Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 5484–5488, July 1991
Biochemistry

Functional activation of the T-cell antigen receptor induces tyrosine phosphorylation of phospholipase C-γ1

(signal transduction/tyrosine kinase/T-cell activation/phosphatidylinositol)

ARTHUR WEISS*, GARY KORETZKY*, RANDALL C. SCHATZMAN†, AND THERESA KADLECEK*

*Howard Hughes Medical Institute, Departments of Medicine and of Microbiology and Immunology, University of California, San Francisco, CA 94143; and †Institute of Cancer and Developmental Biology, Syntex, Palo Alto, CA 94304

Communicated by J. Michael Bishop, March 20, 1991 (received for review January 16, 1991)

ABSTRACT **Stimulation of the T-cell antigen receptor (TCR), which itself is not a protein-tyrosine kinase (PTK), activates a PTK and phospholipase C (PLC). Using the human T-cell leukemic line Jurkat and normal peripheral blood lymphocytes, we demonstrate that stimulation of the TCR specifically induces the recovery of PLC activity in eluates from anti-phosphotyrosine immunoprecipitates. Stimulation of the human muscarinic receptor, subtype 1, when expressed in Jurkat activates PLC through a guanine nucleotide binding protein but does not induce the recovery of PLC activity in eluates from anti-phosphotyrosine immunoprecipitates. Western blot analysis reveals that PLC-γ1 is tyrosine-phosphorylated in response to TCR stimulation. Nearly all of the PLC activity recovered in eluates from anti-phosphotyrosine immunoprecipitates was depleted by anti-PLC-γ1 antibodies. Stimulation of the TCR on mutants derived from Jurkat that are defective in TCR-induced PLC activation results in markedly reduced, if any, PLC activity recovered in phosphotyrosine immunoprecipitates and in no detectable PLC-γ1 tyrosine phosphorylation. Thus, the TCR functions like PTK growth factor receptors, but through an indirect interaction, to induce tyrosine phosphorylation of PLC-γ1. Since other studies have implicated two members of the src family of PTKs in TCR-mediated signal transduction, our findings suggest that the induction of tyrosine phosphorylation of PLC-γ1 by a mechanism involving a src-like kinase may be the means by which the TCR regulates PLC activity in T cells.**

The T-cell antigen receptor (TCR) functions to recognize antigen and to transduce signals across the plasma membrane. The TCR activates a protein-tyrosine kinase (PTK) and phospholipase C (PLC) (1, 2). The PTK activated by the TCR is not intrinsic to the structure of the TCR itself (3, 4). Candidates for the TCR-regulated PTK include fyn and lck, members of the src family of PTKs. The TCR was recently coimmunoprecipitated with fyn from T cells (5). CD4 functions as a coreceptor with the TCR (6) and is associated with lck through its cytoplasmic tail (7, 8). The physiologic substrates of these kinases have not been identified, although the ζ chain of the TCR is phosphorylated after stimulation of either the TCR (1) or CD4 (9).

A number of distinct isozymes of PLC have been described (10). However, the isozyme of PLC that is activated after TCR stimulation has not been identified. Although some studies have suggested that the TCR stimulates PLC activity through a guanine nucleotide binding protein (G protein) (11, 12), studies involving PTK inhibitors (13) and kinetic analyses (14) have suggested that the PTK stimulated by the TCR regulates PLC activation. A mechanism by which the TCR-activated PTK may regulate PLC activity is suggested from studies involving PTK growth factor receptors, the platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) receptors. Stimulation of these receptors induces their association with the γ1 isozyme of PLC as well as the tyrosine phosphorylation of PLC-γ1 (15–18). Moreover, tyrosine phosphorylation of PLC-γ1 induced by the EGF receptor increases PLC catalytic activity (19). Using the human T-cell line Jurkat, normal peripheral blood lymphocytes (PBLs), and signal transduction mutants derived from Jurkat, we show that the TCR functions like PTK growth factor receptors, but through an indirect interaction with a PTK, to induce tyrosine phosphorylation of PLC-γ1. This tyrosine phosphorylation of PLC-γ1 is associated with functional coupling of the TCR to the inositol phospholipid pathway.

MATERIALS AND METHODS

Cells and Monoclonal Antibodies. Jurkat (clone E6-1), J.CaM1, J.CaM2, J-HM1-2.2, and J45.01 have been described (20–23). Plastic nonadherent PBLs were isolated from normal volunteers as described (24).

The following mouse monoclonal antibodies (mAbs) were used in these studies: C305 reacts with the Jurkat TCR (20); Leu4 (Becton Dickinson) and 235 (25) react with CD3; w6/32 reacts with class I major histocompatibility complex molecules (American Type Culture Collection); 9.6 reacts with CD2 (26); and 4G10 reacts with phosphotyrosine (27). A pool of mAbs that specifically react with PLC-γ1 was kindly provided by Sue Goo Rhee (National Institutes of Health) (28). A rabbit heteroserum against the carboxyl-terminal 226 amino acids of PLC-γ1, which specifically recognizes the PLC-γ1 isozyme, was used in depletion studies.

PLC Assay of Eluates from Phosphotyrosine Immunoprecipitates. Jurkat or its derivative cells, 2×10^7 cells per sample, were stimulated at 37°C for 30 sec or as indicated in a total volume of 100 μl. PBLs were stimulated for 45–60 sec according to the maximal calcium responses observed (29). mAbs were used at saturating concentrations. Other stimuli used were carbachol (Sigma), ionomycin (Calbiochem), and phorbol 12-myristate 13-acetate (PMA; Sigma). Cells were rapidly sedimented and lysed in 200 μl of 1% Nonidet P-40/Tris-buffered saline, with protease and phosphatase inhibitors as described (22). Phosphotyrosine-containing proteins were immunoprecipitated with the 4G10 mAb immobilized on protein A-coupled Sepharose beads. The published specificity of this mAb (27) was confirmed by inhibition studies (22). Calcium-dependent PLC activity in 10 mM phenyl phosphate eluates was assessed as described (15, 17). All of the PLC activity was inhibited by a molar excess of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TCR, T-cell antigen receptor; PTK, protein-tyrosine kinase; InsP_3, inositol trisphosphate; PLC, phospholipase C; G protein, guanine nucleotide binding protein; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; PBL, peripheral blood lymphocyte; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; Hm1, human muscarinic subtype 1 receptor.

EGTA. In immunodepletion experiments, phenyl phosphate eluates were incubated with Sepharose beads coated with normal mouse IgG, anti-PLC-γ1 mAbs, or rabbit anti-PLC-γ1 carboxyl-terminal heteroserum prior to assay of PLC activity. The inositol phosphates generated in this assay were shown to represent inositol trisphosphate ($InsP_3$) by ion-exchange chromatography (data not shown).

Western Blot Analysis. Cells (2×10^7 cells per sample) were stimulated for 30 sec with medium or C305. Lysates, immunoprecipitates, and Western blots were prepared as described (22).

RESULTS

To determine whether stimulation of the TCR induces tyrosine phosphorylation of proteins associated with PLC activity, Jurkat leukemic T cells were stimulated with mAb C305, reactive with the TCR β chain (20). Lysates were prepared from 5 sec to 10 min after stimulation. Tyrosine-containing phosphoproteins were immunoprecipitated and then eluted with phenyl phosphate. The eluted material was assessed for calcium-dependent PLC activity. Recovery of PLC activity within the tyrosine phosphoprotein fraction could be readily detected within 15 sec of TCR stimulation, peaked at 30 sec, and declined thereafter but remained elevated even 10 min after stimulation (Fig. 1*a*). This approximates the kinetics of the increase in cytoplasmic free calcium observed in this system (2, 20). A 6- to 20-fold induction of PLC activity recovered in the tyrosine phosphoprotein fraction was routinely detected. This activity could be immunoprecipitated with three distinct anti-phosphotyrosine mAbs, but mAb 4G10 was the most efficient (data not shown). Similarly, PLC activity could be recovered in anti-phosphotyrosine immunoprecipitates isolated from normal PBLs stimulated with an agonist anti-CD3 mAb (Fig. 1*b*). Thus, the TCR on Jurkat and normal T cells induces tyrosine phosphorylation of a protein with PLC activity or of a protein that coprecipitates with PLC.

To assess the specificity of this response, Jurkat cells were stimulated with mAb reactive with several other proteins expressed on Jurkat. Stimulation of the TCR by mAb reactive with either the Ti α/β heterodimer or the associated CD3 complex induced the recovery of comparable PLC activity in the tyrosine phosphoprotein fraction (Fig. 2*a*). In contrast, stimulation with an HLA class I-reactive mAb or with a single CD2 mAb failed to induce recovery of PLC activity among

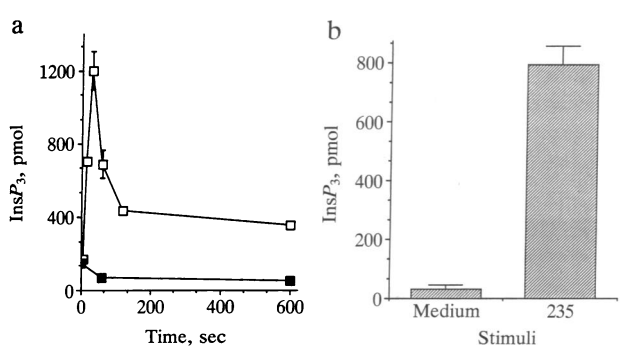

FIG. 1. (*a*) PLC activity in the tyrosine phosphoprotein fraction at various times after stimulation of Jurkat cells with medium (■) or the anti-Ti β mAb C305 (□). Calcium-dependent PLC activity recovered in phenyl phosphate eluates of anti-phosphotyrosine immunoprecipitates isolated from 2×10^7 cells was assayed. Results represent the mean ± SEM of triplicate samples in pmol of $InsP_3$ produced in 15 min. (*b*) PLC activity recovered from the tyrosine phosphoprotein immunoprecipitates isolated from nonadherent PBLs, 2×10^7 cells per sample, after stimulation for 45 sec with medium or the anti-CD3 mAb 235.

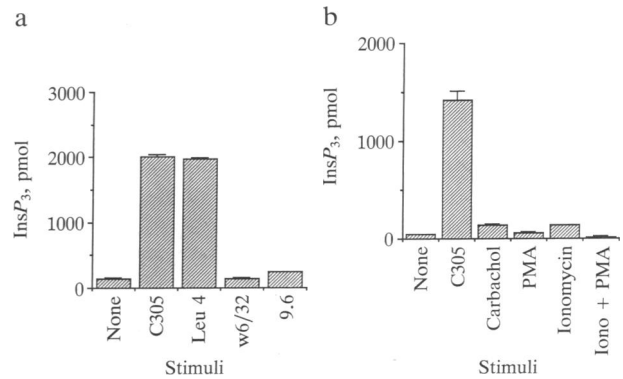

FIG. 2. (*a*) PLC activity in eluates of tyrosine phosphoproteins isolated from Jurkat cells stimulated for 30 sec with the following mAbs: medium (none), anti-Ti β (C305), anti-CD3 (Leu4), anti-HLA class 1 (w6/32), and anti-CD2 (9.6). (*b*) PLC activity in tyrosine phosphoprotein eluates isolated from J-HM1-2.2 cells stimulated for 30 sec with medium (none), anti-Ti β (C305), 500 μM carbachol, PMA at 50 ng/ml, 1 μM ionomycin, or ionomycin plus PMA (Iono + PMA).

tyrosine phosphoproteins. These latter mAbs fail to induce PLC activation *in vivo* under the conditions used. Stimulation with a pair of CD2 mAb that induces slower PLC activation *in vivo* (23) induced smaller increases in PLC activity recovered in tyrosine phosphoproteins and with delayed kinetics compared with TCR stimulation (data not shown). Thus, recovery of PLC activity in tyrosine phosphoprotein immunoprecipitates is associated with PLC activation *in vivo*.

We addressed further the mechanism and specificity of recovery of PLC activity in tyrosine phosphoprotein immunoprecipitates by examining the effects of activation of PLC by a G-protein-coupled receptor. The human muscarinic subtype 1 receptor (Hm1), a member of the seven-transmembrane-domain receptors that couple to effector mechanisms through G proteins, activates the inositol phospholipid pathway in its normal environment in cardiac, smooth muscle, and neuronal cells (30). J-HM1-2.2 is a transfectant of Jurkat that expresses Hm1 (22). Hm1 appears to be coupled to a G protein in J-HM1-2.2 since the affinity of Hm1 for the muscarinic agonist carbachol is influenced by guanine nucleotides (M. Graber and A.W., unpublished results). Stimulation of J-HM1-2.2 with carbachol activates PLC leading to the generation of the second messengers of the inositol phospholipid pathway and to T-cell activation (22). However, stimulation of Hm1 does not activate the PTK pathway (22). Stimulation of Hm1 fails to induce the recovery of PLC activity in the tyrosine phosphoprotein fraction (Fig. 2*b*), despite the fact that it induces PLC activity *in vivo* (22). Moreover, the calcium ionophore ionomycin, a protein kinase C activating phorbol ester, or their combination, which mimics the downstream events associated with PLC activation, fails to induce the recovery of PLC activity in the tyrosine phosphoproteins. Therefore, TCR-induced recovery of PLC activity in the tyrosine phosphoproteins is not a consequence of PLC activation, elevation of intracellular calcium, or protein kinase C activation.

These data do not distinguish whether an isozyme of PLC is being phosphorylated or whether PLC is associated with a tyrosine phosphoprotein. PLC activity can be mediated by a large number of structurally distinct enzymes (10). Stimulation of the PTK growth factor receptors for PDGF and EGF is associated with the tyrosine phosphorylation and association of the PLC-γ1 isozyme with these receptors (15–18). Therefore, we determined whether the PLC-γ1 isozyme is tyrosine-phosphorylated after TCR stimulation. Fig. 3 demonstrates that PLC-γ1 is detectable in Jurkat as a 150-kDa

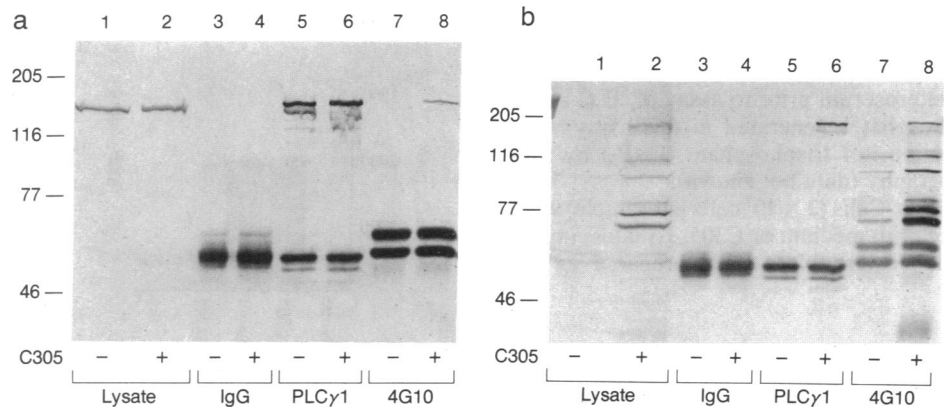

FIG. 3. Jurkat cells were stimulated for 30 sec with medium (lanes 1, 3, 5, and 7) or the anti-Ti β mAb C305 (lanes 2, 4, 6, and 8). Lysates (lanes 1 and 2) or immunoprecipitates, which were isolated with normal mouse IgG (lanes 3 and 4), anti-PLC-γ1 mAb (lanes 5 and 6), or anti-phosphotyrosine mAb (4G10, lanes 7 and 8) were analyzed on Western blots after electrophoresis in 8% polyacrylamide gels. Western blots were probed with anti-PLC-γ1 mAb (*a*) or anti-phosphotyrosine 4G10 mAb (*b*). The 50- to 60-kDa bands seen in lanes 3–8 represent immunoglobulin heavy chains from the immunoprecipitating mAbs.

protein and is tyrosine-phosphorylated after TCR stimulation. The 4G10 anti-phosphotyrosine mAb, which was used to isolate phosphoproteins in the PLC assays, is able to immunoprecipitate PLC-γ1 from TCR-stimulated cells (Fig. 3*a*, lanes 7 and 8). In a reciprocal manner, only PLC-γ1 immunoprecipitated from stimulated cells was detected on a blot with the anti-phosphotyrosine mAb (Fig. 3*b*, lanes 5 and 6). Two-dimensional phosphoamino acid mapping studies in which PLC-γ1 was immunoprecipitated from ^{32}P-labeled cells also demonstrate that TCR stimulation induces PLC-γ1 tyrosine phosphorylation (data not shown). Thus, stimulation of the TCR induces tyrosine phosphorylation of a large number of proteins, among which is PLC-γ1 (Fig. 3*b*, lanes 1 and 2). The relative amount of PLC-γ1 that is tyrosine-phosphorylated is not known; however, in Fig. 3*a*, a comparison of lanes 6 and 8 would suggest it is substoichiometric.

T cells contain several isozymes of PLC. Studies with Jurkat cells have shown the presence of transcripts for PLC-γ1, PLC-γ2, and PLC-α isozymes (31). Other isozymes such as PLC-β may also be expressed. Thus, the PLC activity detected among the tyrosine phosphoproteins may only partially be accounted for by tyrosine-phosphorylated PLC-γ1. To assess this, phenyl phosphate eluates of 4G10 immunoprecipitates isolated from TCR-stimulated Jurkat cells were immunodepleted with PLC-γ1-isozyme-specific mAbs or with a rabbit heteroserum reactive with the carboxyl terminus of PLC-γ1. The anti-carboxyl-terminal antibody helps to distinguish PLC-γ1 from PLC-γ2 since it is not known if the mAbs can distinguish these related two isozymes in immunoprecipitation studies. Significantly, Jurkat cells express much lower levels of PLC-γ2 transcripts compared with PLC-γ1 transcripts (31). Immunodepletion of eluates with either the anti-PLC-γ1 mAbs or the rabbit heteroserum removed greater than 95% of the induced PLC activity detected among tyrosine phosphoproteins after TCR stimulation in Jurkat cells (Table 1) and normal PBLs (data not shown). Thus, PLC-γ1 accounts for essentially all of the induced PLC activity recovered from the phosphotyrosine immunoprecipitates isolated after TCR stimulation. Additional immunodepletion experiments revealed that the 150-kDa tyrosine phosphoprotein observed in stimulated Jurkat lysates represents tyrosine-phosphorylated PLC-γ1 (data not shown). Since 4G10 detects the tyrosine-phosphorylated form of PLC-γ1 by immunoprecipitation, we would also conclude from these studies and the experiment in Fig. 2*b* that although stimulation of the TCR induces PLC-γ1 tyrosine-phosphorylation, Hml does not. This is supported further by the observation that stimulation of Hml fails to induce other tyrosine phosphoproteins except for a 42-kDa protein believed to represent microtubule-associated protein kinase 2 (22).

The functional significance of tyrosine phosphorylation of PLC-γ1 was addressed by using three signal transduction mutants of Jurkat. J.CaM1 and J.CaM2 express high levels of the TCR but fail to increase intracellular calcium or inositol phosphates in response to anti-Ti mAbs (20, 21). The TCR on J.CaM1, unlike that on Jurkat, fails to activate the PTK pathway (Fig. 4*a*, lanes 3 and 4 vs. 1 and 2). However, the TCR on J.CaM2 does partially activate a PTK since some, but not all, phosphotyrosine substrates are detectable after TCR stimulation (lanes 5 and 6). The 150-kDa tyrosine phosphoprotein induced in Jurkat, which represents PLC-γ1, is not observed in J.CaM2. Tyrosine phosphorylation of the TCR ζ chain, not visualized in this blot, is induced in J.CaM2 (data not shown). J45.01 is a Jurkat-derived CD45-tyrosine-phosphatase-deficient mutant (32) that, like a characterized HPB-ALL mutant (23), fails to activate PLC in response to TCR stimulation and also fails to activate the PTK pathway (Fig. 4*a*, lanes 7 and 8). These three mutants define distinct defects based on a heterokaryon complementation analysis (21, 32). A quantitative defect in PLC-γ1 expression does not explain the signal transduction deficiency in any of these cells (Fig. 4*b*).

We determined whether stimulation of the TCR in these cells that are defective in activation of PLC by the TCR correlates with the observed tyrosine phosphorylation of PLC-γ1. Unlike Jurkat, stimulation of the TCR on J.CaM1

Table 1. Immunodepletion of PLC activity from phenyl phosphate eluates of phosphotyrosine immunoprecipitates from stimulated Jurkat and J.CaM2 cells

		InsP_3, pmol		
Exp.	Cell	IgG	Anti-PLC-γ1 mAb	Anti-PLC-γ1 heteroserum
1	Jurkat	605	0 (100)	0 (100)
2	Jurkat	293	11 (96)	ND
	J.CaM2	30	0 (100)	ND

After stimulation of the cells with medium or the anti-TCR β mAb C305, phosphotyrosine proteins were immunoprecipitated with the 4G10 mAb. Tyrosine phosphoproteins were eluted with phenyl phosphate, and an equal portion of each eluate was subjected to immunodepletion with an excess of the indicated antibodies immobilized on protein A-coupled Sepharose beads. The residual induced PLC activity was assayed. The percentage reduction is shown in parentheses. ND, not done.

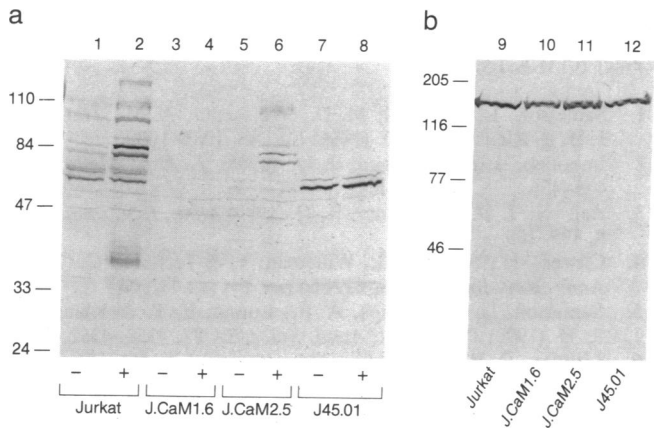

FIG. 4. Lysates of Jurkat (lanes 1, 2, and 9), J.CaM1.6 (lanes 3, 4, and 10), J.CaM2.5 (lanes 5, 6, and 11), and J45.01 (lanes 7, 8, and 12) were prepared from unstimulated cells (lanes 1, 3, 5, 7, 9–12) or from cells stimulated with anti-Ti β mAb (C305; lanes 2, 4, 6, and 8) and analyzed with anti-phosphotyrosine 4G10 mAb (*a*) or anti-PLC-γ1 mAb (*b*) on Western blots. Cells were stimulated for 30 sec and lysates were prepared and analyzed as in Fig. 2 with the exception that 10% acrylamide gels were used for *a*.

and J45.01 fails to induce detectable tyrosine phosphorylation of any PLC activity in the anti-phosphotyrosine immunoprecipitates (Fig. 5). Stimulation of the TCR on J.CaM2 reproducibly induces some, albeit a markedly reduced level, of PLC activity in the tyrosine phosphoprotein fraction (10–23% of wild-type levels). All of this induced PLC activity isolated from J.CaM2 can be immunodepleted with anti-PLC-γ1 mAbs (Table 1). However, no evidence of PLC-γ1 phosphorylation can be detected by Western blot analysis of PLC-γ1 immunoprecipitates after stimulation of the TCR on J.CaM2 (data not shown), suggesting that the enzyme assay for PLC activity is more sensitive than the Western blot analysis. These data demonstrate that tyrosine phosphorylation of PLC-γ1 is markedly impaired in mutants defective in TCR-mediated induction of PLC activation.

DISCUSSION

The activation of PLC activity in T cells has been correlated with a number of important later cellular responses (for review, see ref. 33). Therefore, understanding the mechanism by which the TCR activates PLC is of considerable interest. Studies with cholera toxin and permeabilized cell systems suggested a G protein couples the TCR to PLC (11, 12). However, more recent inhibitor kinetic analyses and studies

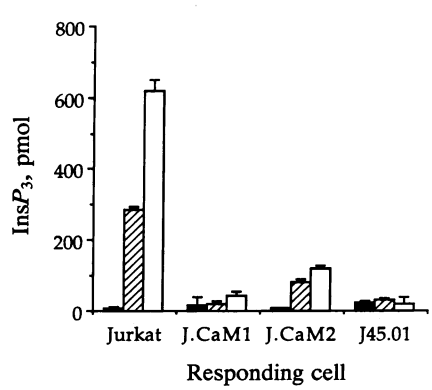

FIG. 5. Tyrosine phosphorylation of PLC activity was analyzed in the indicated cells at 30 sec after no stimulation (solid bars) or stimulation with anti-Ti β mAb C305 (hatched bars) or anti-CD3 mAb Leu4 (open bars).

with CD45-tyrosine-phosphatase-deficient mutants have suggested the importance of a regulatory tyrosine phosphorylation event (11–14, 23). The studies reported here demonstrate that stimulation of the TCR, but not a receptor (Hm1) that activates PLC through a G-protein mechanism, induces the tyrosine phosphorylation of PLC-γ1. This tyrosine phosphorylation of a PLC isozyme represents a potential regulatory event in TCR-mediated activation of PLC activity.

Stimulation of the TCR on both Jurkat cells and PBLs induced the recovery of PLC activity in anti-phosphotyrosine immunoprecipitates. There are multiple isozymes of PLC expressed in T cells. In addition to PLC-γ1, Jurkat cells express high levels of PLC-α and very low levels of PLC-γ2 transcripts (31). Other isozymes of PLC, including the broadly expressed β isozyme, may also be expressed in these cells. The expression of the various isozymes of PLC in normal T cells has not been examined. In addition, many tyrosine phosphoproteins were detected in our anti-phosphotyrosine immunoprecipitates (Fig. 3*b*, lane 8). Several phosphoproteins have been coimmunoprecipitated with PLC-γ1 from fibroblasts stimulated through the PDGF receptor (18). Thus, in addition to tyrosine phosphorylation of a PLC isozyme, it was formally possible that the appearance of PLC activity in our phosphotyrosine immunoprecipitates could have been a consequence of the tyrosine phosphorylation of proteins that associate with PLC. However, by Western blot analysis, the induction of tyrosine phosphorylation of a PLC-γ isozyme was detected after TCR stimulation. No other prominent tyrosine phosphoproteins were detected in the anti-PLC-γ1 immunoprecipitates isolated from stimulated cells arguing against associated tyrosine phosphoproteins (Fig. 3*a*, lane 6). Moreover, the ability to nearly completely deplete PLC activity from eluates of phosphotyrosine immunoprecipitates with mAbs reactive with PLC-γ1 or a heteroserum to the PLC-γ1 carboxyl terminal end demonstrates that the majority of PLC activity detected in the phosphotyrosine immunoprecipitates is accounted for by PLC-γ1.

Other than the TCR ζ chain, substrates in the PTK pathway activated by the TCR have not been identified (1, 14). Here, we demonstrate that PLC-γ1 is a substrate of a PTK in T cells. The phosphorylation of PLC-γ1 does not occur as a consequence of PLC activation based on the failure to detect PLC activity in anti-phosphotyrosine immunoprecipitates isolated from Hm1-stimulated cells or after stimulation with a calcium ionophore and phorbol ester. Nor is the 150-kDa tyrosine phosphoprotein, representing PLC-γ1, detected in lysates obtained after Hm1 stimulation, which leads to PLC activity in a Jurkat Hm1 transfectant (22). Rather, PLC-γ1 tyrosine phosphorylation appears to be a direct or indirect consequence of TCR stimulation. Our studies do not establish whether the observed induction of tyrosine phosphorylation is due to the activation of a PTK or inhibition of a tyrosine phosphatase. One such phosphatase that appears to play a role in the activation of PLC in T cells is CD45 (23, 32). However, deficiency of CD45 in J45.01 is not associated with a general increase in tyrosine phosphoproteins or in the tyrosine phosphorylation of any PLC. These data plus data that have been obtained with PTK inhibitors (13) support a model in which it is the TCR-induced activation of a PTK that leads to PLC-γ1 tyrosine phosphorylation. Regardless, since the TCR induces the activation of PLC activity in T cells, the observed tyrosine phosphorylation of PLC-γ1 may represent the regulatory activation event. This is supported by a recent study demonstrating increased catalytic activity of tyrosine-phosphorylated PLC-γ1 isolated from EGF-treated cells (19). Thus, PLC-γ1 appears to represent an important physiologic substrate of the TCR-activated PTK.

We further examined the functional importance of the tyrosine phosphorylation of PLC-γ1 using signal transduc-

tion mutants that fail to activate PLC activity after TCR stimulation. Both the J.CaM1 and J.CaM2 mutants are deficient in their ability to activate PLC (20, 21). The two mutants have distinct complementing defects (21). Although the TCR on J.CaM1 is deficient in its ability to induce PTK activity, J.CaM2 does exhibit PTK activity after TCR stimulation. Despite the activity of the TCR-induced PTK in J.CaM2, only small amounts of PLC activity (10–23% of wild-type levels) could be detected among tyrosine phosphoproteins after TCR stimulation. It is of interest to consider the defect in J.CaM2 in greater detail in light of the observed small degree of apparent tyrosine phosphorylation of a protein with PLC activity. Immunodepletion with anti-PLC-γ1 mAbs removes all of the tyrosine-phosphorylated PLC activity detected in J.CaM2. Therefore, all of the PLC activity recovered in the tyrosine phosphoprotein fraction isolated from stimulated J.CaM2 cells is attributable to the PLC-γ1 isozyme. There are several possible explanations for the failure of J.CaM2 to activate detectable PLC activity *in vivo*. (*i*) Tyrosine phosphorylation of PLC-γ1 in J.CaM2 may not be of sufficient magnitude to result in detectable PLC activity *in vivo*. Immunoblots have failed to detect induction of tyrosine-phosphorylated PLC-γ1 in J.CaM2 (data not shown), suggesting that the enzymatic assay is more sensitive than Western blot analysis. (*ii*) The phosphorylation of PLC-γ1 in J.CaM2 may qualitatively differ; i.e., different sites of tyrosine phosphorylation may have distinct functional consequences. A detailed phosphoamino acid mapping study will be required to address this point. (*iii*) PLC-γ1 may not function as efficiently because of mutations within PLC or a regulatory factor. (*iv*) Though unlikely, the tyrosine phosphorylation of PLC-γ1 may not be relevant to its activation. Further studies to examine the residues of PLC-γ1 phosphorylated in J.CaM2 may provide insight into the mechanism by which tyrosine phosphorylation contributes to PLC activation.

These studies demonstrate that the TCR, a receptor without intrinsic PTK activity, induces tyrosine phosphorylation of PLC-γ1. Studies of the Jurkat signal transduction mutants and the Hm1 receptor suggest that this phosphorylation event is functionally relevant and is not a consequence of the activation of PLC activity. Thus with the previous inhibitor studies (13) and kinetic analyses (14), our studies provide an important link between the TCR-activated PTK pathway and inositol phospholipid pathway. In the simplest model, stimulation of the TCR activates a PTK, which then through direct or indirect mechanisms induces the tyrosine phosphorylation and activation of PLC-γ1. The coimmunoprecipitation of the TCR and fyn (5), a member of the src family of kinases, suggests that fyn may represent the kinase responsible for the regulation of the PTK pathway and resultant PLC-γ1 phosphorylation. Likewise, lck, another src family member, may contribute to the response by associating with the T-cell surface molecules CD4 or CD8 that may function as coreceptors with the TCR (7). Finally, the recent finding that the PDGF receptor associates with and activates src, fyn, and yes (34) raises the possibility that the src family of kinases may play a direct or indirect role in the regulation of PLC-γ1 tyrosine phosphorylation and its activation.

We thank Dr. T. Daniel for his very helpful advice regarding the PLC assay. We are grateful to Drs. S. M. Fu, D. Morrison, B. Drucker, T. Roberts, and S. G. Rhee for providing mAbs used in these studies. This work was supported in part by a grant from the National Institutes of Health (to A.W.) and by the Arthritis Foundation (to G.K.) and by the Rosalind Russel Arthritis Research Center (to G.K.).

1. Samelson, L. E., Patel, M. D., Weissman, A. M., Harford, J. B. & Klausner, R. D. (1986) *Cell* **46**, 1083–1090.
2. Imboden, J. B. & Stobo, J. D. (1985) *J. Exp. Med.* **161**, 446–456.
3. Ashwell, J. D. & Klausner, R. D. (1990) *Annu. Rev. Immunol.* **8**, 139–168.
4. Clevers, H., Alarcon, B., Willeman, T. & Terhorst, C. (1988) *Annu. Rev. Immunol.* **6**, 629–662.
5. Samelson, L. E., Phillips, A. F., Luong, E. T. & Klausner, R. D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4358–4362.
6. Littman, D. R. (1987) *Annu. Rev. Immunol.* **5**, 561–584.
7. Veillette, A., Bookman, M. A., Horak, E. M. & Bolen, J. B. (1988) *Cell* **55**, 301–308.
8. Turner, J. M., Brodsky, M. H., Irving, B. A., Levin, S. D., Perlmutter, R. M. & Littman, D. R. (1990) *Cell* **60**, 755–765.
9. Veillette, A., Bookman, M. A., Horak, E. M., Samelson, L. E. & Bolen, J. B. (1989) *Nature (London)* **338**, 257–259.
10. Rhee, S. G., Suh, P.-G., Ryu, S.-H. & Lee, S. Y. (1989) *Science* **244**, 546–550.
11. Imboden, J. B., Shoback, D. M., Pattison, G. & Stobo, J. D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5673–5677.
12. Schrezenmeier, H., Ahnert-Hilger, G. & Fleischer, B. (1988) *J. Immunol.* **141**, 3785–3790.
13. June, C. H., Fletcher, M. C., Ledbetter, J. A., Schieven, G. L., Siegel, J. N., Phillips, A. F. & Samelson, L. E. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7722–7726.
14. June, C. H., Fletcher, M. C., Ledbetter, J. A. & Samelson, L. E. (1990) *J. Immunol.* **144**, 1591–1599.
15. Wahl, M. I., Daniel, T. O. & Carpenter, G. (1988) *Science* **241**, 968–970.
16. Wahl, M. I., Nishibe, S., Suh, P.-G., Rhee, S. G. & Carpenter, G. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1568–1572.
17. Kumjian, D. A., Wahl, M. I., Rhee, S. G. & Daniel, T. O. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8232–8236.
18. Meisenhelder, J., Suh, P.-G., Rhee, S. G. & Hunter, T. (1989) *Cell* **57**, 1109–1122.
19. Nishibe, S., Wahl, M. I., Hernandez-Sotomayor, S. M., Tonk, N. K., Rhee, S. G. & Carpenter, G. (1990) *Science* **250**, 1253–1256.
20. Goldsmith, M. A. & Weiss, A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6879–6883.
21. Goldsmith, M. A., Dazin, P. E. & Weiss, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8613–8617.
22. Desai, D., Newton, M. E., Kadlecek, T. & Weiss, A. (1990) *Nature (London)* **348**, 66–69.
23. Koretzky, G., Picus, J., Schultz, T. & Weiss, A. (1990) *Proc. Natl. Acad. Sci. USA* **88**, 2037–2041.
24. Laing, T. J. & Weiss, A. (1988) *J. Immunol.* **140**, 1056–1062.
25. Hara, T. & Fu, S. M. (1985) *J. Exp. Med.* **161**, 641–656.
26. Kamoun, M., Martin, P. J., Hansen, J. A., Brown, M. A., Siadak, A. W. & Nowinski, R. C. (1981) *J. Exp. Med.* **153**, 207–212.
27. Drucker, B., Mamon, T. & Roberts, T. (1989) *N. Eng. J. Med.* **321**, 1383–1391.
28. Suh, P.-G., Ryu, S. H., Moon, K. H., Suh, H. W. & Rhee, S. G. (1988) *Cell* **54**, 161–169.
29. Weiss, A., Dazin, P., Shields, R., Fu, S. M. & Lanier, L. L. (1987) *J. Immunol.* **139**, 3245–3250.
30. Nathanson, N. M. (1987) *Annu. Rev. Neurosci.* **10**, 195–236.
31. Goldfein, R., Seaman, W., Hempl, W. & Imboden, J. (1991) *J. Immunol.*, in press.
32. Koretzky, G. A., Picus, J., Thomas, M. L. & Weiss, A. (1990) *Nature (London)* **346**, 66–68.
33. Weiss, A. & Imboden, J. B. (1987) *Adv. Immunol.* **41**, 1–38.
34. Kypta, R. M., Goldberg, Y., Ulug, E. T. & Courtneidge, S. A. (1990) *Cell* **62**, 481–492.