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Clarifying the Role of G Protein Signaling in HIV Infection: New Approaches to an Old Question

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Abstract

Whether or not HIV gp120-elicited signal transduction through the coreceptors CCR5 and CXCR4 is required for productive viral replication has long been a subject of controversy. The complexity and diversity of G protein signal transduction initiated by chemokine receptor activation has hindered efforts to understand the contributions of these pathways to the HIV life cycle. Several recent studies have demonstrated an important role for G proteins in mediating signaling events through both CCR5 and CXCR4 that are necessary for productive HIV infection. In addition to gp120-mediated G protein activation, there is still much to learn about the impact of G protein signaling during HIV infection, including the role of T-cell receptor/CXCR4 cross-talk, regulation of G protein expression during infection and the contribution of G protein subunit genetic polymorphisms to disease progression. This review will describe the effects of G protein signaling in immune cells, summarize the current understanding of CCR5 and CXCR4-initiated signal transduction in HIV replication, and discuss important gaps that still remain in our understanding of G protein signaling and its contribution to HIV pathogenesis.

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Key words

HIV. Signal transduction. GTP-binding proteins. CC chemokine receptors.

Introduction

Infection of a CD4+ T-cell by HIV-1 requires the binding of viral gp120 to both the host CD4 receptor and a coreceptor, most commonly the chemokine receptors CCR5 or CXCR4. In addition to facilitating membrane fusion, these interactions have the capacity to initiate intracellular signaling pathways through both receptors. Signaling through CD4 is mediated by downstream effectors that include p56 lck and zeta-chain-associated protein kinase 70 (ZAP-70), and the CD4-gp120 interaction initiates signals that not only affect HIV replication (via NFκB activation[1]) but also susceptibility to infection and the rate of disease progression[2,3] (Oyugi unpublished data). In contrast, the coreceptors CCR5 and CXCR4 are G protein-coupled receptors (GPCR) that initiate signaling pathways mediated by heterotrimeric G proteins. Early signal transduction studies indicated that not only can HIV gp120 induce signals through its coreceptors, but that it activates specific signaling pathways that differ from those induced by natural chemokine ligands, possibly due to differing ligand binding sites or affinities[4-11].

For many years, G protein-mediated signaling induced by the chemokine coreceptors was largely considered dispensable for viral entry and replication, and the precise requirement for these signals remains controversial and misunderstood[12,13]. New technical approaches and a better understanding of the diversity of signaling events transduced by GPCR and G proteins has suggested a growing role for G protein signaling in facilitating HIV infection[14,15]. It is important to

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understand the contribution of G protein signaling to HIV replication and disease progression, as it may alter the fate of the infecting virus as well as the pathogenesis and spread of infection. Identification of signaling pathways activated uniquely by gp120 may also offer new therapeutic targets for the disruption of the HIV lifecycle with minimal detrimental side effects. This review will summarize current knowledge regarding the role of CCR5/CXCR4-elicited G protein signaling pathways in HIV replication and disease pathogenesis and will highlight important roadblocks, new approaches, and remaining gaps in our knowledge.

G protein signaling in the immune system

In order to understand the inconsistencies and limitations of studies addressing the role of G proteins in HIV infection, it is important to note the diversity and complexity of G protein-based signal transduction. Like all GPCRs, CCR5 and CXCR4 are 7-transmembrane-spanning cell surface receptors that bind an extracellular ligand and induce signaling cascades mediated by intracellular, membrane-associated heterotrimeric G proteins (herein referred to as G proteins). The G proteins form a trimeric complex composed of one each of an α, β, and γ subunit. The β and γ subunits form a constitutive dimer that binds the α subunit-GDP complex. Activation of the GPCR recruits the G protein complex and results in the exchange of guanosine 5'-triphosphate (GTP) for guanosine 5'-diphosphate (GDP), inducing the dissociation of the $\beta\gamma$ dimer from the α subunit, and initiating downstream signaling pathways from all subunits. The intrinsic guanosine triphosphatase activity of the α subunit cleaves the GTP into GDP and promotes the reformation of the G protein trimeric complex (Fig. 1 A). Signaling specificity is partially achieved by combinations of subunit isoforms with varying properties (Table 1). Currently, 16 α subunit, 5 β subunit, and 12 γ subunit genes have been identified.

Both GPCR and G proteins play crucial roles in immune cells as exemplified by GPCR chemokine receptors, which initiate many cellular events including lymphocyte chemotaxis. The majority of these signaling cascades are mediated by Gαi proteins[16,17], which are commonly identified/inhibited *in vitro* with pertussis toxin (PTX; via adenosine diphosphate [ADP]-ribosylation of the Gα subunit). Recent advances in the understanding of chemokine receptor activation, however, have shown that additional signaling occurs through Gαq and/or Gα12/13 pathways[18,19]. Cross-talk between CXCR4-associated Gαi activation and CD4/T-cell receptor (TCR)-associated signaling proteins (lck, ZAP-70, and potentially JAK2/3) has also been observed, and is described more fully later in this review[20-22]. As discussed in the section "Consequences of gp120-induced G protein signaling during infection", it is the diversity of G protein subunit specificity and downstream signaling that complicates the analysis of chemokine receptor signaling in HIV infection. Careful distinctions must also be made between studies of various immune cell subsets, as stimulation of CXCR4 on dendritic cells and neutrophils initiates signaling pathways that are CD38, Gαi, and Gαq dependent, and therefore distinct from B- and T-cell pathways[23].

There are several major effectors of G protein subunits in lymphocytes (Fig. 1 B). The Gα effectors include adenylyl cyclase (AC), phosphodiesterase (PDE), phospholipase C (PLC) and Rho guanine exchange factors (RhoGEF) (see a more specific description[24]). The $\beta\gamma$ dimer is capable of regulating a variety of effectors, including AC, PLCβ and PLCε, class IB phosphatidylinositol-3-kinase γ (PI3Kγ), G protein-regulated kinases (GRK) and ion channels, including voltage-dependent Ca^{2+} channels (VDCC) and G protein-activated inwardly rectifying K^+ channels (GIRK)[25]. Activation of PI3Kγ produces the second messenger PIP_3, which binds pleckstrin homology domain-containing proteins like Akt/PKB, ultimately promoting cell survival (reviewed by Rommel, et al.[26]). The PLC activation promotes Ca^{2+} release via second messengers as well as PKC activation, which leads to the induction of the mitogen-activated protein kinase (MAPK) pathway (Erk, JNK, p38 MAPK) and activation of focal adhesion kinase Pyk2. Given the ability of gp120 to bind to CCR5 and CXCR4, and given that many of these G protein-mediated signaling pathways ultimately lead to transcription factor activation (including HIV transcriptional regulators NFκB and AP-1), many studies have attempted to determine the capacity of gp120 to elicit intracellular signaling responses similar to those of the natural chemokine ligands.

G protein signaling initiated by gp120, a viral chemokine

Despite controversy over the contribution of gp120-elicited G protein signals to the HIV lifecycle, it is well established that HIV gp120 acts as a "viral chemokine" to activate intracellular signaling pathways through its coreceptors[27]. In macrophages, gp120-GPCR interactions

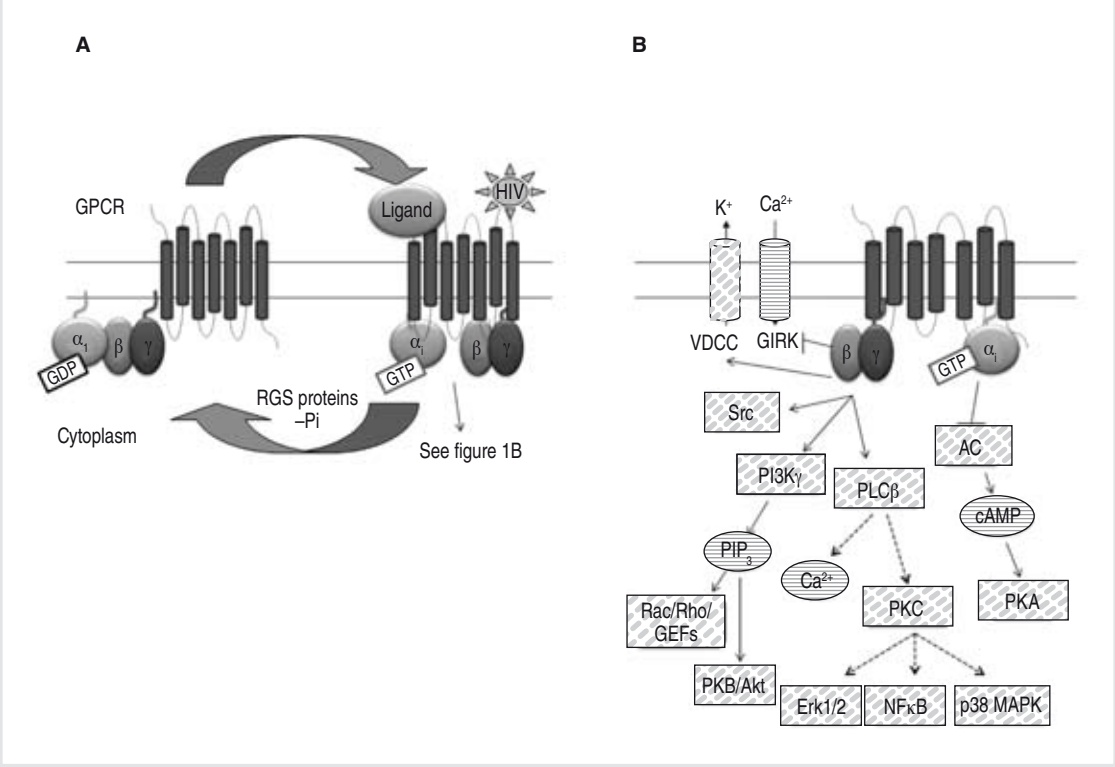

Figure 1. ***A:*** *Process of G protein activation following G protein-coupled receptor stimulation by ligand or HIV. Activation of the receptor results in G protein recruitment, exchange of guanosine 5'-triphosphate for guanosine 5'-diphosphate at the α subunit, dissociation of the α subunit from the βγ dimer and initiation of downstream signaling pathways. Regulators of G protein signaling (RGS) and the guanosine triphosphatase activity of the α subunit results in re-association of the trimeric G protein complex.* ***B:*** *Schematic of major signaling pathways initiated by Gαi protein signaling in response to endogenous ligand. represents effector proteins, represents second messengers. Dashed lines indicate pathways with multiple intermediate effectors. GPCR: G protein-coupled receptor; PI3K: phosphatidylinositol-3-kinase; PLC: phospholipase C; AC: adenylyl cyclase; GEF: guanine exchange factor; PKB: protein kinase B; PKC: protein kinase C; PKA: protein kinase A.*

Table 1. G protein subunit isoforms and notable characteristics

Subunit type	Families/ isoforms	Characteristics
Gα	Gαs	Stimulates adenylyl cyclase
	Gαi/o	Inhibits adenylyl cyclase, most common Gα subunit coupled to chemokine receptors; inhibited *in vitro* by pertussis toxin
	Gαq/11	Can couple to CXCR4 receptor and mediate T-cell receptor activation[20]; activates phospholipase C-β
	Gα12/13	Interaction with RhoGEF; mediate T-cell adhesion/motility (in mice[99])
Gβ	Gβ1 Gβ2	Similar to Gβ2 and Gβ4 subunits
	Gβ3 Gβ4	GNB3 C825T single nucleotide polymorphism associated with multiple diseases (see Future Directions)
	Gβ5	Associates with regulator of G protein signaling (RGS) domains; localized to multiple intracellular membranes
Gγ	Gγ1-12	More overall sequence diversity between γ subunits than between β subunits

result in the induction of ion channel currents (K^+, Cl^-), intracellular Ca^{2+} release and chemokine expression via MAPK activation[8] (see review of gp120-mediated macrophage activation[28]). Interestingly, the ion currents and Ca^{2+} mobilization patterns elicited by R5 gp120 were quantitatively distinct from those elicited by X4 gp120, suggesting that viral tropism may affect the nature of the signaling response following coreceptor binding. In lymphocytes, gp120-initiated G protein signals have a number of functional consequences, as well as notable differences compared to normal chemokine signaling. Gp120 is sufficient to activate G proteins in primary unstimulated peripheral blood mononuclear cells (PBMC) and causes a rapid Ca^{2+} flux in CD4+ T-cells[27]. Pulsing of CD4+ T-cells with gp120 is also sufficient to induce phosphorylation of Erk1/2, to activate PI3K, and to lead to membrane recruitment and phosphorylation of Akt, which was increased compared to stromal derived factor (SDF/CXCL12)-induced signaling. The differences in chemokine- and gp120-elicited Ca^{2+} flux may result in differential transcription factor activation in T-cells, including modulation of NFκB, suggesting a possible influence gp120-elicited signaling on viral replication and long terminal repeat (LTR) activation[29,30]. The Gαi signaling in response to CXCR4-gp120 interactions also stimulates actin polymerization – a process important for HIV infection of target cells and necessary for proper nuclear localization. Finally, gp120 has been repeatedly shown to induce T-cell chemotaxis through activation of both CCR5 and CXCR4[9,27,31,32]. This Gαi-dependent process shows similar kinetics to SDF-induced chemotaxis, but with a prolonged response. Because most of these observations arose from *in vitro* experiments, some studies have questioned whether such signaling could occur under physiological conditions.

Although it is unclear whether gp120 can reach a sufficient concentration in the blood to elicit these effects, studies suggest that it is possible in lymphoid organs[31,33]. To determine the capacity of physiological levels of gp120 to elicit signaling responses through CCR5 and CXCR4, Melar, et al. measured Ca^{2+} mobilization following chemokine and gp120 stimulation of primary PBMC[34]. Coreceptor-mediated Ca^{2+} fluxing was observed upon binding of 2-4 viral particles per cell, suggesting that gp120 mediates signaling (by either virion-associated or free gp120) under physiological conditions, especially in lymphoid organs where gp120 is highly concentrated during chronic infection[33].

Consequences of gp120-induced G protein signaling during infection

Given the capacity of gp120 to mimic the signaling activity of endogenous chemokines and to activate numerous intracellular signaling pathways through CCR5 and CXCR4, there are two ways in which HIV-induced G protein signaling is suggested to affect infection. Firstly, high antigen levels can cause an inappropriate chemotactic lymphocyte response that can promote viral spread; secondly, signaling may directly affect intracellular steps of the HIV lifecycle. These two possibilities are discussed below.

Contribution of gp120-induced G protein signals to immune dysregulation and pathogenesis

In macrophages, the release of many chemokines and cytokines is dependent on G protein signals, which lead to the membrane recruitment of multiple proteins into a signaling complex. Gp120 elicits CCR5-Gαi signals that induce the release of the proinflammatory cytokine interleukin 1b (IL-1b) via a pathway dependent on PI3K, the src kinase Lyn, and the focal adhesion kinase Pyk2[35]. Gp120 binding to CCR5 also induces both tumor necrosis factor alpha (TNF-α) through a PI3K-MAPK (ERK1/2 and p38)-dependent pathway[36] and CCL2 secretion via phosphatidylcholine-specific phospholipase C (PC-PLC) and NFκB activation, a signaling pathway that is not normally activated by the CCL4 ligand[7]. The inappropriate activation of chemotaxis by viral gp120 may play an important role in recruiting uninfected cells to sites of viral replication, thus facilitating the spread of the virus[31,32]. These signals may also lead to dysregulation of cellular function in lymphatic organs, as well as activation of T-cells[32]. Gp120-CXCR4 interactions also appear to promote naive T-cell anergy through a protein kinase A (PKA)-dependent mechanism, although the role of G proteins in this pathway was not specifically investigated[37]. The ability of gp120 to mimic chemokine signaling therefore has the potential to alter both immune activation and cell behavior, especially in late-stage HIV infection.

Contribution of gp120-induced G protein signals to the HIV lifecycle

Viral entry

Following the characterization of CCR5 and CXCR4 as the primary HIV coreceptors *in vivo*, many studies

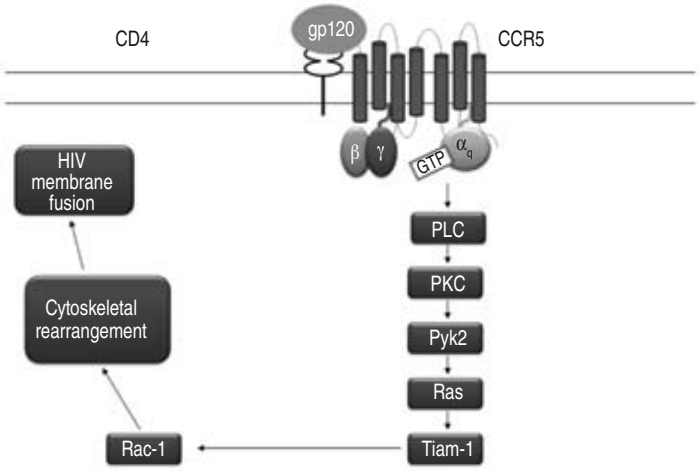

Figure 2. *The Gαq signaling pathway is required for Rac-1-mediated cytoskeletal rearrangements that facilitate HIV entry (Harmon, et al.[44]). PLC: phospholipase C; PKC: protein kinase C.*

investigated whether receptor signaling activity is required for coreceptor function. Regardless of the techniques used to uncouple the receptor and Gαi proteins, all initial studies concluded that G protein signaling was dispensable for coreceptor function[11,38-42]. These techniques, however, were primarily aimed at dissociating coreceptor function from Gαi signaling pathways, which are PTX (an inhibitor of Gαi activity)-sensitive and mediated by the second intracellular loop of CCR5. In contrast, Gαq signaling is mediated by the third intracellular loop of CCR5[43] and is PTX-independent. Using siRNA against Gαq proteins, Harmon and Ratner[44] demonstrate a requirement for Gαq signaling through CCR5 for viral entry (Fig. 2). This signaling pathway is dependent on PLCβ, PKC, Pyk2 and Ras, and is required for Rac-1 mediated actin reorganization that facilitates HIV membrane fusion. This study demonstrates a crucial role for CCR5-mediated G protein signaling during HIV infection and highlights the need to account for all types of signaling pathways activated by chemokine receptors in addition to the classical Gαi pathways. It should be noted that G protein signaling events following gp120-coreceptor binding could affect viral entry regardless of whether viral entry occurs via fusion at the plasma membrane or following receptor-mediated endocytosis, as some recent evidence suggests[45].

Viral replication

Early studies characterizing post-entry blocks of HIV and SIV infection in human vs. macaque cells suggested that the viral gp120, in combination with another viral protein and the host coreceptor, interact to abolish a pre-integration, post-entry block to replication in human T-cell lines[46]. CCR5 signaling may also be required for viral replication in macrophages; R5 viruses with envelopes that render them unable to replicate in macrophages are also unable to mobilize Ca^{2+} and require the addition of macrophage inflammatory protein (MIP)-1α (a CCR5 ligand) to remove the observed replication block, suggesting that CCR5 signaling events initiated by gp120 (including Ca^{2+} mobilization) are needed for replication[47]. Despite these early observations, controversy over the precise role of and requirement for G protein signaling during the viral lifecycle has persisted, with conflicting reports of the capacity of gp120 to elicit G protein-mediated signals during entry and the effect of those signaling cascades. In addition to Harmon, et al., several recent studies have provided important evidence for the contribution G protein signaling during viral replication and are discussed below.

Role for CCR5 signaling

In macrophages, several lines of evidence point to a role for CCR5-mediated G protein signaling in HIV replication. Pre-incubation of primary monocytes and monocyte-derived macrophages with CCR5 ligands enhances subsequent HIV infection in a PTX-dependent manner, suggesting that G protein-mediated signals promote a cellular environment that is highly permissive to viral replication[48]. It is somewhat unclear whether gp120-induced Pyk2 phosphorylation is Gαi sensitive, as suggested by studies in cell lines, or independent, as studies in primary macrophages suggest[49,50]. However, it has been suggested that Gαi-Pyk2 activation may activate MAPK cell survival pathways that allow HIV to replicate in HL60 cell lines[49]. Specific inhibition of G protein-mediated chemotaxis pathways by a novel peptide that does not block ligand-receptor interactions[51,52] was shown to block R5-tropic HIV replication in THP-1 cells, but not X4-tropic viral infection of Jurkat T-cells[13]. The point of restriction was not determined, but was reported to likely occur post-entry, suggesting a critical role for CCR5-mediated Gαi signaling events in promoting R5-tropic viral replication. Later studies have provided potential mechanisms to explain these observations. Paruch, et al.[53] show that MIP-1β or gp120 binding to CCR5 results in activation of Erk1/2 and phospholipase D (PLD), which promotes transactivation of Tat and the HIV LTR (possibly via NFκB) *in vitro*. Knockdown of either PLD1 or PLD2 in THP-1 monocytic cells strongly inhibits HIV infection. Although this study demonstrated overall inhibition of HIV replication following abrogation of Gαi signaling, it did not specifically show dependence of the PLD pathway on G protein activation. Others have shown that gp120 can also induce PTX-sensitive activation of Ca^{2+} release and PI3K and Akt activation in primary activated T-cells and macrophages[54]. This activity appears to remove a block to replication at an unspecified point after reverse transcription (RT) but before integration, again suggesting a role for G protein signaling in facilitating viral replication in macrophages.

Evidence points to a similar role for G protein signaling in CD4+ T-cells, where high cell surface density of CCR5 correlates with an increased level of HIV replication. At high CCR5 density, a twofold increase in viral particles entering the cytosol results in a 30-80-fold increase in viral replication at the late RT/integration phase. This increase is inhibited by the addition of PTX, which suggests the replication increase is due to G protein signaling[55]. These observations were further supported in primary T-cells, where CCR5 mutants incapable of signaling through Gαi proteins poorly supported HIV replication[56]. These results employed non-activated T-cells in order to prevent excessive cellular activation from masking the role of these signaling pathways. Furthermore, while the overexpression of wild-type CCR5 protein in the human osteoblast (HOS) cell line and in primary PBMC increases HIV replication, overexpression of signaling-defective mutants has no effect. Both PTX and Gαi-specific siRNA treatments suggest that this is due to loss of Gαi signals, although it is unclear which stage of the viral lifecycle is affected[57]. This work not only supports a requirement for Gαi signals during the HIV lifecycle, but also suggests that the activation state of primary cells (and transformed cell lines) may strongly affect the outcome of experiments designed to assess the role of G protein signaling in viral replication. This idea is further supported by data demonstrating MAPK pathway activation by gp120 in the context of TCR stimulation but not IL-7 stimulation[58]. Additional evidence that CCR5 signaling may be important post-viral entry comes from the observation that the addition of RANTES (a CCR5 ligand) to primary cells facilitates X4-tropic HIV replication in a Gαi-dependent manner without increasing CXCR4 expression on the cell surface[59]. In contrast, however, at least one study suggests that beta chemokine signaling through Gαi inhibits HIV replication by decreasing cyclic adenosine monophosphate and PKA activation in primary lymphocytes, an observation that should be tested further[60]. Compelling mechanistic evidence for the role of CCR5 (and CXCR4) Gαi signals in post-entry viral replication comes from a report showing that both R5- and X4-tropic viruses are capable of inducing MAPK/Erk (but not p38/Jnk) activation in unstimulated PBMC[61]. It was shown that Erk activation is dependent on Gαi signals and is required for the late stages of reverse transcription (Fig. 3 A). This report contradicts the previous suggestions that R5-tropic virus can activate p38 MAPK and Jnk[4], that the ERK pathway does not affect R5 viral replication[6] and that X4-tropic virus is unable to activate the Erk/MAPK pathway[9]. These discrepancies may be due, in part, to the use of less suitable cells lines (Jurkat) and SIV models in older studies.

Role for CXCR4 signaling

Despite some studies' assertion that only R5-tropic virus requires G protein activity for replication, mounting

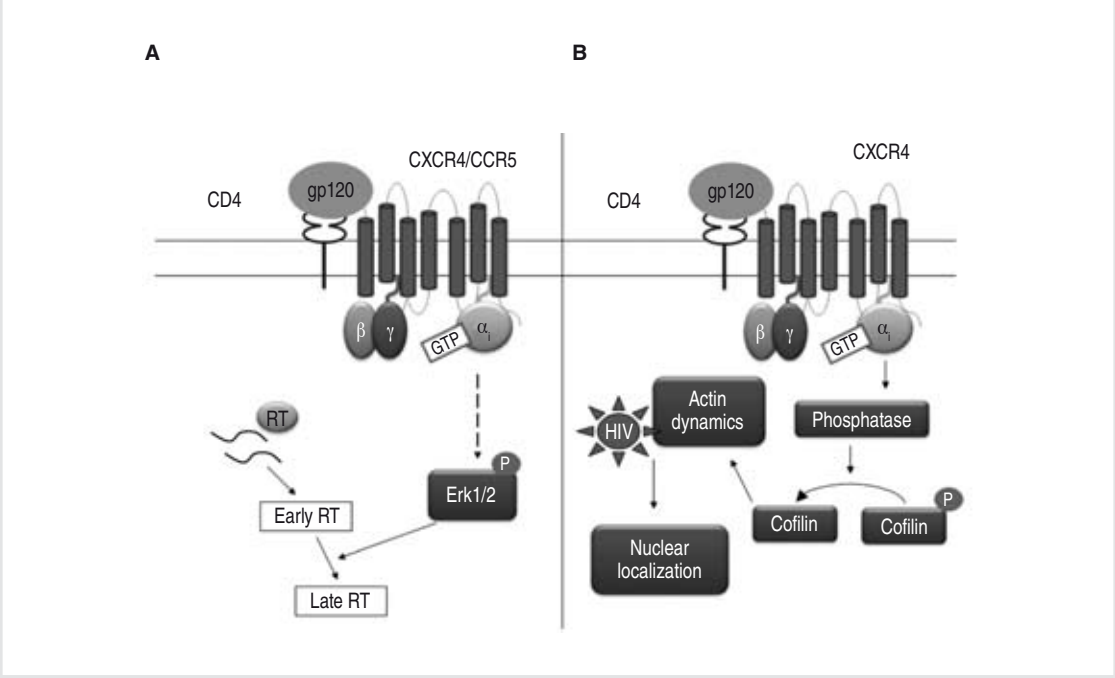

Figure 3. ***A:*** *gp120-Gαi signaling in T-cells activates Erk1/2, which is required for full completion of late reverse transcription* (adapted from Mettling, et al.[61]). ***B:*** *gp120-Gαi signaling activates cofilin in resting T-cells. Cofilin is a regulator of actin dynamics that allows HIV to overcome a post-entry block and complete nuclear localization* (adapted from Yoder, et al.[65]). *RT: reverse transcriptase; P: phosphorylation event.*

evidence suggests that X4-tropic strains have similar requirements. As in the case for CCR5 signaling, addition of the CXCR4 ligand SDF-1α to infected Jurkat cells activates Erk in a Gαi-dependent fashion and enhances viral replication[62]. Pre-incubation of cells with PTX also strongly inhibits X4-tropic infection in primary PBMC[63]. CXCR4 G protein signals have been implicated in facilitating stimulation of the HIV LTR, as soluble gp120-elicited Tat-Sf1 expression in primary T-cells is abrogated by PTX treatment[64]. Most recently, CXCR4-mediated Gαi signals have been shown to overcome actin-based restriction of HIV infection in resting T-cells[65]. The Gαi pathways activate cofilin, an actin depolymerization protein, to allow viral nuclear import. Inhibition of this pathway strongly inhibits latent infection of resting T-cells, and demonstrates an important role for G protein signaling during HIV infection (Fig. 3 B). Together, these recent studies suggest that G protein signaling in both macrophages and T-cells (through either CCR5 or CXCR4) plays a crucial role in facilitating HIV replication at multiple steps during the viral replication cycle.

Apoptosis

Gp120 signaling is also known to induce apoptosis in T-cells, a process which primarily involves CD4 signaling but which can be enhanced by involvement of the coreceptors[66]. Many studies, however, suggest that these signaling pathways are primarily G protein-independent[67,68], as determined by PTX treatment. This, of course, does not test the involvement of G proteins other than Gαi. Disagreement exists about the ability of gp120 to induce pro- and anti-apoptotic signals in T-cells; some studies show Akt activation by gp120[54] whereas others show p38 activation but not Akt[67]. CXCR4 engagement appears to be important in p38 signaling, but in a PTX-independent manner[67]. Fas-independent apoptosis may also be triggered through CXCR4 via mitochondrial release of cytochrome C[69]. Although there is currently no evidence to suggest the involvement of G protein signals in mediating gp120-induced apoptosis, the impact of Gα pathways other than Gαi has not yet been well characterized.

Future directions

Specific inhibition of G protein activity in vitro

A full and accurate understanding of the effects of G protein signaling during HIV infection requires the specific inhibition of G protein activity with minimal off-target effects. Historically, many studies that aim to dissect the impact of G protein signals use PTX to inactivate Gαi activity and measure the resulting HIV replication. This strategy has two downfalls: it is now understood that chemokine receptors signal through other Gα subunit classes that are not inhibited by PTX and it is also known that PTX has multiple inhibitory effects on lymphocytes. As previously mentioned, Gαq and Gα12/13 subunits are not ADP-ribosylated by PTX and therefore will not be inhibited by its treatment. Other techniques, such as siRNA as used by Harmon, et al., can specifically inhibit these pathways. Additionally, the PTX-B subunit may inhibit HIV replication through a CD14-dependent mechanism that does not appear to involve G proteins[70]. The PTX treatment of T-cells also appears to activate the TCR pathway, including lck and ZAP-70, with resulting downregulation of CXCR4 expression and signaling[71,72]. Finally, PTX may also stimulate the activation and proliferation of various immune cells, which can confound the interrogation of G protein pathways[73]. Care must therefore be taken when ascribing the effects of PTX treatment to G protein activity.

Despite the care needed to choose specific inhibitors of G protein signaling, it is important to directly demonstrate the reliance of gp120-coreceptor signaling on G protein activity. In some studies, the effects of signaling pathways on HIV replication are attributed only to CCR5-mediated signaling events without further identification of downstream effectors. This does not, however, necessarily implicate G protein pathways in these observations, as chemokine receptors also signal through G protein-independent pathways. The use of newly developed inhibitors such as those described by Grainger may offer specific inhibition of G protein-mediated chemotactic pathways without off-target effects or the inhibition of receptor-ligand binding.

Contribution of non-coreceptor GPCR and G protein signals to HIV pathogenesis

Due to the prominent role of CCR5 and CXCR4 as primary HIV coreceptors *in vivo*, most studies of G protein signaling during HIV infection have focused on these receptors. It is important to consider the potential impact of signaling through other chemokine receptors that may be expressed on primary T-cells. One such candidate receptor is CCR6 and its potential impact on the activation of innate restriction factor APOBEC3G. Recently, Lafferty, et al.[74] reported that CCR6 and its ligand human beta defensin 2 (hBD2) signal via a Gαi-dependent mechanism to upregulate APOBEC3G in primary cells. CCR6 is expressed on CCR5+ memory peripheral blood T-cells as well as Th17 and α4β7+ gut lymphocytes. This finding is particularly interesting in light of other evidence showing that (i) CCR4+CCR6+ and CXCR3+CCR6+ primary cells are highly permissive to HIV infection and may recruit additional CCR6+ cells to sites of viral replication[75] and (ii) CCR5+CCR6+ Th17 cells may be preferentially targeted by R5-tropic HIV *in vivo*[76]. It has also previously been reported that CCL3 ligation of CCR5 on primary CD4+ T-cells results in the upregulation of APOBEC3G mRNA[77]. Taken together, these data suggest that a subset of highly infectible cells may also have the ability, due to expression of CCR6 and/or CCR5, to induce an antiviral state following HIV binding. A more comprehensive understanding of the effects of hBD2/CCR6-mediated G protein signaling during HIV infection in specialized cell subsets such as Th17 may yield therapeutic targets that could be exploited to protect these cells during early CD4 depletion.

CXCR4 G protein cross-talk with CD4/T-cell receptor pathways

Although the classical G protein-activated signaling pathways triggered by CCR5 and CXCR4 are well understood, the high degree of signaling cross-talk that can occur between chemokine receptor activation and TCR/CD4 activation in lymphocytes has only come to light more recently[78]. Despite some discrepancies in kinetics and order of activation/recruitment of particular signaling mediators, multiple studies show evidence of transactivation of the TCR following CXCR4 engagement[79,80] and the recruitment of lck, zap70, Itk and CD45, all of which have the capacity to respond to both CD4/TCR- and CXCR4-activated pathways.

Several studies have shown physical interactions between and transactivation of CD4, TCR/CD3 and CXCR4. Basmaciogullari, et al.[81] demonstrated that both CD4 and CD8a are capable of binding to

CXCR4, and mapped the CD4/CXCR4 interaction to a cytoplasmic domain on CD4, although the physical association was not required for proper binding of HIV. A physical association between CXCR4 and the TCR has also been shown with the suggestion that TCR immunoreceptor tyrosine-based activation motifs (ITAM) mediate stromal cell-derived factor-1α (SDF-1α)-induced signaling pathways, including Ca^{2+} flux and activation of Erk/MAPK, AP-1, and chemokine secretion[80]. A separate study extended these results, revealing that CD3 phosphorylation following CXCR4 engagement is required for SDF-1α-mediated internalization, Erk phosphorylation, and chemotaxis. Given this evidence, it is clear that gp120-mediated activation of G protein pathways likely includes CD4-associated signaling events and mediators, a topic which has not been well explored in HIV replication studies.

Given the association between TCR and CXCR4, several proteins known to be involved in TCR signaling are also crucial for proper SDF-mediated chemotaxis and CXCR4 signaling, including p56 lck, ZAP-70, and the Tec kinase Itk. Although its role in CXCR4 signaling is not yet fully resolved, mutagenesis studies point to the SH3 domain of lck as playing a strong role in SDF-induced chemotaxis[82]. A more recent report demonstrates that lck and Gαi signals initiate the formation of a signaling complex that also includes ZAP-70 and the adaptor protein p52Shc, suggesting that lck may be responsible for initiating the tyrosine phosphorylation cascade that occurs following CXCR4 activation[79,82]. The precise mechanism by which lck is activated by CXCR4 is currently unknown, but may involve direct interaction of lck with G protein subunits[83] or CXCR4 itself[79,82].

Downstream effectors of TCR signaling include lck-mediated phosphorylation of the Syk kinase ZAP-70[84], which has been shown to be required for SDF-mediated trans-endothelial migration[85]. Interestingly, ZAP-70 has been implicated in facilitating viral synapse formation during HIV infection and promoting cell-to-cell viral spread[86], raising the question of whether gp120-CXCR4 binding could also promote ZAP-70 activation to facilitate this process. ZAP-70 phosphorylation requires the Gαi-dependent activity of the adaptor protein p52Shc, which likely mediates CXCR4-lck-ZAP-70 coupling. Adaptor protein p52Shc not only plays a role in mediating chemotactic responses but also in ligand-induced receptor internalization[79].

Downstream targets of ZAP-70 include the Tec kinase Itk, another TCR-associated signaling protein. Itk is phosphorylated following CXCR4 activation in a PI3K/Src-dependent manner[87] and is recruited to the membrane in a PTX-dependent manner, suggesting a dependence on Gai signaling[88]. Itk is involved in SDF-mediated cell migration and Rac activation, which ultimately regulates actin reorganization[87,88]. Recently, the protein LAD (an Itk and lck binding protein), which is known to mediate T-cell activation, was shown to directly bind G protein β subunits 1, 4, and 5. In both SDF and RANTES-stimulated chemotaxis, LAD was shown to facilitate chemotaxis, associate with lck and ZAP-70, and be required for Pyk2 phosphorylation[89]. Together, these data suggest the recruitment of lck, ZAP-70, and Itk following CXCR4 activation, a process dependent on Gαi signals and adaptor proteins such as p52Shc and LAD. Given the roles in HIV replication now being ascribed to CXCR4-mediated Gαi activity, it will be interesting to determine the contribution of cross-talk with CD4-associated signaling effectors.

Other proteins that suggest extensive cross-talk between chemokine receptor and TCR pathways include PI3Kγ and CD45. PI3Kγ (or PI3K class I_B) is normally only associated with GPCR, while PI3K class I_A associates with protein tyrosine kinases such as CD3. Alcazar, et al.[90] show, however, that PI3Kγ is stimulated by TCR activation, interacts with the TCR, Gαq/11, lck and ZAP-70 and modulates Rac activity, suggesting a potential role in mediating G protein activity during HIV infection. Stromal cell-derived factor binding to CXCR4 also enhances CD45 phosphorylation and its association with CXCR4 in lipid rafts[91]. The presence of CD45 markedly increases the chemotactic response, possibly via regulation of Pyk2, lck, ZAP-70, and SLP76 phosphorylation. Given the extensive overlap and cross-talk of G protein-activated and TCR/CD4-associated pathways, future studies on the importance of coreceptor signaling during infection should include the modulation of these effectors by G proteins in order to fully understand the consequences of receptor activation (Fig. 4).

Subunit polymorphisms and susceptibility/progression

Studies of disease progression in patients with genetic polymorphisms have elucidated the importance of multiple host proteins in HIV infection, including CCR5 and RANTES. Similar insights into the role of signaling pathways may be gained from the identification of single nucleotide polymorphisms (SNP) in other HIV receptors and their downstream effectors. Recently, our lab identified a SNP in the CD4 gene

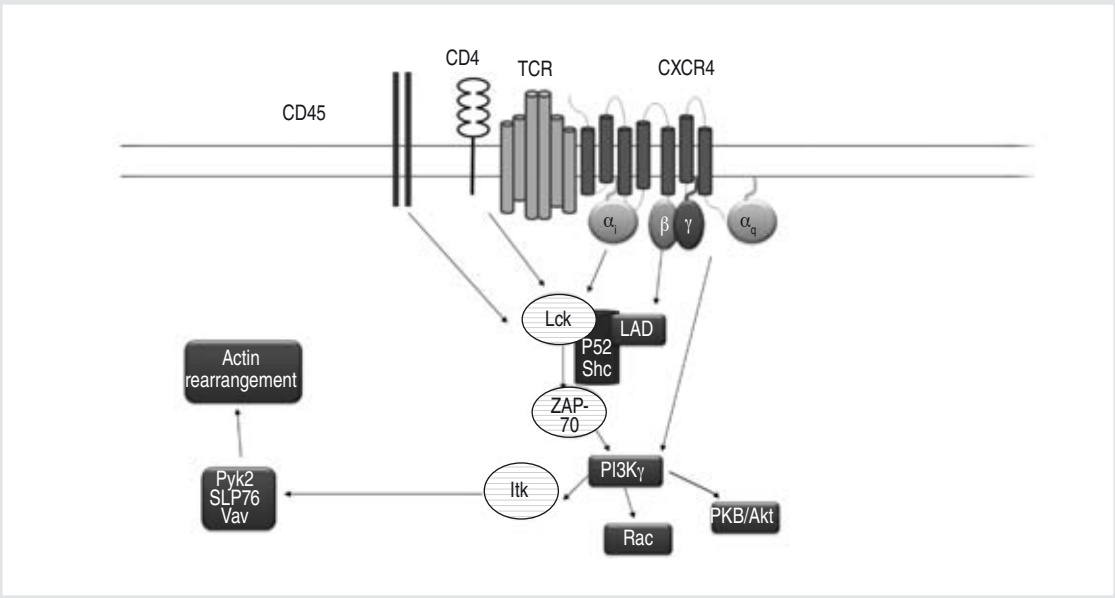

Figure 4. *Cross-talk between TCR/CD4 pathways and CXCR4/G protein subunits. Major mediators include recruitment of lck, ZAP-70 and Tec kinase Itk (shown in ◯) following Gαi activation via adaptor proteins p52Shc and LAD. Downstream effectors include PI3K, Rac and Pyk2. TCR: T-cell receptor; PI3K: phosphatidylinositol-3-kinase; ZAP-70: zeta-chain-associated protein kinase 70.*

associated with increased risk of seroconversion and accelerated disease progression, likely through a mechanism involving increased signal transduction through lck-initiated pathways[2] (Oyugi, unpublished). Currently, the impact of G protein polymorphisms in HIV acquisition and disease progression remains largely unexplored. Although many polymorphisms in G protein subunit genes result in tissue-specific disorders (reviewed by Weinstein, et al.[92]), several have been documented to have wide-ranging pleiotropic effects. In addition to a variety of hormonal pathologies, gain and loss of function mutations in the GNAS locus (the complex gene locus encoding Gαs proteins) have been associated with a more severe course of malarial infection, and a silent SNP in GNAS exon 5 is associated with hypertension, nervous system dysfunction, and differences in lymphocytic cancer progression and survival. In these cases, the T allele is associated with more stable mRNA and has been shown to affect apoptotic signaling pathways[93,94]. To date, no published studies have tested any associations between GNAS polymorphisms and HIV susceptibility or progression. In contrast, a well described SNP in the GNB3 gene (C825T) has been associated with several diseases including favorable responses to HIV antiretroviral treatment[95,96]. The T allele of this SNP is associated with the production of mRNA splice variants that result in more active G protein activity, possibly due to decreased stability of the Gα-Gβγ complex. Although no mechanisms have been demonstrated to explain the link between the SNP and antiretroviral response, the association warrants further investigation.

Regulation of G protein expression

Other insights into the role and regulation of G protein signaling during cellular infection may come from gene expression analysis following HIV infection with different viruses of different tropisms. Microarray studies of peripheral blood cells infected with HIV have shown that various G protein subunits (GNB1, GNB3, GNAS) are among some of the most highly upregulated genes following infection[97,98], but the importance of this upregulation is currently unknown. Additionally, a comparison of genes affected by PBMC incubation with either R5 or X4 envelopes shows that viral tropism results in significantly different gene expression profiles that depend almost exclusively on coreceptor engagement (i.e. not CD4). Incubation with R5 gp120

appears to preferentially modulate the p38 MAPK pathway and STAT-4 transcription factor, whereas X4 gp120 modulates the GATA-3 transcription factor, which binds the HIV LTR[98]. These differences in downstream transcription factor activation may result from differential G protein pathway activation through each coreceptor, and further studies may provide insight into the differences in pathogenesis dependent on viral tropism.

Conclusions

Despite the long-standing controversy and dispute over the relevance of G protein signaling in HIV infection and replication, improved techniques and a more thorough understanding of G protein pathways are beginning to elucidate the diverse and integral role of these signaling cascades in the viral lifecycle. Viral entry requires Gαq induction through the chemokine coreceptor; nuclear import in resting T-cells relies on cofilin activation by Gαi pathways and reverse transcription is dependent on Gαi-mediated Erk induction. Additionally, the role of G proteins in mediating chemotaxis and chemokine/cytokine release plays an important role in the enhancement of viral pathogenesis and spread.

In order to fully appreciate the role of these signaling events during viral infection, it is crucial to thoroughly test the role of specific signaling cascades and to appreciate the confounding diversity of G protein-based signaling; receptor-dependent events may be mediated by either G protein-dependent or G protein-independent pathways, and G protein-dependent pathways may utilize Gai subunits (PTX-sensitive) and/or Gaq subunits (PTX-insensitive), each of which bind different receptor motifs. Additionally, inhibitors such as PTX may have pleiotropic effects that must be considered, and failure to control for the activation state of a cell may mask important signaling effects. By taking these factors into consideration, the field will more easily be able to identify and appreciate the importance of G protein-mediate signaling in HIV infection and take advantage of its potential therapeutic effects.

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