Rap1-mediated Lymphocyte Function-associated Antigen-1 Activation by the T Cell Antigen Receptor Is Dependent on Phospholipase C-γ1*

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The small GTPase, Rap1, is a potent activator of leukocyte integrins and enhances the adhesive activity of lymphocyte function-associated antigen-1 (LFA-1) when stimulated by the T cell receptor (TCR) or chemokines. However, the mechanism by which Rap1 is activated remains unclear. Here, we demonstrate that phospholipase C (PLC)-γ1 plays a critical role in the signaling pathway leading to Rap1 activation triggered by the TCR. In Jurkat T cells, TCR cross-linking triggered persistent Rap1 activation, and SDF-1 (CXCL12) activated Rap1 transiently. A phospholipase C inhibitor, U73122, abrogated Rap1 activation triggered by both the TCR and SDF-1 (CXCL12). PLC-γ1-deficient Jurkat T cells showed a marked reduction of TCR-triggered Rap1 activation and adhesion to intercellular adhesion molecule-1 (ICAM-1) mediated by LFA-1. In contrast, SDF-1-triggered Rap1 activation and adhesion were not affected in these cells. Transfection of these cells with an expression plasmid encoding PLC-γ1 restored Rap1 activation by the TCR and the ability to adhere to ICAM-1, accompanied by polarized LFA-1 surface clustering colocalized with regulator of adhesion and polarization enriched in lymphoid tissues (RAPL). Furthermore, when expressed in Jurkat cells, CalDAG-GEFI, a calcium and diacylglycerol-responsive Rap1 exchange factor, associated with Rap1, and resulted in enhanced Rap1 activation and adhesion triggered by the TCR. Our results demonstrate that TCR activation of Rap1 depends on PLC-γ1. This activity is likely to be mediated by CalDAG-GEFI, which is required to activate LFA-1.

The small GTPase, Rap1, is a potent signaling molecule capable of activating integrins (1). The constitutively active point mutant, Rap1V12, stimulates adhesion mediated by LFA-1, VLA-4, and VLA-5 by modulating integrin affinity and avidity (2–4). Rap1V12 also augments the affinity modulation of αLβ2 (5). Rap1 is activated by a number of external stimuli, including antigens and chemokines, and plays a pivotal role in mediating “inside-out signals” to activate integrins. Rap1 activation by TCR regulates the strength of the LFA-1/ICAM-1-mediated interaction between the T cell and antigen-presenting cells, which has decisive effects on the T cell response to antigen (4, 6, 7). Rapid Rap1 activation in chemokine-stimulated lymphocytes induces lymphocyte polarization, attachment to endothelial cells, and motility across endothelial barriers under flow (8). Thus, lymphocyte Rap1 is the key modulator of T cell activation and trafficking.

Rap1 cycles between an active, GTP-bound form and an inactive, GDP-bound form. The GDP-GTP cycle is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (9). The GTP-bound, active form of Rap1 is thought to exert its biological effects. Recently, we have identified a novel Rap1-binding molecule, RAPL, that is an essential regulator in LFA-1 activation and operates via spatial regulation of LFA-1 distribution (10). However, the signaling pathways by which the TCR and chemokine receptors increase GTP-bound Rap1 have not been defined. GEFs promote the dissociation of GDP from Rap1, thereby allowing its association with GTP. Rap1 activation has been shown to be mediated by GEFs, such as C3G, CalDAG-GEF, and Epac, which respond to specific intracellular signals (1, 9). In T cells, C3G has been shown to form a complex with Cbl via CrkL upon TCR stimulation (11). This tertiary complex was formed constitutively in anergic T cells, in which Rap1-GTP also accumulated (11). This study suggests that the Cbl-CrkL-C3G pathway regulates Rap1 activation triggered by the TCR. On the other hand, PLC-γ2 has been shown to be critical for Rap1 activation in chicken DT 40 B cells, as PLC-γ2-deficient DT 40 B cells were found to be defective in Rap1 activation by BCR. Notably, PLC-γ2 deficiency did not affect a BCR-triggered Cbl-CrkL-C3G association, suggesting a dominant role of PLC-γ2 in regulating Rap1 in B cells (12, 13). To date, it is unclear whether PLC-γ1, the major isotype of PLC-γ in T cells, could contribute to TCR-triggered Rap1 activation and also play a critical role in regulating integrin-mediated adhesion. Here, we report that PLC-γ1 plays a major role in Rap1 activation by TCR, resulting in LFA-1 activation. We further demonstrate that Rap1 is associated with CalDAG-GEFI and augments LFA-1-mediated adhesion upon TCR cross-linking.
Experimental Procedures

Antibodies—Anti-Rap1 (Transduction Laboratory), anti-FLAG (Sigma), anti-PLC-γ1 (Upstate Biotechnology), anti-T7 (Novagen), and horseradish peroxidase-conjugated goat anti-rabbit IgG (Cell Signaling) were used for immunoprecipitation and Western blotting. Anti-human LFA-1 (TS2/4) (American Type Culture Collection) conjugated with AlexaFluor 546 (Molecular Probes) and fluorescein isothiocyanate-conjugated anti-human CD44 (G44-20) (Pharmingen) were used for immunostaining.

Cell Culture and Transfection—Jurkat T cells and PLC-γ1-deficient Jurkat cells (American Type Culture Collection) were maintained as described previously (14). The cells were transfected by electroporation with FLAG-tagged PLC-γ1 (kindly donated by P.G. Suh, Pohang University of Science and Technology, Korea) and pEFpurov vector, CalDAG-GEFI (provided by M. Matsuda, Osaka University), pcDNA3.1/Hygro vector (Invitrogen), and T7-tagged Rap1 and Rap1V12 cDNAs, as described previously (2). Transfected cells were selected with 1 µg/ml puromycin (Sigma) or 0.25 mg/ml hygromycin B (Wako). T cells were purified from mouse lymph nodes with MidiMACS (Miltenyi Biotech) using CD90 (Pharmingen). These T cells were stimulated with anti-TCR antibodies (OKT3 or 2C11) or SDF-1 (R & D Systems) for the indicated time.

Pull-down Assays—Jurkat T cells or LN T cells suspended at 2 × 10^6/ml in RPMI 1640 were stimulated with anti-TCR antibody or 100 nM SDF-1 at 37 °C for the indicated times. After stimulation, cells were resuspended in 1% Triton X-100 containing lysis buffer (2). Active, GTP-bound Rap1 was measured using a GST-RalGDS-RBD fusion protein, as described previously (15).

Cell Adhesion Assay—Adhesion assays were performed as described previously (16). 500 ng/ml of recombinant human ICAM-1-IgG, Fc (hICAM-1-Fc) was used to coat 96-well plate wells (17). Following blocking in 1% BSA, cells were labeled with 2',7'-bis-(2-carboxyethyl)-5(6)-carbofluorescein (Molecular Probes) and resuspended in RPMI 1640 containing 10 mM Hepes (pH 7.4) and 150 mM NaCl, 1 mM MgCl2, 15% glycerol, 1% Hapes (pH 7.4), 150 mM NaCl, 1 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin). After centrifugation, the cell lysates were precleared with protein G-Sepharose 4B (Amersham Biosciences) at 4 °C for 1 h. Precleared lysates were immunoprecipitated with the indicated antibodies and protein G-Sepharose 4B.

Immunoprecipitation and Western Blotting—Transfected cells were lysed with 1% Nonidet P-40 buffer (1% Nonidet P-40, 10 mM MgCl2, 15% glycerol, 1% Hepes (pH 7.4), 150 mM NaCl, 1 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin). After centrifugation, the cell lysates were precleared with protein G-Sepharose 4B (Amersham Biosciences) at 4 °C for 1 h. Precleared lysates were immunoprecipitated with the indicated antibodies and protein G-Sepharose 4B. The beads were washed four times with lysis buffer. Cell lysates or immunoprecipitates were subjected to Western blotting as described previously (2).

Detection of CalDAG-GEFI Expression by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)—CalDAG-GEFI expression was detected by RT-PCR from mRNA isolated from Jurkat cells. Two sets of primers were designed based on the human CalDAG-GEFI sequence (GenBank™ accession number NP005816) to amplify the fragments specific for CalDAG-GEFI by 5'-1553-GTTACAGGCTCTCGGCAAG-3' and 5'-2049-CAGCCCGACTCCAGCCG-3' (primer set 1) or by 5'-1553-GTTACAGGCTCTCGGCAAG-3' and 5'-1567-ATTGCCCTCATCTCAGGACG-3' (primer set 2). The PCR products were cloned into a TOPO vector (Invitrogen) and verified by sequencing.

RESULTS

The Effects of Signal Inhibitors on Rap1 Activation by TCR and SDF-1—To explore the signal transduction pathways leading to Rap1 activation triggered by TCR and chemokines, we measured active Rap1-GTP using GST-RalGDS-RBD, which

![FIG. 1. TCR-induced activation of Rap1 via PLC. A. Jurkat cells were incubated with 2 µg/ml OKT3, 100 nM SDF-1, or medium alone for the indicated lengths of time. GTP-bound Rap1 was detected with pull-down assays using GST-RalGDS-RBD (upper). Total Rap1 is shown (lower). B, Jurkat cells were pretreated with 1 µM U73122 or U73343, 50 µM BAPTA-AM, and 50 µM PP1 for 10 min. The cells were then stimulated by the TCR for 10 min or SDF-1 for 30 s and analyzed for Rap1 activation as in A. C, mouse lymph node T cells were pre-treated with 1 µM U73122 or U73343, 100 nM SDF-1, or medium alone for 10 min, then stimulated by the TCR for 10 min or SDF-1 for 30 s and analyzed for Rap1 activation as in A.](http://www.jbc.org/)
binds specifically to Rap1-GTP (15). Both TCR cross-linking and SDF-1 induced strong Rap1 activation in Jurkat T cells. TCR-triggered Rap1 activation persisted for as long as 30 min. In contrast, SDF-1 induced a transient Rap1 activation, which peaked at 0.5 min and diminished rapidly thereafter (Fig. 1A). The kinetics of Rap1 activation in Jurkat T cells are similar to those in primary lymphocytes (8) (data not shown). Medium alone did not activate Rap1 in Jurkat cells (Fig. 1A), indicating that Rap1 activation was specific for TCR and SDF-1 stimulation and not due to the mixing in the tube (18). To identify the signaling pathway that couples TCR to Rap1 activation, Jurkat T cells were treated with specific signal inhibitors and then stimulated for Rap1 activation. As shown in Fig. 1B, Rap1 activation by TCR or SDF-1 was completely inhibited by the PLC inhibitor, U73122. The inactive analog, U73343, did not affect Rap1 activation (Fig. 1B). U73122 also inhibited Rap1 activation in primary mouse T cells (Fig. 1C). A calcium chelator, BAPTA-AM, did not block Rap1 activation at all (Fig. 1B).

**Activation of Rap1 by the TCR Requires PLC-γ1**—Because the inhibitor experiment suggested that protein-tyrosine kinase-dependent PLC activity is critical for TCR-mediated Rap1 activation, we examined the involvement of PLC-γ1 using a mutant Jurkat cell line, in which there is no expression of PLC-γ1 (14) (Fig. 2). Because these cells do not express PLC-γ2, they are unable to produce IP3 and diacylglycerol (DAG) in response to TCR engagement, resulting in defective calcium influx and IL-2 production (14). We found that TCR-induced activation of Rap1 was markedly reduced in the PLC-γ1-deficient Jurkat cells as compared with wild-type cells (Fig. 2B, left). In contrast, SDF-1-induced Rap1 activation was normal, ruling out the possibility that Rap1 itself is defective in the mutant cells (Fig. 2B, right). To further confirm the role of PLC-γ1 in Rap1 activation by the TCR, we conducted rescue experiments to restore PLC-γ1 expression. FLAG-tagged PLC-γ1 was introduced into the mutant cells at a level similar to that in wild-type cells (Fig. 2A). The expression of PLC-γ1 resulted in almost fully restored Rap1 activation by the TCR (Fig. 2B, left), while introduction of PLC-γ1 did not affect the level of Rap1-GTP when stimulated with SDF-1 (Fig. 2B, right). These results demonstrated the critical involvement of PLC-γ1 in Rap1 activation triggered by TCR engagement, but not SDF-1.

**PLC-γ1 Regulates LFA-1/ICAM-1-mediated Adhesion**—Because Rap1 mediates inside-out signaling from TCR to activate LFA-1 binding of ICAM-1 (6), we examined whether PLC-γ1 deficiency could impair LFA-1-mediated adhesion to ICAM-1. As shown in Fig. 3A, TCR stimulation did not induce ICAM-1 adhesion in PLC-γ1-deficient cells. The expression of LFA-1 in the PLC-γ1-deficient cells was similar to that in wild-type cells by fluorescence-activated cell sorter analysis (data not shown). In contrast, PMA stimulated adhesion of PLC-γ1-deficient Jurkat cells, which was comparable with that of the wild type. This result rules out the possibility that the adhesion defect is due to defects downstream of PLC-γ1. The re-expression of PLC-γ1 in the mutant cells restored TCR-induced LFA-1 adhesive activity to ICAM-1 at a level similar to that in wild-type cells, indicating that PLC-γ1 deficiency impaired TCR-triggered adhesion. Importantly, SDF-1-triggered adhesion to ICAM-1 in PLC-γ1-deficient Jurkat cells was comparable with that of the wild-type and was not changed significantly by introducing PLC-γ1 (Fig. 3B). This result is consistent with the result that showed that Rap1 activation occurred normally upon SDF-1 treatment in PLC-γ1-deficient Jurkat cells (Fig. 2B). Therefore, these results indicate that Rap1 activation by PLC-γ1 is crucial in adhesion by TCR. In addition, the involvement of PLC-γ1 downstream of Rap1 is unlikely, because Rap1V12 induced spontaneous adhesion of PLC-γ1-deficient Jurkat cells, comparable with that of wild type cells (Fig. 3C).

We next examined whether the redistribution of RAPL depended on PLC-γ1, because RAPL is the Rap1 effector molecule critical for Rap1-mediated adhesion, and RAPL associates with LFA-1 and relocates LFA-1 to the leading edge upon Rap1 activation (10). We compared the distribution of LFA-1 and RAPL in adhesion-defective, PLC-γ1-deficient Jurkat cells with cells reconstituted with PLC-γ1. TCR engagement has been found to induce a patch-like distribution of LFA-1 at the leading edge, and CD44 to a uropod, and RAPL colocalized with the patches of LFA-1 (10). In PLC-γ1-deficient Jurkat cells, the uniform distribution of LFA-1 and CD44 on the cell surface was not changed significantly upon TCR engagement (Fig. 3D), as seen in unstimulated wild-type cells (data not shown). In contrast, the introduction of PLC-γ1 induced segregation of LFA-1 and CD44 at opposite sides upon TCR engagement. RAPL colocalized with polarized surface LFA-1. This result is consistent with the results in an earlier report (10), showing colocalization of LFA-1 and RAPL at the leading edge is critical for LFA-1 activation. These further results demonstrated that PLC-γ1 was indispensable for the redistribution of LFA-1 and RAPL and support the notion that PLC-γ1 deficiency results in defective Rap1 activation, which in turn impairs spatial regulation of LFA-1 distribution mediated by RAPL.
CalDAG-GEFI Enhances TCR-induced Rap1 Activation and Adhesion—Because PLC-γ1 generates IP₃ and DAG, the Rap1 GEF family, having Ca²⁺ and DAG binding domains, is likely to be involved in Rap1 activation downstream of PLC-γ1. To explore this possibility, we examined whether CalDAG-GEFI, which is exclusively expressed in the hematopoietic system, the immune system, and the brain (19), could be involved in Rap1 activation triggered by TCR. CalDAG-GEFI mRNA was detected by RT-PCR in Jurkat cells (Fig. 4A). FLAG-tagged CalDAG-GEFI was overexpressed in wild-type Jurkat cells (Fig. 4B), and the effects on Rap1 activation by TCR were examined. As shown in Fig. 4C, Rap1 activation following TCR engagement was increased ~2-fold in CalDAG-GEFI-expressing cells, compared with the level of activation in control cells. Consistently, the introduction of CalDAG-GEFI into Jurkat cells resulted in significantly more adhesion to ICAM-1 than was observed in parent cells upon TCR engagement (Fig. 4D). The treatment by U73122, but not control U73343, inhibited TCR-stimulated adhesion. Thus, forced expression of CalDAG-GEFI enhanced TCR-induced endogenous Rap1 activation and adhesion to ICAM-1. In addition, we found that CalDAG-GEFI could associate with Rap1. As shown in Fig. 4E, when intro-
duced into Jurkat cells, T7-tagged Rap1 and FLAG-tagged CalDAG-GEFI coimmunoprecipitation experiments identified an association with either anti-T7 or anti-FLAG antibody. Furthermore, this association was augmented upon TCR engagement. However, this coimmunoprecipitation was not detected with control mouse IgG and not with either anti-T7 or anti-FLAG antibodies using each of the single transfectants (Fig. 4E). Taken together, these data indicate that CalDAG-GEFI could be physically and functionally linked with TCR-triggered Rap1 activation.

**Fig. 4. Involvement of CalDAG-GEFI in Rap1 activation by the TCR.**
A, RT-PCR analysis of CalDAG-GEFI using mRNA isolated from Jurkat cells with (+) or without (−) reverse transcriptase (RTase). The specific fragments of CalDAG-GEFI were amplified by two primer sets (primer set 1 and set 2). The RT-PCR products (450 and 430 bp) in agarose gel electrophoresis are indicated. B, vector alone, or FLAG-tagged CalDAG-GEFI, were introduced into wild-type Jurkat cells. The amounts of FLAG-CalDAG-GEFI were detected by anti-Flag antibody. C, wild-type Jurkat cells transfected with the vector or CalDAG-GEFI were stimulated with OKT3 for 10 min and analyzed for Rap1 activation, as in Fig. 1A. D, wild-type Jurkat cells transfected with the vector or CalDAG-GEFI, with or without pretreatment with 1 μM U73122 or U73343 for 10 min, were stimulated with OKT3 at 37°C on ICAM-1-coated plates and analyzed as described under “Experimental Procedures.” The average and S.E. values of triplicate experiments are shown. **, p < 0.05, compared with control wild-type cells stimulated by the TCR. E, wild-type Jurkat cells transfected with T7-tagged wild-type Rap1 and FLAG-CalDAG-GEFI (T/F) or Jurkat cells transfected with T7-tagged wild-type Rap1 (T) or FLAG-CalDAG-GEFI (F) were stimulated with OKT3 for 5 and 10 min and lysed. The proteins were immunoprecipitated (IP) with anti-T7 or anti-FLAG antibody, control mouse IgG (Ig), and detected with anti-FLAG or anti-T7 antibody, respectively (upper). Total FLAG-CalDAG-GEFI or T7-tagged wild-type Rap1 was shown (lower).
Rap1 Activation by TCR Depends on PLC-γ1

DISCUSSION

In this study, we have examined critical signal transducers for Rap1 activation triggered by TCR and SDF-1 using specific inhibitors and PLC-γ1-deficient Jurkat cells. Our results have demonstrated that PLC-γ1 plays an essential role in Rap1 activation by the TCR but not in activation by SDF-1. The inability to activate Rap1 due to PLC-γ1-deficiency resulted in the failure of TCR-triggered adhesion to ICAM-1. However, little effect on SDF-1-induced adhesion was observed. Our results further support the idea that CalDAG-GEFII could be an important Rap1 exchange factor downstream of PLC-γ1 activity triggered by TCR.

Using variant Jurkat cells, we have demonstrated that PLC-γ1 was required for Rap1 activation and LFA-1-mediated adhesion upon TCR engagement. The defective Rap1 activation was associated with a downstream failure in polarized redistribution and colocalization of LFA-1 with RAPL in PLC-γ1-deficient Jurkat cells. This result is consistent with our previous study, which demonstrated that Rap1-triggered RAPL association with LFA-1 is critical for LFA-1 activation. Reconstitution experiments with PLC-γ1 rescued TCR-triggered Rap1 activation and adhesion, concomitant with clustered LFA-1 colocalized with RAPL at the leading edge. Taken together, these results confirm that PLC-γ1 activation is indeed, the critical signaling point leading to spatial regulation of LFA-1 mediated by Rap1 and RAPL.

The molecular mechanism of TCR-triggered activation of PLC-γ1 has been extensively studied. The consensus from many studies is that a complex of LAT (linker for activated T cells), adaptor proteins such as Gads and SLP-76, and a member of the Tec family of tyrosine kinases, Itk, regulate PLC-γ1 activation in response to TCR signaling (20). Vav1 is also involved in PLC-γ1 activation following TCR stimulation, probably through association with SLP-76 and Itk/Tec activation (21). Signaling molecules required for PLC-γ1 activation by TCR, such as ZAP-70, SLP-76, and Itk, are critical for the activation of β1 integrins by the TCR (22, 23). In agreement with this, PLC-γ1-deficient Jurkat cells also failed to show an adhesive response through VLA-4 to fibronectin (data not shown). Vav1-deficient thymocytes and peripheral T cells were also shown to be defective in LFA-1 patches and adhesion to ICAM-1, extracellular matrix proteins, and antigen-presenting cell upon TCR stimulation (24). This adhesion defect was considered to be due to deficient actin cytoskeleton. However, LFA-1 localization at immunological synapses appears to be independent of an actin cytoskeleton (25). Our study suggests the alternative possibility that defective PLC-γ1 activation in Vav1-deficient T cells impairs Rap1-induced integrin activation. Thus, PLC-γ1 likely plays a pivotal role in TCR-triggered adhesion mediated by β1 and β2 integrins through Rap1.

In response to external stimuli, PLC hydrolyzes phosphatidylinositol 4,5-biphosphate to produce DAG and IP3, which in turn increases the level of intracellular free Ca2+. There is a distinct family of GEF that contain both Ca2+ and DAG binding domains (1). Members of this family that can act on Rap1 in response to Ca2+ and DAG are CalDAG-GEFI and CalDAG-GEFIII. CalDAG-GEFI is highly enriched in the brain and hematopoietic cells and is more specific for Rap1 than Rap2 (19). In contrast, CalDAG-GEFIII is ubiquitously expressed and has a broad specificity for other Ras family members. We showed that Rap1 could associate with CalDAG-GEFI in Jurkat cells, as reported from PC12 cells stimulated with carbachol (20). Our results have demonstrated that forced expression of CalDAG-GEFI activated endogenous Rap1 and augmented adhesion to ICAM-1 in response to TCR stimulation. This result is in accord with a previous study showing that CalDAG-GEFI is a possible regulator of Rap1β1 and agonist-induced fibrinogen binding to α1β3 in megakaryocytes (5). Thus, CalDAG-GEFI, and possibly also CalDAG-GEFIII, likely act as Rap1 exchange factors downstream of PLC-γ1 in response to TCR stimulation.

It has been reported previously that in anergic alloantigen-specific human T cells Rap1 activation by the TCR was correlated with the recruitment of the C3G/CrkL complex to the plasma membrane via phosphorylated Cbl and Fyn (11). Recent studies from our laboratory, and others (4, 6), have demonstrated that activated Rap1 was required for productive T cell responses through regulated integrin-mediated adhesion. Rap1 was persistently activated by antigen-presented antigen-presenting cell (6) or TCR crosslinking, as shown in this study. PLC-γ1 deficiency decreased Rap1 activation markedly; activation was restored by the introduction of PLC-γ1. These results are consistent with a study using chicken DT40 B cells in which BCR-triggered Rap1 activation was dependent on PLC-γ2. Because PLC-γ1-deficient Jurkat T cells still showed a slight increase in Rap1 activation by TCR, alternative pathways, such as the Cbl-C3G-CrkL pathway, might play some role. In addition, it was suggested that the Cbl-C3G-CrkL pathway may play an important role in immature thymocytes (27). These results imply that Rap1 activation mechanisms are differently regulated in distinct stages of T cell differentiation.

Treatment with the PLC inhibitor, U73122, abolished Rap1 activation by TCR and SDF-1. However, SDF-1 was able to normally induce Rap1 activation in PLC-γ1-deficient Jurkat cells, a result that contrasts with the indispensable role of PLC-γ1 in TCR-induced Rap1 activation. Treatment of B cells with SDF-1 resulted in the rapid activation of Rap1 and Rap2; this activation was also blocked by U73122 (28). Pertussis toxin-sensitive Gt proteins have been shown to couple chemokine receptors with PLC-β (29, 30). In mouse deficient for both PLCβ2 and PLCβ3, cell migration toward chemokine gradients was defective in neutrophils but not in lymphocytes (31, 32). Thus, multiple PLC isoforms likely contribute to chemokine-induced Rap1 activation in lymphocytes.

This study demonstrates the critical role of PLC-γ1 as a regulator of integrin-mediated adhesion through Rap1. This finding gives new insights in the understanding of the in vivo functions of PLC-γ1 during T cell development and the immune response.

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