large European study that found a 35% reduction of +/Δ32 in HIV-1-infected versus HIV-1-uninfected individuals (143); (*b*) a small study of HIV-1 discordant couples that found an increased frequency of +/Δ32 in seronegative heterosexual, but not homosexual, individuals versus their seropositive partners (158); and (*c*) a study of 34 vertically infected Austrian infants in which the frequency of +/Δ32 was significantly reduced (159).

Identification of an EU who was heterozygous for CCR5 Δ32 and whose PBMC were resistant in vitro to R5 HIV-1 initially suggested another exception. However, further analysis revealed that the second allele, designated CCR5 m303, harbored a chain-terminating single nucleotide polymorphism (T → A) at nucleotide 303 of the open reading frame (160), and did not encode a functional coreceptor. CCR5 m303 is inherited as a single mendelian trait; only three of 209 healthy blood donors tested were heterozygous for this allele, which is not an allelic frequency sufficient for meaningful epidemiologic studies using existing HIV-1 cohorts.

Incomplete protection by CCR5 Δ32/Δ32 Rare HIV-1-infected Δ32/Δ32 individuals have been reported (148, 161, 162), thus demonstrating that CCR5 is not absolutely required for HIV-1 transmission. In all cases, disease was progressive and the viral isolates appeared to be syncytium-inducing. In one of these individuals, X4 virus was exclusively and persistently detected (163); although isolates were not available at the time of seroconversion, this result raises the speculation that CXCR4 was the coreceptor responsible for initiating infection.

Origin of CCR5 Δ32 CCR5 Δ32 is common in Caucasians and found at lower frequencies in the Middle East and India, but only sporadically among native Africans, Amerindians and East Asians (142–145, 147, 164–166). The allele frequency in North American Caucasians is typically ∼10%; heterozygotes and homozygotes represent ∼20% and ∼1% of this population, respectively, consistent with Hardy-Weinberg expectations and the absence of any effect of Δ32 on reproductive fitness. Consistent with this, Δ32/Δ32 homozygotes who

have been examined have no obvious health problems, suggesting that normal CCR5 function is dispensable, perhaps because of compensating function by other chemokine receptors with a similar leukocyte distribution. Haplotype analysis indicates that the Δ32 allele originated quite recently, ~700 years ago (range 275–1, 875 years) at a single point in northeastern Europe (165, 166). A cline of CCR5 Δ32 allele frequencies in a north to south gradient has been found in Europe, with the highest frequencies in Finnish and Mordvinian populations (16%), and the lowest in Sardinia (4%). These properties of CCR5 Δ32 suggest that it was rapidly enriched in Caucasians because it conferred an advantage against some relatively recent and strong selective factor, possibly a catastrophic epidemic. Based on the time and place of fixation and the historical record, bubonic plague has been proposed as a plausible selective factor (165). Together the data provide a particularly compelling example of Darwinian evolution, or natural selection working on variation, and a population-based proof of the importance of CCR5 in HIV-1 transmission.

CHEMOKINE BLOCKADE OF TRANSMISSION Although the Δ32/Δ32 genotype is enriched in EU populations, the great majority of EUs do not have this genotype and their PBMCs are infectable with R5 HIV-1, indicating that the EU phenotype is heterogenous and that other resistance mechanisms must exist (145, 149). Indeed, genetic mechanisms unrelated to coreceptors have been proposed to influence susceptibiltiy to HIV-1 infection and disease (reviewed in 167). However, at least one other mechanism mediated by the coreceptor/chemokine system may influence transmission. Several reports have suggested an association between the EU phenotype, both in homosexuals and in hemophiliacs, and high levels of the endogenous CC chemokines MIP-1α, MIP-1β and RANTES; this suggests that a "chemokine condom" could contribute to clinical resistance in some cases (149, 168). In fact, elevated secretion of CC chemokines by PBMCs from Δ32/Δ32 homozygotes has been noted, suggesting a negative feedback loop controlling chemokine production (149). It has been proposed that resistance to HIV-1 infection may arise from a combination of high levels of inhibitory chemokines and low level expression of CCR5 (169).

Role of Coreceptors in HIV-1 Disease Progression

Primary HIV-1 infection is associated with a burst of plasma viremia and an acute febrile illness, characterized by nonspecific symptomatology and spontaneous resolution. Plasma viremia then drops to relatively low levels, and the individual enters a prolonged asymptomatic period known as clinical latency. Numerous factors have been proposed to control viral replication during this period, including neutralizing antibody, virus-specific cytotoxic T cells, cytokines, chemokines, and availability of HIV-1 coreceptors (170).

The rate of disease progression is very heterogeneous among individuals, and is affected by many factors including age, associated diseases, immune activation, nutritional status, and viral strain. However, even when these factors are normalized, progression rates differ dramatically; in the extreme some rare individuals, known as long-term nonprogressors, do not appear to progress at all. These observations raise the possibility that progression rate may be influenced by factors that affect HIV-1 coreceptor levels or function. Directly correlating in vivo coreceptor levels with clinical progression rate is problematic, mainly due to the variability found in the levels on primary cells from different individuals. Associating in vivo levels of blocking chemokines with rates of disease progression is also difficult, due to problems in quantitating levels in plasma and tissues.

A more informative approach has been to correlate genetic polymorphisms that may affect gene expression with clinical progression rate, using survival analysis and disease category analysis. Survival analysis requires large numbers of well-characterized HIV-1 seroconvertors (individuals for whom it is known accurately both the time of infection and the time when AIDS-defining criteria were met) (144, 157, 171, 172). Results from seroprevalent cohorts (individuals found to be HIV-1 seropositive when first tested), which have been used in some studies, can be misleading due to bias introduced by unintended exclusion of rapid progressors. Results are analyzed by Cox proportional hazards or Kaplan-Meier techniques using various endpoints, including the time to AIDS and death. Disease category analysis tests for distortion in expected genotypic frequencies in cohorts of HIV-1-infected subjects with specific outcomes, such as long-term survival (144, 145).

Fortunately, several relatively large, well-characterized and well-managed cohorts of seroconvertors were initiated during the early years of the HIV-1 epidemic. So far four coreceptor/chemokine genetic polymorphisms have been identified and correlated with delayed HIV-1 disease progression rate using these cohorts: CCR5 Δ32 (144, 145, 147, 152), CCR5 59029 G/A (173), CCR2-64I (157, 174, 175), and SDF-1 3′UTR-801G-A (abbreviated SDF-1 3′A) (157, 176). Several polymorphisms in the gene encoding CXCR4 have been found, but none has proven informative (177). Table 2 summarizes the allelic polymorphisms in coreceptors and their ligands that have been linked to HIV-1 disease.

CCR5 59029 G/A, CCR2-64I, and SDF-1 3′A are single nucleotide polymorphisms (SNP) in the CCR5 promoter, CCR2 ORF and SDF-1 3′ untranslated region (UTR), respectively. The mechanisms by which the genotypes produce their associated clinical effects are not clearly established. In this regard, it is important to note that CCR5 Δ32, CCR2-64I, and CCR5 59029 G/A are in linkage disequilibrium, due to the adjacent location of CCR2 and CCR5 genes

Table 2 Inherited polymorphisms in genes for chemokines and HIV-1 coreceptors that alter susceptibility to HIV-1 infection and progression

Molecule	Polymorphism	Site	Type	Freq[a]	Phenotypes −/−	Phenotypes +/−	Mechanism
CCR5	CCR5 Δ32	ORF	Del	10%	R	DP	Truncation
	m303	ORF	SNP	1%	R	ND	Truncation
	59029G/A	Pro	SNP	50%	DP	—	ND
CCR2	CCR2-64I	ORF	SNP	10%	ND	DP	ND
SDF-1	SDF1-3′ A	3′ UTR	SNP	21%	DP	—	ND

[a]The allelic frequencies listed are approximations based on genotyping North American Caucasians. Frequencies vary by racial group (see text for details and references).
Abbreviations: Freq, Allelic frequency; −/−, homozygous for the given allele; +/−, heterozygous for the given allele; ORF, open reading frame; SNP, single nucleotide polymorphism; Del, deletion; Pro, promoter; 3′ UTR, 3′ untranslated region of the mRNA; R, HIV-1 resistance; DP, delayed progression to AIDS relative to other genotypes; ND, not determined. NE, no effects; ?, not determined.

within a chemokine receptor gene cluster on human chromosome 3p21-p24, that also includes CCR1, CCR3, CCR4, CCR8, and CX3CR1 (178). This must be kept in mind when considering statistical associations between particular polymorphisms and clinical outcome, since the identified polymorphism may just be a marker linked to other polymorphisms that have a direct effect on disease outcome.

THE CCR5 Δ32 ALLELE Too few HIV-1-infected Δ32/Δ32 homozygotes have been found to meaningfully analyze the effect of this genotype on progression, except to say that individuals with this genotype have been identified who have progressed to AIDS. In adult populations of HIV-1-infected seroconvertors who are +/Δ32, there is a delay of 0–2 years in mean time to AIDS compared to wild-type controls, depending on the AIDS definition and particular cohorts that are used (144, 145, 147, 152). In the largest study, seroconvertors were pooled from three different cohorts, including hemophiliacs and homosexual men, and a ∼2 year delay was observed (144). In addition, several cohorts of long-term nonprogressors have been reported in which a 50% increase in +/Δ32 genotypic frequency relative to other HIV-1-infected populations and normals was observed, although immunologic and viral parameters broadly overlapped in individuals with similar rates of progression and discordant CCR5 genotypes (144, 145, 179). Significant protection from progression by this genotype has been difficult to demonstrate in studies of seroconvertors that have relied on only a single cohort.

Perhaps contributing to delayed progression among heterozygotes, the mean values of CCR5 by flow cytometry of peripheral blood T cells from HIV-1 negative CCR5 +/Δ32 individuals are significantly lower than would be

predicted by a simple gene dosage effect (~10% of wild type controls, with considerable overlap) (58). There is biochemical evidence suggesting that this may be partly due to a transdominant effect of Δ32 through production of dysfunctional heterodimers composed of normal and truncated receptor subunits, which become trapped in the endoplasmic reticulum (143, 146). Functional consequences of the CCR5 +/Δ32 genotype have been demonstrated both in vitro and in vivo. Thus, R5 HIV-1 entry and replication have been reported to be reduced in CCR5 +/Δ32 versus +/+ PBMCs and monocyte-derived macrophages infected in vitro (58, 180); R5 HIV-1 exhibits delayed replication in SCID-hu mice reconstituted with CCR5 +/Δ32 versus +/+ PBLs (181); and median viral load has been reported to be lower in CCR5 +/Δ32 versus +/+ HIV-1-infected individuals (172). Taken together, these results suggest that CCR5 levels can be limiting, and that the +/Δ32 genotype slows disease progression by causing reduced viral replication through reduced expression of CCR5. There is some evidence that the CCR5 +/Δ32 genotype does not operate throughout the course of disease. In fact, paradoxically, it has been asssociated with accelerated progression to death after AIDS defining criteria were met (182).

THE CCR2-64I ALLELE The CCR2-64I polymorphism (174) causes a conservative amino acid change, valine to isoleucine, at position 64 in the first transmembrane domain of CCR2, a region that has complete amino acid sequence conservation with CCR5. The allele is found in all racial groups tested at the following frequencies: Caucasians, 10%; African Americans, 15%; Hispanics, 17%; and Asians, 25% (174). CCR2-64I and CCR5 Δ32 occur on separate haplotypes, meaning they are never inherited together on the same chromosome.

CCR2-64I has no effect on initial HIV transmission. However, several studies have shown that seroconvertors bearing the CCR2-64I allele progress to AIDS significantly slower (by ~2–3 years) than do CCR2 +/+ HIV-1 seroconvertors (157, 174, 175, 183). Like CCR5 Δ32, CCR2-64I is enriched among long-term nonprogressors and reduced in rapid progressors (174, 175, 183). Moreover, the two alleles appear to exert an additive protective effect on progression rate in individuals who carry both (174, 175). The effect of CCR2-64I has not been observed in seroprevalent cohorts (175, 184), probably because it is masked by exclusion of rapid progressors from these cohorts (175). Also, one example of variability among seroconvertor cohorts has been reported in which the association between CCR2-64I and delayed progression was found for African-Americans but not for Caucasians in one study (157), and in the converse pattern in another (174). A study of African sex workers found that the CCR2-64I allele correlated with delayed progression to AIDS (185).

Consistent with its association with delayed progression in seroconvertor cohorts, CCR2-64I has also been associated with significantly lower viral load at 9–12 months after seroconversion (175); the early viral load is a "set point" highly predictive of progression rate (186). Nevertheless, there is substantial doubt that the mechanism of action of CCR2-64I directly involves CCR2, since this coreceptor can be used by relatively few HIV-1 isolates in vitro (18, 29) and has not been consistently demonstrated to mediate HIV-1 infection in primary cells (14, 69, 115). Moreover, the CCR2-64I mutation does not affect coreceptor expression either in primary cells or in transfected cells, nor does it appear to affect chemokine binding or HIV-1 coreceptor activity (187). Instead, it has been suggested that CCR2-64I may be linked to another polymorphism that affects CCR5 expression or function (174). One candidate has been proposed, a C → T SNP at position 59653 in the CCR5 promoter (numbering as in GenBank #U95626) which is in 100% linkage with CCR2-64I, i.e. the two mutations are always inherited together (157, 175); however, so far there is no evidence that this polymorphism affects CCR5 expression (175). Instead, and quite surprisingly, an association has been found between the +/CCR2-64I genotype and reduced levels of CXCR4 on PBMCs from healthy donors (187). The mechanism cannot involve cis genetic effects since the gene encoding CXCR4 is on a different chromosome (2q) than CCR2 (3p).

THE CCR5 59029 G/A POLYMORPHISM CCR5 59029 G/A is a G versus A SNP at base pair 59029 in the CCR5 promoter. Neither allele can be considered as "wild type," since both are very common in all racial groups; the G allele is found at 43%–68% frequency depending on race (173). Haplotype analysis indicates that the 59029 A allele is in complete linkage disequilibrium with both CCR5 Δ32 and CCR2-64I; that is, all chromosomes bearing either CCR5 Δ32 or CCR2-64I also have 59029 A, although most chromosomes bearing 59029 A lack both CCR5 Δ32 and CCR2-64I. In the one cohort study reported so far, the Multicenter AIDS Cohort Study (MACS) of homosexual and bisexual men, in individuals selected for absence of CCR5 Δ32 and CCR2-64I the mean time to AIDS for 59029 G/G individuals was 3.8 years longer than for 59029 A/A individuals, $p = 0.004$ (173). This is the largest distortion of HIV-1 progression rate found so far for any polymorphism tested in a single cohort. For comparison, in the same cohort the +/64I and +/Δ32 genotypes were each associated with an ∼1 year delay in mean time to AIDS, neither of which reached statistical significance (DA McDermott and PM Murphy, unpublished data).

Consistent with the epidemiologic difference, promoter fragments differing in sequence only at 59029 G versus A had differential activity in a reporter gene assay, with the 59029 A promoter ∼50% more active than 59029 G

(173). This suggests that cells from 59029 G/G individuals may have decreased transcription of the CCR5 gene and decreased expression of CCR5, although this has not yet been demonstrated directly. Many other CCR5 promoter and open reading frame SNPs have been found, but associations with disease outcome have not been reported (157, 175, 177, 188, 189).

THE SDF-1 3′A ALLELE The polymorphism designated SDF1-3′A is a G → A transition at bp 809 of the 3′-UTR of the mRNA encoding one of the two known chemokine ligands for CXCR4, SDF-1β. The second ligand, designated SDF-1α, produced by alternative splicing of a common SDF-1 gene, does not contain the SDF-1 3′A polymorphism. SDF-1 3′A is found in all racial groups tested, with high allele frequency in Caucasians (21%), Hispanics (16%), and Asians (26%), and relatively low frequency in African Americans (6%) (176).

A clear picture has not yet emerged for the role of SDF-1 3′A in HIV-1 disease. In the initial report (176), which was based on analysis of 639 seroconvertors pooled from two cohorts of homosexual men (MACS and San Francisco City Cohort), one cohort of hemophiliacs (Multicenter Hemophilia Cohort Study), and one cohort of IV drug abusers (the ALIVE cohort), a strong association was described between the homozygous 3′A/3′A genotype and delayed onset of AIDS. A statistically significant effect was even observed in the MACS cohort tested separately from the others. In the pooled analysis, the effect was reported to be increased in later stages of HIV-1 infection, twice as strong as that found for CCR2-64I or CCR5 Δ32 separately, and additive, possibly even synergistic, to them. In contrast, in a study of 470 seroconvertors from a single cohort (Tri-Service HIV-1 Natural History Study), the 3′A/3′A was associated with accelerated disease progression (157). Neither study found an altered rate of disease progression in heterozygotes, yet counterintuitively the first study found a significant increase in +/3′A in a group of 79 high-risk ELLs from the MACS, suggesting a protective effect from initial infection. The significance of this is unclear report however, since the same, did not find distortion of expected genotypic frequencies for either +/3′A or 3′A/3′A in a second group of 435 ELLs or in HIV-1 + individuals. The frequency of 3′A/3′A in the group of 79 ELLs was not reported.

The differences between these two studies could result from differences in cohort composition or definition, or the low number of homozygotes available for statistical analysis (3′A/3′A individuals represent only ∼5% of Caucasians). It is quite possible that effects of SDF-1 3′A on progression rate could vary in different cohorts through differential environmental factors acting on the same postulated mechanism (176), namely posttranscriptional modulation of SDF-1 levels leading to effects on CXCR4 coreceptor activity.

Major Questions About Coreceptors in HIV-1 Disease

With the coreceptor discoveries, some of the most perplexing questions about HIV-1 disease can now be posed in molecular terms:

1. What are the mechanisms underlying the predominance of R5 HIV-1 variants during establishment of initial infection as well as throughout the asymptomatic period?
2. What are the mechanisms involved in the emergence of X4 and R5X4 variants at later stages?
3. Why are CXCR4-using viruses detected in some AIDS individuals but not in others?
4. Is there a causal relationship between CXCR4 usage and the decline of $CD4^+$ T cells and, if so, what are the mechanisms?
5. Does signaling induced by Env interaction with coreceptors play any role in HIV-1 disease?
6. Does Env impairment of normal chemokine-mediated signaling play any role in HIV-1 disease?
7. Are coreceptor levels limiting for transmission and disease progression?
8. To what extent does regulation of the levels of coreceptors and/or their chemokine ligands modulate infection susceptibility and disease progression rates?
9. What additional genetic influences on HIV-1 disease are mediated by the coreceptor/chemokine system, and what are their mechanisms of action?
10. Which members of the coreceptor repertoire are important for HIV-1 disease?

The selective mechanisms observed during virus transmission are particularly bewildering. CCR5, the coreceptor used by HIV-1 variants detected shortly after transmission, is expressed on target cells and tissues that may be important for the establishment of initial infection, (i.e. $CD4^+$ T cells, monocyte/macrophages, dendritic cells, Langerhans cells, vagina, cervix, rectum, and colon). Yet CXCR4, the coreceptor used by HIV-1 variants that are rarely detected at early stages of infection, is also expressed at many of these sites. The findings that CXCR4 is expressed at lower levels than CCR5 in colonic (137) and cervical (141a) mucosa may provide a partial explanation for the

predominance of R5 variants after sexual transmission. It is also possible that in order to gain a foothold after entering the body, the infection must become established in some uncharacterized compartment that is enriched in CCR5 but not CXCR4, or that has high levels of blocking chemokine ligands for CXCR4 but not CCR5. However, none of these considerations would explain the enigma of the low transmission frequency of R5X4 viruses, since these can readily use CCR5. It is presently unknown whether this apparent negative selection against CXCR4-using viruses is due to the interaction of the respective Envs with CXCR4 per se, or to other properties of the Envs that accompany functionality with CXCR4. An intriguing speculation is that those very features that confer CXCR4 functionality to Env might render the corresponding virus or infected cell susceptible to elimination by immune mechanisms (humoral and/or cellular).

Similarly perplexing is the enrichment for CXCR4-using (R5X4 and X4) viruses during the course of disease progression. There are many possible selective mechanisms, including the elevation of CXCR4 and depression of CCR5 levels upon activation of $CD4^+$ T lymphocytes (190, 191), the presence of R5-blocking CC chemokines at sites of virus replication (192, 193), enhanced production of CC chemokine caused by X4 virus infection (194), the ability of CC chemokines to stimulate replication of CXCR4-using viruses by inducing colocalization of CXCR4 with CD4 (77), limiting levels of CCR5, e.g. as occurs in CCR5 $+/\Delta 32$ heterozygotes (195), and upregulation of CXCR4 induced by CC chemokines (196) and associated with bacterial infection (197).

Just as the basis for the emergence of CXCR4-using variants is not understood, it is similarly unclear why CXCR4-using viruses are detected in only a subset of AIDS subjects (5). Since this notion is based mainly on analyses of isolates from peripheral blood, it is possible that the fraction would be significantly higher if variants are examined from a broader range of tissues. It is also possible that the frequency of CXCR4-using variants might be underestimated due to difficulties in detection; this concern is raised by the finding that in cells from some individuals, TCL-tropic viruses are only observed upon culture in the presence of CC chemokines (77).

Another fundamental problem is the relationship of coreceptor usage to depletion of $CD4^+$ T cells. R5X4 and X4 HIV-1 strains are generally more cytopathic than R5 strains in vitro, raising the possibility that CXCR4 usage might contribute to target cell killing, directly or indirectly. An ex vivo correlate has come from analysis of lymphoid tissue histocultures, where $CD4^+$ T cell depletion occurred much more with X4 than with R5 viruses (198). The in vivo kinetics of plasma viremia in SCID-hu mice reconstituted with human peripheral blood leukocytes was found to correlate with the phenotype of the innoculated virus, with R5 strains exhibiting high viral titers and low $CD4^+$

T cell depletion, and X4 viruses the converse (199). A critical question for future studies is to determine whether these phenomena reflect an inherently greater cytopathicity of X4 strains, or simply a greater fraction of $CD4^+$ T cells expressing CXCR4 compared to CCR5.

A related problem is the possible physiologic significance of Env/coreceptor interactions beyond those involved in virus entry. Soluble Env has been demonstrated to induce signaling events via interaction with CCR5 (200) or CXCR4 (201), and apoptosis has been observed upon gp120 engagement of CXCR4 (202, 203). Furthermore, gp120 reportedly antagonizes CXCR4 and CCR5 signaling induced by their respective chemokine ligands, in a CD4-dependent fashion (204). Interaction between gp41 and coreceptors has also been suggested, based on the ability of gp41 to induce downmodulation of chemoattractant receptors (including HIV-1 coreceptors) on monocytes (205). The relevance of these processes for HIV-1 disease is presently unknown.

While the importance for HIV-1 disease has clearly been established for CCR5, and to a lesser extent for CXCR4, the data are much less compelling for other members of the coreceptor repertoire. The genetic evidence has defined a central role for CCR5 in transmission; however, it does not rule out the possibility that other coreceptors may act in concert with CCR5, perhaps in a specialized physiological compartment critical for establishment of clinical infection. Other coreceptors may also be important for tissue-specific virus replication that might have profound consequences for the progression and manifestations of disease. This notion suggests the importance of analyzing coreceptor usage by primary virus isolates obtained from diverse target cells and tissues. An intriguing example is CCR8, which is expressed at minimal levels on peripheral blood T cells and monocytes, but at high levels in thymus (206, 207); perhaps this coreceptor plays an important role in the profound thymic abnormalities associated with HIV-1 infection (208). Similarly, STRL33/BONZO is abundantly expressed in placenta (34, 35), raising the possibility that it might play a role in vertical transmission.

CORECEPTOR-BASED THERAPEUTIC STRATEGIES

The coreceptor discoveries have engendered new concepts to combat HIV-1 disease, at the levels of both treatment of infected subjects and prevention of transmission. Most are still at the developmental stage, although a few have progressed to clinical trials.

Coreceptor Blocking Agents

Several classes of coreceptor blocking agents have been described. For each of these, the fact that different HIV-1 strains "see" a given coreceptor differently

(see sections above) raises the possibility that escape variants capable of using that coreceptor in the presence of the blocking agent will be selected for.

CHEMOKINES AND DERIVATIVES The most straightforward coreceptor blocking agents are the natural chemokines that bind to and inhibit fusion/entry/infection mediated by the corresponding coreceptors, as has been shown with the ligands for CXCR4 (19, 20), CCR5 (10, 14–16), CCR3 (17), CCR8 (30), and CX_3CR1 (33). However, concern has been raised that native chemokines may have unwanted activities due to their coupling to signaling pathways; thus, the CC chemokine stimulation of HIV-1 replication in macrophages under certain conditions is dependent on G protein signaling (67), as is the CC chemokine enhancement of TCL-tropic virus replication in T cells (77). Derivatized chemokine variants (93, 95, 209–212) and chemokine-based synthetic peptides (213, 214) have been reported with properties that may be favorable for anti-HIV therapy, including reduced agonist activity, enhanced blocking of fusion/entry/infection, and improved selectivity for the desired coreceptor. A variant of MIP-1α was found to be well-tolerated in Phase II cancer trials aimed at protecting hematopoietic stem cells during chemotherapy (212); however, no significant effects on viral load or CD4 counts were observed in a Phase I trial in HIV-infected subjects (LG Czaplewski, unpublished).

ANTI-CORECEPTOR ANTIBODIES Anti-coreceptor monoclonal antibodies that inhibit HIV-1 entry represent another class of blocking agent. Murine monoclonal antibodies with HIV-1 inhibitory activity have been described for CXCR4 (68), CCR5 (58, 117), and CCR3 (73). Such antibodies can be humanized and tested for potential clinical utility.

LOW MOLECULAR WEIGHT COMPOUNDS Perhaps the most promising class of blockers is low molecular weight compounds that bind directly to the coreceptors and inhibit their function. In general, members of the G protein–coupled receptor superfamily have proven to be ripe targets for pharmacologic intervention, and the chemokine receptors were already under investigation for development of antiinflammatory drugs prior to their implication in HIV-1 entry. Several low molecular weight blockers specific for CXCR4 have been described (215–217), as has been a more broadly active compound that blocks CCR5, CXCR4, and CCR3 (218). One CXCR4-blocking agent, the bicyclam AMD3100, was shown to inhibit HIV-1 replication in SCID-hu mice (219) prior to the realization that it is a specific inhibitor of entry via CXCR4 (216).

Ex Vivo Modulation of Coreceptor Expression

An interesting therapeutic approach involves treatments that modulate coreceptor expression. An example is ex vivo activation of $CD4^+$ T lymphocytes with

antibodies to CD3 and CD28 adsorbed on beads (190). Cells treated in this fashion are refractory to infection with R5 strains but susceptible to X4 virus, consistent with the pattern of coreceptor expression (negligible CCR5, high CXCR4). This strategy is being investigated in the context of immune reconstitution with syngeneic $CD4^+$ T lymphocytes, since the cells are refractory to infection by the R5 viruses that predominate in individuals at earlier stages of infection. Phase I clinical trials have been initiated (220).

Gene Therapy Approaches

Several strategies are suggested in the context of gene therapy, with the goal of depleting coreceptors from the surface of the target cells. One approach is to target coreceptor RNA (e.g. using ribozymes, antisense, DNA-enzymes). Some progress has been reported with a hammer-head ribozyme and a DNA-enzyme targeted to CCR5 mRNA; expression of the latter agent in target cells reduced their capacity to fuse with cells expressing an R5 Env in vitro (221).

Alternatively, the coreceptor protein can be targeted. An intriguing strategy involves the expression of a so-called "intrakine," a genetically engineered chemokine with a carboxy-terminal endoplasmic reticulum retention sequence; the intrakine traps the newly synthesized coreceptor and prevents its expression on the surface, thereby rendering the target cell refractory to HIV-1 infection. Intrakines have been described for downregulation of CXCR4 (222) and CCR5 (223).

Genetically Engineered Enveloped Virus Particles

The coreceptors have also been used to design genetically engineered enveloped virus particles displaying CD4 and coreceptor in place of their native envelope glycoproteins. This concept has been applied to rabies virus (224), vesicular stomatitus virus (225), and an HIV-1 vector (226). The engineered viruses fuse selectively with cells productively infected with HIV-1 and expressing surface Env. The idea is that the engineered virus will kill the HIV-infected cells, either by a viral cytopathic effect or by delivering an antiviral gene.

Major Questions about Coreceptor-Based Therapeutic Strategies

As with any novel therapeutic modality, the approaches described above are subject to obvious concerns about efficacy, toxicity, and practicality. Several interrelated questions arise in view of the current understanding of the coreceptor system:

1. Will the loss of normal chemokine receptor function of a specific coreceptor be tolerated?

2. Will impairment of CCR5 usage accelerate disease progression by enhancing selection of X4 and R5X4 variants?

3. How many members of the coreceptor repertoire must be blocked in order to achieve a therapeutic effect?

Coreceptor-based therapeutic strategies have the appeal of targeting relatively invariant host determinants, in contrast with anti-HIV-1 agents directed against components of the rapidly mutating virus population. The limited in vivo data demonstrating slower HIV-1 disease progression in CCR5 $+/\Delta 32$ heterozygotes and in CCR5 59029 G/G homozygotes provide support for a potential beneficial effect of targeting CCR5. Moreover, these data argue that the positive effects of lower CCR5 levels outweigh negative effects, including possible enhanced selection for CXCR4-using variants. One caveat, however, is that blocking CCR5 in the face of established HIV-1 disease may not be equivalent to expressing limiting CCR5 levels from the time of disease onset.

There are reasonable grounds for optimism that interference with coreceptor function might be well tolerated. Individual components of the chemokine/receptor system have limited rather than pleiotropic physiologic effects, in contrast with elements of other cytokine/receptor systems; moreover, the redundancy of the chemokine/receptor system offers the possibility that blockade of one will be compensated by the activity of others. Again, the genetic data for CCR5 are encouraging, since no adverse medical consequences have been noted in CCR5 $\Delta 32/\Delta 32$ homozygotes. However, the concern remains that individuals with CCR5 genetic defects may have compensating developmental changes in other components of the chemokine/receptor system, and that such compensations may not occur when CCR5 activity is modulated by the treatment modalities noted above.

The situation with CXCR4 may be more problematic. SDF-1 3′A homozygosity has been associated with slower HIV-1 disease progression, but the effect is controversial and the mechanism is unknown. Moreover, there is cause for concern about undesired side effects of blocking CXCR4 function. Knockout mice lacking either SDF-1 (227) or CXCR4 (228, 229) die during embryogenesis, with evidence of hematopoietic, cardiac, vascular, and cerebellar defects. Thus, the SDF-1/CXCR4 interaction appears to be reciprocally monogamous, and may participate in wide-ranging developmental functions. From an optimistic viewpoint, it is possible that CXCR4 function is dispensable after embryogenesis, and that impairment of its activity would be tolerated.

CONCLUSION

The discovery of HIV-1 coreceptors is an example of how basic research can link previously unrelated fields in totally unexpected ways to create a powerful

new research paradigm with immediate clinical relevance. We now know that chemokine receptors, long studied for their roles in leukocyte trafficking in anti-microbial host defense, have been converted to pro-HIV-1 factors. As a result, we now have a more refined model for HIV-1 entry into target cells, a molecular basis for target cell tropism, new insights into the mechanisms underlying diverse aspects of HIV-1 disease, and new targets for therapeutic intervention.

Demonstration of HIV-1 disease-modifying polymorphisms in CCR5 and other coreceptor genes represents a landmark in our understanding of the influence of human genetic factors on outcome in HIV-1 infection, and it supports genetic analysis as a general approach for understanding the heterogeneity of outcome characteristic of all infectious diseases. The observation that protective coreceptor mutations and polymorphisms are well-tolerated provides the proof of principle needed to justify development of coreceptor-based preventive and therapeutic interventions for the AIDS epidemic, and reason for optimism that they may succeed.

ACKNOWLEDGMENTS

We dedicate this review to the memory of Meta Ann Snyder, who, despite her illness, worked tirelessly to help others with AIDS. The insightful comments of Dr. Paolo Lusso are gratefully acknowledged. We regret that limitations of space prevented citation of all relevant articles in this field.

NOTE ADDED IN PROOF

A newly described CCR5 promoter allele, designated P1, has been reported to show an epidemiological association with rapid progression to AIDS (230). Alleles at position 59029 were not assessed in that report; it is possible they may track the same phenotype due to linkage with P1.

Literature Cited

1. Unutmaz D, Kewalramani VN, Littman DR. 1998. G protein-coupled receptors in HIV and SIV entry: new perspectives on lentivirus-host interactions and on the utility of animal models. *Semin. Immunol.* 10:225–36
2. Marx PA, Chen ZW. 1998. The function of simian chemokine receptors in the replication of SIV. *Semin. Immunol.* 10:215–23
3. Wyatt R, Sodroski J. 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science* 280: 1884–88
4. Berger EA. 1997. HIV entry and tropism: the chemokine receptor connection. *AIDS* 11(Suppl. A):S3–S16
5. Miedema F, Meyaard L, Koot M, Klein MR, Roos MTL, Groenink M, Fouchier RAM, Van't Wout AB, Tersmette M, Schellenkens PTA, Schuitemaker H. 1994. Changing virus-host interactions in the course of HIV-1 infection. *Immunol. Rev.* 140:35–72

6. Feng Y, Broder CC, Kennedy PE, Berger EA. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272:872–77
7. Baggiolini M, Dewald B, Moser B. 1997. Human chemokines: an update. *Annu. Rev. Immunol.* 15:675–705
8. Murphy PM. 1996. Chemokine receptors: structure, function, and role in microbial pathogenesis. *Cytokine Growth Fact. Rev.* 7:47–64
9. Levy JA, Mackewicz CE, Barker E. 1996. Controlling HIV pathogenesis: the role of the noncytotoxic anti-HIV response of CD8(+) T cells. *Immunol. Today* 17:217–24
10. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. 1995. Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by $CD8^+$ T cells. *Science* 270:1811–15
11. Samson M, Labbe O, Mollereau C, Vassart G, Parmentier M. 1996. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. *Biochemistry* 35:3362–67
12. Combadiere C, Ahuja SK, Tiffany HL, Murphy PM. 1996. Cloning and functional expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1α, MIP-1β, and RANTES. *J. Leukocyte Biol.* 60:147–52
13. Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF. 1996. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1β, and MIP-1α. *J. Biol. Chem.* 271:17161–66
14. Deng HK, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR. 1996. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 381:661–66
15. Dragic T, Litwin V, Allaway GP, Martin SR, Huang YX, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA. 1996. HIV-1 entry into $CD4^+$ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 381:667–73
16. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA. 1996. CC CKR5: a RANTES, MIP-1α MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 272:1955–58
17. 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. 1996. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 85:1135–48
18. Doranz BJ, Rucker J, Yi YJ, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW. 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* 85:1149–58
19. Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA. 1996. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 382:829–33
20. 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. 1996. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 382:833–35
21. Zhang LQ, Huang YX, He T, Cao YZ, Ho DD. 1996. HIV-1 subtype and second-receptor use. *Nature* 383:768
22. Connor RI, Sheridan KE, Ceradini D, Choe S, Landau NR. 1997. Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. *J. Exp. Med.* 185:621–28
23. Bjorndal A, Deng H, Jansson M, Fiore JR, Colognesi C, Karlsson A, Albert J, Scarlatti G, Littman DR, Fenyö EM. 1997. Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype. *J. Virol.* 71:7478–87
24. Scarlatti G, Tresoldi E, Bjorndal A, Fredriksson R, Colognesi C, Deng HK, Malnati MS, Plebani A, Siccardi AG, Littman DR, Fenyo EM, Lusso P. 1997. In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nature Med.* 3:1259–65
25. Bazan HA, Alkhatib G, Broder CC, Berger EA. 1998. Patterns of CCR5, CXCR4 and CCR3 usage by envelope glycoproteins from human immunodeficiency virus type 1 primary isolates. *J. Virol.* 72:4485–91
26. Trkola A, Paxton WA, Monard SP, Hoxie JA, Siani MA, Thompson DA, Wu L, Mackay CR, Horuk R, Moore JP. 1998. Genetic subtype–independent inhibition of human immunodeficiency

virus type 1 replication by CC and CXC chemokines. *J. Virol.* 72:396–404

27. Tscherning C, Alaeus A, Fredriksson R, Bjorndal A, Deng HK, Littman DR, Fenyö EM, Albert J. 1998. Differences in chemokine coreceptor usage between genetic subtypes of HIV-1. *Virology* 241:181–88
28. Berger EA, Doms RW, Fenyö EM, Korber BTM, Littman DR, Moore JP, Sattentau QJ, Schuitemaker H, Sodroski J, Weiss RA. 1998. A new classification for HIV-1. *Nature* 391:240
29. Rucker J, Edinger AL, Sharron M, Samson M, Lee B, Berson JF, Yi Y, Margulies B, Collman RG, Doranz BJ, Parmentier M, Doms RW. 1997. Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses. *J. Virol.* 71:8999–9007
30. Horuk R, Hesselgesser J, Zhou Y, Faulds D, Halks-Miller M, Harvey S, Taub D, Samson M, Parmentier M, Rucker J, Doranz BJ, Doms RW. 1998. The CC chemokine I-309 inhibits CCR8-dependent infection by diverse HIV-1 strains. *J. Biol. Chem.* 273:386–91
31. Jinno A, Shimizu N, Soda Y, Haraguchi Y, Kitamura T, Hoshino H. 1998. Identification of the chemokine receptor TER1/CCR8 expressed in brain-derived cells and T cells as a new coreceptor for HIV-1 infection. *Biochem. Biophys. Res. Commun.* 243:497–502
32. Choe H, Farzan M, Konkel M, Martin K, Sun Y, Marcon L, Cayabyab M, Berman M, Dorf ME, Gerard N, Gerard C, Sodroski J. 1998. The orphan seven-transmembrane receptor ApJ. supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. *J. Virol.* 72:6113–18
33. Combadiere C, Salzwedel K, Smith ED, Tiffany HL, Berger EA, Murphy PM. 1998. Identification of CX_3CR1: a chemotactic receptor for the human CX_3C chemokine fractalkine, and a fusion coreceptor for HIV-1. *J. Biol. Chem.* 273:23799–804
34. Liao F, Alkhatib G, Peden KWC, Sharma G, Berger EA, Farber JM. 1997. STRL33, a novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med.* 185:2015–23
35. Deng H, Unutmaz D, Kewalramani VN, Littman DR. 1997. Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature* 388:296–300
36. Farzan M, Choe H, Martin K, Marcon L, Hofmann W, Karlsson G, Sun Y, Barrett P, Marchand N, Sullivan N, Gerard N, Gerard C, Sodroski J. 1997. Two orphan seven-transmembrane segment receptors which are expressed in CD4-positive cells support simian immunodeficiency virus infection. *J. Exp. Med.* 186:405–11
37. Edinger AL, Hoffman TL, Sharron M, Lee BH, Yi YJ, Choe W, Kolson DL, Mitrovic B, Zhou YQ, Faulds D, Collman RG, Hesselgesser J, Horuk R, Doms RW. 1998. An orphan seven-transmembrane domain receptor expressed widely in the brain functions as a coreceptor for human immunodeficiency virus type 1 and simian immunodeficiency virus. *J. Virol.* 72: 7934–40
38. Pleskoff O, Treboute C, Brelot A, Heveker N, Seman M, Alizon M. 1997. Identification of a chemokine receptor encoded by human cytomegalovirus as a cofactor for HIV-1 entry. *Science* 276:1874–78
39. Kledal TN, Rosenkilde MM, Coulin F, Simmons G, Johnsen AH, Alouani S, Power CA, Luttichau HR, Gerstoft J, Clapham PR, Clark-Lewis I, Wells TNC, Schwartz TW. 1997. A broad-spectrum chemokine antagonist encoded by Kaposi's sarcoma-associated herpesvirus. *Science* 277:1656–59
40. Boshoff C, Endo Y, Collins PD, Takeuchi Y, Reeves JD, Schweickart VL, Siani MA, Sasaki T, Williams TJ, Gray PW, Moore PS, Chang Y, Weiss RA. 1997. Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. *Science* 278:290–94
41. Owman C, Garzino-Demo A, Cocchi F, Popovic M, Sabirsh A, Gallo RC. 1998. The leukotriene B-4 receptor functions as a novel type of coreceptor mediating entry of primary HIV-1 isolates into CD4-positive cells. *Proc. Natl. Acad. Sci. USA* 95:9530–34
42. Samson M, Edinger AL, Stordeur P, Rucker J, Verhasselt V, Sharron M, Govaerts C, Mollereau C, Vassart G, Doms RW, Parmentier M. 1998. ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. *Eur. J. Immunol.* 28:1689–1700

43. Pal R, Garzino-Demo A, Markham PD, Burns J, Brown M, Gallo RC, DeVico AL. 1997. Inhibition of HIV-1 infection by the beta-chemokine MDC. *Science* 278:695–98
44. DeVico AL, Pal R, Markham PD, Garzino-Demo A, Gallo RC. 1998. β-chemokine MDC and HIV-1 infection. *Science* 281:487a
45. Struyf S, Proost P, Sozzani S, Mantovani A, Wuyts A, De Clercq E, Van Damme J. 1998. Enhanced anti-HIV-1 activity and altered chemotactic potency of NH2-terminally processed macrophage-derived chemokine (MDC) imply an additional MDC receptor. *J. Immunol.* 161:2672–75
46. Lee B, Rucker J, Doms RW, Tsang M, Hu X, Dietz M, Bailer R, Montaner LJ, Gerard C, Sullivan N, Sodroski J, Stantchev TS, Broder CC. 1998. β-chemokine MDC and HIV-1 infection. *Science* 281:487a
47. Arenzana-Seisdedos F, Amara A, Thomas D, Virelizier JL, Baleux F, Clark-Lewis I, Legler DF, Moser B, Baggiolini M. 1998. β-chemokine MDC and HIV-1 infection. *Science* 281: 487a
48. Imai T, Chantry D, Raport CJ, Wood CL, Nishimura M, Godiska R, Yoshie O, Gray PW. 1998. Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. *J. Biol. Chem.* 273:1764–1768
49. Tachibana K, Nakajima T, Sato A, Igarashi K, Shida H, Iizasa H, Yashida N, Yoshie O, Kishimoto T, Nagasawa T. 1997. CXCR4/fusin is not a species-specific barrier in murine cells for HIV-1 entry. *J. Exp. Med.* 185:1865–70
50. Bieniasz PD, Fridell RA, Anthony K, Cullen BR. 1997. Murine CXCR-4 is a functional coreceptor for T-cell-tropic and dual-tropic strains of human immunodeficiency virus type 1. *J. Virol.* 71:7097–7100
51. Parolin C, Barsetti A, Choe H, Farzan M, Kolchinsky P, Heesen M, Ma Q, Gerard C, Palu G, Dorf ME, Springer T, Sodroski J. 1998. Use of murine CXCR-4 as a second receptor by some T-cell-tropic human immunodeficiency viruses. *J. Virol.* 72:1652–56
52. Atchison RE, Gosling J, Monteclaro FS, Franci C, Digilio L, Charo IF, Goldsmith MA. 1996. Multiple extracellular elements of CCR5 and HIV-1 entry: dissociation from response to chemokines. *Science* 274:1924–26
53. Doranz BJ, Lu ZH, Rucker J, Zhang TY, Sharron M, Cen YH, Wang ZX, Guo HH, Du JG, Accavitti MA, Doms RW, Peiper SC. 1997. Two distinct CCR5 domains can mediate coreceptor usage by human immunodeficiency virus type 1. *J. Virol.* 71:6305–14
54. Picard L, Simmons G, Power CA, Meyer A, Weiss RA, Clapham PR. 1997. Multiple extracellular domains of CCR-5 contribute to human immunodeficiency virus type 1 entry and fusion. *J. Virol.* 71:5003–11
55. Ross TM, Bieniasz PD, Cullen BR. 1998. Multiple residues contribute to the inability of murine CCR-5 to function as a coreceptor for macrophage-tropic human immunodeficiency virus type 1 isolates. *J. Virol.* 72:1918–24
56. Connor RI, Paxton WA, Sheridan KE, Koup RA. 1996. Macrophages and $CD4^+$ T lymphocytes from two multiply exposed, uninfected individuals resist infection with primary non-syncytium-inducing isolates of human immunodeficiency virus type 1. *J. Virol.* 70:8758–64
57. Rana S, Besson G, Cook DG, Rucker J, Smyth RJ, Yi Y, Turner JD, Guo H-H, Du J-D, Peiper SC, Lavi E, Samson M, Libert F, Liesnard C, Vassart G, Doms RW, Parmentier M, Collman RG. 1997. Role of CCR5 in infection of primary macrophages and lymphocytes by macrophage-tropic strains of human immunodeficiency virus: resistance to patient-derived and prototype isolates resulting from the delta CCR5 mutation. *J. Virol.* 71:3219–27
58. Wu L, Paxton WA, Kassam N, Ruffing N, Rottman JB, Sullivan N, Choe H, Sodroski J, Newman W, Koup RA, Mackay CR. 1997. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. *J. Exp. Med.* 185:1681–91
59. Simmons G, Wilkinson D, Reeves JD, Dittmar MT, Beddows S, Weber J, Carnegie G, Desselberger U, Gray PW, Weiss RA, Clapham PR. 1996. Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. *J. Virol.* 70:8355–60
60. Naif HM, Li S, Alali M, Sloane A, Wu L, Kelly M, Lynch G, Lloyd A, Cunningham AL. 1998. CCR5 expression correlates with susceptibility of maturing monocytes to human immunodeficiency virus type 1 infection. *J. Virol.* 72:830–36

61. Dittmar MT, McKnight A, Simmons G, Clapham PR, Weiss RA, Simmonds P. 1997. HIV-1 tropism and co-receptor use. *Nature* 385:495–96
62. Coffey MJ, Woffendin C, Phare SM, Streiter RM, Markovitz DM. 1997. RANTES inhibits HIV-1 replication in human peripheral blood monocytes and alveolar macrophages. *Am. J. Physiol.* 272:L1025–29
63. Capobianchi MR, Abbate I, Antonelli G, Turriziani O, Dolei A, Dianzani F. 1998. Inhibition of HIV type 1 BaL replication by MIP-1 alpha, MIP-1 beta, and RANTES in macrophages. *AIDS Res. Hum. Retrovir.* 14:233–40
64. Schmidtmayerova H, Sherry B, Bukrinsky M. 1996. Chemokines and HIV replication. *Nature* 382:767
65. Moriuchi H, Moriuchi M, Combadiere C, Murphy PM, Fauci AS. 1996. $CD8^+$ T-cell-derived soluble factor(s), but not β-chemokines RANTES, MIP-1α, and MIP-1β, suppress HIV-1 replication in monocyte/macrophages. *Proc. Natl. Acad. Sci. USA* 93:15341–45
66. Oravecz T, Pall M, Wang J, Roderiquez G, Ditto M, Norcross MA. 1997. Regulation of anti-HIV-1 activity of RANTES by heparan sulfate proteoglycans. *J. Immunol.* 159:4587–92
67. Kelly MD, Naif HM, Adams SL, Cunningham AL, Lloyd AR. 1998. Dichotomous effects of beta-chemokines on HIV replication in monocytes and monocyte-derived macrophages. *J. Immunol.* 160:3091–95
68. McKnight A, Wilkinson D, Simmons G, Talbot S, Picard L, Ahuja M, Marsh M, Hoxie JA, Clapham PR. 1997. Inhibition of human immunodeficiency virus fusion by a monoclonal antibody to a coreceptor (CXCR4) is both cell type and virus strain dependent. *J. Virol.* 71: 1692–96
69. Yi Y, Rana S, Turner JD, Gaddis N, Collman RG. 1998. CXCR-4 is expressed by primary macrophages and supports CCR5-independent infection by dual-tropic but not T-tropic isolates of human immunodeficiency virus type 1. *J. Virol.* 72:772–77
70. Verani A, Pesenti E, Polo S, Tresoldi E, Scarlatti G, Lusso P, Siccardi AG, Vercelli D. 1998. CXCR4 is a functional coreceptor for infection of human macrophages by CXCR4-dependent primary HIV-1 isolates. *J. Immunol.* 161: 2084–88
71. Schmidtmayerova H, Aflano M, Nuovo G, Bukrinsky M. 1998. Human immunodeficiency virus type 1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-mediated pathway: Replication is restricted at a post-entry level. *J. Virol.* 72:4633–42
72. Kozak SL, Platt EJ, Madani N, Ferro FE Jr, Peden K, Kabat D. 1997. CD4, CXCR-4, and CCR-5 dependencies for infections by primary patient and laboratory-adapted isolates of human immunodeficiency virus type 1. *J. Virol.* 71:873–82
73. He J, Chen Y, Farzan M, Choe H, Ohagen A, Gartner S, Busciglio J, Yang X, Hofmann W, Newman W, Mackay CR, Sodroski J, Gabuzda D. 1997. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* 385:645–49
74. Rubbert A, Combadiere C, Ostrowski M, Arthos J, Dybul M, Machado E, Cohn MA, Hoxie JA, Murphy PM, Fauci AS, Weissman D. 1998. Dendritic cells express multiple chemokine receptors used as coreceptors for HIV entry. *J. Immunol.* 160:3933–41
75. Lapham CK, Ouyang J, Chandrasekhar B, Nguyen NY, Dimitrov DS, Golding H. 1996. Evidence for cell-surface association between fusin and the CD4-gp120 complex in human cell lines. *Science* 274:602–5
76. Ugolini S, Moulard M, Mondor I, Barois N, Demandolx D, Hoxie J, Brelot A, Alizon M, Davoust J, Sattentau QJ. 1997. HIV-1 gp120 induces an association between CD4 and the chemokine receptor CXCR4. *J. Immunol.* 159:3000–8
77. Kinter A, Catanzaro A, Monaco J, Ruiz M, Justement J, Moir S, Arthos J, Oliva A, Ehler L, Mizell S, Jackson R, Ostrowski M, Hoxie J, Offord R, Fauci AS. 1998. CC-chemokines enhance the replication of T-tropic strains of HIV-1 in $CD4^+$ T cells: role of signal transduction. *Proc. Natl. Acad. Sci. USA* 95: 11880–85
78. Iyengar S, Hildreth JEK, Schwartz DH. 1998. Actin-dependent receptor colocalization required for human immunodeficiency virus entry into host cells. *J. Virol.* 72:5251–55
79. Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, Borsetti A, Cardoso AA, Desjardin E, Newman W, Gerard C, Sodroski J. 1996. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature* 384:179–83
80. Trkola A, Dragic T, Arthos J, Bin-

ley JM, Olson WC, Allaway GP, Cheng-Mayer C, Robinson J, Maddon PJ, Moore JP. 1996. CD4-dependent antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. *Nature* 384:184–87
81. Hill CM, Deng H, Unutmaz D, Kewalramani VN, Bastiani L, Gorny MK, Zolla-Pazner S, Littman DR. 1997. Envelope glycoproteins from human immunodeficiency virus types 1 and 2 and simian immunodeficiency virus can use human CCR5 as a coreceptor for viral entry and make direct CD4-dependent interactions with this chemokine receptor. *J. Virol.* 71:6296–304
82. Bandres JC, Wang QF, O'Leary J, Baleaux F, Amara A, Hoxie JA, Zolla-Pazner S, Gorny MK. 1998. Human immunodeficiency virus (HIV) envelope binds to CXCR4 independently of CD4, and binding can be enhanced by interaction with soluble CD4 or by HIV envelope deglycosylation. *J. Virol.* 72:2500–4
83. Sullivan N, Sun Y, Sattentau Q, Thali M, Wu D, Denisova G, Gershoni J, Robinson J, Moore J, Sodroski J. 1998. CD4-induced conformational changes in the human immunodeficiency virus type 1 gp120 glycoprotein: Consequences for virus entry and neutralization. *J. Virol.* 72:4694–4703
84. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 393:648–59
85. Rizzuto CD, Wyatt R, Hernandez-Ramos N, Sun Y, Kwong PD, Hendrickson WA, Sodroski J. 1998. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* 280:1949–53
86. Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, Sodroski JG. 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* 393:705–11
87. Farzan M, Choe H, Martin KA, Sun Y, Sidelko M, Mackay CR, Gerard NP, Sodroski J, Gerard C. 1997. HIV-1 entry and macrophage inflammatory protein-1β-mediated signaling are independent functions of the chemokine receptor CCR5. *J. Biol. Chem.* 272:6854–57
88. Gosling J, Monteclaro FS, Atchison RE, Arai H, Tsou CL, Goldsmith MA, Charo IF. 1997. Molecular uncoupling of C-C chemokine receptor 5–induced chemotaxis and signal transduction from HIV-1 coreceptor activity. *Proc. Natl. Acad. Sci. USA* 94:5061–66
89. Alkhatib G, Locati M, Kennedy PE, Murphy PM, Berger EA. 1997. HIV-1 coreceptor activity of CCR5 and its inhibition by chemokines: independence from G protein signaling and importance of coreceptor downmodulation. *Virology* 234:340–48
90. Aramori I, Zhang J, Ferguson SS, Bieniasz PD, Cullen BR, Caron MG. 1997. Molecular mechanism of desensitization of the chemokine receptor CCR-5: receptor signaling and internalization are dissociable from its role as an HIV-1 co-receptor. *EMBO. J.* 16:4606–16
91. Hu H, Shioda T, Hori T, Moriya C, Kato A, Sakai Y, Natsushima K, Uchiyama T, Nagai Y. 1998. Dissociation of ligand-induced internalization of CXCR-4 from its co-receptor activity for HIV-1 Env-mediated membrane fusion. *Arch. Virol.* 143:851–61
92. Amara A, Gall S, Schwartz O, Salamero J, Montes M, Loetscher P, Baggiolini M, Virelizier J-L, Arenzana-Seisdedos F. 1997. HIV coreceptor downregulation as antiviral principle: SDF-1α-dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J. Exp. Med.* 186:139–46
93. Crump MP, Gong JH, Loetscher P, Rajarathnam K, Amara A, Arenzana-Seisdedos F, Virelizier JL, Baggiolini M, Sykes BD, Clark-Lewis I. 1997. Solution structure and basis for functional activity of stromal cell-derived factor-1; dissociation of CXCR4 activation from binding and inhibition of HIV-1. *EMBO J.* 16:6996–7007
94. Signoret N, Oldridge J, Pelchen-Matthews A, Klasse PJ, Tran T, Brass LF, Rosenkilde MM, Schwartz TW, Holmes W, Dallas W, Luther MA, Wells TN, Hoxie JA, Marsh M. 1997. Phorbol esters and SDF-1 induce rapid endocytosis and down modulation of the chemokine receptor CXCR4. *J. Cell. Biol.* 139:651–664
95. Mack M, Luckaw B, Nelson PJ, Cihak J, Simmons G, Clapham PR, Signoret N, Marsh M, Stangassinger M, Borlat F, Wells TN, Schlondorff D, Proudfoot AE. 1998. Aminooxypentane-RANTES induces CCR5 internalization but inhibits recycling: a novel inhibitory mechanism of HIV infectivity. *J. Exp. Med.* 187:1215–24
96. Tarasova NI, Stauber RH, Michejda CJ.

1998. Spontaneous and ligand-induced trafficking of CXC-chemokine receptor 4. *J. Biol. Chem.* 273:15883–86

97. Endres MJ, Clapham PR, Marsh M, Ahuja M, Turner JD, McKnight A, Thomas JF, Stoebenau-Haggarty B, Choe S, Vance PJ, Wells TNC, Power CA, Sutterwala SS, Doms RW, Landau NR, Hoxie JA. 1996. CD4-independent infection by HIV-2 is mediated by Fusin/CXCR4. *Cell* 87:745–56
98. Reeves JD, McKnight A, Potempa S, Simmons G, Gray PW, Power CA, Wells T, Weiss RA, Talbot SJ. 1997. CD4-independent infection by HIV-2 (ROD/B): use of the 7-transmembrane receptors CXCR-4, CCR-3, and V28 for entry. *Virology* 231:130–34
99. Potempa S, Picard L, Reeves JD, Wilkinson D, Weiss RA, Talbot SJ. 1997. CD4-independent infection by human immunodeficiency virus type 2 strain ROD/B: the role of the N-terminal domain of CXCR-4 in fusion and entry. *J. Virol.* 71:4419–24
100. Reeves JD, Schulz TF. 1997. The CD4-independent tropism of human immunodeficiency virus type 2 involves several regions of the envelope protein and correlated with a reduced activation threshold for envelope-mediated fusion. *J. Virol.* 71:1453–65
101. Martin KA, Wyatt R, Farzan M, Choe H, Marcon L, Desjardin E, Robinson J, Sodroski J, Gerard C, Gerard NP. 1997. CD4-independent binding of SIV gp120 to rhesus CCR5. *Science* 278:1470–73
102. Edinger AL, Markowski JL, Doranz BJ, Margulies BJ, Lee B, Rucker J, Sharron M, Hoffman TL, Berson JF, Zink MC, Hirsch VM, Clements JE, Doms RW. 1997. CD4-independent, CCR5-dependent infection of brain capillary endothelial cells by a neurovirulent simian immunodeficiency virus strain. *Proc. Natl. Acad. Sci. USA* 94:14742–47
103. Hesselgesser J, Halks-Miller M, DelVecchio V, Peiper SC, Hoxie J, Kolson DL, Taub D, Horuk R. 1997. CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons. *Curr. Biol.* 7:112–21
104. Dumonceaux J, Nisole S, Chanel C, Quivet L, Amara A, Baleux F, Briand F, Hazan U. 1998. Spontaneous mutations in the env gene of the human immunodeficiency virus type 1 NDK isolate are associated with a CD4-independent entry phenotype. *J. Virol.* 72:512–19
105. Cocchi F, DeVico AL, Garzino-Demo A, Cara A, Gallo RC, Lusso P. 1996. The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine-mediated blockade of infection. *Nature Med.* 2:1244–47
106. Bieniasz PD, Fridell RA, Aramori I, Ferguson SS, Caron MG, Cullen BR. 1997. HIV-1-induced cell fusion is mediated by multiple regions within both the viral envelope and the CCR-5 co-receptor. *EMBO J.* 16:2599–2609
107. Pleskoff O, Sol N, Labrosse B, Alizon M. 1997. Human immunodeficiency virus strains differ in their ability to infect $CD4^+$ cells expressing the rat homolog of CXCR-4 (fusin). *J. Virol.* 71: 3259–62
108. Speck RF, Wehrly K, Platt EJ, Atchison RE, Charo IF, Kabat D, Chesebro B, Goldsmith MA. 1997. Selective employment of chemokine receptors as human immunodeficiency virus type 1 coreceptors determined by individual amino acids within the envelope V3 loop. *J. Virol.* 71:7136–39
109. Cho MW, Lee MK, Carney MC, Berson JF, Doms RW, Martin MA. 1998. Identification of determinants on a dualtropic human immunodeficiency virus type 1 envelope glycoprotein that confer usage of CXCR4. *J. Virol.* 72:2509–15
110. Ross TM, Cullen BR. 1998. The ability of HIV type 1 to use CCR-3 as a coreceptor is controlled by envelope V1/V2 sequences acting in conjunction with a CCR-5 tropic V3 loop. *Proc. Natl. Acad. Sci. USA* 95:7682–86
111. Smythe RJ, Yi YJ, Singh A, Collman RG. 1998. Determinants of entry cofactor utilization and tropism in a dualtropic human immunodeficiency virus type 1 primary isolate. *J. Virol.* 72:4478–84
112. Wang WK, Dudek T, Zhao YJ, Brumblay HG, Essex M, Lee TH. 1998. CCR5 coreceptor utilization involves a highly conserved arginine residue of HIV type 1 gp120. *Proc. Natl. Acad. Sci. USA* 95: 5740–45
113. Xiao LZH, Owen SM, Goldman I, Lal AA, deJong JJ, Goudsmit J, Lal RB. 1998. CCR5 coreceptor usage of non-syncytium-inducing primary HIV-1 is independent of phylogenetically distinct global HIV-1 isolates: delineation of consensus motif in the V3 domain that predicts CCR5 usage. *Virology* 240:83–92
114. Hoffman TL, Stephens EB, Narayan O, Doms RW. 1998. HIV type I envelope determinants for use of the CCR2b,

CCR3, STRL33, and APJ. coreceptors. *Proc. Natl. Acad. Sci. USA* 95:11360–65
115. Frade JMR, Llorente M, Mellado M, Alcami J, Gutierrez-Ramos JC, Zaballos A, Real G, Martinez-A C. 1997. The amino-terminal domain of the CCR2 chemokine receptor acts as coreceptor for HIV-1 infection. *J. Clin Invest* 100:497–502
116. Rucker J, Samson M, Doranz BJ, Libert F, Berson JF, Yi YJ, Smyth RJ, Collman RG, Broder CC, Vassart G, Doms RW, Parmentier M. 1996. Regions in beta-chemokine receptors CCR5 and CCR2b that determine HIV-1 cofactor specificity. *Cell* 87:437–46
117. Wu L, Larosa G, Kassam N, Gordon CJ, Heath H, Ruffing N, Chen H, Humblias J, Samson M, Parmentier M, Moore JP, Mackay CR. 1997. Interaction of chemokine receptor CCR5 with its ligands: multiple domains for HIV-1 gp120 binding and a single domain for chemokine binding. *J. Exp. Med.* 186: 1373–81
118. Alkhatib G, Berger EA, Murphy PM, Pease JE. 1997. Determinants of HIV-1 coreceptor function on CC chemokine receptor 3: importance of both extracellular and transmembrane/cytoplasmic regions. *J. Biol. Chem.* 272:20420–26
119. Lu Z, Berson JF, Chen Y, Turner JD, Zhang T, Sharron M, Jenks MH, Wang Z, Kim J, Rucker J, Hoxie JA, Peiper SC, Doms RW. 1997. Evolution of HIV-1 coreceptor usage through interactions with distinct CCR5 and CXCR4 domains. *Proc. Natl. Acad. Sci. USA* 94: 6426–31
120. Edinger AL, Amedee A, Miller K, Doranz BJ, Endres M, Sharron M, Samson M, Lu ZH, Clements JE, Murphey-Corb M, Peiper SC, Parmentier M, Broder CC, Doms RW. 1997. Differential utilization of CCR5 by macrophage and T cell tropic simian immunodeficiency virus strains. *Proc. Natl. Acad. Sci. USA* 94:4005–10
121. Strizki JM, Turner JD, Collman RG, Hoxie J, Gonzalez-Scarano F. 1997. A monoclonal antibody (12G5) directed against CXCR-4 inhibits infection with the dual-tropic human immunodeficiency virus type 1 isolate HIV-1(89.6) but not the T-tropic isolate HIV-1(HxB). *J. Virol.* 71:5678–83
122. Alkhatib G, Ahuja SS, Light D, Mummidi S, Berger EA, Ahuja SK. 1997. CC chemokine receptor 5-mediated signaling and HIV-1 co-receptor activity share common structural determinants: critical residues in the third extracellular loop support HIV-1 fusion. *J. Biol. Chem.* 272:19771–76
123. Kuhmann SE, Platt EJ, Kozak SL, Kabat D. 1997. Polymorphisms in the CCR5 genes of African green monkeys and mice implicate specific amino acids in infections by simian and human immunodeficiency viruses. *J. Virol.* 71:8642–56
124. Willett BJ, Picard L, Hosie MJ, Turner JD, Adema K, Clapham PR. 1997. Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses. *J. Virol.* 71:6407–15
125. Brelot A, Heveker N, Pleskoff O, Sol N, Alizon M. 1997. Role of the first and third extracellular domains of CXCR-4 in human immunodeficiency virus coreceptor activity. *J. Virol.* 71:4744–51
126. Picard L, Wilkinson DA, McKnight A, Gray PW, Hoxie JA, Clapham PR, Weiss RA. 1997. Role of the amino-terminal extracellular domain of CXCR-4 in human immunodeficiency virus type 1 entry. *Virology* 231:105–11
127. Dragic T, Trkola A, Lin SW, Nagashima KA, Kajumo F, Zhao L, Olson WC, Wu LJ, Mackay CR, Allaway GP, Sakmar TP, Moore JP, Maddon PJ. 1998. Amino-terminal substitutions in the CCR5 coreceptor impair gp120 binding and human immunodeficiency virus type 1 entry. *J. Virol.* 72:279–85
128. Rabut GEE, Konner JA, Kajumo F, Moore JP, Dragic T. 1998. Alanine substitutions of polar and nonpolar residues in the amino-terminal domain of CCR5 differently impair entry of macrophage- and dualtropic isolates of human immunodeficiency virus type 1. *J. Virol.* 72:3464–68
129. Wang ZX, Berson JF, Zhang TY, Cen YH, Sun Y, Sharron M, Lu ZH, Peiper SC. 1998. CXCR4 sequences involved in coreceptor determination of human immunodeficiency virus type-1 tropism. Unmasking of activity with M-tropic Env glycoproteins. *J. Biol. Chem.* 273:15007–15
130. Farzan M, Choe H, Vaca L, Martin K, Sun Y, Desjardins E, Ruffing N, Wu LJ, Wyatt R, Gerard N, Gerard C, Sodroski J. 1998. A tyrosine-rich region in the N terminus of CCR5 is important for human immunodeficiency virus type 1 entry and mediates an association between gp120 and CCR5. *J. Virol.* 72:1160–64
131. Reeves JD, Heveker N, Brelot A, Alizon M, Clapham PR, Picard L. 1998.

The second extracellular loop of CXCR4 is involved in CD4-independent entry of human immunodeficiency virus type 2. *J. Gen. Virol.* 79:1793–99

132. Chan DC, Fass D, Berger JM, Kim PS. 1997. Core structure of gp41 from the HIV envelope glycoprotein. *Cell* 89: 263–73
133. Weissenhorn W, Dessen A, Harrison SC, Skehel JJ, Wiley DC. 1997. Atomic structure of the ectodomain from HIV-1 gp41. *Nature* 387:426–30
134. Caffrey M, Cai ML, Kaufman J, Stahl SJ, Wingfield PT, Covell DG, Gronenborn AM, Clore GM. 1998. Three-dimensional solution structure of the 44 kDa ectodomain of SIV gp41. *EMBO J.* 17:4572–84
135. Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. 1997. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc. Natl. Acad. Sci. USA* 94:1925–30
136. Rottman JB, Ganley KP, Williams K, Wu L, Mackay CR, Ringler DJ. 1997. Cellular localization of the chemokine receptor CCR5. Correlation to cellular targets of HIV-1 infection. *Am. J. Pathol.* 151:1341–51
137. Zhang LQ, He T, Talal A, Wang G, Frankel SS, Ho DD. 1998. In vivo distribution of the human immunodeficiency virus simian immunodeficiency virus coreceptors: CXCR4, CCR3, and CCR5. *J. Virol.* 72:5035–45
138. Granelli-Piperno A, Moser B, Pope M, Chen D, Wei Y, Isdell F, O'Doherty U, Paxton W, Koup R, Mojsov S, Bhardwa JN, Clark-Lewis I, Baggiolini M, Steinman RM. 1996. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. *J. Exp. Med.* 184:2433–38
139. Ayehunie S, Garcia-Zepeda EA, Hoxie JA, Horuk R, Kupper TS, Luster AD, Ruprecht RM. 1997. Human immunodeficiency virus-1 entry into purified blood dendritic cells through CC and CXC chemokine coreceptors. *Blood* 90:1379–86
140. Dittmar MT, Simmons G, Hibbitts S, O'Hare M, Louisirirotchanakul S, Beddows S, Weber J, Clapham PR, Weiss RA. 1997. Langerhans cell tropism of human immunodeficiency virus type 1 subtype A through F isolates derived from different transmission groups. *J. Virol.* 71:8008–13
141. Zaitseva M, Blauvelt A, Lee S, Lapham CK, Klaus-Kovtun V, Mostowski H, Manischewitz J, Golding H. 1997. Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. *Nature Med.* 3:1369–75

141a. Patterson BK, Landay A, Andersson J, Brown C, Behbahani H, Jiyamapa D, Burki Z, Stanlaslawski D, Czerniewski MA, Garcia P. 1998. Repertoire of chemokine receptor expression in the female genital tract: implications for human immunodeficiency virus transmission. *Am. J. Pathol.* 153:481–90

142. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, Macdonald ME, Stuhlmann H, Koup RA, Landau NR. 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86:367–77
143. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, Muyldermans G, Verhofstede C, Burtonboy G, Georges M, Imai T, Rana S, Yi YJ, Smyth RJ, Collman RG, Doms RW, Vassart G, Parmentier M. 1996. Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 382:722–25
144. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, Dornfield 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. 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CKR5* structural gene. *Science* 273:1856–62
145. Zimmerman PA, Buckler-White A, Alkhatib G, Spalding T, Kubofcik J, Combadiere C, Weissman D, Cohen O, Rubbert A, Lam G, Vaccarezza M, Kennedy PE, Kumaraswami V, Giorgi JV, Detels R, Hunter J, Chopek M, Berger EA, Fauci AS, Nutman TB, Murphy PM. 1997. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. *Mol. Med.* 3:23–35
146. Benkirane M, Jin DY, Chun RF,

Koup RA, Jeang KT. 1997. Mechanism of transdominant inhibition of CCR5-mediated HIV-1 infection by ccr5delta32. *J. Biol. Chem.* 272:30603–6

147. Huang YX, Paxton WA, Wolinsky SM, Neumann AU, Zhang LQ, He T, Kang S, Ceradini D, Jin ZQ, Yazdanbakhsh K, Kunstman K, Erickson D, Dragon E, Landau NR, Phair J, Ho DD, Koup RA. 1996. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nature Med.* 2:1240–43
148. O'Brien TR, Winkler C, Dean M, Nelson JR, Carrington M, Michael NL, White GC II. 1997. HIV-1 infection in a man homozygous for CCR5 delta 32. *Lancet* 349:1219
149. Paxton WA, Martin SR, Tse D, O'Brien TR, Skurnick J, VanDevanter NL, Padian N, Braun JF, Kotler DP, Wolinsky SM, Koup RA. 1996. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures. *Nature Med.* 2:412–17
150. Edelstein RE, Arcuino LA, Hughes JP, Melvin AJ, Mohan KM, King PD, McLellan CL, Murante BL, Kassman BP, Frenkel LM. 1997. Risk of mother-to-infant transmission of HIV-1 is not reduced in CCR5/delta32ccr5 heterozygotes. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* 16:243–46
151. Rousseau CMS, Just JJ, Abrams EJ, Casabona J, Stein Z, King MC. 1997. CCR5del32 in perinatal HIV-1 infection. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* 16:239–242
152. Michael NL, Chang G, Louie LG, Mascola JR, Dandero D, Birx DL, Sheppard HW. 1997. The role of viral phenotype and CCR-5 gene defects in HIV-1 transmission and disease progression. *Nature Med.* 3:338–40
153. Misrahi M, Teglas JP, N'Go N, Burgard M, Mayaux MJ, Rouzioux C, Delfraissy JF, Blanche S. 1998. CCR5 chemokine receptor variant in HIV-1 mother-to-child transmission and disease progression in children. *J. Am. Med. Assoc.* 279:317–18
154. Malo A, Rommel F, Bogner J, Gruber R, Schramm W, Goebel FD, Riethmuller G, Wank R. 1998. Lack of protection from HIV infection by the mutant HIV coreceptor CCR5 in intravenously HIV infected hemophilia patients. *Immunobiology* 198:485–88
155. Mangano A, Prada F, Roldan A, Picchio G, Bologna R, Sen L. 1998. Distribution of CCR-5 delta32 allele in Argentinian children at risk of HIV-1 infection: its role in vertical transmission. *AIDS* 12:109–10
156. Shearer WT, Kalish LA, Zimmerman PA. 1998. CCR5 HIV-1 vertical transmission. *J. Acquired Immune Defic. Syndr.* 17:180–81
157. Mummidi S, Ahuja SS, Gonzalez E, Anderson SA, Santiago EN, Stephan KT, Craig FE, O'Connell P, Tryon V, Clark RA, Dolan MJ, Ahuja SK. 1998. Genealogy of the CCR5 locus and chemokine system gene variants associated with altered rates of HIV-1 disease progression. *Nature Med.* 4:786–93
158. Hoffman TL, MacGregor RR, Burger H, Mick R, Doms RW, Collman RG. 1997. CCR5 genotypes in sexually active couples discordant for human immunodeficiency virus type 1 infection status. *J. Infect. Dis.* 176:1093–96
159. Mandl CW, Aberle SW, Henkel JH, Puchhammer-Stockl E, Heinz FX. 1998. Possible influence of the mutant CCR5 allele on vertical transmission of HIV-1. *J. Med. Virol.* 55:51–55
160. Quillent C, Oberlin E, Braun J, Rousset D, Gonzalez-Canali G, Metais P, Montagnier L, Virelizier J-L, Arenzana-Seisdedos F, Beretta A. 1998. HIV-1 resistance phenotype conferred by combination of two separate inherited mutations of *CCR5* gene. *Lancet* 351:14–18
161. Biti R, Ffrench R, Young J, Bennetts B, Stewart G, Liang T. 1997. HIV-1 infection in an individual homozygous for the CCR5 deletion allele. *Nature Med.* 3:252–53
162. Theodorou I, Meyer L, Magierowska M, Katlama C, Rouzioux C. 1997. HIV-1 infection in an individual homozygous for CCR5 delta 32. *Lancet* 349:1219–20
163. Michael NL, Nelson JAE, Kewalramani VN, Chang G, O'Brien SJ, Mascola JR, Volsky B, Louder M, White GC, Littman DR, Swanstrom R, O'Brien TR. 1998. Exclusive and persistent use of the entry coreceptor CXCR4 by human immunodeficiency virus type 1 from a subject homozygous for CCR5 Delta 32. *J. Virol.* 72:6040–47
164. Martinson JJ, Chapman NH, Rees DC, Liu YT, Clegg JB. 1997. Global distribution of the CCR5 gene 32-basepair deletion. *Nature Genet* 16:100–3
165. Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, Carrington M, Winkler C, Huttley GA, Allikmets R, Schriml L, Gerrard B, Malasky M, Ramos MD, Morlot S, Tzetis M, Oddoux

C, di Giovine FS, Nasiolas G, Chandler D, Aseev M, Hanson M, Kalaydjieva L, Glavac D, Gasparini P, Kanavakis E, Claustres M, Kambouris M, Ostrer H, Duff G, Baranov V, Sibul H, Metspalu A, Goldman D, Martin N, Duffy D, Schmidtke J, Estivill X, O'Brien SJ, Dean M. 1998. Dating the origin of the CCR5-Delta 32 AIDS-resistance allele by the coalescence of haplotypes. *Am. J. Hum. Genet.* 62:1507–15

166. Libert F, Cochaux P, Beckman G, Samson M, Aksenova M, Cao A, Czeizel A, Claustres M, de la Rua C, Ferrari M, Ferrec C, Glover G, Grinde B, Guran S, Kucinskas V, Lavinha J, Mercier B, Ogur G, Peltonen L, Rosatelli C, Schwartz M, Spitsyn V, Timar L, Beckman L, Parmentier M, Vassart G. 1998. The Delta ccr5 mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in Northeastern Europe. *Hum. Mol. Genet.* 7:399–406
167. Simonsen JN, Fowke KR, MacDonald KS, Plummer FA. 1998. HIV pathogenesis: mechanisms of susceptibility and disease progression. *Curr. Opin. Microbiol.* 1:423–29
168. Zagury D, Lachgar A, Chams V, Fall LS, Bernard J, Zagury JF, Bizzini B, Gringeri A, Santagostino E, Rappaport J, Feldman M, O'Brien SJ, Burny A, Gallo RC. 1998. C-C chemokines, pivotal in protection against HIV type 1 infection. *Proc. Natl. Acad. Sci. USA* 95:3857–61
169. Paxton WA, Liu R, Kang S, Wu LJ, Gingeras TR, Landau NR, Mackay CR, Koup RA. 1998. Reduced HIV-1 infectability of CD4(+) lymphocytes from exposed-uninfected individuals: association with low expression of CCR5 and high production of beta-chemokines. *Virology* 244:66–73
170. Fauci AS. 1996. Host factors and the pathogenesis of HIV-induced disease. *Nature* 384:529–34
171. Eugen-Olsen J, Iversen AKN, Benfield TL, Koppelhus U, Garred P. 1998. Chemokine receptor CCR2b 64I polymorphism and its relation to CD4 T-cell counts and disease progression in a Danish cohort of HIV-infected individuals. *J. Acquired Immune Defic. Syndr.* 18:110–16
172. Meyer L, Magierowska M, Hubert JB, Rouzioux C, Deveau C, Sanson F, Debre P, Delfraissy JF, Theodorou I, the SEROCO Study Group. 1997. Early protective effect of CCR-5 delta 32 heterozygosity on HIV-1 disease progression: relationship with viral load. *AIDS* 11:F73–F78
173. McDermott DH, Zimmerman PA, Guignard F, Kleeberger CA, Leitman SF, Murphy PM. 1998. CCR5 promoter polymorphism and HIV-1 disease progression. *Lancet* 352:866–70
174. Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, Goedert JJ, O'Brien TR, Jacobson LP, Kaslow R, Buchbinder S, Vittinghof E, Vlahov D, Hoots K, Hilgartner MW, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study, O'Brien SJ. 1997. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. *Science* 277:959–64
175. Kostrikis LG, Huang Y, Moore JP, Wolinsky SM, Zhang LQ, Guo Y, Deutsch L, Phair J, Neumann AU, Ho DD. 1998. A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation. *Nature Med.* 4:350–53
176. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, Dean M, Honjo T, Tashiro K, Yabe D, Buchbinder S, Vittinghoff E, Goedert JJ, O'Brien TR, Jacobson LP, Detels R, Donfield S, Willoughby A, Gomperts E, Vlahov D, Phair J, ALIVE Study, Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), O'Brien SJ. 1998. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. *Science* 279: 389–93
177. Cohen OJ, Paolucci S, Bende SM, Daucher M, Moriuchi H, Moriuchi M, Cicala C, Davey RT, Baird B, Fauci AS. 1998. CXCR4 and CCR5 genetic polymorphisms in long-term nonprogressive human immunodeficiency virus infection: lack of association with mutations other than CCR5-Delta 32. *J. Virol.* 72:6215–17
178. Samson M, Soularue P, Vassart G, Parmentier M. 1996. The genes encoding the human CC-chemokine receptors CC-CKR1 to CC-CKR5 (CMKBR1-CMKBR5) are clustered in the p21. 3-p24 region of chromosome 3. *Genomics* 36:522–526

179. Cohen OJ, Vaccarezza M, Lam GK, Baird BF, Wildt K, Murphy PM, Zimmerman PA, Nutman TB, Fox CH, Hoover S, Adelsberger J, Baseler M, Arthos J, Davey RT Jr, Dewar RL, Metcalf J, Schwartzentruber DJ, Orenstein JM, Buchbinder S, Saah AJ, Detels R, Phair J, Rinaldo C, Margolick JB, Fauci AS. 1997. Heterozygosity for a defective gene for CC chemokine receptor 5 is not the sole determinant for the immunologic and virologic phenotype of HIV-infected long-term nonprogressors. *J. Clin. Invest.* 100:1581–89
180. Blaak H, Vantwout AB, Brouwer M, Cornelissen M, Kootstra NA, Albrechtvanlent N, Keet RPM, Goudsmit J, Coutinho RA, Schuitemaker H. 1998. Infectious cellular load in human immunodeficiency virus type 1 (HIV-1)-infected individuals and susceptibility of peripheral blood mononuclear cells from their exposed partners to non-syncytium-inducing HIV-1 as major determinants for HIV-1 transmission in homosexual couples. *J. Virol.* 72:218–24
181. Picchio GR, Gulizia RJ, Mosier DE. 1997. Chemokine receptor CCR5 genotype influences the kinetics of human immunodeficiency virus type 1 infection in human PBL-SCID mice. *J. Virol.* 71:7124–27
182. Garred P, Eugen-Olsen J, Iversen AK, Benfield TL, Svejgaard A, Hofmann B. 1997. Dual effect of CCR5 delta 32 gene deletion in HIV-1-infected patients. Copenhagen AIDS Study Group. *Lancet* 349:1884
183. Rizzardi GP, Morawetz RA, Vicenzi E, Ghezzi S, Poli G, Lazzarin A, Pantaleo G. 1998. CCR2 polymorphism and HIV disease. *Nature Med.* 4:252–53
184. Michael NL, Louie LG, Rohrbaugh AL, Schultz KA, Dayhoff DE, Wang CE, Sheppard HW. 1997. The role of *CCR5* and *CCR2* polymorphisms in HIV-1 transmission and disease progression. *Nature Med.* 3:1160–62
185. Anzala AO, Ball TB, Rostron T, O'Brien SJ, Plummer FA, Rowland-Jones S, Univ. Nairobi Collab. HIV Res. 1998. CCR2-64I allele and genotype association with delayed AIDS progression in African women. *Lancet* 351:1632–33
186. Mellors JW, Rinaldo CR, Gupta P, White RM, Todd JA, Kingsley LA. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272:1167–70
187. Lee B, Doranz BJ, Doms RW. 1998. Influence of the CCR2-V64I polymorphism on human immunodeficiency virus type 1 coreceptor activity and on chemokine receptor function of CCR2b, CCR3, CCR5 and CXCR4. *J. Virol.* 72: 2509–15
188. Mummidi S, Ahuja SS, McDaniel BL, Ahuja SK. 1997. The human CC chemokine receptor 5 (CCR5) gene: multiple transcripts with 5′-end heterogeneity, dual promoter usage, and evidence for polymorphisms within the regulatory regions and noncoding exons. *J. Biol. Chem.* 272:30662–71
189. Carrington M, Kissner T, Gerrard B, Ivanov S, O'Brien SJ, Dean M. 1997. Novel alleles of the chemokine-receptor gene CCR5. *Am. J. Hum. Genet.* 61: 1261–67
190. Carroll RG, Riley JL, Levine BL, Feng Y, Kaushal S, Ritchey DW, Bernstein W, Weislow OS, Brown CR, Berger EA, June CH, St. Louis DC. 1997. Differential regulation of HIV-1 fusion cofactor expression by CD28 costimulation of $CD4^+$ T cells. *Science* 276:273–76
191. Valentin A, Lu WH, Rosati M, Schneider R, Albert J, Karlsson A, Pavlakis GN. 1998. Dual effect of interleukin 4 on HIV-1 expression: implications for viral phenotypic switch and disease progression. *Proc. Natl. Acad. Sci. USA* 95:8886–91
192. Tedla N, Palladinetti P, Kelly M, Kumar RK, DiGirolamo N, Chattophadhay U, Cooke B, Truskett P, Dwyer J, Wakefield D, Lloyd A. 1996. Chemokines and T lymphocyte recruitment to lymph nodes in HIV infection. *Am. J. Pathol.* 148:1367–73
193. Trumfheller C, Tenner-Racz K, Racz P, Fleischer B, Frosch S. 1998. Expression of macrophage inflammatory protein (MIP)-1 alpha, MIP-1 beta, and RANTES genes in lymph nodes from HIV+ individuals: correlation with a Th1-type cytokine response. *Clin. Exp. Immunol.* 112:92–99
194. Margolis LB, Glushakova S, Grivel J-C, Murphy PM. 1998. Blockade of CC chemokine receptor 5 (CCR5)-tropic human immunodeficiency virus-1 replication in human lymphoid tissue by CC chemokines. *J. Clin. Invest.* 101:1876–80
195. D'Aquila RT, Sutton L, Savara A, Hughes MD, Johnson VA. 1998. CCRS/Δ ccr5 heterozygosity: a selective pressure for the syncytium-inducing human immunodeficiency virus type 1 phenotype. *J. Infect. Dis.* 177:1549–53
196. Dolei A, Biolchini A, Serra C, Cur-

reli S, Gomes E, Dianzani F. 1998. Increased replication of T-cell-tropic HIV strains and CXC-chemokine receptor-4 induction in T cells treated with macrophage inflammatory protein (MIP)-1 alpha, MIP-1 beta and RANTES beta-chemokines. *AIDS* 12: 183–90

197. Ostrowski MA, Justement SJ, Catanzaro A, Hallahan CA, Ehler LA, Mizell SG, Kumar PN, Mican JA, Chun TW, Fauci AS. 1998. Expression of chemokine receptors CXCR4 and CCR5 in HIV-1-infected and uninfected individuals. *J. Immunol.* 161:3195–3201
198. Glushakova S, Baibakov B, Zimmerberg J, Margolis LB. 1997. Experimental HIV infection of human lymphoid tissue: correlation of CD4+ T cell depletion and virus syncytium-inducing/non-syncytium-inducing phenotype in histocultures inoculated with laboratory strains and patient isolates of HIV type 1. *AIDS Res. Hum. Retroviruses* 13:461–71
199. Picchio GR, Gulizia RJ, Wehrly K, Chesebro B, Mosier DE. 1998. The cell tropism of human immunodeficiency virus type 1 determines the kinetics of plasma viremia in SCID mice reconstituted with human peripheral blood leukocytes. *J. Virol.* 72:2002–9
200. Weissman D, Rabin RL, Arthos J, Rubbert A, Dybul M, Swofford R, Venkatesan S, Farber JM, Fauci AS. 1997. Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor. *Nature* 389:981–85
201. Davis CB, Dikic I, Unutmaz D, Hill CM, Arthos J, Siani MA, Thompson DA, Schlessinger J, Littman DR. 1997. Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5. *J. Exp. Med.* 186:1793–98
202. Hesselgesser J, Taub D, Baskar P, Greenberg M, Hoxie J, Kolson DL, Horuk R. 1998. Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 alpha is mediated by the chemokine receptor CXCR4. *Curr. Biol.* 8:595–98
203. Herbein G, Mahlknecht U, Batliwalla F, Gregersen P, Pappas T, Butler J, O'Brien WA, Verdin E. 1998. Apoptosis of CD8(+) T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* 395:189–94
204. Madani N, Kozak SL, Kavanaugh MP, Kabat D. 1998. gp120 Envelope glycoproteins of human immunodeficiency viruses competitively antagonize signaling by coreceptors CXCR4 and CCR5. *Proc. Natl. Acad. Sci. USA* 95:8005–10
205. Ueda H, Howard OMZ, Grimm MC, Su SB, Gong WH, Evans G, Ruscetti FW, Oppenheim JJ, Wang JM. 1998. HIV-1 envelope gp41 is a potent inhibitor of chemoattractant receptor expression and function in monocytes. *J. Clin. Invest.* 102:804–12
206. Tiffany HL, Lautens LL, Gao J-L, Pease J, Locati M, Combadiere C, Modi W, Bonner TI, Murphy PM. 1997. Identification of CCR8: a human monocyte and thymus receptor for the CC chemokine I-309. *J. Exp. Med.* 186:165–70
207. Napolitano M, Zingoni A, Bernardini G, Spinetti G, Nista A, Storlazzi CT, Rocchi M, Santoni A. 1996. Molecular cloning of TER1, a chemokine receptor-like gene expressed by lymphoid tissues. *J. Immunol.* 157:2759–63
208. McCune JM. 1997. Thymic function in HIV-1 disease. *Semin. Immunol.* 9:397–404
209. Arenzana-Seisdedos F, Virelizier J-L, Rousset D, Clark-Lewis I, Loetscher P, Moser B, Baggiolini M. 1996. HIV blocked by chemokine antagonist. *Nature* 383:400
210. Simmons G, Clapham PR, Picard L, Offord RE, Rosenkilede NM, Schwartz TW, Buser R, Wells TNC, Proudfoot AE. 1997. Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science* 276:276–79
211. Struyf S, De Meester I, Scharpe S, Lanaerts JP, Menten P, Wang JM, Proost P, Van Damme J. 1998. Natural truncation of RANTES abolishes signaling through the CC chemokine receptors CCR1 and CCR3, impairs its chemotactic potency and generates a CC chemokine inhibitor. *Eur. J. Immunol.* 28:1262–71
212. Clemons MJ, Marshall E, Durig J, Watanabe K, Howell A, Miles D, Earl H, Kiernan J, Griffiths A, Towlson K, DeTakats P, Testa NG, Dougal M, Hunter MG, Wood LM, Czaplewski LG, Millar A, Dexter TM, Lord BI. 1998. Randomized phase-II study of BB-10010 (macrophage inflammatory protein-1 alpha) in patients with advanced breast cancer receiving 5-fluorouracil, adriamycin, and cyclophosphamide chemotherapy. *Blood* 92:1532–40
213. Heveker N, Montes M, Germeroth L,

Amara A, Trautman A, Alizon M, Schneider-Mergener J. 1998. Dissociation of the signalling and antiviral properties of SDF-1-derived small peptides. *Curr. Biol.* 8:369–76

214. Nishiyama Y, Murakami T, Kurita K, Yamamoto N. 1998. Synthesis of some peptides corresponding to the active region of RANTES for chemotaxis and evaluation of their anti-human immunodeficiency virus-1 activity. *Chem. Pharmacolog. Bull.* 45:2125–27
215. Doranz BJ, Grovit-Ferbas K, Sharron MP, Mao SH, Goetz MB, Daar ES, Doms RW, O'Brien WA. 1997. A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor. *J. Exp. Med.* 186:1395–1400
216. Schols D, Struyf S, Van Damme J, Este JA, Henson G, De Clercq E. 1997. Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J. Exp. Med.* 186:1383–88
217. Murakami T, Nakajima T, Koyanagi Y, Tachibana K, Fujii N, Tamamura H, Yoshida N, Waki M, Matsumoto A, Yoshie O, Kishimoto T, Yamamoto N, Nagasawa T. 1997. A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection. *J. Exp. Med.* 186:1389–93
218. Howard OMZ, Openheim JJ, Hollingshead MG, Covey JM, Bigelow J, McCormack JJ, Buckheit RW, Clanton DJ, Turpin JA, Rice WG. 1998. Inhibition of in vitro and in vivo HIV replication by a distamycin analogue that interferes with chemokine receptor function: a candidate for chemotherapeutic and microbicidal application. *J. Med. Chem.* 41:2184–93
219. Datema R, Rabin L, Hincenbergs M, Moreno MB, Warren S, Linquist V, Rosenwirth B, Seifert J, McCune JM. 1996. Antiviral efficacy in vivo of the anti-human immunodeficiency virus bicyclam SDZ SID 791 (JM 3100), an inhibitor of infectious cell entry. *Antimicrob. Agents Chemother.* 40:750–54
220. Levine BL, Cotte J, Carroll RG, Riley JL, Small CS, Francomano TN, Weislow OS, St. Louis DC, Bernstein W, June CH. 1997. A phase I dose-escalation study of polyclonal CD4 T cell ex vivo expansion for immune system restoration of HIV infection. *J. Aller. Clin. Immunol.* 99, Part 2, Suppl.:1625
221. Goila R, Banerjea AC. 1998. Sequence specific cleavage of the HIV-1 coreceptor CCR5 gene by a hammer-head ribozyme and a DNA-enzyme: inhibition of the coreceptor function by DNA-enzyme. *FEBS Lett.* 436:233–38
222. Chen JD, Bai X, Yang AG, Cong Y, Chen SY. 1997. Inactivation of HIV-1 chemokine co-receptor CXCR-4 by a novel intrakine strategy. *Nature Med.* 3:1110–16
223. Yang AG, Bai X, Huang XF, Yao C, Chen S. 1997. Phenotypic knockout of HIV type 1 chemokine coreceptor CCR-5 by intrakines as potential therapeutic approach for HIV-1 infection. *Proc. Natl. Acad. Sci. USA* 94:11567–72
224. Mebatsion T, Finke S, Weiland F, Conzelmann KK. 1997. A CXCR4/CD4 pseudotype rhabdovirus that selectively infects HIV-1 envelope protein-expressing cells. *Cell* 90:841–47
225. Schnell MJ, Johnson JE, Buonocore L, Rose JK. 1997. Construction of a novel virus that targets HIV-1-infected cells and controls HIV-1 infection. *Cell* 90:849–57
226. Endres MJ, Jaffer S, Haggarty B, Turner JD, Doranz BJ, O'Brien PJ, Kolson DL, Hoxie JA. 1997. Targeting of HIV- and SIV-infected cells by CD4-chemokine receptor pseudotypes. *Science* 278:1462–64
227. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutnai H, Kishimoto T. 1996. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* 382: 635–38
228. Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, Kitamura Y, Matsushima K, Nishikawa S, Kishimoto T, Nagasawa T. 1998. The chemokine rceptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* 393:591–94
229. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. 1998. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* 393:595–99
230. Martin MP, Dean M, Smith MW, Winkler C, Gerrard B, Michael NL, Lee B, Doms RW, Margolick J, Buchbinder S, Goedert JJ, O'Brien TR, Hilgartner MW, Vlahov D, O'Brien SJ, Carrington M. 1998. Genetic acceleration of AIDS progression by a promoter variant of CCR5. *Science* 282:1907–11

Annual Review of Immunology
Volume 17, 1999

CONTENTS