Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2745–2749, April 1991
Immunology

Tyrosine phosphorylation of phospholipase C induced by membrane immunoglobulin in B lymphocytes

(calcium/inositol phosphate/tyrosine kinase/phospholipase C-γ1)

ROBERT H. CARTER*†, DO JOON PARK‡, SUE GOO RHEE‡, AND DOUGLAS T. FEARON*§

*Department of Medicine and §Graduate Program in Immunology, the Johns Hopkins University School of Medicine, Baltimore, MD 21205; and ‡Laboratory of Biochemistry, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by K. Frank Austen, January 2, 1991

ABSTRACT **Ligation of membrane IgM on B lymphocytes causes activation of a protein-tyrosine kinase(s) (PTK) and of phospholipase C (PLC). To determine whether these are elements of a common signal-transduction pathway, the effect of three PTK inhibitors on the rise in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in human B-lymphoblastoid cell lines was assessed. Tyrphostin completely suppressed the increase in $[Ca^{2+}]_i$ and the generation of inositol phosphates induced by ligation of membrane immunoglobulin (mIg) M. Herbimycin and genistein reduced by 30% and 50%, respectively, the rise in $[Ca^{2+}]_i$ caused by optimal ligation of mIgM, and they abolished it in cells activated by suboptimal ligation of mIgM. Tyrphostin had no effect on the capacity of aluminum fluoride to increase $[Ca^{2+}]_i$. To determine whether a function of PTK is the phosphorylation of PLC, immunoprecipitates obtained with anti-phosphotyrosine from detergent lysates of B-lymphoblastoid cells were assayed for PLC activity. Ligation of mIgM increased immunoprecipitable PLC activity 2-fold by 90 sec and 4-fold by 30 min. Specific immunoprecipitation and Western blot analysis identified tyrosine phosphorylation of the γ1 isoform of PLC after 60 sec of stimulation. Activation of PLC in B cells by mIgM requires PTK function and is associated with tyrosine phosphorylation of PLC-γ1, suggesting a mechanism of PLC activation similar to that described for certain receptor PTKs.**

The membrane immunoglobulin (mIg) complex links the recognition of antigen by B lymphocytes to activation or, conversely, to suppression of proliferation of these cells. The complex is composed of protein products of clonally distributed, rearranged, variable regions of heavy- and light-chain genes in association with nonpolymorphic subunits of 32–39 kDa (1, 2). Among early intracellular events triggered by ligation of this complex is the activation of phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate inositol trisphosphate (IP_3) and diacylglycerol, which, in turn, produce an increase in the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$) and translocation of protein kinase C to the membrane (3–6). Analysis of possible mechanisms coupling the mIg complex to PLC have emphasized a role for a guanine nucleotide-binding protein based on the enhancing and suppressing effects of guanosine 5′-[γ-thio]triphosphate and guanosine 5′-[β-thio]diphosphate, respectively, on mIgM-induced PIP_2 hydrolysis (7–9). The guanine nucleotide-binding component has not been identified, but one candidate may be $p21^{ras}$, which cocaps with mIgM and shifts to a GTP-bound state following ligation of the antigen receptor complex of a developmentally related cell type, the T lymphocyte (10, 11).

Ligation of the mIg complex also activates a protein-tyrosine kinase (PTK) that rapidly phosphorylates several unidentified cellular proteins (12, 13); candidates for this PTK include the products of *hck*, *lyn*, and a B-lymphocyte-specific member of the *src* family, *blk* (14–17). A role for this reaction in the activation of PLC has not been demonstrated in the B lymphocyte, but in other cell types two receptor PTKs, the platelet-derived growth factor (PDGF) receptor and the epidermal growth factor (EGF) receptor, mediate activation and tyrosine phosphorylation of the γ1 isoform of PLC (18–22).

We have examined the role of tyrosine kinases in the activation of PLC by mIgM in B cells. Tyrosine kinase activity is required for PLC stimulation by mIgM; immunoprecipitates of lysates of mIgM-stimulated cells obtained with anti-phosphotyrosine contain increased PLC activity; and ligation of mIgM stimulates tyrosine phosphorylation of PLC-γ1. Thus, activation of PTK and of PLC by mIgM in the B cell are linked rather than independent events, resembling in this respect the activation of PLC by the growth factor tyrosine kinase receptors in other cell types.

MATERIALS AND METHODS

Effect of PTK Inhibitors on $[Ca^{2+}]_i$. B-lymphoblastoid cells were incubated for 16 hr with tyrphostin (RG50864; Rorer Pharmaceutical, King of Prussia, PA) or herbimycin (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute), or for 15 min with genistein (Calbiochem), or with vehicle (dimethyl sulfoxide) only. Analysis of $[Ca^{2+}]_i$ was as previously described (23), except for the use of a FACStar Plus flow cytometer with the CHRONYS program (Becton Dickinson). Cells were loaded with indo-1 acetoxymethyl ester (Molecular Probes), incubated with Fab′ DA4.4 mouse anti-human IgM (24), and washed. Intracellular fluorescence was monitored by flow cytometry, and after a 20-sec baseline period, affinity-purified $F(ab')_2$ anti-mouse IgG (Organon Tenika–Cappel) was added and the analysis continued for a total of 180 sec. The data were converted to mean $[Ca^{2+}]_i$ by the method of Rabinovitch *et al.* (25).

Effect of PTK Inhibitors on Inositol Phosphates. B-lymphoblastoid cells loaded with [^{3}H]inositol were cultured for 16 hr with tyrphostin or herbimycin or the vehicle (dimethyl sulfoxide) only. The cells were then incubated with Fab′ DA4.4 mouse anti-human IgM or an isotype-matched control antibody (MOPC-21), washed, and stimulated with $F(ab')_2$ anti-mouse IgG. After 10 min the cells were lysed. [^{3}H]Inositol phosphates were extracted, separated by chromatography on AG 1-X8 (Bio-Rad), and quantified by scintillation spectroscopy (26, 27). The percent increase in inositol phosphates in

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: $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; EGF, epidermal growth factor; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; mIg, membrane immunoglobulin; PDGF, platelet-derived growth factor; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PTK, protein-tyrosine kinase.

†To whom reprint requests should be addressed.

stimulated cells over replicate samples of unstimulated cells cultured with either dimethyl sulfoxide or inhibitor was calculated.

Anti-Phosphotyrosine-Precipitable PLC Activity. B-lymphoblastoid cells were incubated with Fab' DA4.4 mouse anti-human IgM or isotype-matched control antibody, stimulated with $F(ab')_2$ anti-mouse IgG for timed intervals, and lysed. By using the method of Wahl *et al.* (18, 19), immunoprecipitates were obtained with anti-phosphotyrosine, eluted with phenyl phosphate, and assayed for hydrolysis of $[^3H]PIP_2$ in the presence of 1 μM free Ca^{2+} or excess EGTA for 15 min at 37°C. Less than 70 pmol of PIP_2 was hydrolyzed in any sample in the presence of excess EGTA. In similar experiments the inositol phosphate products were separated as described above. More than 95% of the increase in inositol phosphates produced in the presence of 1 μM Ca^{2+} relative to those obtained in the presence of excess EGTA was due to IP_3 in both control and anti-IgM-stimulated cells.

Analysis of Tyrosine Phosphorylation of PLC-γ1. B-lymphoblastoid cells were stimulated as described above for timed intervals, pelleted rapidly, and lysed in 1% Triton X-100/20 mM Hepes, pH 7.2/10% glycerol/50 mM sodium fluoride/1 mM phenylmethylsulfonyl fluoride/1 mM sodium vanadate with leupeptin at 10 μg/ml. After centrifugation and preclearance with Pansorbin (Calbiochem), immunoprecipitates were obtained as previously described by incubating the lysates with a mixture of monoclonal anti-PLC-γ1 antibodies followed by Pansorbin (28). Eluates released by heating in sample buffer at 95°C for 5 min were resolved by SDS/6% PAGE and transferred to nitrocellulose, which was blocked by incubation with bovine serum albumin and then probed with anti-phosphotyrosine (Upstate Biotechnical, Lake Placid, NY) followed by alkaline phosphatase-coupled anti-mouse IgG (Promega).

RESULTS

PTK Dependence of PLC Activation. To determine whether a PTK function is required for the activation of PLC in human B lymphocytes, mIgM was crosslinked on Daudi B-lymphoblastoid cells with saturating and subsaturating concentrations, respectively, of anti-IgM in the presence or absence of incremental concentrations of three unrelated PTK inhibitors having distinct mechanisms of action: tyrphostin, herbimycin, and genistein. $[Ca^{2+}]_i$ was monitored by flow cytometry of the cells, which had been loaded with indo-1. The PTK inhibitors in the concentrations used did not diminish the viability of the cells, which was >95%. Preincubation of the Daudi cells for 16 hr with 80 μM or 160 μM tyrphostin fully inhibited the response in B cells activated by either high- or low-dose anti-IgM (Fig. 1). These concentrations of tyrphostin are in the range of those reported to suppress the increase in $[Ca^{2+}]_i$ induced by the EGF receptor in A431 epidermoid carcinoma cells (20). Herbimycin at 1.0 μM partially decreased the response of the B-lymphoblastoid cells to high-dose anti-IgM, and at 0.2 μM and 1.0 μM it essentially abolished the increase in $[Ca^{2+}]_i$ caused by low-dose anti-IgM. In previous studies this range of concentrations of herbimycin suppressed the function of the products of the nonreceptor PTK oncogenes *src*, *yes*, *fps*, and *erbB* (29). Genistein resembled herbimycin in being more effective in blocking the rise in $[Ca^{2+}]_i$ in submaximally stimulated Daudi cells, and the inhibitory concentrations of 150 μM and 300 μM were comparable to those suppressing PLC activation in T cells (30).

Release of intracellular Ca^{2+} without detectable activation of PLC in B cells (31) and suppression of the rise in $[Ca^{2+}]_i$ by PTK inhibitors without diminished inositol phosphate levels in T cells (32) have been reported. Therefore, inhibition by tyrphostin and herbimycin of PLC activation was assessed

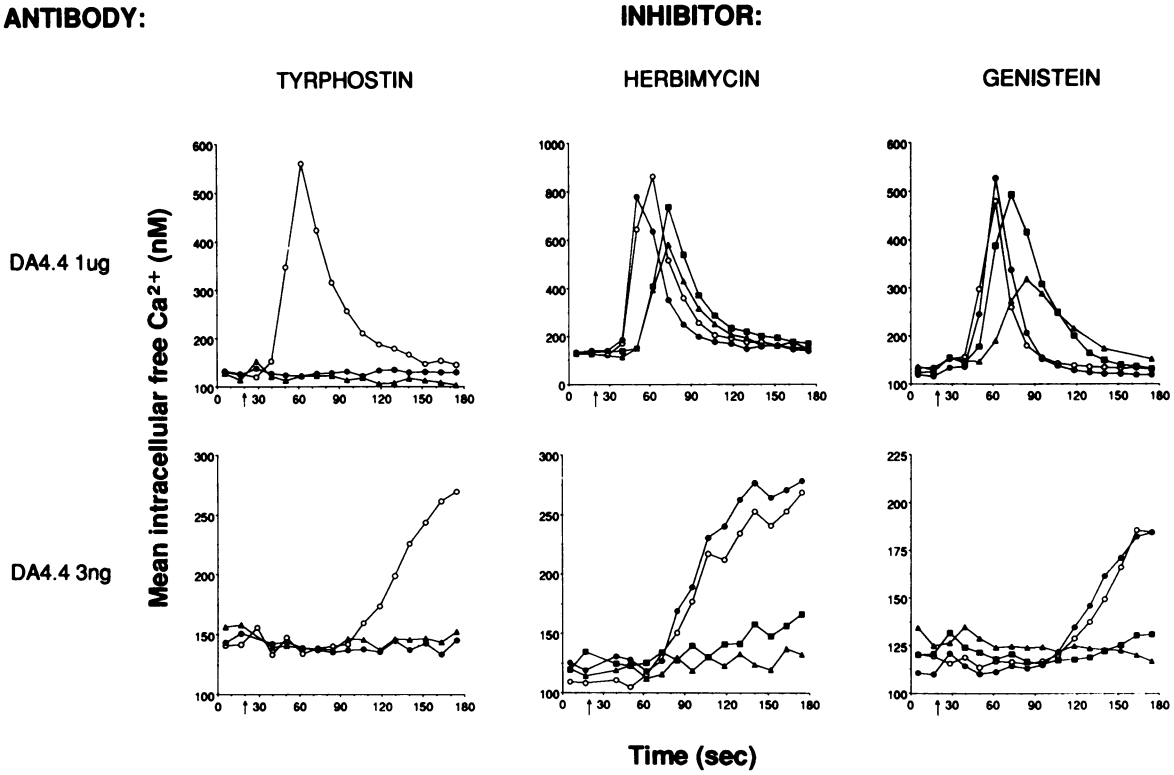

FIG. 1. PTK inhibitors suppress the increase in $[Ca^{2+}]_i$ stimulated by mIgM. Daudi B-lymphoblastoid cells were treated with tyrphostin (RG50864) (80 μM, ●; 160 μM, ▲) for 16 hr, herbimycin (40 nM, ●; 200 nM, ■; 1000 nM, ▲) for 16 hr, or genistein (75 μM, ●; 150 μM, ■; 300 μM, ▲) for 15 min or with the vehicle for the inhibitor, dimethyl sulfoxide (○). The cells were incubated with Fab' DA4.4 anti-human IgM at optimal (1 μg/ml) or suboptimal (3 ng/ml) concentrations. Intracellular fluorescence was monitored by flow cytometry, and after a 20-sec baseline period, $F(ab')_2$ anti-mouse IgG was added (arrow), and the analysis continued for a total of 180 sec.

Table 1. PTK inhibitors suppress the increase in inositol phosphates stimulated by mIgM

Antibody	IP_2 increase, %		IP_3 increase, %	
	DMSO	Inhibitor	DMSO	Inhibitor
Experiment 1 (inhibitor = tyrphostin)				
MOPC-21 (1 μg/ml)	2 ± 4	2 ± 3	0 ± 3	1 ± 2
DA4.4 (3 ng/ml)	72 ± 6	20 ± 4	62 ± 3	11 ± 2
DA4.4 (1 μg/ml)	346 ± 16	137 ± 8	292 ± 20	61 ± 6
Experiment 2 (inhibitor = herbimycin)				
DA4.4 (3 ng/ml)	114 ± 8	31 ± 6	159 ± 10	24 ± 26
DA4.4 (1 μg/ml)	333 ± 11	207 ± 11	261 ± 11	181 ± 7

L4 (experiment 1) or Daudi (experiment 2) cells loaded with [^{3}H]inositol were cultured for 16 hr with 160 μM tyrphostin RG50864 (experiment 1), 1.0 μM herbimycin (experiment 2), or an equal volume of vehicle only (dimethyl sulfoxide, DMSO). The cells were incubated with optimal (1 μg/ml) or suboptimal (3 ng/ml) concentrations of Fab' DA4.4 anti-IgM or with MOPC-21 (control antibody, 1 μg/ml) and stimulated for 10 min by addition of $F(ab')_2$ goat anti-mouse IgG (20 μg/ml). Values are means ± SD for triplicate samples of 2×10^6 cells.

also by measuring the products of PIP_2 hydrolysis, inositol bisphosphate (IP_2) and IP_3, by Daudi cells and the L4 subclone of Ramos B-lymphoblastoid cells that had been stimulated with high or low concentrations of anti-IgM. The L4 cells, unlike Daudi cells, are not infected by the Epstein–Barr virus. Tyrphostin diminished the generation of IP_2 and IP_3 in cells triggered by both concentrations of anti-IgM, whereas herbimycin was more inhibitory with cells that had been activated with the low concentration of anti-IgM (Table 1), suggesting that the inhibitors suppressed PLC activation or activity.

To exclude an inhibitory effect of tyrphostin on the activity of PLC, cells were stimulated with aluminum fluoride, which directly activates a GTP-binding protein coupled to PLC (27, 33). L4 cells that had been pretreated with 80 or 160 μM tyrphostin increased $[Ca^{2+}]_i$ to the same level as buffer-treated cells following addition of aluminum fluoride, despite being suppressed for mIgM-induced changes in $[Ca^{2+}]_i$ (Fig. 2). This differential response suggests that the function of at least certain PLC isoforms is not directly inhibited by tyrphostin.

Tyrosine Phosphorylation of PLC. The possibility that in the B lymphocyte a PTK mediates activation of PLC by phosphorylating the enzyme, as occurs with the PDGF receptor and the EGF receptor in other cell types, was assayed by the method of Wahl *et al.* (18, 19), in which PLC activity was measured in immunoprecipitates obtained with anti-phosphotyrosine from lysates of B-lymphoblastoid cells activated through mIgM. L4 B-lymphoblastoid cells were activated by crosslinking Fab' DA4.4 anti-IgM, bound at the same optimal (1 μg/ml) and suboptimal (4 ng/ml) concentrations used in the above studies. Detergent lysates of the cells were subjected to immunoprecipitation with immobilized monoclonal anti-phosphotyrosine. Eluates were obtained by treatment of the immunoprecipitates with phenyl phosphate and were assayed for PLC activity by the hydrolysis of PIP_2. Almost 4-fold more PLC activity was recovered with anti-phosphotyrosine from cells activated with high-dose anti-IgM than from cells treated with control antibody; low-dose anti-IgM caused a small but significant increase relative to the control cells (Table 2). Kinetic analysis of this reaction revealed that 90 sec after stimulation of the cells with anti-IgM at 1 μg/ml, the amount of PLC activity recovered with anti-phosphotyrosine was twice that of control cells, and that it continued to rise so that at 30 min it was 4-fold that of the control cells (Fig. 3).

To determine whether the γ1 isoform of PLC was tyrosine-phosphorylated after ligation of mIgM, replicate samples of L4 B-lymphoblastoid cells were lysed without or with crosslinkage of mIgM for timed intervals. PLC-γ1 was specifically immunoabsorbed from the lysates, eluted, resolved by SDS/6% PAGE, transferred to nitrocellulose and probed

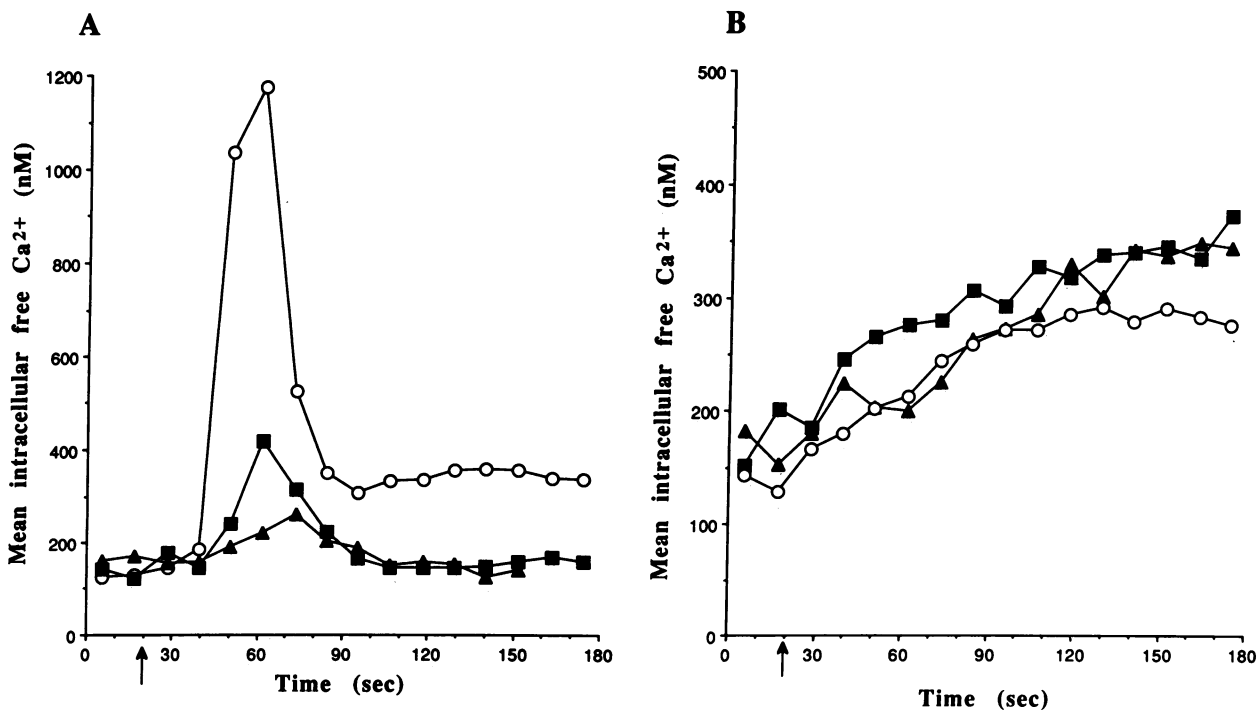

FIG. 2. Tyrphostin does not suppress the rise in $[Ca^{2+}]_i$ stimulated by aluminum fluoride. L4 B-lymphoblastoid cells were treated for 16 hr with tyrphostin (RG50864) at 80 μM (■) or 160 μM (▲) or with the vehicle, dimethyl sulfoxide (○). After loading with indo-1, intracellular fluorescence was monitored by flow cytometry. (*A*) Cells incubated with Fab' DA4.4 anti-human IgM (1 μg/ml) and then stimulated by the addition (arrow) of $F(ab')_2$ goat anti-mouse IgG. (*B*) Cells were stimulated by the addition (arrow) of NaF and $AlCl_3$ to yield final concentrations of 50 mM and 10 μM, respectively.

Table 2. Anti-phosphotyrosine-precipitable PLC activity stimulated by mIgM

Antibody	PLC activity, pmol/15 min
MOPC-21 (1 μg/ml)	510 ± 25
DA4.4 (4 ng/ml)	665 ± 61*
DA4.4 (1 μg/ml)	1884 ± 121*

L4 cells were incubated with Fab' DA4.4 anti-human IgM at 1 μg/ml or 4 ng/ml or MOPC-21 control antibody at 1 μg/ml, stimulated with $F(ab')_2$ goat anti-mouse IgG for 10 min, lysed, and immunoprecipitated with anti-phosphotyrosine-Sepharose. Eluates were assayed for PLC activity, which is expressed as pmol of PIP_2 hydrolyzed per 15 min per 5×10^6 cells (mean ± SD of triplicate samples).
*$P < 0.005$ when compared to the response to MOPC-21.

with anti-phosphotyrosine. A representative experiment is shown in Fig. 4. Faint tyrosine phosphorylation was detectable in unstimulated cells. Increased tyrosine phosphorylation of PLC-γ1 was observed 60 sec after ligation of mIgM. The enhanced tyrosine phosphorylation persisted at 30 min.

DISCUSSION

Each of three PTK inhibitors suppressed the rise in $[Ca^{2+}]_i$ induced by mIgM in B lymphoblastoid cells. Although each may effect other cellular enzymes, the finding that all three were suppressive suggests that this action is a consequence of their shared capacity to inhibit PTK. The tyrphostin RG50864, which was developed on the basis of potency in inhibiting EGF receptor kinase activity, was most effective. The degree of inhibition of $[Ca^{2+}]_i$ correlated with suppression of inositol phosphate production with two inhibitors in two cell lines, suggesting that the inhibition of $[Ca^{2+}]_i$ did reflect decreased activation of PLC. The lack of an effect of tyrphostin on aluminum fluoride-induced PLC activity, as well as previous reports (20, 30), suggest that the inhibitors do not block either PLC enzymatic activity directly or downstream mechanisms necessary for induction of a rise in $[Ca^{2+}]_i$.

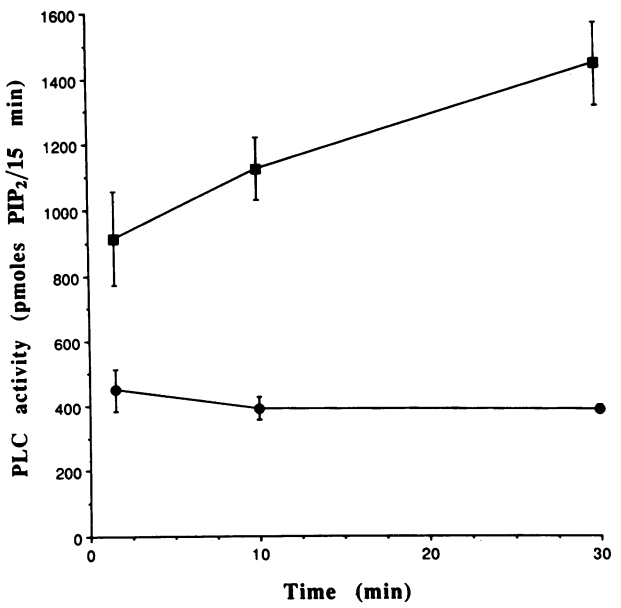

FIG. 3. Kinetics of mIgM-stimulated, anti-phosphotyrosine-precipitable PLC activity. L4 B-lymphoblastoid cells were incubated with DA4.4 anti-human IgM (■) or control antibody MOPC-21 (●) at 1 μg/ml; stimulated with $F(ab')_2$ goat anti-mouse IgG for 90 sec, 10 min, or 30 min; lysed; and analyzed for PLC activity.

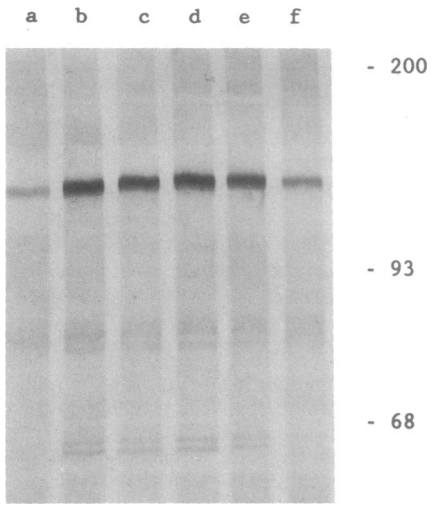

FIG. 4. Tyrosine phosphorylation of PLC-γ1. L4 B-lymphoblastoid cells were incubated with Fab' DA4.4 anti-human IgM (lanes b–e) or control antibody, MOPC-21 (lanes a and f) at 1 μg/ml; stimulated with $F(ab')_2$ goat anti-mouse κ chain; and lysed at 0 min (lane a), 1 min (lane b), 3 min (lane c), 5 min (lane d), or 30 min (lanes e and f). PLC-γ1 was specifically immunoprecipitated, eluted, resolved by SDS/PAGE, transferred to nitrocellulose, and probed with murine anti-phosphotyrosine followed by alkaline phosphatase-coupled goat anti-mouse IgG. Molecular weight standards are indicated at right ($M_r \times 10^{-3}$).

Not only is a PTK essential for PLC activation induced by the mIgM complex, but also one substrate for the PTK is PLC itself. mIgM stimulation increased anti-phosphotyrosine-precipitable PLC activity and induced tyrosine phosphorylation of PLC-γ1. Therefore, the nonreceptor PTK coupled to mIgM phosphorylates the same PLC isoform as do two receptor PTKs, the EGF receptor and the PDGF receptor. The kinetics of this modification of PLC and the active dose range of anti-IgM are consistent with tyrosine phosphorylation being involved in the activation of this enzyme. A potential mechanism by which tyrosine phosphorylation of PLC may enhance its activity has been described. Profilin, an actin-binding protein, binds PIP_2 and reduces availability of this substrate for PLC (34). Phosphorylation of PLC allows full activity even in the presence of profilin, perhaps by enhancing accessibility to PIP_2 (37). The finding that a PTK may function at a distal step in PLC activation by mIgM suggests that the role of guanine nucleotide-binding proteins in this reaction is not the direct coupling of receptor to PLC, as suggested for certain other receptors (35, 36). The effects of GTP analogues on mIgM-induced responses in the B cell (7–9) may be due to modulation by a GTP-binding protein of the PTK activity stimulated by mIgM or may be indirect due to effects on other enzyme pathways.

In the immune response, ligation of the mIgM complex must be capable of eliciting both activation and anergy, or tolerance, depending on the developmental stage of the B lymphocyte, the extent of the crosslinking of mIgM, or the concomitant ligation of other membrane receptors. The noncovalent coupling of the antigen receptor to the PTK mediating PLC activation may contribute to the capacity of the B lymphocyte to respond to ligand with these various biologic outcomes rather than with the single response of cellular proliferation that characterizes receptor PTKs of fibroblasts. Determining how mIgM is coupled to the relevant nonreceptor PTK in B lymphocytes and identifying this PTK will enhance understanding of the immunoregulatory effects of antigen.

We thank Daniel Raben, Raymond Lyall, Matthew Wahl, and Pascal Goldschmidt-Clermont for helpful advice. This work was supported by National Institutes of Health Grants AI22833 and

AI28191. R.H.C. is supported by a Terri Gotthelf Lupus Research Scholar Award.

1. Hornbach, J., Tsubata, T., Leclercq, L., Stappert, H. & Reth, M. (1990) *Nature (London)* **343,** 760–762.
2. Campbell, K. S. & Cambier, J. C. (1990) *EMBO J.* **9,** 441–448.
3. Coggeshall, K. M. & Cambier, J. C. (1984) *J. Immunol.* **133,** 3382–3386.
4. Bijsterbosch, M. K., Meade, C. J., Turner, G. A. & Klaus, G. G. B. (1985) *Cell* **41,** 999–1006.
5. Chen, Z. Z., Coggeshall, K. M. & Cambier, J. C. (1986) *J. Immunol.* **136,** 2300–2304.
6. Harris, L. K. & Cambier, J. C. (1987) *J. Immunol.* **139,** 963–970.
7. Gold, M. R., Jakway, J. P. & DeFranco, A. L. (1987) *J. Immunol.* **139,** 3604–3613.
8. Harnett, M. M. & Klaus, G. G. B. (1988) *J. Immunol.* **140,** 3135–3139.
9. Monroe, J. G. & Haldar, S. (1989) *Biochim. Biophys. Acta* **1013,** 273–278.
10. Downward, J., Graves, J. D., Warne, P. H., Rayter, S. & Cantrell, D. A. (1990) *Nature (London)* **346,** 719–723.
11. Graziadei, L., Riabowol, K. & Bar-Sagi, D. (1990) *Nature (London)* **347,** 396–400.
12. Gold, M. R., Law, D. A. & DeFranco, A. L. (1990) *Nature (London)* **345,** 810–813.
13. Campbell, M. A. & Sefton, B. M. (1990) *EMBO J.* **9,** 2125–2131.
14. Quintrell, N., Lebo, R., Varmus, H., Bishop, J. M., Pettenati, M. J., Le Beau, M. M., Diaz, M. O. & Rowley, J. D. (1987) *Mol. Cell. Biol.* **7,** 2267–2275.
15. Ziegler, S. F., Marth, J. D., Lewis, D. B. & Perlmutter, R. M. (1987) *Mol. Cell. Biol.* **7,** 2276–2285.
16. Yamanash, Y., Mori, S., Yoshida, M., Kishimoto, T., Inoue, K., Yamamoto, T. & Toyoshima, K. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6538–6542.
17. Dymecki, S. M., Niederhuber, J. E. & Desiderio, S. V. (1990) *Science* **247,** 332–336.
18. Wahl, M. I., Daniel, T. O. & Carpenter, G. (1988) *Science* **241,** 968–970.
19. Kumjian, D. A., Wahl, M. I., Rhee, S. G. & Daniel, T. O. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8232–8236.
20. Margolis, B., Rhee, S. G., Felder, S., Mervic, M., Lyall, R., Levitzki, A., Ullrich, A., Zilberstein, A. & Schlessinger, J. (1989) *Cell* **57,** 1101–1107.
21. Meisenhelder, J., Suh, P.-G., Rhee, S. G. & Hunter, T. (1989) *Cell* **57,** 1109–1122.
22. Nishibe, S., Wahl, M. I., Hernandez-Sotomayor, S. M. T., Tonks, N. K., Rhee, S. G. & Carpenter, G. (1990) *Science* **250,** 1253–1256.
23. Carter, R. H., Spycher, M. O., Ng, T. C., Hoffman, R. & Fearon, D. T. (1988) *J. Immunol.* **141,** 457–463.
24. Maruyama, S., Kubagawa, H. & Cooper, M. D. (1985) *J. Immunol.* **135,** 192–199.
25. Rabinovitch, P. S., June, C. H., Grossman, A. & Ledbetter, J. A. (1986) *J. Immunol.* **137,** 952–961.
26. Berridge, M. J. (1983) *Biochem. J.* **212,** 849–858.
27. Mizuguchi, J., Yong-yong, J., Nakabayaschi, H., Huang, K.-P., Beaven, M. A., Chused, T. & Paul, W. E. (1987) *J. Immunol.* **139,** 1054–1059.
28. Kim, U.-H., Kim, J. W. & Rhee, S. G. (1989) *J. Biol. Chem.* **264,** 20167–20170.
29. Uehara, Y., Murakami, Y., Mizuno, S. & Kawai, S. (1988) *Virology* **164,** 294–298.
30. Mustelin, T., Coggeshall, K. M., Isakov, N. & Altman, A. (1990) *Science* **247,** 1584–1587.
31. Monroe, J. G., Seyfert, V. L., Owen, C. S. & Sykes, N. (1989) *J. Exp. Med.* **169,** 1059–1070.
32. Trevillyan, J. M., Lu, Y., Atluru, D., Phillips, C. A. & Bjorndahl, J. M. (1990) *J. Immunol.* **145,** 3223–3230.
33. Sternweis, P. C. & Gilman, A. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4888–4891.
34. Goldschmidt-Clermont, P. J., Machesky, L. M., Baldassare, J. J. & Pollard, T. D. (1990) *Science* **247,** 1575–1578.
35. Chambard, J. C., Paris, S., L'Allemain, G. & Pouyssegur, J. (1987) *Nature (London)* **326,** 800–803.
36. Hasegawa-Sasaki, H., Lutz, F. & Sasaki, T. (1988) *J. Biol. Chem.* **263,** 12970–12976.
37. Goldschmidt-Clermont, P. J., Kim, J. W., Machesky, L. M., Rhee, S. G. & Pollard, T. D. (1991) *Science,* in press.