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HIV and the 7-Transmembrane Domain Receptors

Abstract

The recent discovery of a chemokine receptor, fusin (fusin/CXCR-4), as the long-sought human immunodeficiency virus type 1 (HIV-1) coreceptor opened an entirely new field of aquired immunodeficiency syndrome (AIDS) research on mechanisms of viral entry, tropism and pathogenesis. It was soon followed by the identification of the chemokine receptor CCR-5 as the major macrophage-tropic (M-tropic) HIV-1 coreceptor and the demonstration that other chemokine receptors, CCR-3 and CCR-2b, also may serve as coreceptors, albeit at somewhat lower efficiency. Very recently it was demonstrated that the mechanism of the coreceptor function involves the formation of a complex on the cell surface between the HIV-1 envelope, the primary receptor CD4 and the coreceptor. Thus the prevention of the HIV-1 envelope glycoprotein-mediated fusion by the chemokines RANTES, macrophage inflammatory protein-1α (MIP-1α) and MIP-1β, as well as by the recently identified fusin/CXCR-4 ligand, stromal cell-derived factor-1 (SDF-1) could be explained by disruption of that complex. Interestingly, the identification of the HIV-1 coreceptor CCR-5 not only provided new insights into the mechanisms of viral entry and tropism, but also may help in explaining why some people with genetic alterations in CCR-5 are protected from HIV-1 infection.

Key Words

HIV-1
Receptors
Chemokines
Fusion

Accessory Fusion Molecules Are Required in Addition to CD4 for HIV-1 Entry

Entirely new directions in acquired immunodeficiency syndrome (AIDS) research have been afforded to scientists this year stemming from the milestone discovery by Feng et al. [1] of a human immunodeficiency virus type 1 (HIV-1) entry cofactor. It has long been known that the principle cell types targeted and infected by HIV-1 in vivo are the helper T lymphocytes and cells of the monocyte-macrophage lineage via the CD4 receptor pathway, the primary high affinity receptor for HIV-1 [2, 3]. However, known almost as long is that HIV-1 does not enter and infect most nonhuman cell lines even upon expression of the human CD4 antigen [4–8], whereas expression of the CD4 antigen renders most, but not all, human cell types infectable by HIV-1 [8–13]. These earlier experiments suggested that either an additional component, perhaps a coreceptor, is required, or the block in entry is due to an inhibitory component of the nonpermissive cells. It was later demonstrated that this block could be overcome by forming stable [14] or transient [12, 15, 16] hybrids with human cells and therefore an additional human component(s) was acting as an HIV-1 coreceptor. Experiments with cell lines expressing tailless human CD4 demonstrated that accessory fusion molecules, most likely the same as the additional human component(s), can be downmodulated by phorbol esters and that downmodulation can be primed through an interaction with the gp120 component of the HIV-1 envelope (env) [17, 18]. It was also revealed that membrane vesicles alone could transfer the required human cell component and that this component is relatively stable to protease and heat treatment [19, 20]. These findings further solidified the possibility that there is an essential human-cell cofactor(s) or coreceptor(s) residing in the plasma membrane of human cell that is required for the fusion process rather than the presence of an inhibitor of membrane fusion.

The CXC Chemokine Receptor, Fusin/CXCR-4, Is the Long-Sought HIV-1 Coreceptor

There have been more than a half-dozen molecules proposed to be accessory factors involved in HIV-1 envelope (gp120-gp41)-CD4 mediated membrane fusion and viral entry [reviewed in 21]. However, none have fulfilled the requirements of supporting CD4-gp120-gp41-me-

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diated virus-cell and cell-cell fusion in a normally nonpermissive system [13, 21–28]. The breakthrough came when Feng and coworkers [1] used a highly sensitive screening technique for the functional identification of a cDNA(s) that upon expression could render a nonhuman cell expressing CD4 permissive for HIV-1 envelope-mediated membrane fusion. This screening assay was a recombinant vaccinia virus-based transient expression system in which fusion between env-expressing and receptor-expressing cells leads to activation of a reporter gene *(Escherichia coli lacZ)* [29]. This allowed the functional identification of a seven-transmembrane domain molecule (named initially fusin but recently CXCR-4) that could serve as a fusion cofactor for T cell line-tropic (T-tropic) HIV-1 isolates [1], and this finding was quickly confirmed [30]. As a point of clarification, Feng and coworkers referred to fusin/CXCR-4 as a fusion cofactor not coreceptor. Referring to the molecule as a coreceptor would have implied that binding interactions were occurring between the HIV-1 env, CD4, and fusin/CXCR-4. Although a favored model by many researchers including us, data to support a coreceptor function for fusin/CXCR-4 has only just been obtained (discussed below). We therefore use the terms cofactor and coreceptor interchangeably in this review. Expression of fusin/CXCR-4 in conjunction with CD4 renders a variety of nonhuman cells fully permissive for HIV-1 envelope-mediated membrane fusion and infection, including murine, mink, feline, quail, and simian cell lines, as well as some human cell lines otherwise resistant to HIV-1 entry. Thus, fusin/CXCR-4 fulfills the requirements of a cofactor in the HIV-1 envelope-mediated membrane fusion process. However, fusin/CXCR-4 does not serve as a cofactor for major macrophage-tropic (M-tropic) isolates of HIV-1. The hints for the identification of coreceptors for M-tropic isolates of HIV-1 came from the analysis of the structure of the fusin/CXCR-4 molecule and the finding that chemokines inhibit infection by M-tropic isolates of HIV-1 but not by T-tropic HIV-1.

CCR-5 and Other Chemokine Receptors Serve as Coreceptors for M-Tropic Isolates of HIV-1

Analysis of the fusin/CXCR-4 cDNA revealed that the protein is a member of the superfamily of G protein-coupled receptors possessing seven transmembrane domains. The longest open reading frame of the coding strand is 352 amino acids, and the nucleotide sequence had been identified and reported earlier by several groups [31–35]. Fusin/CXCR-4 is most closely related to the CXC chemokine receptors, having approximately 33% homology [32–35], and the CXC chemokine stromal cell-derived factor-1 (SDF-1) was recently identified as its ligand [36, 37]. Chemokines (chemoattractant cytokines) are a superfamily of homologous serum proteins of between 7 and 16 kD that were originally characterized by their ability to induce migration of leukocytes [reviewed in 38]. The chemokines are divided into two major subgroups, based on structural properties and chromosomal location. The structural hallmark that is conserved in both groups is the general positioning of four cysteine residues. The α-subfamily, (CXC subfamily) is distinguished from the β-subfamily (CC subfamily) by the insertion of a single amino acid between the first and second cysteine residues. The CC chemokines RANTES (regulated on activation normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, and MIP-1β were recently shown [39] to suppress HIV-1 infection in the human PM1 cell line. These chemokines were particularly effective in suppression of M-tropic isolates of HIV-1, and these findings have been confirmed [40]. Taken together, these data led several groups to hypothesize that the suppressive activities exhibited by these CC chemokines were derived from their binding to a chemokine receptor that serves as a fusion cofactor for M-tropic HIV-1 isolates, resulting in the inhibition of env-mediated membrane fusion and viral entry. These groups soon demonstrated that the CC chemokine receptor, CCR-5 [41–43], possessing the chemokine binding specificity profile corresponding to that or suppression of HIV-1 infection [39, 40], could serve as a fusion cofactor for M-tropic HIV-1 isolates [44–48]. As in the case of fusin/CXCR-4 for T-tropic HIV-1 isolates, CCR-5 renders nonhuman cells expressing CD4 completely permissive for M-tropic, but not T-tropic env-mediated membrane fusion and syncytia formation. However, it was also evident that these chemokines not only do not inhibit infection of macrophages by some HIV-1 isolates [48] but can even enhance it [49], suggesting a new yet to be identified coreceptor.

Structural Models of the HIV-1 Coreceptors as 7-Transmembrane Domain Proteins

Despite only 30% amino acid homology including conserved amino acid changes, fusin/CXCR-4 and CCR-5 are likely to share a similar membrane topology, and it may be that the native conformation of these cofactors, as they exist in the plasma membrane, is the critical feature that allows them to serve as fusion cofactors. A general structural model for 7-transmembrane (7TM) domain proteins has been proposed [reviewed in 38, 50] (depicted in fig. 1). The major structural features are an extracellu-

lar amino-terminus (N-terminus), an intracellular carboxyl-terminus (C-terminus), seven α-helical transmembrane domains arranged perpendicularly to the plasma membrane with several conserved proline residues, and three intracellular and three extracellular loops composed of hydrophilic amino acids with highly conserved cysteine residues in the first and second extracellular loops that are predicted to form a disulfide bond thought to stabilize the receptor structure [51]. Interestingly, there are no landmark residues or sequence motifs that are common to all chemokine receptors that can distinguish them from other types of seven-transmembrane domain proteins. However, there are several common features that place them in a distinct family within the seven-transmembrane domain superfamily [reviewed in 38]. Fusin/CXCR-4 and CCR-5 are similar in length, both are 352 amino acids and both possess a highly acidic N-terminus. Fusin/CXCR-4 contains two potential N-linked glycosylation sites, one in the N-terminus where most G protein-coupled receptors also contain such a consensus sequence, and one in the second extracellular loop. CCR-5 possesses only one N-linked glycosylation site in the third extracellular loop. The C termini of both molecules are rich in conserved serine and threonine residues and represent potential phosphorylation sites by the family of G protein-coupled receptor kinases following ligand binding. Also, their RNAs are expressed in leukocytes. There are now nine functional receptors for chemokines known in humans: 4 CXC and 5 CC. Two CXC receptors for interleukin-8 [52] have been described as well as the recently identified receptor for the monokine induced by γ-interferon (Mig) and the interferon-inducible protein 10 [53] and SDF-1 [36, 37]. The remaining five receptors are in the CC subfamily, designated CCR-1 through 5. CCR-1 binds RANTES, MIP-1α, and MCP (monocyte chemoattractant protein)-3 [54]. CCR-2 binds MCP-1, and MCP-3 [55–57]. CCR-3 binds Eotaxin [42]. CCR-4 binds MIP-1α, RANTES, and MCP-1 [58] and CCR-5 binds RANTES, MIP-1α, and MIP-1β [43]. Whether any of these related members can serve as fusion cofactors with many HIV-1 isolates has not been extensively analyzed, though it has recently been demonstrated that CCR-3 as well as CCR-2b exhibit fusion cofactor function with some isolates of HIV-1 [45, 47].

Why Does HIV-1 Use 7TM Proteins as Coreceptors?

Why has HIV-1 acquired this dependence on these 7TM receptors as necessary cofactors? Several viruses use as their receptors cell surface molecules that are members of the Ig gene superfamily: HIV uses CD4, rhinoviruses uses ICAM-1, and poliovirus uses an as yet functionally unknown molecule [reviewed in 59]. In addition, other retroviruses utilize different multiple membrane-spanning proteins. Perhaps HIV-1 has developed a dependence on CD4 and fusin/CXCR-4 or CCR-5 because they, i.e. CD4 and 7TM receptor, are colocalized in the cell membrane. It may be that CD4 and some 7TM receptors have some interacting role in the cells of the immune system. If this is true it might help explain why primary macrophages expressing both fusin/CXCR-4 and CCR-5 are predominately only a target for M-tropic isolates of HIV-1. A possible model is that the very limiting supply of CD4 in a macrophage or monocyte is preferentially associated with CCR-5 and the fusin/CXCR-4 molecule is left unassociated, thereby allowing only the M-tropic isolates a pathway to infect the cell. We have found that primary monocytes are a target for only M-tropic HIV-1 env-mediated membrane fusion when endogenous levels of CD4 are available [Broder et al., unpubl. data]. However, elevation of the CD4 levels by recombinant vaccinia virus vectors renders these monocytes equally susceptible to both T-tropic and M-tropic HIV-1 env-mediated membrane fusion [Broder et al., unpubl. data], suggesting that there is now enough CD4 to associate with both fusin/CXCR-4 and CCR-5. It also seems plausible that the HIV-2 variants known to infect CD4 negative cells [11] are utilizing only the 7TM cofactor as its primary receptor, i.e. fusin/CXCR-4 or CCR-5. This has recently been demonstrated by Hoxie and co-workers [pers. commun]. They found that fusin/CXCR-4 is able to function as an alternative receptor for some isolates of HIV-2 in the absence of CD4. The evidence for this finding was multifold: (1) the CD4-independent infection by these viruses is inhibited by an antifusin/

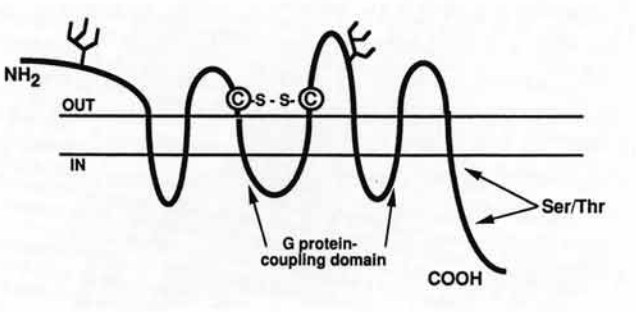

Fig. 1. Typical structural features and a model for the 7TM domain chemokine receptor proteins. The amino terminus (NH_2), carboxy terminus (COOH), -S-S-proposed disulfide bond, 2 potential N-linked glycosylation sites, the serine- and threonine-rich cytoplasmic region, and the conserved cytoplasmic domains proposed to be involved in G protein-coupled signaling are indicated [adapted from 38].

CXCR-4 monoclonal antibody, and (2) recombinant fusin/CXCR-4 expression rendered human and nonhuman CD4-negative cell lines sensitive to HIV-2-induced syncytium induction and/or infection. These data led Hoxie and coworkers to suggest that the HIV env glycoprotein contains a binding site for these proteins and perhaps differences in the affinity and/or the availability of this site can extend the host range of these viruses to include a number of CD4-negative cell types.

How Do the Coreceptor Help in Mediating HIV-1 Entry and Determine Tropism?

In order to participate in the viral entry process the HIV-1 coreceptors must interact directly or indirectly with the HIV-1 envelope glycoprotein (the gp120-gp41 complex) which serves two important functions: gp120 binds to CD4 and gp41 induces membrane destabilization and merging. This high-affinity interaction between gp120 and CD4 is the first step in what is most likely a complex cascade of further events that ultimately results in a pH-independent fusion of the virion and host cell membranes, thus permitting virus entry [reviewed in 21, 60–62]. An analogous mechanism mediates cell-cell fusion between gp120-gp41-expressing and CD4-expressing cells, resulting in the formation of multinucleated giant cells (syncytia). HIV-1 env-mediated fusion may be triggered in part by specific CD4 receptor-induced conformational changes in the gp120-gp41 structure that presumably leads to the exposure of the hydrophobic amino-terminal fusion peptide sequence of the transmembrane subunit gp41. Although the postbinding molecular interactions in the CD4-gp120-gp41-mediated membrane fusion event remain obscure and poorly understood, there have been several notable observations that suggest that specific conformational changes occur in the CD4-gp120-gp41 complex following binding; increased exposure of antibody epitopes on gp120 and gp41 [63, 64], increased cleavage of the V3 loop by exogenous proteinases [63, 65, 66], dissociation of gp120 from the surface of virions and gp120-gp41-expressing cells [63, 67–73], as well as alterations in CD4 structure as detected by immunological and biochemical techniques [74–76].

The identification of two classes of HIV-1 coreceptors mediating entry of either T-tropic or M-tropic isolates indicates a possible mechanism of coreceptor function. It has been known that individual isolates of HIV-1 exhibit distinct tropisms in their abilities to infect different CD4 expressing cell types [reviewed in 21, 77–79]. Most isolates obtained directly from infected individuals during the asymptomatic phase replicate efficiently in primary macrophages but poorly in CD4+ continuous cell lines (e.g., T cell lines, HeLaCD4 transformants), these strains are referred to as M-tropic. Other isolates obtained from patients during the symptomatic phase, and also selected after long-term virus propagation in T cell lines, show the opposite selectivity, replicating more efficiently in CD4+ continuous cell lines than in primary macrophages, such strains are T-tropic. The viral determinants for this cytotropism are located in the env gene, with particular importance assigned to the V3 loop [reviewed in 21, 77]. These distinct cytotropisms, though not absolute [80, 81], have been correlated to different states of disease progression [78] and may be influential during viral transmission between individuals [82]. Utilizing direct assays of env-mediated membrane fusion, it was demonstrated that the observed cytotropism of different virus isolates is due to the inherent membrane fusion selectivities of those isolate env glycoproteins [83]. This led to the speculation that like the observed species restriction in env-mediated membrane fusion, HIV-1 cellular tropism is a reflection of a particular isolate dependency on a particular fusion cofactor [84].

So how and what role do these cofactors play in HIV-1 env-mediated membrane fusion and virus entry? The simplest model is that fusin/CXCR-4 (for T-tropic envs) and CCR-5 (for M-tropic envs) function directly as coreceptors in concert with CD4. The cofactors may interact with different regions of the env oligomer, perhaps binding to the V3 (and V2 and V1) loops (fig. 2). It is well established that CD4 alone can induce conformational alterations in the oligomeric env structure, perhaps these cofactors induce a second set of conformational changes that ultimately trigger env fusion activity. Alternatively, the initial CD4 binding interaction may generate a conformationally unique env oligomer-CD4 complex that in turn binds to the cofactor and triggers the env fusiogenic activity. Evidence for a conformationally unique env-CD4 complex is supported by the identification of monoclonal antibodies raised against CD4 or gp120 that bind better to the gp120-CD4 complex than to the individual proteins [74, 85]. These antibodies may be recognizing either combinatorial epitopes in the complex or conformationally dependent neoepitopes that are generated by the env-CD4 association. A third possibility is that the coreceptor interacts relatively weakly with CD4 and that interaction is increased upon binding to gp120 leading to conformational changes required for fusion. Recent findings by Golding et al. [18] do suggest that there is an association of env-CD4 complexes with additional accessory transmembrane molecules. It was found that the phorbol ester myristate acetate (PMA) inhibits HIV-1 env-mediated membrane fusion by inducing downmodulation of an accessory component in the

CD4-expressing cell in a model involving phosphorylation and endocytosis via clathrin-coated pits [17]. In addition, full-lenght CD4, but not truncated CD4 (no cytoplasmic tail) [86, 17], are downmodulated in a phosphorylation-dependent manner [87]. Interestingly, downmodulation of tailless CD4 by PMA could be achieved if the cells were first preincubated with soluble gp120 env glycoprotein [18], but only when tailless CD4 was expressed in human cells. These findings suggested that the gp120-CD4 complex associates with an accessory transmembrane molecule that is susceptible to PMA-induced downmodulation. Fusin/CXCR-4 does contain conserved cytoplasmic regions predicted to associate with G proteins as well as several conserved phosphorylation sites that may be involved in signal transduction and receptor recycling. It will be interesting to examine whether introduction of fusin/CXCR-4 into a nonhuman cell expressing tailless CD4 could restore the priming effect by soluble gp120. Data to support a model in which interactions occur between HIV-1 env and cofactor has very recently been obtained through the further examination of this accessory transmembrane molecule that is susceptible to PMA-induced downmodulation. We showed that this molecule is in fact fusin/CXCR-4 [88]. Lapham and coworkers [88] were able to coprecipitate HIV-1 env, CD4, and a third 45-kD protein specifically from human cells. They then demonstrated that this 45-kD protein was fusin/CXCR-4 through the use of specific antibody.

What regions in fusin/CXCR-4 or CCR-5 are important for this association to the HIV-1 gp120-gp41-CD4 complex? It was demonstrated by Feng et al. [1] that a polyclonal rabbit antisera to the fusin/CXCR-4 amino terminus could block env-mediated cell fusion and virus infection implicating a possible role for this region in the fusion process. The amino terminus of several chemokine receptors has been shown to be a major determinant in defining ligand specificity [reviewed in 89]. Complementing this work are recent findings by Rucker et al. [90] which demonstrate an important role for the amino terminus of CCR-5 in the env-mediated fusion activity of M-tropic and dual tropic HIV-1 isolates [90]. Through the use of an extensive panel of hybrid constructs and deletion mutants generated from CCR-5 and CCR-2b, it was determined that the first 20 amino-terminal residues of CCR-5 are important for cofactor activity yet individual residues were differentially critical for either M-tropic versus dual tropic isolate envs. In addition, importance of the first extracellular loop of CCR-5 was also evident. These findings suggest that the interactions between HIV-1 env, CD4, and coreceptor are structurally complex and further studies will be required to better define the contact sites and other critical determinants for fusion cofactor activity. A possible model for the interactions between the CD4-gp120-gp41 and the HIV-1 coreceptors leading to fusion is shown in figure 2.

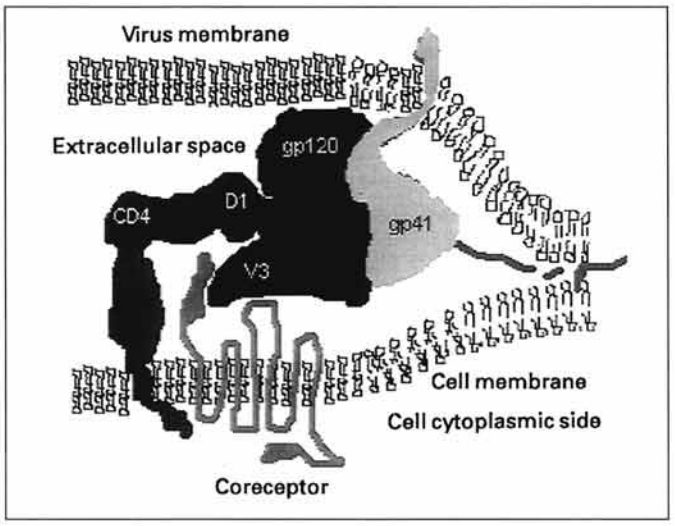

Fig. 2. A sketch of possible interactions between the CD4-gp120-gp41 complex and HIV-1 coreceptors (fusin/CXCR-4, CCR-5 or else) leading to fusion. (On the right side fusion peptides from other gp41 molecules participating in the fusion complex are shown.) The conformational changes induced by those interactions lead to a close approach of the two membranes and their destabilization and fusion induced by the exposure of the fusion peptide. The V3 loop is depicted as the major part of the gp120 interacting with the HIV-1 coreceptors because of the correlation between the virus tropism, V3 loop sequence and type of coreceptors. However, the effect of the V3 loop may be indirect through V2 or V1 loop. In addition, not only the N-terminus of the HIV-1 coreceptor but also the extracellular loops may contribute to the interaction. Also the structure of the HIV-1 coreceptor is more likely to form a circle rather than a linear array of transmembrane domains as shown here for the purpose of illustration [modified from 103].

Yet another possibility is that the activities of the HIV-1 coreceptors may be indirect, and involve G protein signaling and the activation of some effector system. A possible intriguing aspect of this complex interaction is that among the many effector systems downstream of G protein-coupled pathways are the activation of phospholipase C and D which may ultimately play a role in the critical membrane destabilization events which fuse the membranes between the virus and target cell. However, because of the rapid kinetics of virus-cell fusion it seems less likely that such a signaling scenario is taking place, rather there may be some involvement of such an effector system activation pathway in syncytium formation. The kinetics of syncytium formation and the size of syncytia generated are dependent on the cell types used, as is G protein-coupled signaling. Taken together, it seems highly plausible based on the work of

Golding et al. and Hoxie et al., that there are interactions occurring between the HIV env and coreceptor. Exploiting this information in the development of new methods of intervention will be an important area of future research. Other related questions to be addressed are what are the important regions in HIV-1 env, and perhaps CD4, critical for these interactions.

Primary HIV-1 Isolates Can Use Two or More Coreceptors

To date, comparatively little work has been performed on the fusion activity of envs from primary isolates of HIV-1. It is also important to point out that most primary HIV-1 isolates, unlike the laboratory-adapted strains, exhibit some dual tropic characteristics. The dual tropic isolates can infect both primary macrophages and T cell lines [80], and perhaps this reflects their ability to utilize more than one cofactor. We predicted that the env glycoproteins from dual tropic viruses, like HIV-1 89.6, would be able to function with both fusin/CXCR-4 and CCR-5, and this has recently been demonstrated by others [47]. In general, the diversity of env glycoprotein sequences from the various strains of virus and the large number of 7TM domain proteins homologous to fusin/CXCR-4 and CCR-5 also led us to predict that distinct fusion cofactors/coreceptors exist for HIV-2 and simian immunodeficiency virus as well [1]. As mentioned above, it appears that at least some strains of HIV-2 utilize fusin/CXCR-4 as a coreceptor. Also, we have observed some primary clade B isolates utilizing fusin/CXCR-4 and not CCR-5 as a cofactor and both syncytium-inducing and non-syncytium-inducing clade E isolates utilizing CCR-5 and not fusin/CXCR-4 [Broder et al., unpubl. data]. It will therefore be critical to examine the role of fusin/CXCR-4 and CCR-5 in the fusion process mediated by envs from a variety of strains including primary HIV-1 isolates, particularly primary isolates of HIV-1 representing all of the HIV-1 subtypes (clades), as well as examine other related 7TM domain proteins for cofactor activity.

Implications for Pathogenesis and Therapy

The identification of distinct fusion cofactors for HIV-1 isolates with different cytotropisms raises several interesting questions about transmission, pathogenesis, animal models of pathogenesis, and new therapeutics. It has been reported [40] that for some individuals who remain uninfected despite repeated high risk exposure, their CD4+ T lymphocytes are relatively resistant to in vitro infection by M-tropic isolates, but susceptible to T-tropic variants. The viral determinants for this distinction have been mapped to the env glycoprotein. CD4+ T lymphocytes from exposed uninfected individuals secreted greater amounts of RANTES, MIP-1α and MIP-1β compared to nonexposed control subjects. One hypothesis suggested by the authors invoked the involvement of putative second receptors [40]. Because of the resistance to macrophage-tropic isolates, the importance of examining the expression levels and functionality of CCR-5 in cells from such individuals became clear, and it has been recently demonstrated that a homozygous defect in CCR-5 expression correlates with resistance of some multiply-exposed individuals to HIV-1 infection [91, 92]. A similar concept applies in considering possible relationships of fusion cofactors to long-term nonprogression of some HIV-infected individuals [93]. Isolates with the capacity to infect T cell lines generally appear during the transition from the asymptomatic to the symptomatic stage, coincident with the decline of CD4+ T lymphocytes. It was previously speculated that env interaction with CD4 and fusin/CXCR-4 might contribute to this decline by direct or indirect mechanisms [1]; perhaps defects in the expression or activity of fusin/CXCR-4 and/or related fusion cofactors like CCR-5, or overexpression of the ligand(s) (or a mutant form of the ligand(s)) to these cofactors are the basis for the favorable disease course in some nonprogressors.

The discoveries of fusin/CXCR-4 and CCR-5 as HIV-1 human specific fusion cofactors may also allow for a reassessment of transgenic small animal models for HIV-1 infection. Transgenic mice [94–96] and rabbits [97, 98] expressing human CD4 have been generated, but they support productive HIV-1 replication poorly at best. It is clear that there are additional restrictions in the mouse model in addition to virus entry [reviewed in 99] that may become problematic even with a double transgenic mouse having CD4 and a cofactor. However, rabbit cells do not appear to have the same restrictions observed in mouse cells for HIV-1 replication, and this system may hold more promise [100].

Finally, and perhaps most importantly, the identification of the fusion cofactors provide new targets for the design of novel strategies to treat HIV-1 infection. Indeed, the suppressive activity of RANTES, MIP-1α, and MIP-1β led to the proposal that these chemokines might have therapeutic value [39]. Understanding the molecular basis of how these cofactors function in env-mediated fusion/infection is now an important area of further research. If these cofactors are directly involved in the env-CD4 fusion complex then perhaps therapeutic agents may be developed that could block the HIV-1 infection process. The env-CD4 fusion complex is the target for the inhibitory activity of the

soluble form of CD4 (sCD4), CD4 immunoadhesins [101], and the gp41 peptide DP178 [102]. New drugs may be designed that mimic the relevant structures in fusin/CXCR-4 or CCR-5, and because fusin/CXCR-4 and CCR-5 are host cell proteins the virus is less likely to mutate and find alternative coreceptors under the selective pressures of viral inhibition therapies. In summary, in light of the current findings on specific HIV-1 fusion/infection cofactors, it is imperative that a fuller understanding of the nature of these fusion cofactors be attained.

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Note added in proof

Recent papers by Zhang et al. [1] and Simmons et al. [2] suggested that primary syncytium-inducing isolates can use both CXCR-4 and CCR-5 as coreceptors; the paper by Dean et al. [3] suggested that a deletion allele of CCR-5 may restrict HIV-1 infection and delay progression to AIDS in infected individuals; the papers by Wu et al. [4] and Trkola et al. [5] demonstrated that gp120 can interact directly with CCR-5, but that prior interaction with CD4 greatly increases its affinity, and complement our finding that CXCR-4 associates with the gp120-CD4 complex [88].

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