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SAP-Mediated Inhibition of Diacylglycerol Kinase α Regulates TCR-Induced Diacylglycerol Signaling

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Diacylglycerol kinases (DGKs) metabolize diacylglycerol to phosphatidic acid. In T lymphocytes, DGKα acts as a negative regulator of TCR signaling by decreasing diacylglycerol levels and inducing anergy. In this study, we show that upon costimulation of the TCR with CD28 or signaling lymphocyte activation molecule (SLAM), DGKα, but not DGKβ, exits from the nucleus and undergoes rapid negative regulation of its enzymatic activity. Inhibition of DGKα is dependent on the expression of SAP, an adaptor protein mutated in X-linked lymphoproliferative disease, which is essential for SLAM-mediated signaling and contributes to TCR/CD28-induced signaling and T cell activation. Accordingly, overexpression of SAP is sufficient to inhibit DGKα, whereas SAP mutants unable to bind either phospho-tyrosine residues or SH3 domain are ineffective. Moreover, phospholipase C activity and calcium, but not Src-family tyrosine kinases, are also required for negative regulation of DGKα. Finally, inhibition of DGKα in SAP-deficient cells partially rescues defective TCR/CD28 signaling, including Ras and ERK1/2 activation, protein kinase C membrane recruitment, induction of NF-AT transcriptional activity, and IL-2 production. Thus SAP-mediated inhibition of DGKα sustains diacylglycerol signaling, thereby regulating T cell activation, and it may represent a novel pharmacological strategy for X-linked lymphoproliferative disease treatment. The Journal of Immunology, 2011, 187: 5941–5951.

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to transcriptional activation of specific genes whose expression mediates T cell proliferation and differentiation. Activation of Ras and PKC θ triggers key signaling pathways, leading, among others, to the activation of NF-AT and NF-κB and contributing to transcription of the IL-2 gene (8, 9). In T cells, activation of Ras and PKC θ is dependent on the generation of diacylglycerol (DAG) through phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol-4,5-bis-phosphate. DAG recruits RasGRP, the Ras-GEF mainly responsible for TCR-induced Ras activation, and PKC θ to the plasma membrane (10, 11). Notably, engagement of TCR in the absence of costimulation results in a weak and transient activation of both Ras and PKC θ, which drives T cells into anergy, a hyporesponsive status characterized by the inability to produce IL-2 and proliferate (12, 13).

DAG generated upon T cell activation is rapidly metabolized by DAG kinases (DGKs), a multigenic family of enzymes responsible for phosphorylation of DAG to phosphatidic acid (PA). Consistently with the crucial role of DAG signaling in T cell activation, several pieces of evidence indicate that the DGK θ and DGK θ isomers, which are highly expressed in thymus and T cells, act as negative regulators of TCR signaling and immune cell function (14). Specifically, 1) genetic deletion of DGK θ and DGK θ in T cells enhances TCR-induced activation of ERK1/2, resulting in defective induction of anergy (15, 16); 2) DGK θ is strongly induced in anergic T cells (13); 3) overexpression of either DGK θ or DGK θ impairs CD3/CD28-induced activation of Ras signaling (17–19); 4) pharmacological inhibition of DGKs reverses the inability of anergic cells to produce IL-2 in response to TCR stimulation (13); and 5) DGK θ expression is downregulated within a few hours from T cell activation (19). Collectively, these data support the concept that second messengers signaling is highly dependent on the fine tuning of DAG synthesis and degradation rates. Although there is no evidence for regulation of DGK θ upon T cell activation, TCR/CD28 costimulation of T cells results in rapid and sustained recruitment of DGKs to the plasma membrane (19), an event mediated by both Lck-dependent phosphorylation of tyrosine 335 and calcium binding to the EF hand domain of DGK θ (20, 21).

Based on the role of DGK θ as a negative regulator of T cell responses, we investigated the hypothesis that, upon T cell stimulation, DGK θ activity might undergo negative regulation. In this study, we show indeed that the enzymatic activity of DGK θ is inhibited upon costimulation of TCR and CD28 through a SAP-mediated mechanism. Moreover, we found that, in SAP-deficient cells, defective TCR/CD28 signaling and T cell activation can be partially rescued by inhibition of DGK θ.

Materials and Methods

Cell culture

Jurkat A3 cells (LCG Standards) and 293FT cells (Life Technologies) were cultured, respectively, in RPMI 1640 GlutaMAX medium or DMEM GlutaMAX high glucose (Life Technologies), supplemented with 10% FBS (Life Technologies) and antibiotic-antimycotic solution (Sigma-Aldrich) in humidified atmosphere with 5% CO2 before further stimulation. BI-141 TTS-SAP cells were a gift of A. Veillette (Montreal, QC, Canada). Jurkat/SAP-short hairpin RNA (shRNA) cells were obtained by infection with lentiviruses encoding a shRNA specific for murine DGKs in pLKO.1-Puro vector (clone ID TRCN000002 82712 RNAi Consortium through Sigma-Genosys), sequence: 5′-CCGGCAACAGTGTTACTACGGGATAATCCGAGTTATCCTCGAGATACACACCTTACCTAGTCTTTTT-3′. Jurkat/control-shRNA cells were obtained by infection with lentiviruses encoding a shRNA specific for murine DGKs in pLKO.1-Puro vector (clone ID TRCN000002 24825 RNAi Consortium through Sigma-Genosys), sequence: 5′-CCGGGAATCAGTTAAGGTTGATCATCTCGAGATACACACCTTACCTAGTCTTTTT-3′.

Lentivirus production and Jurkat infection were carried out according to the manufacturer’s instructions. Infected Jurkat cells were selected for 14 d in puromycin (1 μg/ml) and used as a bulk population in all experiments.

Reagents

The Abs used recognize the following proteins: pan-Ras (Ab-4; Merck), H-Ras (F235; Cell Signaling Technology), linker for activation of T cells (LAT; Santa Cruz Biotechnology), anti–IL-2α receptor Ab (TAC; Abcam), CD3 antigen (OKT3; provided by: U. Dianzani, Novara, Italy), CD28 agonist (ANC28.1/5D10; Ancell) (except for Fig. 4D, where anti-CD28 was from BD Pharmingen), SLAM agonistic Ab (A12; BioLegend), anti-DGK θ Abs (gift of M. Topham, Salt Lake City, UT), mixture of DGK θ Abs used for immunoprecipitation (22), DGK θ (C-20) and PKC θ (Santa Cruz Biotechnology) used for immunofluorescence, ERK1/2 and phospho-ERK1/2 from Cell Signaling Technology for Supplemental Fig. 2 and from Cell Signaling Technology Laboratories for Fig. 5C; SAP (FL-128; Upstate Biotechnology), α-tubulin (Sigma-Aldrich), secondary HRP-conjugated Abs (PerkinElmer), secondary FITC-conjugated Ab (Dako), and Alexa Fluor 546-phallolidin (Life Technologies). In all experiments involving stimulation with Abs, species-matched preimmune serum (Santa Cruz Biotechnology) was used for controls in equal amounts.

Inhibitors used were from Sigma-Aldrich: R59949, DGKs inhibitor; PP2, Src-family inhibitor; U73122, PLC inhibitor; IPA-3, PAK-specific inhibitor. BAPTA-AM was dissolved in water; other inhibitors were dissolved in DMSO. DMSO was always used in control samples at the same dilution as the inhibitor tested.

Expression vectors and transfections

GFP-SAP-wild type, GFP-SAP-R78A, and GFP-SAP-R55L were a gift of P. Schwartzberg (National Institutes of Health, Bethesda, MD). N-terminal yellow fluorescent protein (YFP)-DGK θ was obtained by cloning DGK θ in PYP-P-DEST (Life Technologies) using the Gateway kit (Life Technologies) according to the manufacturer’s instructions, pNF-AT-TA-luciferase reporter vector and pRL-TK normalization vector were from Clontech. Small interfering RNA (siRNA) and negative control siRNA were from Ambion/Life Technologies: DGK θ siRNA (23 sense, 5′-GGUCA-GUGAGUGUCCUAAGTT-3′, antisense, 5′-CUUAGGACUACUGAC-ACTT-3′). Transient transfections in Fig. 5D and 5E were performed using Lipofectamine 2000 reagent (Life Technologies) according to the manufacturer’s instructions. Microinjection of Jurkat cells for imaging experiments was performed according to the manufacturers’ instructions with the Microporator MP-100 system from Digital Bio Technology (Fig. 2, Supplementary Fig. 3B) or with the Gene Pulser II from Bio-Rad (Fig. 5B).

Cell stimulation, preparation of cell lysates and homogenates, immunoprecipitation, Western blotting, and DGK assay

Cells (3 × 107/ml) were resuspended in RPMI 1640 and incubated for the indicated time with agonist Abs or control species-matched preimmune serum. Jurkat cells (3 × 107) were lysed in 1 ml lysis buffer A (25 mM HEPES [pH 8], 1% Nonidet P-40, 10% glycerol, 150 mM NaCl, 5 mM EDTA, 2 mM EGTA, 1 mM ZnCl2, 50 mM ammonium molybdate, 10 mM NaF, 1 mM sodium orthovanadate, and protease inhibitor mixture from Sigma-Aldrich). An aliquot of cell lysate was retained for Western blot analysis, and the remainder was immunoprecipitated with a mixture of anti-DGK θ Abs as previously described (24). Whole-cell homogenates were prepared by homogenizing 3 × 107 cells in 1 ml cold buffer B (buffer A without detergent) by 20 passages in a 23-gauge syringe. Protein concentration was determined by BCA (Pierce), and equal amounts of proteins were loaded in each lane. SDS-PAGE and Western blots were performed as described previously (25). Western blot results were acquired with a VersaDoc system and quantified using Quantity One software (Bio-Rad). DGK θ activity in cell homogenates (25 μl) and anti-DGK θ immunoprecipitates were assayed by measuring initial velocities (5 min at 30°C) as previously described (24). Radioactive signals were detected and quantified by GS-250 Molecular Imager and Phosphor Analyst software (Bio-Rad).
**Immunofluorescence**

For immunofluorescence on fixed cells with Ab stimulation, cells were seeded on poly-t-lysine-coated glass coverslips (Marienfeld) in 24-well plates for 1 h and then stimulated with 10 μg/ml agonist Abs for 1 h in the presence or absence of the indicated inhibitors. Cells were then fixed with formaldehyde and stained as previously described (26). Confocal images were acquired with a Leica confocal microscope TSP2 (objective, ×63; numerical aperture, 1.32) and analyzed with LCS confocal software (Leica).

For the immunological synapse experiments, Raji cells (used as APCs) were incubated for 2 h with 10 μg/ml staphylococcal enterotoxin E. Raji cells were washed, mixed with Jurkat control-shRNA or Jurkat SAP-shRNA (1:1) for 15 min, and plated on polylysine-coated wells of diagnostic microscope slides (Erie Scientific). Cells were allowed to adhere for 15 min and then fixed in methanol at −20 °C for 10 min. Samples were then washed for 5 min in PBS and incubated with anti-PKCα Ab overnight at 4 °C. After washing in PBS, samples were incubated for 1 h at room temperature with FITC-labeled anti-goat Ab. Images were taken using an Axio Imager Z1 microscope equipped with an HBO 50-W mercury lamp for epifluorescence and with an AxioCam HR cooled charge-coupled camera (Carl Zeiss).

For live cell imaging experiments, Jurkat A3 cells, Jurkat control-shRNA, and Jurkat SAP-shRNA were microporated and serum starved in RPMI 1640 plus 0.2% BSA plus 50 mM HEPES for 2 h. Cells were seeded on glass-bottom dishes coated with poly-t-lysine or with the agonistic Ab anti-CD3, anti-CD1, anti-CD28, or anti-SLAM plus anti-CD28 at the final concentration of 10 μg/ml. Confocal images were acquired at the indicated times with a Zeiss LSM 510 inverted laser scanning microscope using a C-Apochromat ×63 water immersion objective lens (Carl Zeiss). Laser scanning microscope image files were processed using the Zeiss ZEN laser scanning microscope image browser software. When comparisons among images were to be made, the images were taken in identical conditions and equally manipulated using Adobe Photoshop 7.0 software (Adobe Systems).

**Cell fractionation**

Cells (3 × 10^7/ml) were resuspended in RPMI 1640 and incubated for the indicated time with agonist Abs or control species-matched preimmune serum at 37 °C. Whole-cell homogenates were prepared by homogenizing 3 × 10^6 cells as described above and sonicating the homogenates for 1 min. Postnuclear and postmitochondrial fractions (obtained by 10 min centrifugation at 10,000 × g) were further separated by ultracentrifugation (30 min at 100,000 × g, RCF). Supernatants (soluble cytoplasmic fraction) and pellets (insoluble membrane fraction) were collected and SDS-PAGE and Western blots were performed as described previously (25) using anti-DGKα and anti-LAT Abs.

**Biochemical Ras activation assays**

Recombinant GST–Raf-RBD protein was produced in Escherichia coli as described (27). Jurkat cells were serum deprived (2 h in RPMI 1640 supplemented with 0.2% fatty acid-free/endoxin-low BSA and 50 mM HEPES [pH 7.5]). After stimulation, 1 ml cell suspension (10^7 cells) was lysed in 1 ml ice-cold lysis buffer (50 mM HEPES [pH 7.5], 140 mM NaCl, 5 mM MgCl2, 1 mM DTT, 1% Nonidet-40, protease inhibitors) supplemented with 25 μg GST-RBD protein and 100 μM GTP to quench postlytic GTP-loading and GAP-dependent Ras-bound GTP hydrolysis, respectively. Cell extracts were cleared by centrifugation and GST-RBD–Ras-GTP complexes were collected on glutathione-Sepharose, washed once with lysis buffer, and processed for SDS-PAGE analysis.

**Mammalian two hybrid system**

A modified Clontech MatchMaker (BD Biosciences) mammalian two-third–hybrid assay was used. Full-length human SAP and its point-mutated variants were cloned into the pM series vectors as GAL4-binding domain fusions. Either full-length DGKα or the N-terminal DGKα fragment was cloned into a pVP vector to direct expression of VP16-activation domain fusion proteins. These were cotransfected into subconfluent HEK293 cells with a GAL4-luciferase reporter plasmid and a pVAX-based expression plasmid containing full-length human FynT. Luciferase activity was measured after 24 h using a commercial kit (Promega).

**NF-AT assay**

Jurkat cells (4 × 10^6/ml) were cotransfected with pNF-AT-TA-luc and pRL-TK plasmids. After 48 h, cells were stimulated as indicated for 16 h. Luciferase was assayed with a Dual-Luciferase reporter assay system (Promega) according to the manufacturer’s instructions and assessed using a Victor^3^ V multilabel counter (PerkinElmer). NF-AT–driven firefly luciferase activity was normalized for the reference Renilla luciferase activity to take in account differences in transfection and expression efficiency, and all values were expressed as fold increase upon unstimulated controls.

**IL-2 assay**

Jurkat cells (1 × 10^3) were plated in 100 μl medium supplemented with 10% FBS and stimulated as indicated for 72 h. IL-2 released in the media was measured by ELISA (GE Healthcare).

**Statistical analysis**

The data were expressed as means ± SE. Statistical analysis was determined by a Student t test.

**Results**

**Negative regulation of DGKα during T cell activation**

Because DGKα negatively regulates T cell activation (17, 19), we set out to investigate whether it is regulated in the early phase of lymphocyte activation. To this purpose, we assayed the enzymatic activity and subcellular localization of DGKα upon activation of primary lymphocytes (PBLs) and Jurkat leukemic T cells. DGKα activity was measured in vitro in the presence of exogenous substrates in anti-DGKα immunoprecipitates obtained from either control or stimulated lymphocytes. Following 15 min costimulation of PBLs with agonistic anti-CD3 and anti-CD28 Abs, the enzymatic activity of DGKα was reduced by ~60% as compared with unstimulated cells (Fig. 1A), without any change in DGKα protein content (Fig. 1A, lower right panel). Stimulation of PBLs with anti-CD3 Ab alone did not significantly affect DGKα activity (data not shown). Because activation of SLAM family receptors was reported to enhance TCR signaling (28, 29), we investigated whether SLAM might regulate the enzymatic activity of DGKα. Indeed, 15 min costimulation of PBLs with anti-CD3 and anti-SLAM agonist Abs resulted in an even stronger inhibition of DGKα activity without affecting DGKα protein content (Fig. 1A). We then measured DGKα activity in anti-DGKα immunoprecipitates from Jurkat leukemia cells following costimulation with anti-CD3 and either anti-CD28 or anti-SLAM agonist Abs. Similar to the data on PBLs, DGKα enzymatic activity was strongly reduced upon 15 min costimulation via the TCR and either SLAM or CD28 and lasted for at least 1 h, without changes in DGKα protein content (Fig. 1B, 1C). Finally, to address the reported ambiguity of how anti-SLAM Abs may affect SLAM signaling, we used an alternative approach to induce SLAM signaling. We used a chimeric receptor featuring SLAM intracellular domain and the extracellular and transmembrane regions of the human IL-2 receptor α-chain coexpressed with SAP in Bi-141 lymphocytes (30). Crosslinking of the chimeric receptor with TAC triggers SLAM signaling (30) and it was sufficient to induce a strong decrease of DGKα activity without changes in DGKα protein content (Fig. 1D).

This result indicates that signals originating from the intracellular domain of SLAM lead to DGKα inhibition. Taken together, these observations indicate that upon costimulation of the TCR with either CD28 or SLAM, the enzymatic activity of DGKα undergoes a negative regulation, which likely contributes to the accumulation of DAG required for RasGRP-mediated activation of Ras and full T cell activation.

To verify whether this regulation was specific to DGKα, we first examined whether anti-CD3 costimulation with either anti-CD28 or anti-SLAM Abs regulated DGKζ, which, along with DGKα, is highly expressed in T cells. We observed that neither CD3/CD28 nor CD3/SLAM costimulation of T cells did affect the enzymatic activity of DGKζ in anti-DGKζ immunoprecipitates from either
control or costimulated cells (Supplemental Fig. 1). These observations indicate that TCR activation specifically regulates DGKα enzymatic activity while not affecting DGKζ. To verify the contribution of DGKα regulation to the total cellular DGK activity, we measured DGK activity in whole-lymphocyte homogenates using exogenous substrates. Following 15 min TCR/CD28 costimulation of either PBLs or Jurkat cells, total DGK activity was not significantly affected, even when the costimulation was sufficient to activate ERK1/2 (Supplemental Fig. 2A, 2B). Conversely, upon 15 min TCR/SLAM costimulation, total DGK activity was significantly reduced (Supplemental Fig. 2C, 2D). Given the specific subcellular localization of DGK isoforms, these observations suggest that DGKα inhibition does not affect the bulk of DAG metabolism while selectively promoting DAG accumulation at specific compartments.

As DGKα recruitment from the cytoplasm to the plasma membrane is highly regulated both upon growth factor stimulation of epithelial cells and TCR/CD28-mediated costimulation of lymphocytes (20, 24), we assessed DGKα localization following costimulation of the TCR with either CD28 or SLAM. Both endogenous DGKα in CD3+ PBLs and YFP-DGKα transiently expressed in Jurkat cells localize diffusely in the nucleus and in the cytoplasm of unstimulated or TCR-stimulated cells. Upon 1 h costimulation of the TCR with either CD28 or SLAM, DGKα was almost entirely excluded from the nucleus and recruited to the cell periphery in both PBLs and Jurkat cells (Fig. 2A, 2B, Supplemental Fig. 3A, 3B). Whereas inhibition of DGKα enzymatic activity was an early event, starting 5 min following costimulation, reaching maximal inhibition at 15 min, and lasting up to 1 h (Fig. 1C), translocation of DGKα became detectable 15 min after costimulation, reached its maximum at 30 min, and lasted for several hours (Fig. 2B).

To distinguish between plasma membrane and cytoplasmic localization, we labeled plasma membrane with either K-Ras-V12/A28 (31) or wheat germ agglutinin. Upon T cell costimulation, DGKα only partially colocalized with K-Ras-V12/A28 (Fig. 2B) or with wheat germ agglutinin (Supplemental Fig. 3B). Accordingly, ~10% of cytoplasmic DGKα sedimented in the 100,000 RCF fraction of CD3/CD28-costimulated Jurkat cells (Fig. 2C). These findings indicate that, upon lymphocyte activation, DGKα undergoes both negative regulation of its enzymatic activity and translocation from the nucleus to the cell periphery, although with different kinetics.

Regulation of DGKα inhibition and recruitment to the cell periphery

We explored whether translocation to the cell periphery and negative regulation of DGKα were regulated by common signaling pathways. DGKα activity and localization are regulated by Src-mediated tyrosine phosphorylation (21, 24, 25), calcium binding (17, 32), and D-3 phosphoinositides (33). Pharmacological inhibition of PLC by U73122 and calcium chelation by BAPTA-AM blunted DGKα translocation from the nucleus to the cell periphery induced by costimulation of TCR with either SLAM or CD28.

FIGURE 1. DGKα is inhibited upon T cell activation. PBLs (A) and Jurkat A3 cells (B) were stimulated for 15 min with 10 μg/ml indicated Abs and lysed. Anti-DGKα immunoprecipitates were assayed for DGK enzymatic activity while an aliquot of whole-cell lysate was analyzed by Western blot with anti-DGKα Ab to ensure equal loading. A representative experiment is shown (lower panel) together with a graph showing the mean ± SE of four independent experiments shown as percentage of control (upper panel). *p < 0.05, t test versus control. C, Jurkat A3 cells were stimulated with 10 μg/ml anti-CD3 and anti-SLAM Abs and lysed at the indicated times. Anti-DGKα immunoprecipitates were assayed for DGK enzymatic activity while not affecting DGKζ activity and localization are regulated by Src-mediated tyrosine phosphorylation (21, 24, 25), calcium binding (17, 32), and D-3 phosphoinositides (33). Pharmacological inhibition of PLC by U73122 and calcium chelation by BAPTA-AM blunted DGKα translocation from the nucleus to the cell periphery induced by costimulation of TCR with either SLAM or CD28.

FIGURE 2. DGKα localization is regulated by TCR/CD28 costimulation. A, B, PBLs (A) and Jurkat A3 cells (B) were stimulated for 15 min with 5 μg/ml anti-TAC and 4 μg/ml anti-IgG and lysed. Anti-DGKα immunoprecipitates were assayed for DGK enzymatic activity while not affecting DGKζ activity and localization are regulated by Src-mediated tyrosine phosphorylation (21, 24, 25), calcium binding (17, 32), and D-3 phosphoinositides (33). Pharmacological inhibition of PLC by U73122 and calcium chelation by BAPTA-AM blunted DGKα translocation from the nucleus to the cell periphery induced by costimulation of TCR with either SLAM or CD28.
FIGURE 2. YFP-DGKα localization upon T cell stimulation. A, Jurkat A3 cells were transfected with YFP-DGKα (green) and after 24 h were serum starved for 2 h and seeded on either poly-l-lysine, anti-CD3, anti-CD3 plus anti-SLAM, or anti-CD3 plus anti-CD28 (10 μg/ml each)-coated glass-bottom dishes and microscope images were acquired. Representative images are shown along with a quantification from three independent experiments. *p < 0.0005, t test versus control. Scale bar, 5 μm. B, Jurkat A3 cells were transfected with YFP-DGKα (green) and DS-Red-K-Ras V12/A38 (red) and after 72 h were serum starved for 2 h and seeded on anti-CD3 plus anti-SLAM agonistic Ab (10 μg/ml each)-coated glass-bottom dishes and images were acquired at the indicated times. Representative images are shown. Scale bar, 5 μm. C, Jurkat A3 cells were stimulated with anti-CD3 and anti-CD28 agonistic Abs (10 μg/ml each) and homogenized 1 h later. The postnuclear and postmitochondrial fraction was separated by centrifugation (100,000 RCF) in a soluble fraction and in a membrane-associated fraction. One fiftieth of the soluble fraction and the entire membrane-associated fraction were analyzed by Western blotting for DGKα in a soluble fraction and in a membrane-associated fraction. One fiftieth of the soluble fraction and the entire membrane-associated fraction were analyzed by Western blotting for DGKα and LAT content.

The role of SAP in DGKα activity was not inhibited following stimulation of TCR and SLAM (Fig. 4A), consistent with the essential role of SAP in DGKα-induced signaling. Surprisingly, in SAP-deficient cells, DGKα activity was not inhibited by TCR/CD28 costimulation. This observation suggests that SAP is not only required for SLAM signaling, but may also play a more direct role in promoting negative regulation of DGKα enzymatic activity (Fig. 4B). Indeed, overexpression of SAP and myc-DGKα in Jurkat cells resulted in the reduction of DGKα activity by 60% as measured in anti-myc immunoprecipitates, whereas myc-DGKα protein content was not affected (Fig. 4C). Conversely, in the same assay SAP mutants unable to bind either SH3 domains (SAP-R78A) or both tyrosine-phosphorylated proteins and SH3 domains (SAP-R55L) (3, 35) failed to inhibit DGKα (Fig. 4C). These findings indicate that SAP overexpression is sufficient to inhibit DGKα through a mechanism that requires SH3-binding ability of SAP.

The sequence surrounding tyrosine 335 of DGKα (SIY335PSV) features a high similarity to the SAP-SH2 binding motif on SLAM (ITY281AQQ) (36), suggesting that DGKα might bind directly to SAP. However, we could not detect a direct physical association between SAP and DGKα in a mammalian two-hybrid assay (Supplemental Fig. 4A) or in coimmunoprecipitation assays using transfected 293T cells (Supplemental Fig. 4B), even when the two proteins were coexpressed with SLAM and Fyn. Taken together, these results indicate that SAP does not inhibit DGKα by directly binding to it, but through the SAP-mediated recruitment of a yet unidentified SH3-containing protein.

The role of SAP in DGKα membrane recruitment in Jurkat cells was investigated by shRNA-mediated stable knockdown of SAP. SAP silencing selectively impaired the recruitment of DGKα to the cell periphery induced by TCR/SLAM costimulation, but not by TCR/CD28 costimulation (Fig. 4D). Similar results were obtained upon transient siRNA-mediated downregulation of SAP in Jurkat
A3 cells were treated with 10 μM independent experiments. * and anti-DGK experiments for each inhibitor. The graph shows the mean activity. The graph shows the mean of at least three independent experiments with super Ag-loaded APCs was impaired (Fig. 5A). Both pharmacological inhibition (Fig. 5A) and siRNA-mediated silencing of DGKα (Fig. 5B) nearly completely rescued the defective translocation of PKCθ to the immune synapse observed in SAP-deficient Jurkat cells, pointing to a rescue of DAG-mediated signaling.

Upon TCR/CD28 costimulation, both T cells from XLP patients and Jurkat cells made SAP-deficient by siRNA-mediated down-regulation exhibit defective ERK1/2 activation (5, 39), suggesting that DAG-mediated Ras-GTP signaling is impaired. Indeed, upon TCR/CD28 costimulation, Jurkat SAP-shRNA cells showed both a decrease in ERK1/2 phosphorylation and a marked reduction of Ras-GTP loading, as measured by Ras-GTP pull-down with GST-RBD (Fig. 5C). Pharmacological inhibition of DGKα with R59949 fully restored both ERK1/2 phosphorylation and Ras-GTP loading (Fig. 5C). These findings confirm that SAP is required for Ras activation in human T cells and provide further support to the hypothesis that negative regulation of DGKα is a critical step in the activation of the Ras pathway downstream of TCR/CD28.

Interestingly, R59949 raised basal levels of ERK1/2 phosphorylation without significantly affecting Ras-GTP loading, suggesting that under these conditions ERK1/2 phosphorylation may be enhanced through a Ras-independent mechanism, likely through DAG-dependent PKCθ activation (40).

Activation of PKCθ and Ras pathways upon TCR/CD28 costimulation triggers NF-AT transcriptionsal activity, which plays a central role in cytokine production (8, 41). Moreover, NF-AT is activated upon SAP overexpression in Jurkat cells (42). Consistently, SAP down-regulation in Jurkat cells impaired TCR/CD28-induced stimulation of NF-AT activity, as measured by luciferase reporter system (Fig. 5D, 5E). In SAP-deficient cells, pharmacological inhibition of DGKα with 1 μM R59949 fully restored TCR/CD28-induced activation of NF-AT without affecting basal NF-AT activity (Fig. 5D), whereas siRNA-mediated DGKα silencing resulted only in partial rescue (Fig. 5E). These data suggest either that DGKα along with other R59949-sensitive DGKs mediate NF-AT activation downstream from SAP or that the low quantity of DGKα remaining after RNA interference may still transduce the signaling.

Upon T cell stimulation, activation of Ras, PKCθ, and NF-AT signaling pathways leads to IL-2 production (41, 43, 44), which has been reported to be reduced in lymphocytes from XLP patients (5). Indeed, in Jurkat cells, shRNA-mediated SAP silencing reduced TCR/CD28-induced IL-2 secretion (Fig. 5F). Pharmacological inhibition of DGKα by R59949 enhanced TCR/CD28-induced IL-2 production in control cells and fully rescued the defective IL-2 secretion of SAP-deficient Jurkat cells. These findings suggest that SAP-mediated negative regulation of DGKα is a key event in the modulation of T cell activation.

**Discussion**

In this study, we demonstrate that within minutes following costimulation of the TCR with either CD28 or SLAM, the enzymatic activity of DGKα, as assayed in immunoprecipitates in the pres-

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**FIGURE 3.** PLC and calcium mediate TCR-induced regulation of DGKα. A, Jurkat A3 cells were transfected with YFP-DGKα and after 24 h were serum starved for 2 h, seeded on 1 h on poly-L-lysine, CD3 plus SLAM, or CD3 plus CD28 agonistic Ab (10 μg/ml each)-coated glass-bottom dishes in the presence or absence of the indicated inhibitors (50 μM PP2, 5 μM U73122, 10 μM BAPTA-AM, or 100 nM wortmannin) and images were acquired. The quantification of three independent experiments is shown. *p < 0.0005, t test versus control. B, Jurkat A3 cells were treated with the indicated inhibitors (10 μM PP2, 5 μM U73122, 10 μM BAPTA-AM, 10 μM IPA-3) or vehicle for 30 min before stimulation with 10 μg/ml anti-CD3 and anti-SLAM Abs. After 15 min, cells were lysed and anti-DGKα immunoprecipitates were assayed for DGK enzymatic activity. The graph shows the mean ± SE of at least three independent experiments for each inhibitor. *p < 0.05, t test versus control. C, Jurkat A3 cells were treated with 10 μM PP2 or vehicle for 30 min before stimulation with 10 μg/ml anti-CD3 and anti-SLAM Abs. After 15 min, cells were lysed and anti-DGKα immunoprecipitates were assayed for DGK enzymatic activity. The graph shows the mean ± SE of at least three independent experiments. *p < 0.05, t test versus control.

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cells (data not shown). Thus, following engagement of the TCR and SLAM, SAP is required for DGKα enzymatic inhibition and recruitment to the cell periphery. In contrast, whereas SAP is required for TCR/CD28-induced enzymatic inhibition of DGKα, it is not essential for translocation of DGKα to the cell periphery. These findings indicate that the localization and enzymatic activity of DGKα are regulated through distinct processes and kinetics, although the mechanisms involved are still partially unknown.

**Inhibition of DGKα rescues the functional defects caused by SAP deficiency in XLP**

Collectively, these data demonstrate that SAP is essential for regulation of DGKα activity upon T cell activation via the TCR/SLAM or TCR/CD28. We therefore reasoned that, similarly to SAP-deficient Jurkat cells, T cells of XLP patients lacking functional SAP might be defective in the negative regulation of DGKα, thereby contributing to the defective lymphocyte respon-

ses observed in both XLP patients and in SAP-null mice. To address this hypothesis, we first characterized the signaling capacity of SAP-deficient Jurkat cells following stimulation via the TCR and CD28. We then assessed whether pharmacological inhibition of DGKα by R59949, or its siRNA-mediated downregulation, might rescue those aberrant T cell responses.

DAG-dependent recruitment of PKCθ to the plasma membrane is defective in T cells from SAP-null mice, it is potentiated upon SAP overexpression (7, 37) and it is negatively regulated by constitutive activation of DGKα (38). Consistently, in SAP-deficient Jurkat cells, PKCθ recruitment to the immune synapse with super Ag-loaded APCs was impaired (Fig. 5A). Both pharmacological inhibition (Fig. 5A) and siRNA-mediated silencing of DGKα (Fig. 5B) nearly completely rescued the defective translocation of PKCθ to the immune synapse observed in SAP-deficient Jurkat cells, pointing to a rescue of DAG-mediated signaling.

Upon TCR/CD28 costimulation, both T cells from XLP patients and Jurkat cells made SAP-deficient by siRNA-mediated down-regulation exhibit defective ERK1/2 activation (5, 39), suggesting that DAG-mediated Ras-GTP signaling is impaired. Indeed, upon TCR/CD28 costimulation, Jurkat SAP-shRNA cells showed both a decrease in ERK1/2 phosphorylation and a marked reduction of Ras-GTP loading, as measured by Ras-GTP pull-down with GST-RBD (Fig. 5C). Pharmacological inhibition of DGKα with R59949 fully restored both ERK1/2 phosphorylation and Ras-GTP loading (Fig. 5C). These findings confirm that SAP is required for Ras activation in human T cells and provide further support to the hypothesis that negative regulation of DGKα is a critical step in the activation of the Ras pathway downstream of TCR/CD28.

Interestingly, R59949 raised basal levels of ERK1/2 phosphorylation without significantly affecting Ras-GTP loading, suggesting that under these conditions ERK1/2 phosphorylation may be enhanced through a Ras-independent mechanism, likely through DAG-dependent PKCθ activation (40).

Activation of PKCθ and Ras pathways upon TCR/CD28 costimulation triggers NF-AT transcriptionsal activity, which plays a central role in cytokine production (8, 41). Moreover, NF-AT is activated upon SAP overexpression in Jurkat cells (42). Consistently, SAP down-regulation in Jurkat cells impaired TCR/CD28-induced stimulation of NF-AT activity, as measured by luciferase reporter system (Fig. 5D, 5E). In SAP-deficient cells, pharmacological inhibition of DGKα with 1 μM R59949 fully restored TCR/CD28-induced activation of NF-AT without affecting basal NF-AT activity (Fig. 5D), whereas siRNA-mediated DGKα silencing resulted only in partial rescue (Fig. 5E). These data suggest either that DGKα along with other R59949-sensitive DGKs mediate NF-AT activation downstream from SAP or that the low quantity of DGKα remaining after RNA interference may still transduce the signaling.

Upon T cell stimulation, activation of Ras, PKCθ, and NF-AT signaling pathways leads to IL-2 production (41, 43, 44), which has been reported to be reduced in lymphocytes from XLP patients (5). Indeed, in Jurkat cells, shRNA-mediated SAP silencing reduced TCR/CD28-induced IL-2 secretion (Fig. 5F). Pharmacological inhibition of DGKα by R59949 enhanced TCR/CD28-induced IL-2 production in control cells and fully rescued the defective IL-2 secretion of SAP-deficient Jurkat cells. These findings suggest that SAP-mediated negative regulation of DGKα is a key event in the modulation of T cell activation.

**Discussion**

In this study, we demonstrate that within minutes following costimulation of the TCR with either CD28 or SLAM, the enzymatic activity of DGKα, as assayed in immunoprecipitates in the prev-
FIGURE 4. SAP negatively regulates DGKα activity. A, Jurkat control-shRNA or Jurkat SAP-shRNA cells were stimulated for 15 min with 10 μg/ml anti-CD3 and anti-SLAM Abs and lysed. Anti-DGKα immunoprecipitates were assayed for DGK enzymatic activity while an aliquot of whole-cell lysate was analyzed by Western blot with anti-DGKα Ab to ensure equal loading and with anti-SAP Ab to verify the downregulation of SAP expression. A representative experiment is shown together with a graph of the mean ± SE of three independent experiments shown as percentage of control. *p < 0.05, t test versus control. B, Jurkat control-shRNA or Jurkat SAP-shRNA cells were stimulated for 15 min with 10 μg/ml anti-CD3 and (Figure legend continues)
ence of saturating DAG substrate concentration, undergoes a strong negative regulation without protein downregulation. This finding is surprising, given accumulating evidence that synthesis of 
P44/20 PA is increased upon T cell stimulation (15, 16, 45) and that DGK activity is increased in whole-cell lysates from 
in-activated T cells (19). However, increased PA synthesis through 
DAG phosphorylation may depend on both positive regulation of 
one or more DGK isoforms and on increased availability of DAG, 
whose production by PLCγ is increased upon TCR/CD28 co-
stimulation (46). A parallel increase of DAG and PA levels upon 
TCR stimulation has been indeed observed (45, 47). Several 
pieces of evidence suggest that most of the PA generated upon 
T cell activation derives from phospholipase D2-mediated phos- 
pholipid hydrolysis and from DGKζ-mediated phosphorylation of 
DAG, whereas deletion of DGKα does not significantly affect PA 
production upon T cell stimulation (15, 16, 45). Nevertheless, 
recent genetic and biochemical data indicating that DGKα is a 
negative regulator of DAG-mediated TCR signaling (15, 17) are 
highly consistent with our finding that enzymatic activity of 
DGKα is reduced upon TCR costimulation with either CD28 or 
SLAM. This regulation appears to be isoform-specific, as DGKζ 
activity is unaffected by TCR triggering (Supplemental Fig. 1). 
Interestingly, the previous finding that stimulation of the sole TCR 
is not sufficient to promote sustained DAG signaling (48) is 
consistent with our observation that TCR activation in PBLs is not 
sufficient to inhibit DGKα activity in the absence of costimula-


Moreover, costimulation of TCR/CD28, compared with TCR 
alone, strongly enhances production of DAG but not of PA (15), 
suggesting a slowdown in the rate of DAG conversion to PA that is 
consistent with a negative regulation of DAG activity. 

The molecular mechanisms underlying the negative regulation of 
DGKα have not yet been elucidated. In this study, we report that 
the adaptor function of SAP is required for DGKα inhibition 
induced by TCR costimulation with either SLAM or CD28. SAP is 
essential for SLAM tyrosine phosphorylation by recruiting the 
Src-related kinase FynT (3, 34); however, a growing body of evi-
dence indicates that SAP is also involved in T cell responses to 
antigenic stimulation (2). Indeed, SAP binds directly to ITAM 
sequences of CD3ζ subunit (29), whereas TCR activation pro-
motes the recruitment of SAP and SLAM family receptors to the 
signalosome (28, 29, 49). Furthermore, genetic deletion of SAP 
in mice results in the impairment of TCR/CD28-induced DAG-
mediated activation of PKC0 and of downstream signaling events 
(7). Moreover, TCR/CD28-induced ERK1/2 activation and IL-2 
production, which are both dependent on DAG-mediated activa-
tion of RasGRP, are impaired in T cells from SAP-deficient XLP 
patients (5). Intriguingly, SAP is physically associated to PKC0, 
and it has been demonstrated that SAP overexpression, which is 
sufficient to inhibit DGKα, promotes PKC0 recruitment to the 
immune synapse (37). Finally, we and others have shown that, in 
Jurkat cells, SAP silencing impairs TCR-induced Ras-GTP load-
ing, ERK1/2 activation, PKC0 recruitment, NF-AT activation, and 
IL-2 production (5–7). Taken together, these observations suggest 
that, upon TCR/CD28 costimulation, SAP is required for optimal 
DAG signaling. The finding that SAP is required for inhibition of 
DGKα might provide a mechanistic link between SAP and the 
regulation of DAG signaling. Thus, we propose that, upon stim-
ulation of T cells from either SAP-deficient XLP patients or SAP-
null mice, DGKα may inappropriately retain a high enzymatic 
activity, thereby converting DAG to PA and decreasing DAG 
signaling. 

If this hypothesis holds true, we would expect that inhibition 
or downregulation of DGKα would rescue, at least partially, the 
defective signaling of SAP-deficient T cells. Accordingly, we 
observed that the inhibition of DGKα enzymatic activity in SAP-
deficient Jurkat cells rescued defective DAG-dependent PKC0 
membrane recruitment, Ras-GTP loading, ERK1/2 and NF-AT 
activation, and IL-2 production. These findings indicate that the 
excess of DGKα activity contributes to the defective signaling 
of SAP-deficient cells and, along with the demonstration that 
SAP overexpression inhibits DGKα, provide further support to the 
hypothesis that SAP negatively regulates DGKα. According to 
these findings, the negative regulation of DGKα activity repre-
sents a key event controlling the early phase of T cell activation 
by contributing to fine tuning of DAG levels required for appro-
appropriate signaling. 

In this study, we observed that costimulation of the TCR with 
either SLAM or CD28 induces DGKα exit from the nucleus and 
accumulation in the cytoplasm with only partial localization at 
the plasma membrane. This finding appears to contrast previous 
studies reporting GFP-DGKα localization at the plasma mem-
brane of CD3/CD28 costimulated Jurkat cells; however, according 
to the same authors, DGKα membrane translocation is rapid and 
transient and can be visualized in conditions that inhibit its re-
localization to the cytoplasm (17, 21). Moreover, DGKα plasma 
membrane localization was clearly induced by stronger stimuli, 
such as the activation of ectopically overexpressed muscarinic 
receptor (20, 21, 50, 51) or Ag challenge in vivo (19). Further 
support to the hypothesis that enzymatic activity of DGKα regu-
lates DAG level at the plasma membrane of T cells derives both 
from our finding that uncoupling of DGKα inhibition from TCR 
stimulation impairs PKC0 recruitment to the immune synapses 
(Fig. 5A) and from the observation that pharmacological inhibition 
of DGKα allows accumulation of DAG at the plasma mem-
brane of T cells, thereby triggering activation of Ras signaling (52). 

Interestingly, stimulation with either SLAM or TCR alone did 
not induce DGKα translocation from the nucleus, indicating that 
the concerted signaling via both receptors is required. Moreover, 
the finding that SAP, which is essential for SLAM tyrosine 
phosphorylation and signaling, is required for translocation in-
duced exclusively by TCR/SLAM, but not by TCR/CD28, sug-
gests that SAP may not directly regulate DGKα subcellular 
localization. Additionally, the fact that upon TCR/CD28 costi-
mulation, SAP is required for inhibition of DGKα activity, but not 

anti-CD28 Abs and lysed. Anti-DGKα immunoprecipitates were assayed for DGK enzymatic activity while an aliquot of whole-cell lysate was analyzed by Western blot with anti-DGKα Ab to ensure equal loading and with anti-SAP Ab to verify the downregulation of SAP expression. A representative ex-

FIGURE 5. DGKα inhibition rescues defective TCR-induced DAG-dependent signaling and IL-2 production of SAP-deficient T lymphocytes. A, Jurkat control-shRNA and Jurkat SAP-shRNA cells (T) were pretreated with R59949 (10 μM 30 min) incubated with super Ag-loaded Raji cells (APCs) for 15 min, fixed, and stained for PKCζ. Representative images are shown. Scale bar, 5 μm. Cells displaying PKCζ at the immune synapse were counted. The histogram shows data from three independent experiments as mean ± SE (*p < 0.05, t test). B, Jurkat control-shRNA and Jurkat SAP-shRNA cells (T) were transfected with DGKα-specific siRNA or control siRNA. After 72 h, cells were lyzed and analyzed by Western blot with anti-DGKα and anti-actin Abs (left panel). At the same time cells were incubated with super Ag-loaded Raji cells (APCs) for 15 min, fixed, and stained for PKCζ. Cells displaying PKCζ at the synapse were counted (right panel). The histogram shows data from three independent experiments as mean ± SE (*p < 0.05, t test). C, Control shRNA Jurkat or SAP shRNA Jurkat cells were stimulated with 1 μg/ml anti-CD3 and 0.1 μg/ml anti-CD28 Abs in the presence or in absence of 1 μM R59949. After 15 min, cells were lysed and Ras-GTP was separated by pull-down with Raf-RBD and quantified by Western blotting with anti pan-Ras Ab. Total Ras, phospho-ERK1/2, and SAP contents were revealed in whole-cell lysates by Western blotting. D, Jurkat control-shRNA and Jurkat SAP-shRNA cells were transfected with a Dual-Luciferase NF-AT reporter system. After 48 h, cells were stimulated with 1 μg/ml anti-CD3 and anti-CD28 Abs in the presence or absence of 1 mM R59949. After 16 h stimulation, cells were lysed and analyzed for NF-AT-driven luciferase activity. Graph shows the mean ± SE of quadruplicates of a representative experiment. *p < 0.05, t test versus control. E, Jurkat control-shRNA or Jurkat SAP-shRNA cells were transfected with a siRNA targeting DGKα or a control siRNA and a Dual-Luciferase NF-AT reporter system. After 48 h, cells were stimulated with 1 μg/ml anti-CD3 and anti-CD28 Abs. After 16 h stimulation, cells were lyzed and analyzed for NF-AT-driven luciferase activity. Graph shows the mean ± SE of quadruplicates of a representative experiment. *p < 0.05, t test versus control. F, Jurkat control-shRNA or Jurkat SAP-shRNA cells were stimulated with 1 μg/ml anti-CD3 and 0.1 μg/ml anti-CD28 Abs. After 72 h, cells were lyzed and the amount of IL-2 released in the medium was measured by ELISA. Graph shows the mean ± SE of four replicates of a representative experiment. *p < 0.05, t test versus control.
for its exit from the nucleus, indicates that enzymatic activity and localization of DGKα are regulated independently of each other, as suggested also by the different kinetics of the two processes. Importantly, these findings also indicate that DGKα exit from the nucleus is not required for the inhibition of its enzymatic activity. The massive exit from the nucleus may reflect a potential increase in the availability of DGKα outside the nucleus for control of DAG signaling both at the plasma membrane and at intracellular vesicles. Indeed, several reports indicate a role of DGKs in T cells intracellular trafficking (50, 53, 54). Conversely, DGKα exit from the nucleus may contribute to regulate nuclear pools of DAG and PA. Indeed, several DGK isoforms have been reported to localize in the nucleus where they contribute to regulate transcription and cell cycle progression (55).

Previous evidence indicates that SFK-induced phosphorylation of DGKα on tyrosine 335 mediates its activation and membrane localization upon growth factor stimulation of epithelial and large cell lymphomas (24, 56). Moreover, in T cells DGKα phosphorylation by LCK on tyrosine 335 mediates CD3/CD28-induced recruitment of DGKα to the plasma membrane (21). Surprisingly, in our study pharmacological inhibition of SFKs did not affect either TCR/CD28-induced inhibition of DGKα or its exit from the nucleus, suggesting that both events are independent from SFK-mediated tyrosine phosphorylation of DGKα. Conversely, PP2 completely blocks DGKα inhibition and exit from the nucleus induced by CD3/SLAM, as SLAM signaling is dependent on Fyn tyrosine kinase.

The mechanism by which SAP regulates DGKα still remains to be elucidated. Based on the high similarity between the sequences surrounding tyrosine 335 of DGKα and tyrosine 281 of SLAM, we investigated the hypothesis that SAP may regulate DGKα by associating with it in a complex. However, we could not detect any direct or indirect physical interaction between the two proteins, even in a reconstituted association assay in a mammalian two-hybrid system. Our data demonstrate that SAP ability to inhibit DGKα requires the interaction with a yet unidentified SH3 domain-containing protein. The finding that inhibition of DGKα is independent of activity by SFKs suggests that the SAP interactor required for DGKα inhibition is not Fyn. The previous observation that SAP overexpression activates Cdc42 signaling by interacting with SH3-containing βPIX and independently of Fyn suggests that DGKα may be regulated by Cdc42-dependent PAK activation. However, the PAK-specific inhibitor IPA-3 did not affect the inhibition of DGKα following TCR/SLAM costimulation (Fig. 3B). Finally, upon TCR stimulation of Jurkat cells, SAP silencing results in defective tyrosine phosphorylation of several proteins, including LAT and SLP76 (39). As both LAT and SLP76 regulate PLCγ activation (57), it is possible to speculate that SAP regulates DGKα by controlling PLCγ activity. Consistent with this possibility, PLCγ activity and cytosolic-free calcium are both required for DGKα inhibition and membrane recruitment. However, lack of SAP in T cells of both SAP-null mice and XLP patients does not affect PLCγ-mediated intracellular calcium increase (5, 58). Moreover, cell stimulation with a calcium ionophore and phorbol ester failed to inhibit DGKα activity and to recruit it to the plasma membrane, indicating that activation of PLC is necessary but not sufficient to regulate both enzymatic activity and membrane localization (data not shown). Furthermore, the requirement for PLC activity in regulating DGKα suggests that the two enzymes may act as a bicomponent unit able to finely modulate the extent and the duration of DAG signaling.

In conclusion, our findings suggest that the coordinated, but independent, control of DGKα enzymatic activity and of its localization regulates both its access to DAG and its rate of concentration to PA. Upon T cell stimulation, such a coordinated and complementary mechanism of regulation might finely tune the intensity and the duration of DAG-mediated signaling. Indeed, SAP silencing, by uncoupling TCR/CD28 costimulation from DGKα inhibition, results in the impairment of TCR/CD28-induced DAG-mediated signaling, providing further evidence that the SAP-mediated negative regulation of DGKα is crucial for the ability of T cells to trigger DAG-mediated responses.

Similar to cAMP signaling, which is triggered by G protein-coupled receptors by reciprocal regulation of both adenylate cyclase and phosphodiesterase activities (59), the findings presented in this study suggest that TCR/CD28 controls DAG signaling both by means of PLCγ activation and DGKα inhibition. Similarly, genetic and biochemical studies in Caenorhabditis elegans motor-neurons and murine hepatocytes showed that DAG-mediated signaling is controlled by G protein-coupled receptor-dependent reciprocal regulation of both PLC and DGKα (60–62).

In summary, our findings demonstrate that SAP-mediated DGKα inhibition is an early event in TCR signaling, which might be required for efficient T cell activation. The impaired regulation of DGKα activity in SAP-deficient lymphocytes may contribute to their defective TCR-induced responses, suggesting that pharmacological inhibition of DGKα could be useful in the treatment of certain manifestations of XLP.

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Disclosures

The authors have no financial conflicts of interest.

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