Stromal Cell-Derived Factor-1 Signaling via the CXCR4-TCR Heterodimer Requires Phospholipase C-β3 and Phospholipase C-γ1 for Distinct Cellular Responses

Kimberly N. Kremer, Ian C. Clift, Alexander G. Miamen, Adebowale O. Bamidele, Nan-Xin Qian, Troy D. Humphreys and Karen E. Hedin

J Immunol 2011; 187:1440-1447; Prepublished online 24 June 2011; doi: 10.4049/jimmunol.1100820
http://www.jimmunol.org/content/187/3/1440

Supplementary Material
http://www.jimmunol.org/content/suppl/2011/06/22/jimmunol.1100820.DC1

Why The JI?
• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Speedy Publication! 4 weeks from acceptance to publication

*average

References
This article cites 42 articles, 24 of which you can access for free at:
http://www.jimmunol.org/content/187/3/1440.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Stromal Cell-Derived Factor-1 Signaling via the CXCR4-TCR Heterodimer Requires Phospholipase C-β3 and Phospholipase C-γ1 for Distinct Cellular Responses

Kimberly N. Kremer, Ian C. Clift, Alexander G. Miamen, Adebowale O. Bamidele, Nan-Xin Qian,¹ Troy D. Humphreys,² and Karen E. Hedin

The CXCR4 chemokine receptor is a G protein-coupled receptor that signals in T lymphocytes by forming a heterodimer with the TCR. CXCR4 and TCR functions are consequently highly cross regulated, affecting T cell immune activation, cytokine secretion, and T cell migration. The CXCR4-TCR heterodimer stimulates T cell migration and activation of the ERK MAPK and downstream AP-1–dependent cytokine transcription in response to stromal cell-derived factor-1 (SDF-1), the sole chemokine ligand of CXCR4. These responses require Gi-type G proteins as well as TCR ITAM domains and the ZAP70 tyrosine kinase, thus indicating that the CXCR4-TCR heterodimer signals to integrate G protein-coupled receptor-associated and TCR-associated signaling molecules in response to SDF-1. Yet, the phospholipase C (PLC) isoforms responsible for coupling the CXCR4-TCR heterodimer to distinct downstream cellular responses are incompletely characterized. In this study, we demonstrate that PLC activity is required for SDF-1 to induce ERK activation, migration, and CXCR4 endocytosis in human T cells. SDF-1 signaling via the CXCR4-TCR heterodimer uses PLC-β3 to activate the Ras-ERK pathway and increase intracellular calcium ion concentrations, whereas PLC-γ1 is dispensable for these outcomes. In contrast, PLC-γ1, but not PLC-β3, is required for SDF-1–mediated migration via a mechanism independent of LAT. These results increase understanding of the signaling mechanisms employed by the CXCR4-TCR heterodimer, characterize new roles for PLC-β3 and PLC-γ1 in T cells, and suggest that multiple PLCs may also be activated downstream of other chemokine receptors to distinctly regulate migration versus other signaling functions. The Journal of Immunology, 2011, 187: 1440–1447.

CXCR4, a G-protein coupled receptor (GPCR), initiates migration, calcium mobilization, ERK activation, survival signals, gene transcription, and cytokine production upon binding its ligand, stromal cell-derived factor-1 (SDF-1; also called CXCL12). CXCR4 signaling in multiple cell types is important for embryonic development, homeostasis, vascularization, immune regulation, HIV-1 infection, and cancer progression (1, 2). CXCR4 uses numerous signal transmission strategies to regulate these downstream events, including activation of several G proteins and heterodimerization with other types of cell-surface receptors (3–5). The molecular mechanisms involved in mediating these diverse signaling outcomes have begun to be characterized. Of particular interest is the identification of signaling molecules that could be therapeutically targeted to disrupt SDF-1–mediated migration without altering SDF-1–induced gene transcription, because this would be helpful for treating the numerous diseases involving CXCR4. In this study, we show that migration and ERK activation in response to SDF-1/CXCR4 signaling in T cells is mediated by two distinct phosphatidylinositol-specific phospholipase C (PLC) isoforms.

We previously showed that upon SDF-1 binding to CXCR4 in T cells, CXCR4 heterodimerizes with the TCR to stimulate increased calcium ion concentrations ([Ca²⁺]i), prolonged ERK activation, gene transcription, and cytokine production. We further showed that these outcomes of CXCR4-TCR signal transduction occur via pathways that use several traditional TCR signaling molecules including ZAP70 and SLP-76 as well as G proteins (6–8). Others have similarly demonstrated that CXCR4 signaling in T cells uses the TCR to mediate CXCR4 endocytosis and SDF-1–mediated migration via p52Shc (9). Thus, the SDF-1–induced heterodimerization of CXCR4 with the TCR is critical for many T cell functions and allows CXCR4 to access additional signaling molecules to achieve CXCR4’s diverse signaling outcomes. Although both the TCR and GPCRs including CXCR4 have been shown to signal via various PLC isoforms, the particular PLC isoforms involved in mediating the distinctive signaling pathways arising from SDF-1 signaling via the CXCR4-TCR heterodimer in T cells have not been fully characterized.

PLC enzymes, upon their activation by signals derived from upstream receptors, catalyze the cleavage of phosphatidylinositol 4,5-bisphosphate into 1,4,5-inositol triphosphate and diacylglycerol, and these second messengers subsequently lead to calcium mobilization and activation of protein kinase C. Mammals express 11 PLC isoforms, all of which contain the X and Y domains that comprise the catalytic core of the enzyme. Different PLC isoforms vary in the types of regulatory domains they contain, which allow...
the upstream receptors to signal to activate only particular PLC isoforms (10). For example, lysophosphatidic acid receptors signal to activate PLC-β3, whereas bradykinin signals to activate PLC-β1, with these PLC isoforms being activated by distinct upstream signaling mediators that bind their distinct PDZ-binding motifs to mediate their respective signaling functions within the same cell type (11).

The predominant PLC isoforms expressed in T lymphocytes include PLC-γ1, PLC-β2, and PLC-β3. PLC-γ isoforms were originally thought to be only activated downstream of receptor tyrosine kinases, whereas PLC-β isoforms are predominantly activated by GPCRs (10). Direct stimulation of the TCR activates PLC-γ1, which is responsible for the subsequent calcium mobilization and diacylglycerol production, leading to the ERK activation, gene transcription, cytokine production, and proliferation necessary for T cell development and T cell activation (12). PLC-β3 has been shown to be phosphorylated in response to SDF-1 in leukemia cells (13), and mice lacking both PLC-β2 and PLC-β3 display a partial defect in migration in response to SDF-1 (14). PLC-γ1 was recently shown to be required for T cell migration in response to signaling by the CCR7 chemokine receptor (15), and PLC-γ2 was shown to be required for B cell migration in response to SDF-1 (16), suggesting that chemokine receptors can also use PLC-γ isoforms for signaling leading to cellular migration. Yet, the particular PLC isoforms required for CXCR4 signaling apart from migration are poorly understood, and the role(s) for PLC-γ1 in SDF-1–mediated signaling in T cells has not been defined.

In this study, we demonstrate that the CXCR4-TCR heterodimer uses multiple PLCs, each with a distinct role, to mediate the diverse signaling outcomes of SDF-1 treatment of T cells. In addition, we describe new roles for both PLC-β1 and PLC-β3 in CXCR4-TCR signaling. These findings identify molecules that can regulate one pathway from a receptor without altering another and thus may allow for the selective therapeutic targeting of different CXCR4–derived responses.

Materials and Methods

Cells, stimulation conditions, reagents, and statistical analysis

Jurkat T cells were maintained as described (6, 8). Jurkat sublines deficient in PLC-γ1 (J.gammam1, 100% deficient in PLC-γ1; P98, >90% deficient in PLC-γ1) (17) or 100% deficient in LAT (ANJ-3) (18) were gifts of R. Abraham (Pfizer, Pearl River, NY). Stimulations were performed at 37°C with 5 mM CaCl2 (Calbiochem, San Diego, CA) or vehicle (ethanol) for 15–30 min. CXCR4 (R&D Systems) and TCR (CD3ε; BD Biosciences, San Diego, CA) mAbs were used for flow cytometry. Statistical analysis was via two-tailed t test (Microsoft Excel; Microsoft). The means of two distributions were considered significantly different if p ≤ 0.05.

Assays of active, phosphorylated ERK1 and ERK2 and short hairpin RNA-mediated protein depletion

For analysis of active ERK, cells were stimulated and assayed via flow cytometry or Western blot as described (8). Raw data from representative flow cytometric determinations of ERK activation is shown in Supplemental Fig. 1. Where indicated, 72 h prior to assay, cells were transiently transfected as described (8) with either a control plasmid or a plasmid encoding an H1 promoter-driven short hairpin RNA (shRNA) specific for human PLC-β3. The shRNA encoding plasmid also encoded either m. Cherry or GFP via a separate SV40 promoter. Flow cytometric data were gated to compare ERK activation in cells expressing similar levels of m. Cherry. The PLC-β3 shRNA-encoding plasmid was made by annealing and ligating DNA oligonucleotides into either pCM54.H1.p.mCherry (5) or the p1012 plasmid vector, a modified version of pCMS4.eGFP.H1p (19) (gifts of D. Billadeau, Mayo Clinic, Rochester, MN) lacking the FLAG tag. PLC-β3 shRNA oligos were: 5'-GATCCCGCGGTTGGAAAGGAGCTCTTATTCAATTCAAGAGATTTGAATAGCTTCAAGAGACCTTATTGGAAAA-3' and 5'-AGCTTTTCAAAAAAGGGCTTGAGGCTACTTGTCACTTGAAT-

TGATAAGCTCTCCATACCCGG-3'. Protein depletion was confirmed by immunoblotting whole-cell lysates with anti–PLC-β3 (Santa Cruz Biotechnology, Santa Cruz, CA). Controls were the same blots immunoblotted for total ERK2 (Santa Cruz Biotechnology).

Migration assay

Where indicated, Jurkat T cells were transfected with either p1012 or p1012 encoding shRNA specific for PLC-β3. Seventy-two hours later, migration assays were performed using the GFP fluorescence expressed by these plasmids to detect migrating cells. For migration assays using mutant Jurkat sublines, the cells were stained with 2 μM calcein-AM (Invitrogen, Carlsbad, CA) for 30 min, washed, and assayed for migration. All migration assays used 96-well Chemotaxis plates (Neuroprobe, Gaithersburg, MD) with 5-μm pore filters coated with fibronectin (Invitrogen). Cells (500,000) were diluted in Migration Buffer (RPMI 1640 without phenol red supplemented with 0.5% BSA and 1% DMSO) and placed in the upper chamber of each well. After migrating at 37°C for 1 h toward lower chambers containing the indicated concentrations of SDF-1, cells remaining in the upper chambers were removed, and cells that migrated into the lower chambers were quantified by measuring GFP or calcein-AM (485 nm excitation/530 nm emission) fluorescence using a Cytofluor 4000 spectrometer (PerSeptive Biosystems, Framingham, MA).

Assays of active, GTP-bound N-Ras and K-Ras, CXCR4–TCR interaction by fluorescence resonance energy transfer, and [Ca2+]

Active, GTP-bound Ras was measured using an activated Ras assay kit (Upstate Biotechnology, Waltham, MA) and immunoblotting with specific Abs to N-Ras or K-Ras (Santa Cruz Biotechnology). As controls, cell lysates were immunoblotted for N-Ras or actin. The interaction between CXCR4 and TCR in response to 20 min of SDF-1 treatment was assayed by fluorescence resonance energy transfer (FRET) as described (8, 20), with gating on GFP+ cells following transfection with P1012. [Ca2+], assays were performed as described (6).

Immunoprecipitation of PLC-γ1 and LAT

Cells were stimulated with SDF-1 for the indicated time, lysed in Buffer A (25 mM Tris HCl, 150 mM NaCl, 5 mM EDTA, 50 mM β-glycerophosphate, and 1% each of Nonidet P-40, leupeptin, microcystin-LR, aprotinin, and sodium orthovanadate), and endogenous signaling proteins were immunoprecipitated with Abs to PLC-γ1 or LAT (Santa Cruz Biotechnology). Immunoprecipitated proteins were separated by SDS-PAGE and immunoblotted with p-Tyr mAb (4G10; Upstate Biotechnology). LAF-phospho-Tyr171 (Cell Signaling Technology, Danvers, MA), PLC-γ1, or LAT.

Results

SDF-1/CXCR4 signaling requires a PLC to stimulate the ERK MAPK pathway, T cell migration, and internalization of CXCR4

We previously demonstrated that SDF-1 induces CXCR4 to complex with the TCR to prolong ERK activation, and we showed that these events lead to gene expression and cytokine production in activated T cells (7, 8). In addition, the SDF-1–mediated migration of T cells has been shown to require a mechanism involving CXCR4 use of the TCR (9). Yet, the PLC isoforms used by the CXCR4-TCR heterodimer to mediate these distinct outcomes have not been fully characterized. First, we used the PLC inhibitor U73122 to identify signaling outcomes affected by the loss of PLC activity. Jurkat T cells treated with U73122 activated significantly less ERK in response to SDF-1 as compared with vehicle-treated cells (Fig. 1A). Similarly, inhibiting PLC activity with U73122 substantially decreased the percent of cells migrating in response to SDF-1 (Fig. 1B). Furthermore, the internalization of CXCR4 in response to SDF-1 was also inhibited by pretreatment with U73122 (Fig. 1C, 1D). Together, these results indicate that PLC activity is required for SDF-1–induced signaling following formation of the CXCR4–TCR complex that mediates ERK activation and T cell migration as well as CXCR4 internalization.
SDF-1/CXCR4 signaling does not require PLC-γ1 to mobilize calcium, activate N-Ras, K-Ras, or ERK, or internalize CXCR4

We previously showed that calcium mobilization in response to SDF-1 requires the TCR, ZAP70, and SLP-76 (6, 8). PLC-γ1, which is known to use these same signaling molecules to mobilize calcium in response to direct TCR stimulation (21), seemed likely to participate in mediating calcium mobilization in response to SDF-1. Yet, the PLC-γ1–deficient cell line, J.gamma1, mobilized calcium in response to SDF-1 in a manner similar to the parental wild-type Jurkat T cell line (Fig. 2A). Moreover, ERK activation in response to SDF-1, which also requires the TCR, ZAP70, and SLP-76 (6, 8), was defective in J.gamma1 cells as assayed by a flow cytometric ERK activation assay. In fact, J.gamma1 cells showed a >50% increase in ERK activation in response to SDF-1 as compared with the parental Jurkat T cell line (Fig. 2B). The enhanced SDF-1–induced ERK activation in the J.gamma1 cells was nevertheless sensitive to inhibition with U73122, suggesting a role for a different PLC in this pathway (Fig. 2B). ERK activation assayed by Western blot similarly showed no defect in SDF-1–dependent ERK activation in J.gamma1 cells as compared with normal Jurkat cells (Fig. 2C). To determine which Ras isoforms are potentially mediating the increase in ERK activation in PLC-γ1–deficient cells, we examined the activation of the Ras isoforms N-Ras and K-Ras. We recently showed that these Ras isoforms are responsible for mediating signaling from the CXCR4–TCR heterodimer in response to SDF-1 (Kimberly N. Kremer, Ashok Kumar, and Karen E. Hedin, submitted for publication). N-Ras was activated in response to SDF-1 treatment of the J.gamma1 cells to levels similar to that seen in the parental Jurkat cell line (Fig. 2D), and K-Ras activation was actually increased in the J.gamma1 cell line (Fig. 2D). Thus, PLC-γ1 is not required for either N-Ras or K-Ras activation in response to SDF-1. Moreover, compared with wild-type cells, CXCR4 on J.gamma1 cells internalized normally in response to SDF-1 (Fig. 2E, 2F). In addition, PLC-γ1 is not required for the SDF-1–dependent signaling that leads to formation of the CXCR4–TCR heterodimer. Endogenous, cell-surface CXCR4 and TCR molecules were labeled via mAbs, stimulated with SDF-1, and assayed for FRET signals via flow cytometry (as in Refs. 8, 20). The results show that PLC-γ1–deficient Jurkat cells increased CXCR4–TCR FRET (FL3) fluorescence in response to SDF-1 in a manner similar to that observed in the parental Jurkat cell line (Fig. 2G, 2H). Thus, the results in Figs. 1 and 2 show that PLC-γ1 is not required for CXCR4–TCR complex formation in response to SDF-1 and that PLC-γ1 is not the PLC required for calcium mobilization, activation of N-Ras, K-Ras, or ERK or for internalization of CXCR4 in response to SDF-1 treatment of T cells.

PLC-β3 mediates SDF-1–induced calcium mobilization and activation of N-Ras, K-Ras, and ERK but not internalization of CXCR4

We next looked at the role of PLC-β3 in mediating the downstream signaling events of SDF-1. Jurkat T cells were transfected with either a control plasmid or a plasmid encoding shRNA targeted against PLC-β3, which yielded an 80–95% depletion of the PLC-β3 protein compared with vector-transfected cells (Fig. 3A). Both plasmids also express the fluorescent protein, m.Cherry, allowing detection and assay of shRNA-containing cells. Compared to cells transfected with the control plasmid, cells transfected with the plasmid encoding PLC-β3 shRNA displayed significantly lower SDF-1–dependent calcium mobilization (Fig. 3A), as well as lower SDF-1–mediated ERK activation as assayed by flow cytometry and Western blot (Fig. 3B, 3C). Consistent with the lower ERK activation in these cells, PLC-β3–deficient Jurkat cells also displayed defective activation of N-Ras and K-Ras in response to SDF-1 (Fig. 3D). In contrast to its effects on Ras and ERK activation, PLC-β3 depletion did not alter the internalization of CXCR4 in response to SDF-1 (Fig. 3E, 3F). Furthermore, PLC-β3 depletion did not inhibit the SDF-1–mediated CXCR4–TCR complex formation, as assayed by FRET (Fig. 3G, 3H). Thus, PLC-β3 is required for ERK activation downstream of the formation of the CXCR4–TCR heterodimeric complex in response to SDF-1. Together, these results indicate that PLC-β3 is required for SDF-1–mediated calcium mobilization and for the activation of N-Ras, K-Ras, and ERK in response to SDF-1 while indicating that other PLCs participate in mediating the internalization of CXCR4 in response to SDF-1.

PLC-γ1, but not PLC-β3, is required for the migration of T cells in response to SDF-1

We next determined the effect of depleting PLC-β3 on the migration of Jurkat T cells in response to SDF-1. Despite the requirement for PLC-β3 in the signaling pathways that lead to calcium mobilization and ERK activation in response to SDF-1 (Fig. 3), cells deficient in PLC-β3 did not show a defect in migration in response to SDF-1. In fact, the Jurkat cells transfected with the PLC-β3 shRNA showed a slight increase in migration in response to SDF-1 compared with cells transfected with the vector control (Fig. 4A). We next assayed the role of PLC-γ1 in migration by using the PLC-γ1–deficient cell line J.gamma1. In contrast to PLC-β3 deficiency, PLC-γ1 deficiency in the J.gamma1 cell
line resulted in a substantial decrease in migration in response to SDF-1 (Fig. 4B). To confirm this finding, we also used the P98 cell line, an independently obtained somatic mutant of Jurkat that is only ∼90% deficient in PLC-γ1, in contrast to the J.γamma1 cell line, which is 100% deficient in PLC-γ1 (17). As seen for J. gamma1 cells, P98 cells displayed defective migration in response to SDF-1 (Fig. 4C). The defective migration of PLC-γ1-deficient cells did not arise from defective cell-surface levels of CXCR4 (Fig. 4B, inset histograms). Cell-surface TCR levels were lower on J. gamma1 but not P98 cells (Fig. 4B, 4C, inset histograms); however, these PLC-γ1–deficient cells were similarly defective in migration toward SDF-1 (Fig. 4A–C). Together, these results indicate that PLC-γ1, but not PLC-β3, is essential for the SDF-1–mediated migration of T cells.

PLC-γ1 has not previously been shown to mediate T cell migration in response to SDF-1. Upon direct stimulation of the TCR, PLC-γ1 is recruited to the plasma membrane via the multi-molecular signaling complex nucleated by LAT and SLP-76 and is then rapidly phosphorylated by Itk, which allows PLC-γ1 to mediate its downstream signaling outcomes (21). Because migration in response to SDF-1 has been shown to depend on the transactivation of the TCR by CXCR4 (9), we determined if SDF-1 treatment induces the phosphorylation of PLC-γ1. In contrast to the results of directly stimulating the TCR with anti-CD3 mAb, SDF-1 treatment failed to detectably increase the tyrosine phosphorylation of PLC-γ1 (Fig. 4D). The phosphorylation of LAT on Tyr171 is involved in PLC-γ1 activation and downstream signaling in response to direct stimulation of the TCR (22); thus, we also assayed LAT phosphorylation on Tyr171 in response to SDF-1. Tyr171 of LAT failed to be inducibly phosphorylated in response to SDF-1 treatment, despite being phosphorylated in response to direct TCR stimulation in the same experiment (Fig. 4E). To confirm that LAT is not involved in PLC-γ1–dependent migration in response to SDF-1, we also assayed migration of a LAT-deficient somatic mutant of the Jurkat T cell line. Indeed, LAT-deficient Jurkat cells migrated in a manner similar to wild-type Jurkat cells (Fig. 4F). Thus, SDF-1 uses PLC-γ1, not PLC-β3, to mediate migration of T cells via a mechanism that is distinct from that arising from direct ligation of the TCR and that does not require tyrosine phosphorylation of either PLC-γ1 or LAT-Tyr171 and is independent of LAT protein expression.

**SDF-1 signaling via the CXCR4-TCR heterodimer requires different PLCs to mediate distinct cellular outcomes**

Based on our results presented in this study, and together with results reported previously by our group and others, we propose that CXCR4-TCR heterodimer signals in T cells in response to SDF-1 via the signaling pathway shown in Fig. 5. We propose that SDF-1 treatment induces the formation of the CXCR4-TCR heterodimer that mediates the activation of Gαi proteins and ZAP70 (6, 8, 23, 24) and that this event either directly or indirectly leads to the activation of several PLCs including both PLC-γ1 and PLC-β3. PLC-γ1 activity is required to mediate the migration of T cells via a mechanism that does not require either LAT (shown in this study) or SLP-76 (24) and is also not impaired by the lack of PLC-β3. Based on others’ observations, PLC-β2 (not shown) may also
mediate migration via signaling cross-talk with PLC-γ1 (14). In contrast to PLC-γ1 activation, PLC-β3 activation leads to ERK activation and gene transcription via a mechanism that previous results show does require SLP-76 but is independent of LAT (6). Thus, PLC-γ1 and PLC-β3 perform distinct, nonredundant functions in response to SDF-1. In addition to PLC-γ1 and PLC-β3, our results also indicate that SDF-1 treatment of T cells activates one or more other PLCs that are responsible for mediating CXCR4 endocytosis and the downregulation of CXCR4 from the cell surface in response to SDF-1.

Discussion
Since its discovery in 1994 (25), CXCR4 has been shown to be one of the widest-expressed and most biologically critical of all of the chemokine receptors. CXCR4 mediates a diverse array of signaling pathways in a large number of cell types, regulating crit-
The CXCR4-TCR heterodimer to integrate the signaling of other PLCs. Finally, we showed that PLC-γ1 mediates migration in response to SDF-1. PLC-γ1 is able to integrate signaling downstream of other GPCRs and is also activated by the TCR in a manner independent of SLP-76 (24). GPCR kinase interacting protein-1 has been shown to regulate PLC-γ1 signaling downstream of other GPCRs and is also activated by the TCR in a manner independent of SLP-76 (33, 34). PLC-γ1 signaling via the CXCR4-TCR heterodimer may therefore use GPCR kinase interacting protein-1 as an alternative mechanism of PLC-γ1 activation to regulate T cell migration. Furthermore, and as we show in this paper, PLC-γ1 can mediate cellular functions without the commonly seen inducible phosphorylation of its tyrosines (35). Bach et al. (14) have shown that depletion of PLC-γ1 can mediate cellular functions in mouse T cells partially inhibits T cell migration in response to SDF-1. PLC-γ1 may therefore function to regulate migration via a mechanism that integrates its signals with PLC-β2, possibly via forming a PLC dimer as has been seen for other PLC isoforms (36, 37). Thus, CXCR4-TCR signaling uses a distinct mechanism to integrate the functions of GPCR-associated and TCR-associated signaling molecules to mediate PLC-γ1-dependent migration.

Internalization of CXCR4 has been extensively studied because the functions of CXCR4 critically depend on its cell-surface expression levels. A key mechanism regulating this pathway includes the phosphorylation of CXCR4 by GPCR kinases and protein kinase C followed by the recruitment of β-arrestins (38). A role for PLC activity in the internalization of CXCR4 has not previously been described. Inhibition of Gi proteins (23) or the depletion of ZAP70 (K.N. Kremer and T.D. Humphreys, unpublished observations) failed to inhibit internalization of CXCR4 in response to SDF-1, consistent with neither PLC-γ1 nor PLC-β3 being required for this process. Further studies will be required to determine which

![Diagram](http://www.jimmunol.org/)

**FIGURE 5.** SDF-1 signaling via the CXCR4-TCR heterodimer requires different PLCs to mediate distinct cellular outcomes. Based on our results presented in this study, and together with results reported previously by our group and others, we propose that CXCR4-TCR heterodimer appears to signal in T cells in response to SDF-1 via the signal transduction pathway model shown. SDF-1 treatment induces the formation of the CXCR4-TCR heterodimer, which mediates the activation of Gi proteins and ZAP70, and this either directly or indirectly leads to the activation of several PLCs, including both PLC-γ1 and PLC-β3. PLC-γ1 activity is required to mediate the migration of T cells via a mechanism that does not require either LAT (shown) or SLP-76 and is also independent of PLC-β3. In contrast, PLC-β3 activation leads to ERK activation and gene transcription via a mechanism that previous results show does require SLP-76 but is independent of LAT. In addition to PLC-γ1 and PLC-β3, the CXCR4-TCR heterodimer appears to activate a different PLC(s) to participate in regulating CXCR4 endocytosis in response to SDF-1.
PLC isoforms are critically required for the internalization of CXCR4 in response to SDF-1. Together, the results in this paper indicate that multiple PLCs are able to integrate signaling from the CXCR4-TCR heterodimer to act as key mediators of SDF-1-mediated T cell functions. The PLC-dependent T cell functions mediated by SDF-1 described in this study are critical for many immunological events. Both PLC-β-deficient mice and mice specifically deficient in T cell PLC-γ display spontaneous autoimmune phenotypes involving mononuclear infiltrates, particularly into the skin and ear (12, 39). Furthermore, T cell-specific PLC-γ-deficient mice were characterized by a paucity of peripheral T cells, possibly arising from abnormal T cell migration, as well as by the impaired development and function of regulatory T cells (12). We previously demonstrated that SDF-1 costimulates IL-10 production in regulatory T cells by activating prolonged ERK and AP-1-mediated transcription (7). Thus, it seems possible that the SDF-1 signaling that is impaired by the lack of PLC-γ may inhibit the appropriate migration of regulatory T cells, whereas SDF-1 signaling impaired by the lack of PLC-β3 inhibits the costimulation of IL-10 production by regulatory T cells. In addition, both CXCR4 and PLC-γ have been implicated as regulators of the metastasis of various cancer cell types (2, 40–42). Our results therefore raise the possibility that PLC-γ1 helps mediate the SDF-1-induced metastasis of these tumor cells. The novel integration of multiple PLCs with distinct functions downstream of SDF-1-mediated signaling described in this study may thereby be responsible for the diverse roles CXCR4 plays in immunity and pathology.

Acknowledgments

We are grateful to Dr. R. Abraham for providing mutant Jurkat sublines and Dr. D. Billadeau for the pCMSt4.mCherry.H1p and pCMSt4.eGFP.H1p constructs.

Disclosures

The authors have no financial conflicts of interest.

References


