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The role of glycosphingolipids in HIV signaling, entry and pathogenesis

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Although HIV uses CD4 and coreceptors (CCR5 and CXCR4) for productive infection of T cells, glycosphingolipids (GSL) may play ancillary roles in lymphoid and non-lymphoid cells. Interactions of the HIV Envelope Glycoprotein (Env) with GSL may help HIV in various steps of its pathogenesis. Physical-chemical aspects of the interactions between HIV Env and GSL leading to CD4-dependent entry into lymphocytes, the role of GSL in HIV transcytosis, and CD4-independent entry into non-lymphoid cells are reviewed. An overview of signaling properties of HIV receptors is provided with some speculation on how GSL may play a role in these events by virtue of being in membrane rafts. Finally, we summarize how interactions between HIV and coreceptors leading to signaling and/or fusion can be analyzed by the use of various tyrosine kinase and cytoskeletal inhibitors.
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Introduction

In order to better understand the mechanism of HIV entry and pathogenesis, it is essential to identify all the participants involved and elucidate the way they contribute to these processes. HIV-1 delivers its genetic material into the cell by direct fusion of the viral membrane with the plasma membrane of the host cells [1]. The Env gene of HIV-1 encodes a polypeptide (gp160) of about 840–860 amino acids, the precise length depending upon the isolate [2]. HIV-1 gp160 is folded in the endoplasmic reticulum into a complex disulphide-linked structure that is anchored in the membrane by virtue of the hydrophobic, membrane-spanning domain in the gp41 moiety. During post-translational processing, the precursor glycoproteins oligomerize and are extensively glycosylated before cellular proteinases cleave the precursor at a characteristic sequence to create the mature glycoproteins gp120 and gp41 [3]. The surface glycoprotein, gp120, forms trimeric spikes [4], which are associated by non-covalent interactions with each subunit of the membrane-anchored gp41.

The triggering mechanisms that activate Env are quite complex involving target cell CD4 and co-receptors [1]. The chemokine receptors CCR5 and CXCR4 are the principal co-receptors used by macrophage M-tropic (CCR5) and T-cell line T-tropic (X4) HIV-1, respectively [5]. HIV-1 attachment to host cells is generally considered to take place via binding between CD4 and gp120. However, the binding of virion-associated gp120 to cellular CD4 is often weak, and some cell types that are permissive for HIV-1 infection express little CD4. Thus, other interactions between the virion and the cell surface could dominate the attachment process [6]. In this review we will focus on glycosphingolipids (GSL) that have been shown to support HIV-1 entry [7]. We will examine whether they play a passive role in enhancing virus attachment, and whether they are also actively involved in mediating HIV-1 entry.

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GSL-HIV envelope interactions in model systems

In their studies aimed at characterizing the possible interaction of HIV envelopes with GSL, Fantini and coworkers [8] have focused on one of the five variable regions of gp120, the V3 loop, which plays a role in determining coreceptors usage [5]. SPC3, a gp120 V3 loop-derived synthetic peptide, has been shown to bind to the GSLs, LacCer, GM3, and GD3 on the target membrane [9]. A specific interaction between HIV-1 and HIV-2 envelope glycoproteins and the GSLs, GalCer, Gb3, and GM3

was documented by measuring variations in surface pressure of GSL monolayers [10,11]. Lingwood *et al*. have also recently shown that a soluble analog of Gb3, adamantylGb3, is a highly effective ligand for gp120 [12].

Recently, Conboy *et al*. [13] have done quantitative measurements of the interaction of certain GSLs with recombinant HIV-1 gp120 using total internal reflection fluorescence (TIRF) spectroscopy. This study compared the binding affinities of LacCer, GlcCer and GalCer to gp120. Among these, only GalCer had high affinity for gp120 ($Ka = 1.6 \times 10^9\ M^{-1}$). Gp120 did not show significant affinity for either GlcCer or LacCer. Also when the fatty acyl chain length of GalCer was varied from C18 to C24, no effect on the binding affinity of GalCer to gp120 was observed, suggesting that the binding depends more on the headgroup than on the chain length. This binding was also independent of the distribution of GalCer in lipid bilayers, since an increase in mole fraction of GalCer in these membranes from 5 to 50% had no effect on the binding affinity of GalCer for gp120. However, protein surface coverage measurements show that this strong binding process accounts for very little of the total protein adsorbed over the entire concentration range studied. At a protein concentration of ca. 20 nM, the surface coverage is only 3% of that achieved at apparent saturation (*i.e*., when the protein concentration is ca. 220 nM). Thus the 'high affinity' binding sites comprise only a small fraction of the total number of binding sites. Studies of GSL-gp120 interactions in model systems have largely been done using monomeric gp120. Gp120, as expressed in HIV-1, exists, however, as a trimer. It remains to be studied whether gp120 trimers [4] have altered binding affinities with respect to GSLs. We will examine how these specific interactions between GSLs and gp120 can participate in different aspects of HIV entry and pathogenesis.

The role of GSL in HIV transcytosis

The first potential cellular targets of HIV-1 are the epithelial cells lining the gastrointestinal, anorectal, and genitourinary tracts [14]. These cells are, however, not infected by HIV-1. The plasma membrane in these cells is divided into two unique domains: the apical membrane, which faces the lumen and the basolateral membrane, which faces the extracellular fluid on the other side. In addition to the endocytic route of uptake, these cells have evolved a specialized transport mechanism, transcytosis, for transport of selective cargo from the lumen to the extracellular fluid bathing the cells underlying the epithelial layer. Bomsel *et al.* reported that the infectious HIV could cross the tight human epithelial cell line barrier by transcytosis [15]. This was ascertained by exposing the apical domain of a sheet of epithelial cells (of intestinal and endometrial origin) to cells chronically infected with either HIV-1 or HIV-2 isolates. Following exposure for varying lengths of time, the basolateral medium was evaluated for the presence of the transcytosed virus. The basolateral medium was found capable of infecting a number of target cells, which included human macrophages, monocytic, or $CD4^+$ T cells. The transport of the virus into the basolateral medium was blocked by colchicines, a class of microtubule network disrupting-drugs, suggesting an involvement of microtubular network of the epithelial cells in the transcytosis. The transfer was also blocked at 4°C, suggesting endocytic uptake as the possible route of transit across the epithelial cells. GalCer had earlier been suggested to be an alternative receptor for HIV-1 infection into cells lacking CD4 receptor molecule [16]. Bomsel *et al.* demonstrated that the envelope glycoprotein of HIV-1, gp120, binds to GalCer, which is enriched at the apical membrane of epithelial cells. Furthermore, Alfsen and Bomsel [17] mapped a binding site for GalCer on the gp41 subunit of HIV-1 corresponding to residues 650–685, a region of gp41 interestingly exposed and accessible for binding to GalCer. They could conclude that in CD4 negative epithelial cells GalCer was the receptor for HIV-1 transcytosis, but the possible participation of a HIV coreceptor was not assessed.

In a recent study it was proposed that the transport of HIV-1 across a Caco-2 monolayer requires both GalCer and CXCR4 receptors, which are expressed on the apical surface of Caco-2 and M cells [18]. However, the same study determined that follicle-associated epithelium covering human gut lymphoid follicles express CCR5 rather than CXCR4. Can CCR5 support the transcytosis of CCR5-utilizing virus HIV-1? When Fotopoulos *et al*. transfected the M-cells with a CCR5 expression plasmid, transcytosis selective for CCR5-utilizing isolates could be observed. This observation was substantiated by studies from another laboratory demonstrating that CCR5-expressing primary intestinal cells could selectively transfer CCR5 utilizing HIV-1. Meng *et al*. showed that HIV-1 uses GalCer and CCR5 expressed on these cells to transcytose across the epithelial cells [19]. These cells were not by themselves infected with HIV-1. The transfer was also reduced by colchicines and blocked at 4°C. Epithelial cells lining the human intestine primarily express CCR5 and GalCer [18]. The selection of CCR5-utilizing HIV-1 virions at the level of transcytosis could thus have an important role in the predominance of CCR5-utilizing HIV-1 viruses in the early stages of HIV pathogenesis. In a similar way the reproductive epithelium has been considered as a transit pathway for HIV infection as the expression of HIV receptors as well as GalCer on human uterine epithelial cells appears to be regulated during the menstrual cycle [20].

The role of GSL in CD4-dependent HIV entry

Upon crossing the epithelial cells, HIV encounters a multitude of cells susceptible of infection. Investigations into the role of GSL in CD4-dependent entry have largely examined the interactions of three main components: CD4, co-receptor (CXCR4/CCR5) and HIV-1 envelope with GSLs. An antibody specific for GalCer and sulphatide was shown to block the binding of HIV-1 envelope. However, this antibody did not block the infection of $CD4^+$ cells [21]. Hence, it was argued that the

role of sulphatides was restricted to the binding to HIV-1 envelope protein. Subsequently, it has been demonstrated that the inhibitor (L-cycloserine) of GSL biosynthesis affected HIV-1 infection through specific downregulation of CD4 [22], which is required for HIV entry. This study, however, did not point to specific interaction between any components of the fusion machinery and GSLs. Studies from our laboratory have shown that pretreatment of $CD4^+$ cells with GSL biosynthesis inhibitors, such as PPMP, which are known to reduce GSL levels in target cells, had profound effects on subsequent fusion and infection mediated by HIV-1 gp120-gp41 [7]. In contrast to cycloserine, PPMP did not change the expression levels of CD4 and CXCR4 on target cells. Furthermore, GSL depletion mediated reduction in viral fusion was found to be independent of receptor or co-receptor expression levels. This was in contrast to the effect of cholesterol-depleting agents on target cells which was found to be strictly dependent on the receptor expression levels on target cell membranes [23]. Reconstitution of Gb3 and GM3 to the membranes of GSL-depleted cells restored fusion with gp120-gp41 expressing cells [7]. Since Gb3, a B cell differentiation antigen (CD77), is not present in T cells [24], it appears that GM3 serves as an accessory factor enabling HIV-1 entry in $CD4^+$ lymphocytes. This hypothesis is supported by the fact that GM3 interacts with CD4 [25] and CXCR4 [26] on the surface of target cells as well as with HIV-1 Env [10].

The precise role of GSLs in the viral entry into host cells expressing CD4 and coreceptor is not clearly understood. Post-CD4 binding, gp120 undergoes a conformational change exposing the coreceptor-binding sites as well as the V3 loop of gp120 [1]. In a recent study it has been suggested that the interaction of GSLs with HIV-1 envelope could occur post CD4 binding [27]. This study utilized a peptide corresponding to the V3 loop of HIV-1 gp120. The linear peptide displayed no affinity for either CD4 or co-receptor. However, this peptide was shown to bind specifically to GM3, Gb3, and GalCer isolated from the host cells. This study suggested that in addition to binding to the coreceptor, the V3 loop could also interact with the GSLs present on the host cells. The effect of this interaction would be to stabilize the fusion complex of HIV-1 envelope, CD4, and co-receptor.

Taken together, interaction of GSLs with various components of the fusion machinery could thus stabilize the various intermediate steps en route to fusion of the virus with plasma membrane. As a result of these interactions, receptors may be arranged in a proper disposition to interact with the HIV Env glycoprotein.

The role of GSL in CD4-independent HIV entry

GalCer and its sulphated derivative are expressed on macrophages [21] on neural cells (neuron and glia) in the brain [16] and colon epithelial cell lines [28]. Harouse *et al.* [16] showed that an antibody specific for GalCer could block entry of HIV-1 into $CD4^-$ cells of neural origin (U373-MG and SK-N-MC). Furthermore, they demonstrated that recombinant gp120 could bind to GalCer and galactosyl sulphatide but not to glucosyl ceramide, GM1, GD1a or GSLs extracted from human erythrocytes that included glucosyl ceramide, lactosyl ceramide, ceramide trihexoside and globoside. In a recent study, cell associated HIV-1 infection has been documented in salivary gland epithelial cells. These cells do not express CD4 but express GalCer and CXCR4 [29].

Although the data suggest that HIV can infect epithelial and neuronal cell lines it is not clear that these tissues are infected *in vivo*. Primary intestinal epithelia are not infected by HIV [19]. However, recent studies by Klotman *et al.* show active viral replication in renal epithelia [30]. The mechanism of HIV infection of these cells is still unclear. However, it is temping to speculate that Gb3, which is the major GSL of the human kidney [31], plays a major role in this process.

HIV receptors in GSL domains (rafts)

GSLs exhibit long saturated acyl chains which are thought to drive self-assembly with cholesterol to form tightly packed liquid ordered domains on the plasma membrane, distinct from the more fluid phase represented by glycerolphospholipids [32,33]. These domains variously termed as rafts, DRM, DIG, DIM, LDTI are endowed of specific receptor and signaling molecules, thus representing functionally active centres involved in several biological processes [34]. Raft-associated molecules are characterized by their insolubility in cold Triton X100 [33]. The existence of a distinct population of lipid rafts in T cells has been demonstrated by the heterogeneous distribution of signaling molecules (such as lck and LAT) [35] and GSL composition. Gomez-Mouton *et al.* have presented evidence that GM3 is localized in rafts in leading edges and GM1 is in rafts in uropods in the course of chemotaxis of polarized T cells [36].

In addition, a direct association of CD4 with GM3 has been observed in T cells [25] and in reconstituted microdomains of GSL (Gb3 and GM3) [8,37]. This interaction could stabilize a localization of CD4 in rafts. Indeed, CD4 is selectively enriched in GSL rafts [38] and co-localized with GM3 in the same lipid rafts in human T-lymphocytes [39]. CCR5 also appears to be localized in rafts [40], whereas a small portion of CXCR4 is found to be raft-associated in un-stimulated cells [41]. However, following treatment of the cells with intact HIV-1 or gp120, an increased presence of CXCR4 in GSL rafts has been observed [23,26,41,42].

Rafts are particularly enriched in signaling molecules and notably members of the Src-tyrosine kinase family (Lyn, Lck, Hck and Fyn). The organization of signaling molecules into discrete membrane-associated microdomains allows the cell to modulate signaling pathways not simply by regulating enzymatic activity, but by regulating substrate access to the various kinases and phosphatases involved in different processes. In lymphoid cells, Harder and Simons [43] showed that raft clustering leads

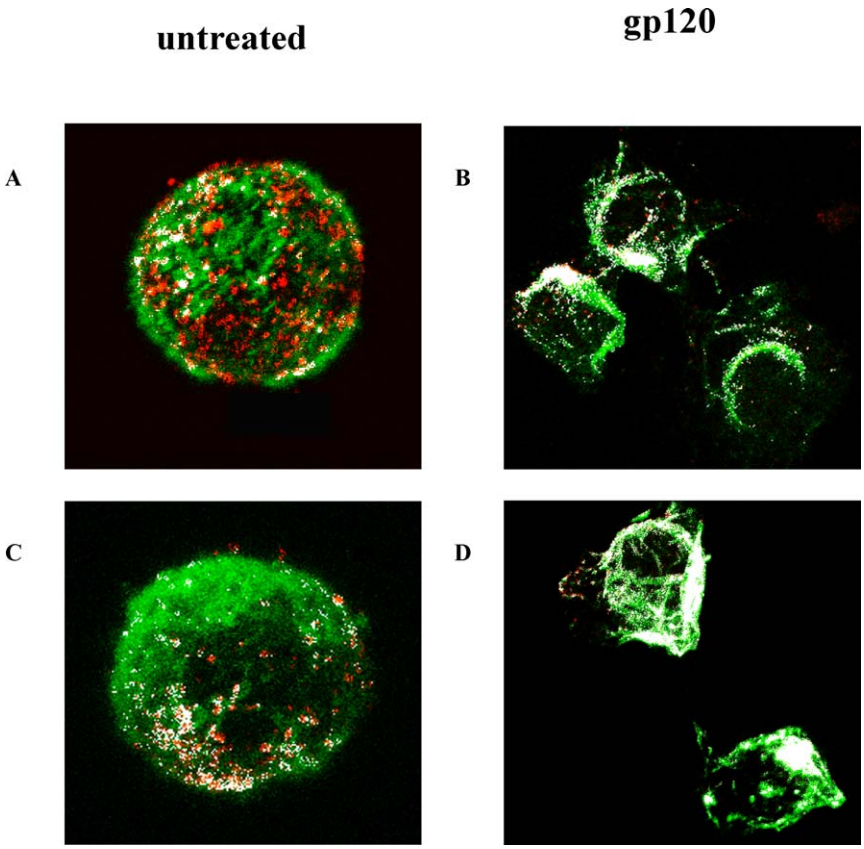

Figure 1. Co-localization of HIV receptors with actin. CEM cells were left untreated (A, C) or treated with 10 μg/ml HIV-1 gp120 at 12°C for 15 min (B, D), then labeled with anti-CD4 (A, B) or anti CXCR4 (C, D), followed by Alexa fluor 594-conjugated antibodies (red) according to Viard *et al.* [23]. Cells were then fixed, permeabilized and stained with Alexa fluor 488-conjugated phalloidin (green), and examined by confocal microscopy. The white color indicates CD4/actin or CXCR4/actin colocalization.

to the accumulation of actin regulated by local tyrosine phosphorylation. F-actin and tyrosine phosphorylation were also shown to control the segregation of the T cell receptor in lipid rafts [44]. It seems therefore that actin and signaling (notably tyrosine kinase) may participate in the formation and/or the stabilization of big complexes. In the case of HIV it has been shown that disruption of the actin filaments prevented gp120-induced colocalization of CD4 and CXCR4 [45]. In order to examine the role of actin in the HIV-1 receptor clustering required for fusion [23], we monitored receptor localization by immunofluorescence and actin localization by staining with fluorescently labeled phalloidin. Figure 1 shows that CD4 and CXCR4 indeed colocalize with actin upon stimulation with HIV-1 gp120.

Signaling properties of HIV receptors

CD4 is a membrane glycoprotein expressed on the surface of T helper cells and to a lesser extent on monocytes/macrophages. Its association with p56lck through the cytoplasmic domain mediates its signaling potential [46]. Lck is a tyrosine kinase protein, anchored to the cytoplasmic side of the membrane through dual acylation at N-terminus [47,48] and endowed with SH2 and SH3 domains that provide a docking site for the recruitment of downstream signaling molecules [49,50].

The chemokine receptors are members of the 7 transmembrane G protein coupled receptor family. All G proteins have a common heterotrimeric structure consisting of α, β and γ subunits. Chemokine binding to the receptor promotes the exchange of GDP for GTP in the G-protein, dissociation of Gα from the Gβ and Gγ subunits, and numerous downstream effector functions including phospholipid hydrolysis and calcium mobilization. The variety of downstream signaling they can elicit has recently been reviewed [51].

The signaling capability of CD4 is mediated by its interaction with p56lck. Once activated, lck triggers a cascade of molecular events through raf-1 association and phosphorylation leading to MAPK/erk activation [52,53]. Importantly, this signaling pathway was found to occur also as a result of gp120 mediated CD4 cross-linking [54]. In fact, truncated CD4 is unable to associate with lck, which impaired the erk activation by HIV-env stimulation [55]. As a consequence a delayed HIV-1 replication and delayed apoptosis was observed [56] although lck activity was recently reported as negatively affecting viral replication [57].

Figure 2 shows that gp120 activation results in MAPK and Pyk2 phosphorylation in accordance with published data

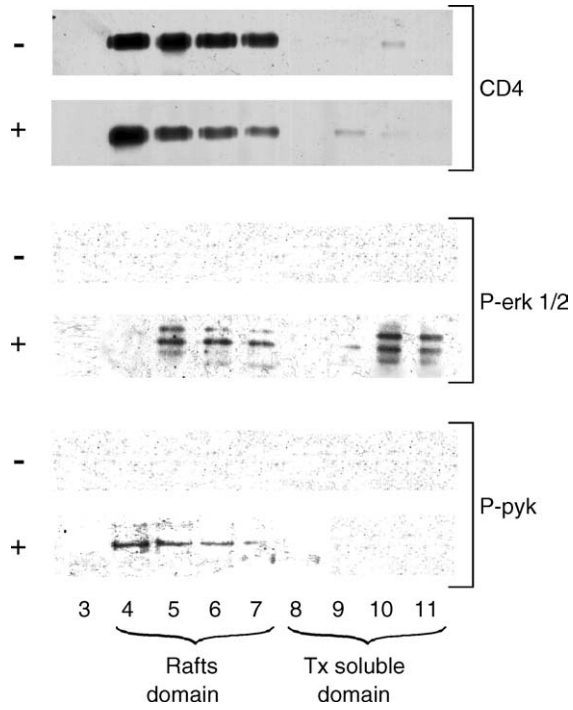

Figure 2. Activated signalling molecules distribute in rafts. Human primary T lymphocytes were left untreated (−) at 37°C or incubated with 10 μg/ml of GP120 for 3 min at 37°C (+), then lysed with 1% TX 100 and subjected to sucrose gradient as described previously [23]. Resulting fractions were protein quantified and 4 μg protein for each fraction was analysed by western blotting with anti-CD4, anti-P-erk1/2, anti-P-Pyk specific antibodies.

[55,58]. To examine whether these molecules become associated with membrane rafts we treated cells with Triton X-100 at 4°C followed by flotation of the cell lysate on a sucrose gradient. The molecules that are not solubilized by Triton X-100 under these conditions are assigned to the 'detergent-resistant membrane (DRM)' domains [33]. The presence of the molecules of interest in the soluble and DRM fractions is then examined by Western Blot analysis. We performed this analysis with the same number of untreated and HIV-1 gp120-treated human primary lymphocytes. Figure 2 shows that CD4 behaves like a typical raft protein [23] in the presence and absence of gp120 treatment in that it localized on the top of the gradient in fractions 4–7. Surprisingly, we found that activated erk1/2 is partially localized in a detergent-insoluble fraction following stimulation of the cells by gp120 for 3 min at 37°C, and furthermore that Pyk2 completely localizes to this domain. This new finding indicates that rafts are the center of complex and diverse signaling events induced by gp120. In support of these results, we found that genistein, a powerful tyrosine kinase inhibitor known to inhibit HIV infection [59] (see Figure 3), inhibits MAPK activation and its recruitment to lipid rafts under gp120 stimulation (data not shown).

In T cells, the down-regulation of CXCR4 was found to be associated with tyrosine phosphorylation of lck, since it was inhibited by treating cells with a tyrosine kinase inhibitor, Herbimycin A [59]. Moreover, the down-regulation was insensitive to pertussis toxin (PTX), suggesting that G protein was not involved in this pathway [51]. Conversely, a functional requirement of G protein signalling in the gp120-elicited pathway on HL60 and T cell line was established by Davis *et al.* [58]. In

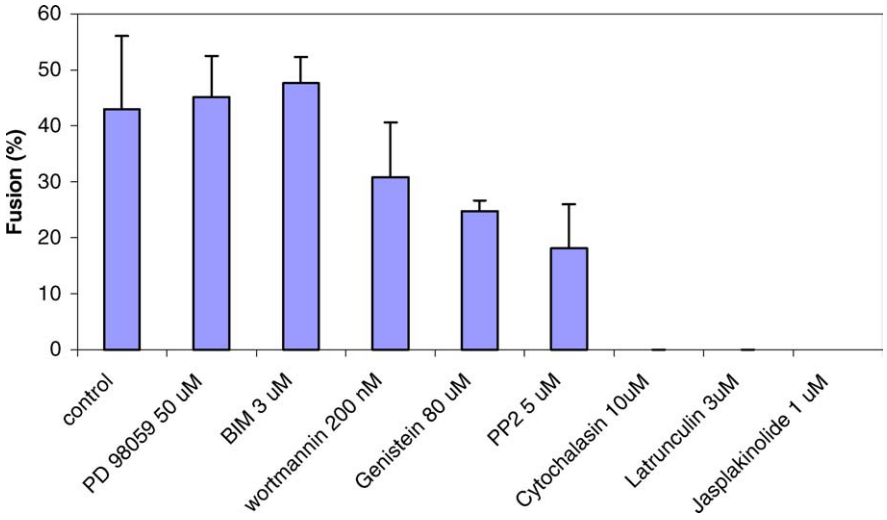

Figure 3. Effects of inhibitors of tyrosine-kinase and actin polymerization on HIV-1 Env-mediated fusion. HIV-1 IIIB Env was expressed on Hela cells using a vaccinia construct and the target cells used were $CD4^+$ PBL stimulated 10 days with IL2 and cell fusion was monitored as described previously [23]. The various tyrosine kinases and disrupters or stabilizers of F-actin are described in the text and were preincubated one hour with the PBL prior to the fusion experiments.

this pathway G protein was required to activate Pyk2 (see Figure 2) that in turn formed a multicomponent signaling complex including G-coupled receptors, GRB, SOS, that feed into the classical MAPK pathway [55]. Importantly, this gp120-induced pathway was also described as occurring in biological phenomena such as augmented intracellular calcium mobilization and β chemokine secretion [60] in primary T cells and macrophages. HIV env also induces phosphorylation of focal adhesion tyrosine kinases (FAK) in T cells [61] or in macrophages [60], indicating that such activation is a prerequisite for the formation of signaling complexes that in turn could lead to actin cytoskeleton rearrangement. Indeed, Pyk2 is a member of the focal adhesion PTK family and is known to associate with paxillin and other cytoskeletal proteins [62]. Interestingly, it has been shown that the interaction of HIV envelope with $CD4^+$ target cells led to phosphorylation of Lck [63]. Moreover, the interactions of the HIV envelope with $CD4^+$ lymphocytes appears to result in tyrosine phosphorylation of FAK and CCR5, redistribution of focal adhesion complexes, and association of FAK with CCR5 [64]. Furthermore, FAK and Pyk2 have been shown to be able to interact with the SH2 domain of Lck [65] and Lck can itself associate with tyrosine phosphorylated paxillin that in turn is constitutively associated with Pyk2 [66]. Therefore, paxillin can at least provide one conceptual link between CD4, coreceptor and actin.

HIV-1 signaling and entry

The ability of each participant of the fusion process to induce signaling and the concentration of multiple signaling molecules in the rafts raises the question whether signaling may play a role in HIV-1 entry. Early studies from Cohen *et al*. [67] showed that the tyrosine kinase inhibitor Herbimycin A inhibited syncytia formation among Jurkat cells infected with HIV-1. Furthermore, several groups showed that tyrosine kinase inhibitors could inhibit HIV-1 entry of various cell lines as well as primary macrophages [51].

By contrast, studies involving mutated CD4 [68] or chemokine receptors [69] that lack signaling properties showed they could still efficiently support HIV-1 entry leading to the notion that signaling was dispensable for HIV-1 entry. Studies with Pertussis toxin (PTX), a reagent widely used to characterize the involvement of heterotrimeric G-proteins in signaling, indicate that signaling properties of chemokine receptors are dispensable for their role in mediating HIV-1 entry in cell lines [70]. The interest in chemokine receptor signaling for entry was revived by a report of Alfano *et al*. [71] showing that the B-oligomer of PTX could inhibit the entry of CCR5 but not X4 HIV strains in primary T cells. The B-oligomer of PTX does not inhibit Gαi signaling but is known to have signaling effects on its own that could lead to improper signaling of CCR5. Thus, despite no alteration of its surface expression or its ability to bind gp120, the inability of CCR5, upon treatment with the B-oligomer of PTX, to support HIV entry was linked to its inability to signal properly. Along with a report from Guntermann *et al.* [72] showing the inhibition of infection of PBMC's treated by PTX by X4 HIV-1 without effect on the level of receptor expression, these studies renewed the question of the passive role of the chemokine receptors in HIV entry. Interestingly, the inability of CCR5 to promote entry upon treatment with the B-oligomer of PTX was associated with a blocking of the co-capping of CD4 and CCR5 induced by R5 HIV-1 in primary T cells [71].

We have examined the effects of inhibitors of various steps in the signaling cascades on HIV-1 Env-mediated cell fusion using a dye-mixing assay developed in our laboratory [73]. This assay distinguishes the effects of these inhibitors on the early steps of HIV-1 entry from those on post entry events. HIV-1 Env was expressed on Hela cells using a vaccinia construct, and the target cells used were $CD4^+$ lymphocytes, stimulated 10 days with IL2. As shown in Figure 3, the MAP kinase inhibitor PD98059 [74] did not seem to have an effect on HIV-1 Env-mediated fusion. However, the MEK inhibitor UO126 was shown to suppress HIV-induced erk 1/2 phosphorylation and lipid raft-dependent HIV macropinocytosis in brain microvascular endothelial cells [75]. The PKC inhibitor, bisindolylmaleimide (BIM) [76], slightly but consistently increased the fusion susceptibility of the cells. Ligands to CD4 have been shown to activate the PI3 kinase pathway in the presence [77] or absence of p56Lck [78]. Inhibition of the PI3 kinase pathway by Wortmannin [79] slightly inhibited fusion whereas the effect of genistein, a potent tyrosine kinase inhibitor [51], and PP2, an inhibitor selective for the src family of kinases that notably affects the function of p56Lck [80], considerably reduced the extent of the fusion. Remarkably, alteration of the integrity of the cytoskeleton with disrupters of actin polymerization, cytochalasin or Latrunculin, or stabilizer of F-actin Jasplakinolide [81] inhibited nearly completely the ability of the cells to fuse. We had previously shown that cytochalasin did not inhibit fusion with cells expressing high levels of CD4 and co-receptors [23]. Similar experiments indicate that the signaling inhibitors do not affect HIV-1 Env-mediated fusion with cells expressing high levels of CD4 and coreceptors (data not shown). These data are consistent with the notion that tyrosine kinases and actin are important factors in the first step of HIV entry into target cells suggesting an active mechanism at least in cells expressing low levels of HIV-1 receptors.

Chemotaxis is initiated by signaling events following the interaction of chemokines with their receptor [82]. In order to examine the role of GSL in these events, cells expressing chemokine receptors have been treated with the inhibitor of GSL biosynthesis, PPMP [83]. We demonstrated that such treatment did not affect chemotaxis mediated by SDF1-α or MIP1-β, but susceptibility to fusion was significantly reduced [7]. This is an example of a lack of correlation between effects of GSL on HIV-1 entry and on chemokine receptor signaling (see Figure 4).

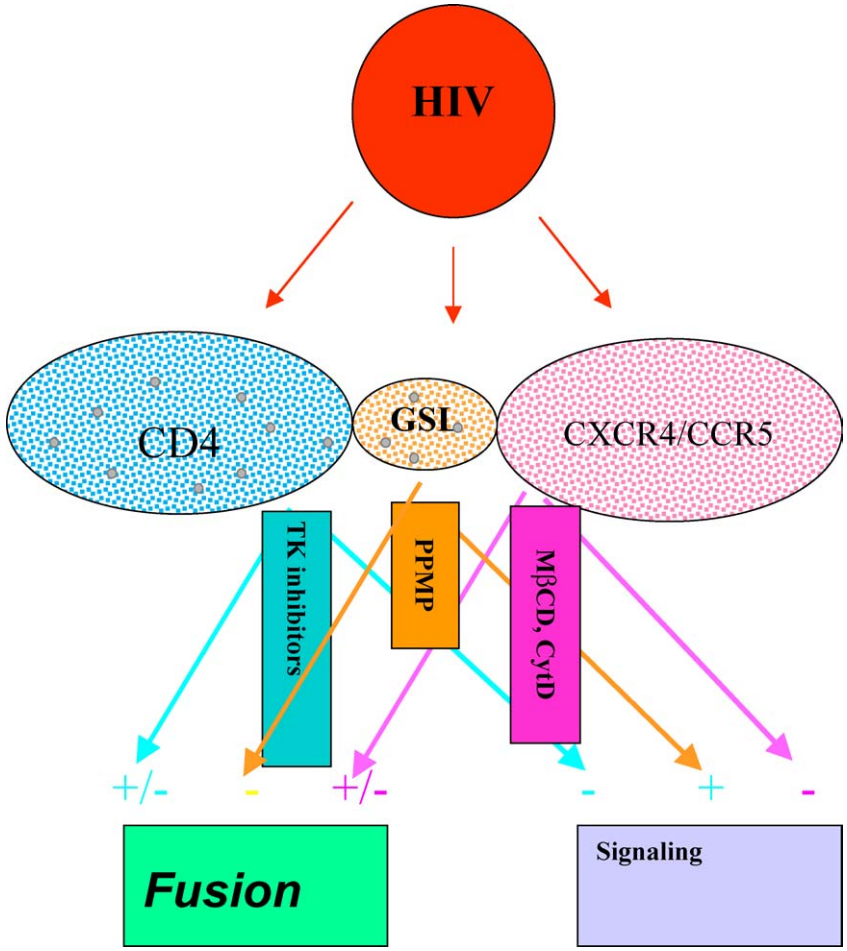

Figure 4. The role of GSL in HIV entry and signaling. The model shows that interactions of HIV with CD4, coreceptors and GSL could result in fusion and/or signaling as indicated by the arrows. These three components may be in different pools that could intermingle as a result of interactions with HIV Env. CD4 and GSL are in domains (rafts) enriched with cholesterol (gray circles). The optimal condition for fusion is when all three components interact. The effects HIV Env on fusion and signaling can be resolved by the use of inhibitors. CD4-mediated tyrosine kinase signaling is inhibited by TK inhibitors (−), which do not always block fusion (+/−) (see Figure 3). Treatment of cells with agents that remove cholesterol (MβCD) or disrupt actin filaments (CytD) leads to a disruption of interactions between these clusters and consequently HIV Env-mediated fusion is inhibited in cells expressing low levels of HIV receptors [23]. However, the effects of MβCD and cytD are not apparent in cells expressing high levels of HIV receptors, but chemokine receptor signaling leading to chemotaxis is blocked by these agents. Treatment of cells with a GSL biosynthesis inhibitor (PPMP) also disrupts these interactions resulting in inhibition of fusion. However, signaling events leading to chemotaxis are not inhibited [7].

Conclusions

HIV signaling causes a myriad of post-entry effects that ultimately may lead to HIV pathogenesis [84,85]. This is of particular significance in HIV-associated dementia [86]. Expression of GSLs and its biosynthetic regulations [87] may have a major role in HIV pathogenesis ranging from possible binding, transcytosis allowing the crossing of barriers, and reaching new compartments for infection as well as entry. GSL can participate in HIV Env-mediated fusion through multiple aspects. They can act as cofactors in a multimeric complex with gp120, CD4 and the coreceptors but may also provide domains on the cell that seem to be favorable for entry and budding of the virion. GSL domains have been shown to be the center of a multitude of signaling events that seem critical for the entry of HIV in cells expressing low levels of receptors and coreceptors [23]. Figure 4 summarizes how interactions between HIV and coreceptors leading to signaling and/or fusion can be analyzed by the use of various inhibitors.

Although we have focused our attention throughout the review on the interaction of HIV envelope with GSLs, their domains and CD4 and coreceptors, the sources of interaction from the virus are multiple [84]. Beyond the proteins encoded by the virus, it is also known that HIV particles bud from raft domains and therefore specifically incorporate components of these domains (HLA1 and 2, ICAM) [88]. Those proteins can

interact with counterparts on the target cells and modify the lateral organization and composition of GSL domains. The signaling of GSLs themselves is poorly known but they have been shown to influence tyrosine kinases and play an important role in cell-cell recognition through multiple signaling events [89]. It would therefore be interesting to see how this impacts upon HIV pathogenesis.

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