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MINIREVIEW

Exploitation of Cellular Signaling by HIV-1: Unwelcome Guests with Master Keys That Signal Their Entry

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It has been well established that HIV-1 entry into the target cell involves formation of a trimolecular complex consisting of HIV-1 gp120 envelope, a CD4 receptor, and a chemokine receptor. Although the sequence of events leading to the formation of this complex is not fully understood, it has been proposed that binding of gp120 to CD4 induces conformational changes in both of these interacting molecules.

CD4 is a membrane glycoprotein that has an important role in the activation of peripheral T cells and differentiation of thymocytes. CD4 closely associates with TCR and acts as a signal transducer during T cell activation. Transmission of CD4-induced signaling occurs through activation of tyrosine kinase Lck, a T cell-specific protein kinase (PTK) which associates with the cytoplasmic tail of the CD4 receptor. Activation of Lck is essential for the signaling pathway that controls the activation and proliferation of thymocytes. However, in addition to its role as a tyrosine kinase, Lck can act as an adaptor protein by providing a docking site for the recruitment of downstream signaling molecules. In fact, Lck has the potential to interact through its SH2 and SH3 domains with various target proteins.

The signaling properties of the CD4 receptor seem to be dispensable for HIV-1 entry; however, binding of HIV-1 virions or gp120 glycoprotein to CD4 receptors triggers a broad spectrum of signaling pathways that can modulate the activation status of the cells and/or affect the postentry stages of HIV-1 replication. Thus, cells expressing the truncated form of CD4, which does not associate with Lck, show delayed HIV-1 replication and consequently delayed cell death by apoptosis (Guillerm *et al.,* 1998).

The mechanism by which Lck generates intracellular signals upon HIV-1 binding and the intermediates in the CD4-mediated signaling cascade have not yet been clearly characterized. It was shown that HIV-1 binding stimulates both phosphatidylinositol-3-kinase (PI3-K) and PI4-K (Prasad *et al.,* 1993; Schmid-Antomarchi *et al.,* 1996); however, only PI3-K was found to interact directly with Lck. It was also shown that activated Lck directly associates with and phosphorylates a serine/threonine kinase Raf-1. This Ras-independent Lck/Raf-1 signaling pathway (Popik and Pitha, 1996) feeds into a classical MAPK ERK pathway. Thus, HIV-1 binding to CD4 induces phosphorylation of MAPK ERK, activation of transcription factors NFkB and AP-1, and consequently expression of cytokine and chemokine genes (Briant *et al.,* 1998; Popik and Pitha 1996; Popik *et al.,* 1998).

The outcome of the T cell signaling induced by HIV-1 binding to CD4 can be modulated by the signals elicited by concomitant engagement of other membrane receptors. Thus, crosslinking of CD4 receptors, which transiently stimulates ERK activity, can interfere with the subsequent TCR/CD3-mediated activation of CD4+ T cells by suppressing CD3-induced activation of ERK (Chirmule *et al.,* 1999). These observations indicate that binding of HIV-1 virions to CD4 receptors results in partial activation of T cells and alters their ability to respond to signaling mediated through TCR. Since HIV-1 gp120 interacts also with chemokine receptors and proteoglycans, it is likely that engagement of these molecules may also modify signaling generated by the gp120–CD4-coreceptor complex.

The conformational changes induced in the HIV gp120 molecule upon its binding to the CD4 receptor increase binding affinity of gp120 to the coreceptor (Sattentau *et al.,* 1993; Trkola *et al.,* 1996). These changes are required for fusion of HIV with a cellular membrane. In addition, interaction of gp120 with a chemokine receptor may result in activation of different cellular signaling pathways.

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Chemokine receptors belong to a family of seven transmembrane-spanning receptors that signal through heterotrimeric G proteins composed of a monomeric Gα and heterodimer G$\beta\gamma$ subunits. Upon stimulation with a specific ligand, the cytoplasmic regions of the receptor undergo conformational changes that allow the receptor to interact with G proteins. This association causes further conformational changes in the G proteins that facilitate an exchange of Gα subunit-bound GDP with GTP and leads to dissociation of Gα and G$\beta\gamma$. The activated G protein subunits then bind to and regulate many different cellular effectors (Hamm, 1998; Gutkind, 1998). However, significant evidence has accumulated which shows that G protein-coupled receptors (GPCRs) can signal not only through interaction with G proteins but also with other cellular proteins (Hall *et al.,* 1999).

A spectrum of chemokine receptors that may serve as HIV-1 entry cofactors has been identified. However, a large body of evidence suggests that two of the receptors, CCR5 and CXCR4, play a dominant role in the HIV-1 entry into primary cells (Zhang *et al.,* 1998). CXCR4 was originally identified as an orphan chemokine receptor which is expressed on myeloid cells, neutrophils, and T lymphocytes and was shown to be a coreceptor for entry of T cell tropic (X4) HIV-1. It was later recognized that lymphocyte chemoattractant SDF-1 is a natural ligand for CXCR4 which can inhibit X4 HIV-1 infection of CD4+ T lymphocytes. CCR5 is expressed in lymphoid organs, peripheral T lymphocytes, and macrophages (Raport *et al.,* 1996) and was found to bind a number of chemokines including MIP-1α, MIP-1β, RANTES, and MCP-2. CCR5 was found to be a major coreceptor for entry of macrophage tropic (R5) HIV-1. Accordingly, MIP-1α, MIP-1β, and RANTES are effective inhibitors of HIV-1 infection.

Despite numerous studies that address the role of CXCR4 and CCR5 in HIV-1 infection, relatively little is known about the signaling pathways mediated by these receptors. It was shown that binding of α chemokine SDF-1 to its cognate receptor CXCR4 resulted in phosphorylation of focal adhesion components, including the related adhesion focal tyrosine kinase RAFTK/Pyk2, Crk, and the cytoskeleton protein paxilin (Ganju *et al.,* 1998). Phosphorylation of RAFTK/Pyk2 could also be induced by binding of β chemokines, RANTES and MIP-1β, to the CCR5 receptor. Interestingly, tyrosine phosphorylation of Pyk2 was also triggered by gp120 derived from X4 and R5 HIV-1 strains (Davis *et al.,* 1997), suggesting that binding of the HIV-1 envelope to CXCR4 and CCR5 may generate signaling pathway(s) similar to those initiated by binding of the natural ligands to these receptors. Similarly, phosphorylation of paxilin was not limited to signaling through the CXCR4 receptor but could also be induced by binding of RANTES and MIP-1β to the CCR5 receptor.

Phosphorylation of the focal adhesion components RAFTK/Pyk2, paxilin, and Crk and their association with each other may result in the formation of signaling complexes that have a potential to reorganize actin cytoskeletal structures. Consequently, this event may affect cell motility and cell polarization. Interestingly, changes in the activation of focal adhesion complexes induced by chemokines α and β can be elicited by both T-tropic and macrophage-tropic HIV-1. This observation suggests that some signaling responses induced by HIV-1 binding to chemokine coreceptors may be independent of cellular tropism of the virus. However, some of these responses may also be generated by engagement of the CD4 receptor alone (Cicala *et al.,* 1999).

Recently, new signaling intermediates activated by binding of MIP-1β to CCR5 were described (Ganju *et al.,* 2000). These included the SH2 domain-containing cytoplasmic tyrosine phosphatases SHP1 and SHP2 as well as Src-related kinase Syk. Association of SHP1 with RAFTK, Syk, and Grb2 resulted in formation of a multicomponent signaling complex. While SHP-1 negatively regulated a number of signaling pathways by dephosphorylation of specific tyrosine residues on proteins, SHP2 functioned as a positive regulator. Whether a similar signaling pathway is also induced upon binding of the HIV-1 envelope to the CCR5 receptor remains unknown.

Studies with the chemokine receptor chimeras suggested the existence of multiple receptor domains important for HIV-1 entry that can be separated from the domains that regulate chemokine-mediated signaling responses. However, there are also common structural determinants required for chemokine receptor-mediated signaling and HIV-1 coreceptor activity (Alkhatib *et al.,* 1997). This suggests that chemokines and HIV-1 gp120 may initiate both common and unique, ligand-specific signaling. Indeed, signals induced by binding of chemokines to the receptors do not always mimic the interaction of the HIV-1 envelope with the chemokine receptors. Thus, binding of SDF-1 to CXCR4 was shown to stimulate the MAPK ERK pathway in CD4+ T cells; however, interaction of X4 HIV-1 with the chemokine receptor did not activate this pathway (Popik *et al.,* 1998). In contrast, viruses using CCR5 for entry efficiently activated ERK as well as JNK and p38 MAP kinases (Popik and Pitha, 1998). Similarly, the ionic signaling response induced by the interaction of HIV-1 gp120 with CCR5 or CXCR4 in primary macrophages and the signaling response induced by the interaction of the receptors with their specific ligands MIP-1β and SDF-1α were not identical (Liu *et al.,* 2000). Surprisingly, neither SDF-1α nor MIP-1β activated nonselective cation channels that were opened by R5 and X4 HIV-1 gp120. These results suggest that signaling generated by gp120 does not merely reflect signaling induced by chemokines but, in some instances, represents a unique signaling pathway that may contribute to pathogenesis of HIV-1.

Association between CD4 and CCR5 in the plasma

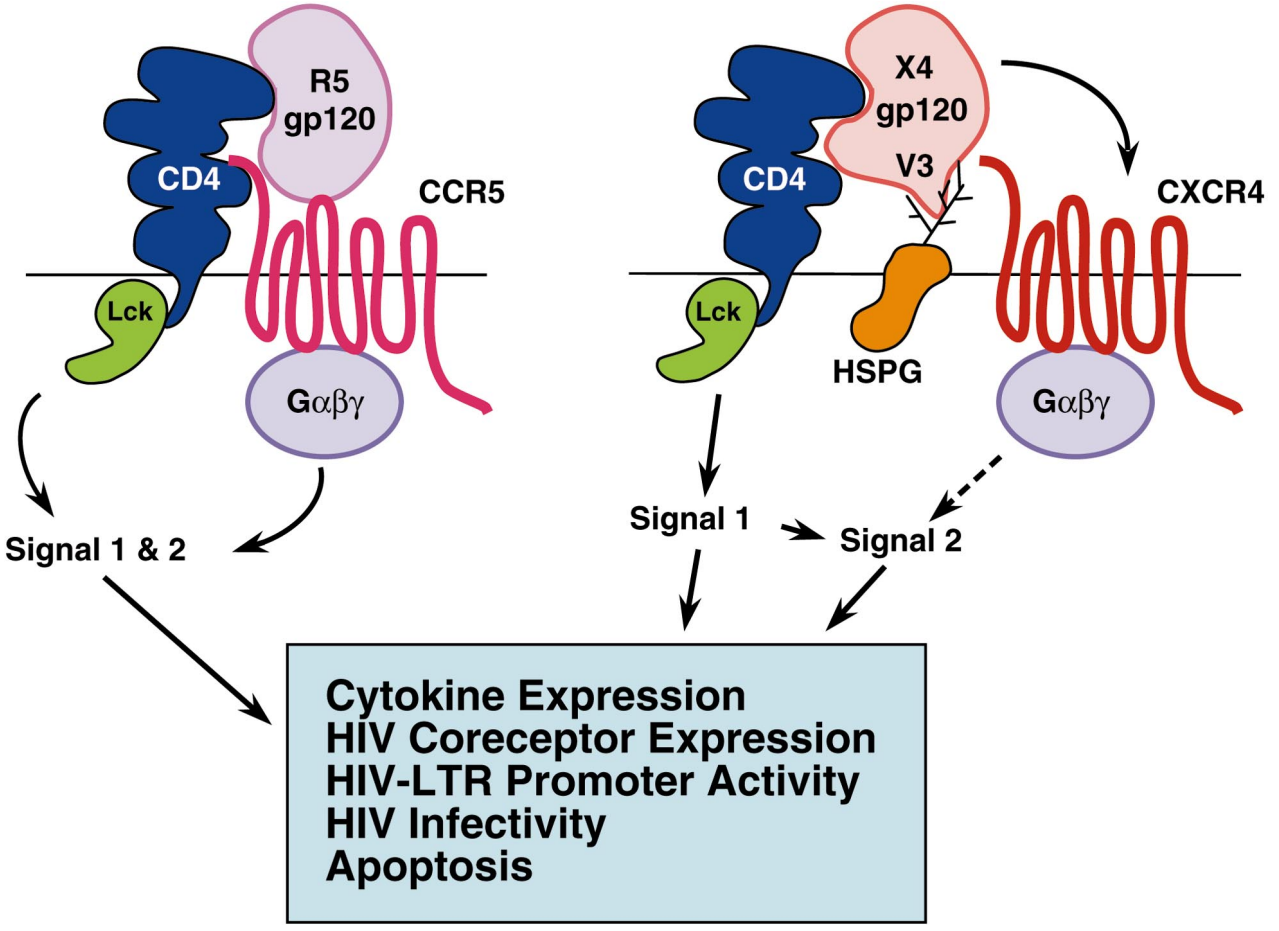

FIG. 1. Interaction of R5 and X4 HIV-1 gp120 with different cell surface molecules may result in stimulation of distinct signaling pathways. R5 HIV-1 gp120 binds to the preassembled CD4–CCR5 complex. This interaction may result in simultaneous stimulation of CD4- and CCR5-mediated signaling (Signal 1 & 2). In contrast, X4 HIV-1 gp120 binds first to CD4 and then to the CXCR4 receptor. Interaction of the X4 gp120 V3 loop with cellular HSPG may interfere with the association between the CD4–gp120 complex and CXCR4. Consequently, the earliest X4 gp120-induced signaling may be generated mostly by the CD4 receptor (Signal 1). Subsequent binding to CXCR4 may induce signal 2. Thus, R5 and X4 HIV-1 binding to target cells may contribute differently to HIV pathogenesis.

membranes has been recently shown (Xiao *et al.,* 1999). This association seems to be required for HIV-1 infection since inhibition of the CD4–CCR5 interaction inhibited R5 HIV-1 envelope-mediated fusion and HIV-1 entry. In contrast, association between CD4 and CXCR4 was negligible, but was substantially increased upon binding of gp120. It is therefore tempting to speculate that binding of the R5 HIV-1 envelope to CD4–CCR5 complexes may initiate a signaling pattern distinct from that induced by either receptor alone or by binding of the X4 HIV-1 envelope (Fig. 1).

HIV-1 entry into target cell is a multistep process that requires interactions between the viral envelope glycoproteins and cellular CD4 and chemokine receptors. An accumulating body of evidence suggests that other cell surface molecules, either independently or in association with CD4 and chemokine receptors, may participate in virus binding and entry. Consequently, these additional interactions between the viral envelope glycoprotein and the proteins at the surface of the plasma membrane may modulate CD4- and/or chemokine receptor-mediated signaling. It was shown that in addition to viral proteins, plasma membrane glycoproteins can be selectively incorporated into HIV-1 virions during budding from a plasma membrane. These include HLA class I and II antigens (Rossio *et al.,* 1995), adhesion molecules LFA-1 and ICAM (Orentas and Hildreth, 1993; Fortin *et al.,* 1997), as well as other cell-type-specific antigens (Lawn *et al.,* 2000). Interaction of these virus-acquired membrane glycoproteins with their respective cellular counterparts may generate signaling that will result in a change in the activation status of the cell (Ni *et al.,* 1999). However, while it has been shown that the interaction between the virion-bound adhesion molecule and its cellular counterpart potentiates HIV-1 infectivity (Fortin *et al.,* 2000), the role of signaling induced in this process remains unknown.

The interactions between virion-incorporated and cellular surface adhesion molecules occur independently of the engagement of the viral gp120 envelope. However,

the HIV-1 envelope can interact not only with CD4 and chemokine receptors but also with cell surface heparan sulfate proteoglycans (HSPGs). This interaction involves the V3 loop of gp120 (Roderiquez *et al.,* 1995; Moulard *et al.,* 2000) as well as the newly characterized conserved coreceptor binding surface on gp120 (Moulard *et al.,* 2000).

The heparin-like moieties of HSPG bind and modulate the activities of several matrix components, growth factors, proteinase inhibitors, and cell–matrix adhesion molecules (Hileman *et al.,* 1998). Substantial evidence suggests that cell surface-associated HSPGs act as "coreceptors" for FGF, EGF, Wnt, and members of the TGF-β superfamily and facilitate the interactions of these ligands with the specific receptors. Thus, HSPGs potentiate the activity of these signal-transducing receptors.

Whether HSPG plays a role in HIV-1-mediated cellular signaling is unknown. It has been shown that dextran sulfate can interfere with the association between X4 HIV-1 gp120 and CXCR4, while it does not have any detectable effect on the gp120–CD4 interaction (Moulard *et al.,* 2000). In contrast, the interaction between polyanions and R5 HIV-1 gp120 was weak or undetectable. These results, together with the earlier observations that showed constitutive association between CD4 and CCR5 and only a marginal association, if any, between CD4 and CXCR4 (Xiao *et al.,* 1999), suggest that signaling induced immediately upon binding of X4 HIV-1 may be generated primarily by the X4 gp120–CD4 interaction. In contrast, binding of R5 HIV-1 gp120 may trigger signaling through both CD4 and CCR5 receptors. It is tempting to speculate that selective interactions of gp120 with cellular proteoglycans may result, at least partially, in a differential signaling outcome induced by X4 versus R5 HIV-1. However, the implications of gp120 binding to HSPG in the outcome of HIV-1 signaling are unclear, since the principal target cells for HIV-1 infection *in vivo,* CD4+ T cells and macrophages, express rather low levels of HSPG on cell membranes compared to endothelial and epithelial cells. It may, however, facilitate the transfer of HIV-1 across the brain–endothelial cell barrier.

The biological activities of chemokines seem to be affected by their association with cellular or extracellular matrix glycosaminoglycans (GAGs). Although the biological role of chemokine–GAG complexes *in vivo* is unclear, many results suggest that GAGs immobilize and locally concentrate the chemokines. This may promote their oligomerization and facilitate their presentation to the receptors. In this respect, it has recently been demonstrated that chemokines SDF-1α (Mbemba *et al.,* 1999; Amara *et al.,* 1999) and RANTES (Trkola *et al.,* 1999) are able to interact with glycosaminoglycans as well as with CD4 and chemokine receptors.

The activation of signal transduction pathways by binding of HIV-1 gp120 to CD4 and chemokine receptors raises the question of whether the modulation of cellular signaling contributes to HIV pathogenesis. Chronic activation of the immune system is commonly observed in AIDS, and aberrant expression of inflammatory cytokines observed during progression of the disease has been implicated in the pathogenicity of HIV-1.

It has been shown that binding of both X4 and R5 HIV-1 virions or corresponding envelopes to the CD4 receptor initiated signaling pathways independent of the binding to chemokine receptors and resulted in an expression of cytokine and chemokine genes (Popik *et al.,* 1998; Popik and Pitha, 1998). Aberrant expression of inflammatory genes *in vivo* may contribute significantly to HIV-1 replication as well as to deregulation of the immune system. Furthermore, crosslinking of CD4 receptors by the HIV-1 envelope may lead to partial T cell activation and interference with the signals mediated by the TCR/CD3 complex and result in functional unresponsiveness resembling T cell anergy.

Progressive depletion of CD4+ T cells is a hallmark of HIV-1 disease. Destruction of these cells may involve apoptosis. It was shown that this process could be induced as a result of binding of gp120 to CD4 (Moutouh *et al.,* 1998; Guillerm *et al.,* 1998). Activation of Lck seems not to be required for the induction of apoptosis since cells expressing the mutated CD4 receptor unable to associate with Lck also undergo apoptosis. However, the role played by the cytoplasmic tail of CD4 in the induction of apoptosis remains obscure.

GPCRs are also able to transmit proapoptotic or antiapoptotic signaling and utilization of CXCR4 by HIV-1 was shown to be sufficient to trigger CD4+ T cell depletion (Penn *et al.,* 1999; Schramm *et al.,* 2000). Also, neuronal apoptosis induced by HIV-1 gp120 and SDF-1 was shown to be mediated by the CXCR4 receptor (Hesselgesser *et al.,* 1998). However, the role of signaling in these processes was not addressed.

The nature of chemokine receptor-mediated signaling in apoptosis is poorly understood. While SDF-1 shows proapoptotic activity in neuronal cells, in T lymphocytes SDF-1 activates PI 3-kinase and Akt/protein kinase B which are both implicated in antiapoptotic pathways (Sotsios *et al.,* 1999). Involvement of the caspase-dependent apoptotic signaling pathway induced by the engagement of CXCR4 by gp120 was recently suggested (Biard-Piechaczyk *et al.,* 2000). However, this process did not involve activation of the Gi protein which couples to and transduces "classical" chemokine receptor-dependent signaling (Berndt *et al.,* 1998; Biard-Piechaczyk *et al.,* 2000).

The ability of X4 HIV-1 gp120 to transduce proapoptotic, Gi-independent signaling may result from the inability of the X4 envelope to generate CXCR4-dependent antiapoptotic signaling rather than from intrinsic properties of the chemokine receptor. Accordingly, stimulation of the MAP kinase MEK/ERK pathway, often involved in antiapoptotic activity, was shown to be induced only by

viruses using CCR5 but not CXCR4 receptors (Popik and Pitha, 1998; Popik *et al.,* 1998).

The effect of chemokine receptor-mediated signaling on HIV-1 infection is unclear. However, the observation that β-chemokines MIP-1α, MIP-1β, and RANTES, which inhibit entry and replication of macrophage-tropic R5 HIV-1, increased replication of T-tropic X4 HIV-1 strains in primary CD4+ T cells (Kinter *et al.,* 1998) suggests that the signaling triggered by the engaged chemokine receptor can stimulate HIV-1 replication. Interestingly, the observed CCR5-mediated enhancement of X4 HIV-1 replication was associated with increased colocalization of CD4 and CXCR4 receptors (Kinter *et al.,* 1998). These results further suggest that colocalization of CD4 and CXCR4 receptors enhances X4 HIV-1 infection (Dimitrov *et al.,* 1999). Further experiments are required to dissect the signaling components involved in this process.

The effect of chemokine receptor signaling on HIV-1 infection was recently substantiated by the finding of a strong correlation between the levels of calcium mobilization induced in macrophages by the engagement of CCR5 by HIV-1 and replication of HIV-1 in these cells (Arthos *et al.,* 2000). Involvement of CCR5-mediated signaling was further confirmed by the observation that replication of the R5 HIV-1 primary isolate unable to replicate in macrophages could be promoted by signaling induced by binding of MIP-1α to CCR5. Similarly, inefficient replication of the primary CXCR4 isolate in macrophages was significantly increased by MIP-1β.

The requirement for activation of the MAP kinase MEK/ERK pathway in the replication of R5 and X4 HIV-1 strains in quiescent primary CD4+ T lymphocytes was recently documented (Popik and Pitha, 2000). In the presence of the MEK/ERK pathway inhibitor, replication of X4 but not R5 HIV-1 was significantly inhibited at the level of nuclear localization of the proviral DNA. These results, together with the earlier observations that R5 but not X4 HIV-1 efficiently activated MEK/ERK as well as JNK and p38 MAP kinases (Popik and Pitha, 1998), suggest that R5 viruses may replicate preferentially in suboptimally activated primary CD4+ T cells. This characteristic of R5 viruses may be one of the factors that gives a replicative advantage to macrophage-tropic over T-tropic HIV-1 isolates during the early stages of infection (Vicenzi *et al.,* 1999).

These data indicate that selection of CD4 and chemokine receptors by HIV-1 was not a random choice but that the HIV-1 virus has been using these receptors to induce signaling that facilitate the early stages of its own replication cycle. The activation of signaling pathways alters the activation status of the host T cells and possibly macrophages as well and results in functional modification of responsiveness to natural stimuli. Thus, understanding of the signaling induced by the engagement of these receptors upon HIV-1 binding could potentially offer new strategies for the interference with HIV-1 replication and virus-mediated immune pathogenicity.

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