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Immunology

Predominant expression and activation-induced tyrosine phosphorylation of phospholipase C-γ2 in B lymphocytes

(B-cell activation/inositol phospholipids/protein tyrosine kinase)

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ABSTRACT **The triggering of T- or B-cell antigen-specific receptors is accompanied by rapid tyrosine phosphorylation of distinct cellular substrates, one of which is the γ1 isoform of inositol phospholipid-specific phospholipase C (PLC-γ1). This phosphorylation event, mediated by a putative protein tyrosine kinase coupled to the antigen receptor, probably stimulates the enzymatic activity of PLC-γ1, thereby promoting inositol phospholipid hydrolysis and other downstream signal transduction events. Recently, another ubiquitously expressed PLC isoform, PLC-γ2 (which shares 50.2% amino acid homology with PLC-γ1), has been identified. PLC-γ2-specific antibodies were used to evaluate the distribution and potential signaling role of this isoform in lymphocytes. Here, we report that, in contrast to T lymphocytes that express predominantly PLC-γ1, the major isoform expressed in murine and human resting B cells is PLC-γ2. Among B-cell tumor lines, all five murine B-lymphoma lines tested and one of six human B-lymphoblastoid cell lines also expressed predominantly PLC-γ2. However, three other human lines preferentially expressed PLC-γ1, and two others displayed similar levels of the two PLC-γ isoforms. Furthermore, the triggering of B-cell surface immunoglobulin by anti-receptor antibodies was accompanied by a rapid tyrosine phosphorylation of PLC-γ2, which peaked after 5 min of stimulation. Conversely, and in agreement with recent reports, triggering of the T-cell antigen receptor complex led to the predominant phosphorylation of PLC-γ1 on tyrosine. These findings identify PLC-γ2 as a substrate for a B-cell putative protein tyrosine kinase coupled to the antigen receptor and suggest that its tyrosine phosphorylation constitutes a critical and early event in B-cell activation and, furthermore, that PLC-γ1 and PLC-γ2 may participate in similar but distinct signal transduction pathways in lymphocytes.**

Triggering of the antigen-specific receptor complexes expressed by either B or T lymphocytes with antigen or anti-receptor antibodies initiates signal transduction cascades associated with the rapid activation of inositol phospholipid-specific phospholipase C (PLC) and the subsequent production of second messengers, namely, inositol phosphates [primarily inositol 1,4,5-trisphosphate (IP_3)] and diacylglycerol (for reviews, see refs. 1 and 2). In both cell types, the activation of PLC is dependent on the prior stimulation of a protein tyrosine kinase (PTK), as indicated by the rapid kinetics of tyrosine phosphorylation (3) or by using PTK inhibitors. In T cells, receptor-stimulated IP_3 formation, a manifestation of PLC activation, and downstream activation events such as Ca^{2+} mobilization, interleukin 2 receptor expression, interleukin 2 secretion, and proliferation are all blocked by several inhibitors that reduce PTK activity by distinct mechanisms (4–6). Likewise, signaling events initiated by triggering of the surface immunoglobulin (sIg) receptor expressed by B cells is blocked by several PTK inhibitors (7–10). These findings support the hypothesis that PTKs play a critical role in receptor-driven PLC activation. However, the identity and precise role of the relevant PTKs in these signaling processes are not well understood.

Recent studies indicated that ligand-induced stimulation of the endogenous enzymatic activity of tyrosine kinase receptors such as the platelet-derived growth factor or epidermal growth factor (EGF) receptors induces phosphorylation of a PLC isoform, PLC-γ1, on tyrosine residues (11–14) and that the enzymatic activity of PLC-γ1 is increased as a result of its tyrosine phosphorylation (15). More recently, triggering of T (16–19) or B (9) lymphocyte antigen receptors was also found to lead to tyrosine phosphorylation of PLC-γ1, probably at similar, if not identical, sites to the ones phosphorylated by the EGF receptor kinase (16). These findings provide a possible mechanistic basis for the receptor-initiated PTK-dependent activation of PLC in lymphocytes. The mode of regulation of other PLC isoenzymes, namely, α, β, and δ (for review, see ref. 20), is not well understood, although PLC-β1 was recently found to be activated by the guanine nucleotide-binding protein G_q (21).

Recent cDNA cloning studies identified an additional PLC-γ isoform, termed PLC-γ2, derived from Epstein–Barr virus-transformed human lymphocytes (22) or rat brain (23). PLC-γ2 transcripts are ubiquitously expressed although the highest levels were found in spleen (23). PLC-γ1 and PLC-γ2 display 50% identity at the amino acid level, and this homology is particularly evident within the X and Y boxes that likely represent the catalytic domains of PLC enzymes (20) and the regulatory SH2 domain (for review, see ref. 24). Based on this moderate level of homology, it was suggested that PLC-γ1 and PLC-γ2 may possess distinct functions and substrate specificities (23).

To address the potential role of PLC-γ2 in lymphocyte signal transduction pathways, we have examined the distribution of PLC-γ2 and the status of tyrosine phosphorylation after receptor triggering in human or murine primary T and B cells or established lines and compared the results with those obtained with PLC-γ1. Here we report that PLC-γ2 is the predominant PLC-γ isoform expressed by B cells and that antibody-mediated sIg triggering induces tyrosine phosphorylation of PLC-γ2 (in addition to PLC-γ1). These results suggest that, in B cells, PLC-γ2 is coupled to a functionally important PTK and may, therefore, play a critical role in signal transduction events leading to B-cell differentiation and proliferation.

MATERIALS AND METHODS

Cells. WEHI-231, a murine B-cell tumor, and DO11.10, a murine T-cell hybridoma, were obtained from D. Green at

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Abbreviations: EGF, epidermal growth factor; IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PTK, protein tyrosine kinase; Tyr(*P*), phosphotyrosine; sIg, surface immunoglobulin; pNPP, *p*-nitrophenyl phosphate.
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this institute. The human B-cell tumor Raji was obtained from A. Theofilopoulos (Scripps Research Institute, La Jolla, CA). The human B-lymphoblastoid cell lines BJAB, DHL9, Louckes, Daudi, and WIL2 were obtained from D. Carson (University of California, San Diego). The CH series of murine B-cell lymphomas (25) were obtained from D. Scott (University of Rochester Medical Center, Rochester, NY). In addition, the human T-leukemia cell line Jurkat was used in the studies. These cell lines were maintained by serial passage in RPMI 1640 medium supplemented with 5% (vol/vol) heat-inactivated fetal bovine serum (GIBCO) and used in their logarithmic growth phase.

Single cell suspensions were prepared from spleens of BALB/c mice (obtained from The Jackson Laboratory). T cells were isolated by passage over mouse T-cell columns (Biotex Laboratories, Edmonton, Canada) as in the manufacturer's instructions. B lymphocytes were prepared by two cycles of complement-mediated lysis of cells pretreated with a mixture of anti-CD4 and anti-CD8 monoclonal antibodies (culture supernatants from B-cell hybridomas GK1.5 and 3.155, respectively, obtained from the American Type Culture Collection). Cells were treated with antibodies for 30 min on ice, washed once, resuspended in appropriately diluted guinea pig complement, and incubated at 37°C for 1 h. T and B cells prepared in this manner contained <2% contaminating sIg^+ or Thy-1^+ cells, respectively, as determined by flow cytometry (data not shown).

B cells were stimulated with $F(ab')_2$ fragments of goat anti-mouse IgG (10 μg/ml; Organon Teknika–Cappel), and T cells were pretreated with hamster anti-murine CD3 monoclonal antibody 145-2C11 (10 μg/ml; ref. 26), followed by crosslinking with $F(ab')_2$ fragments of goat anti-hamster immunoglobulin (25 μg/ml; Organon Teknika–Cappel). The cells were incubated for various periods of time at 37°C and extracted for further analyses.

Immunoprecipitation. Anti-PLC-γ1 or -PLC-γ2 antisera were generated in rabbits immunized with synthetic peptides, representing C-terminal sequences of the two isoforms covalently linked to keyhole limpet hemocyanin. The two peptides, PFEDFRISQEHLADHFDGR and QEKCNRRLREKRVSNSRFYS, correspond to residues 1257–1275 of bovine PLC-γ1 (27) and residues 1246–1265 of rat PLC-γ2 (23), respectively. Sera were prepared after three successive immunizations given in complete or incomplete Freund's adjuvant and alum gel, respectively. Cells were washed twice in 10 mM Hepes-buffered saline (pH 7.3) and lysed in TN1 [150 mM NaCl/20 mM Tris·HCl, pH 7.5/5 mM EDTA/1% Nonidet P-40/leupeptin (20 μg/ml)/aprotinin (20 μg/ml)] at 20–40 × 10^6 cells per ml. After 5 min on ice, the cell lysates were centrifuged at 16,000 × g for 4 min and the supernatant was collected. Anti-PLC-γ1 or -PLC-γ2 serum (5 μl) was added to the supernatant, and the lysate was incubated for 2 h at 4°C. A 10% (vol/vol) Pansorbin suspension (Calbiochem; 25 μl) was added during the final 30 min. The precipitate was washed five times with lysis buffer (without protease inhibitors).

To immunoprecipitate phosphotyrosine [Tyr(*P*)]-containing proteins, cells in culture medium were extracted by adding an equal volume of 2× lysis buffer (100 mM Tris·HCl, pH 8.5/50 mM NaCl/10 mM Na_3VO_4/10 mM EDTA/10 mM NaF/2% Nonidet P-40/protease inhibitors as above). A monoclonal anti-Tyr(*P*) antibody, 4G10 (Upstate Biotechnology, Lake Placid, NY), was added (10 μg per 40 × 10^6 cell equivalents), the lysates were incubated for 4 h at 4°C, and 15 μl of Pansorbin was added for the final 30 min. Tyr(*P*)-containing proteins were eluted by incubating precipitates for 20 min at 4°C in 1× lysis buffer containing 10 mM *p*-nitrophenyl phosphate (pNPP; Sigma) and collected by centrifugation. Anti-PLC or -Tyr(*P*) immunoprecipitates were resuspended in SDS sample buffer (60 mM Tris·HCl, pH 6.8/10% glycerol/2.3% SDS) for immunoblot analysis or in PLC assay buffer for measurements of enzymatic activity.

Immunoblot Analysis. Samples were separated by SDS/PAGE on 7.5% gels, transferred to nitrocellulose membranes (Immobilon-P, Millipore), and blocked for 2 h with 2% (wt/vol) gelatin in 150 mM NaCl/50 mM Tris·HCl, pH 8.0/0.1% NaN_3/0.05% Tween-20 (TBS/Tw). Membranes were treated with anti-PLC-γ antibodies (5 μg/ml in TBS/Tw for 2 h at 37°C), affinity purified from anti-peptide sera on peptide-coupled agarose bead columns and eluted with a low-pH glycine buffer. Similar conditions were used for anti-Tyr(*P*) immunoblots except that anti-Tyr(*P*) antibodies, prepared as described (28) and affinity-purified from hyperimmune rabbit antisera on Tyr(*P*)-coupled agarose bead columns, were used at 1 μg/ml for 4 h at room temperature. Whole cell lysates for generating profiles of total tyrosine-phosphorylated proteins (see Fig. 4) were prepared by addition of an equal volume of the 2× lysis buffer described above, and postnuclear supernatants were diluted with 5× SDS sample buffer prior to SDS/PAGE.

Nitrocellulose membranes were washed six times with TBS/Tw, incubated with ^{125}I-labeled protein A (ICN; 1 μCi/ml; 1 Ci = 37 GBq) for 60 min at room temperature, and washed an additional five or six times in TBS/Tw, and autoradiography was performed with an intensifying screen. In some experiments, the blots were incubated with an alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Zymed Laboratories) and bands were visualized by an enzymatic color reaction.

PLC Assay. PLC was assayed by quantitating IP_3 production as described (14). Briefly, substrate was prepared by drying a mixture of [^3H]phosphatidylinositol 4,5-bisphosphate ([^3H]PIP_2; DuPont/NEN) and unlabeled PIP_2 (Sigma) as a carrier in a SpeedVac concentrator and redissolving the dried material at 1 mM (specific activity, 10 Ci/mol) in 2.5% (wt/vol) octyl glucoside. Substrate (10 μl) was added to anti-PLC immunoprecipitates resuspended in a PLC assay buffer (20 mM Na_2PO_4, pH 6.8/70 mM KCl/0.125% octyl glucoside/0.8 mM EGTA/0.8 mM $CaCl_2$). Duplicate reactions were incubated at 37°C for 30 min and stopped by adding 100 μl of 1% bovine serum albumin and 500 μl of 10% (wt/vol) trichloroacetic acid. After centrifugation at 16,000 × g for 10 min, 500 μl of supernatant was removed, and radioactivity was measured by liquid scintillation spectroscopy. Radioactivity in the supernatant of negative controls, to which no PLC immunoprecipitate was added (generally 0.2% of input counts), was subtracted from all experimental groups.

RESULTS

Rabbit antisera directed against C-terminal peptides of PLC-γ1 or PLC-γ2 were characterized by immunoprecipitating whole murine spleen cell extracts and testing the precipitates in an enzymatic assay and in immunoblots. The results, shown in Fig. 1, demonstrate that both antisera, but not normal rabbit IgG (or preimmune sera, data not shown), immunoprecipitated PLC enzymatic activity, which was detected by the production of IP_3, and that this immunoprecipitation was specific as it was blocked by including an excess of free homologous peptide in the immunoprecipitation reaction mixture (Fig. 1*A*). Addition of a heterologous peptide, corresponding to the opposite PLC-γ sequence, did not block the ability of the antibody to precipitate the enzymatic activity (data not shown). When the same precipitates were analyzed on an immunoblot with affinity-purified anti-peptide antibodies, a major band corresponding in size to PLC-γ (i.e., 145–150 kDa) was detected in each case only by the homologous antibody (Fig. 1 *C* and *D*). Fig. 1*B* shows an immunoblot of a murine spleen cell extract probed with anti-PLC-γ1 (lane a), anti-PLC-γ2 (lane c), or a mixture of the

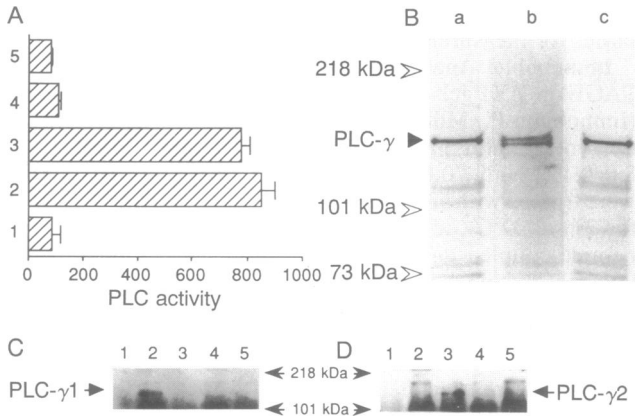

FIG. 1. Characterization of anti-PLC-γ1 and anti-PLC-γ2 antisera. (*A*, *C*, and *D*) Murine splenocytes (30×10^6) were lysed in TN1 buffer and the lysates were immunoprecipitated with normal rabbit immunoglobulin (10 μg; lane 1), anti-PLC-γ1 antiserum (lanes 2 and 4; 5 μl), or anti-PLC-γ2 antiserum (lanes 3 and 5; 5 μl) in the absence (lanes 1–3) or presence (lanes 4 and 5) of 100 μg of homologous immunizing peptide. The immunoprecipitates were washed, resuspended in PLC assay buffer, and assayed for PLC activity by incubation at 37°C for 30 min with [^{3}H]PIP_2 (*A*). Bars indicate the average amount of [^{3}H]IP_3 generated in duplicate samples. PLC activity is expressed as pmol per 30 min per 20×10^6 cells. Parallel immunoprecipitates, separated by SDS/PAGE and transferred to nitrocellulose, were analyzed on an immunoblot with affinity-purified anti-PLC-γ1 (*C*) or anti-PLC-γ2 (*D*) antibodies (5 μg/ml). Immunoblots were exposed to XAR-5 film for 24 h at −70°C. (*B*) Murine spleen cell lysates (15×10^6 cell equivalents, lysed in TN1 buffer and diluted with 5× SDS sample buffer) were separated by SDS/PAGE in a 7.5% gel and analyzed on an immunoblot with anti-PLC-γ1 (lane a), anti-PLC-γ2 (lane c), or a mixture of both antibodies (lane b) and developed by alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin. The position of PLC-γ is indicated (other nonspecific bands were also present in immunoblots lacking the primary anti-PLC-γ antibodies; data not shown).

two antibodies (lane b) to illustrate (*i*) that each antibody reacts with a distinct molecular species and (*ii*) that the two isoforms that differ by ≈5 kDa can be resolved (20, 22, 23, 27). These results establish the specificity of the anti-peptide antibodies for PLC-γ1 or PLC-γ2, respectively, and virtually exclude any possibility of cross-reactivity between them.

Next, the distribution of the two PLC-γ isoforms was examined in B or T cells by immunoblot analysis of cytosolic extracts from freshly prepared murine splenic T and B cells, two T-cell lines, Jurkat (human) and DO11.10 (murine), and two B-cell lines, Raji (human) and WEHI-231 (murine). The results (Fig. 2) reveal an asymmetrical distribution of the two PLC-γ isoforms: T cells predominantly expressed PLC-γ1, whereas B cells displayed mostly PLC-γ2. Purified human peripheral blood-derived T and B cells showed a expression

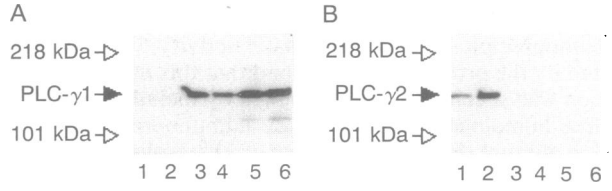

FIG. 2. Expression pattern of PLC-γ1 and PLC-γ2. T (Jurkat and DO11.10) or B (Raji and WEHI-231) cell lines and purified murine splenic B or T cells were lysed by sonication. Cytosolic fractions (250 μg) were separated by SDS/PAGE in 7.5% gels and analyzed on an immunoblot with affinity-purified anti-PLC-γ1 (*A*) or anti-PLC-γ2 (*B*) antibodies. Autoradiograms were obtained by a 2-h exposure to XRP film (room temperature). Lanes: 1, B cells; 2, WEHI-231; 3, Raji; 4, T cells; 5, DO11.10; 6, Jurkat.

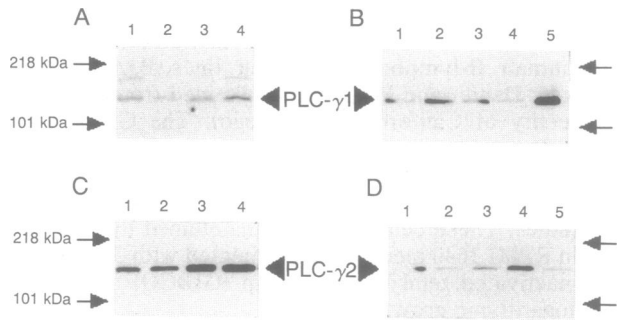

FIG. 3. Expression pattern of PLC-γ1 and PLC-γ2 in human B-lymphoblastoid (*B* and *D*) and murine B lymphoma (*A* and *C*) lines. Cytosolic fractions (250 μg) of the indicated cell lines separated by SDS/PAGE in 7.5% gels were analyzed on an immunoblot with anti-PLC-γ1 (*A* and *B*) or anti-PLC-γ2 (*C* and *D*). Autoradiograms were obtained by exposure to XAR-5 film for 2 h at room temperature. (*A* and *C*) Lanes: 1, CH27; 2, CH31; 3, CH33; 4, CH12. (*B* and *D*) Lanes: 1, BJAB; 2, DHL9; 3, Loucke; 4, Daudi; 5, WIL2.

pattern similar to that of their murine splenic counterparts (data not shown). This expression pattern in cytosolic extracts was representative of that found in total cell extracts. The low-level expression of PLC-γ2 in resting T cells is in agreement with a recent report (29).

Raji, a human B-lymphoblastoid line that, like the T cells tested, expressed predominantly PLC-γ1, was an exception to this pattern of PLC-γ1/2 expression seen in *ex vivo* B and T cells (Fig. 2). To determine whether high PLC-γ1 expression is a general feature of human B-lymphoblastoid cell lines (as opposed to murine B lymphomas), we examined the levels of the two PLC-γ isoforms in additional human and murine B-cell tumor lines. Among the human B-cell lines, only Daudi preferentially expressed PLC-γ2 (Fig. 3 *B* and *D*) whereas the other human B-cell lines tested preferentially expressed PLC-γ1 (WIL2 and DHL9) or expressed the two isoforms almost equally (Loucke and BJAB). In contrast, and in agreement with the expression pattern of *ex vivo* B cells, the murine CH series of lymphomas preferentially expressed PLC-γ2 (Fig. 3 *A* and *C*).

The effect of sIg receptor triggering on tyrosine phosphorylation in purified resting B cells was examined by immunoblot analysis of total cellular extracts by using polyclonal anti-Tyr(*P*) antibodies. By 30 sec after stimulation with goat anti-mouse immunoglobulin antibodies, there was a marked increase in the Tyr(*P*) content of several protein bands and *de*

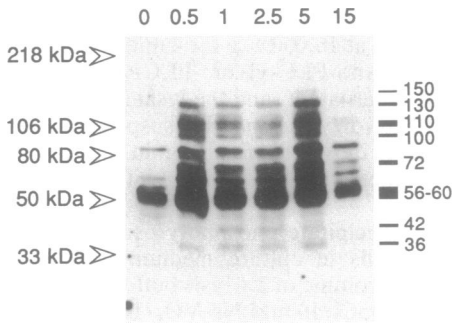

FIG. 4. Profile and kinetics of induction of tyrosine-phosphorylated proteins in anti-immunoglobulin-stimulated murine B cells. Purified splenic B cells (30×10^6) were incubated for the indicated times (in min at the top of the lanes) at 37°C with anti-mouse immunoglobulin (10 μg/ml) before extraction with an equal volume of 2× Nonidet P-40 lysis buffer containing phosphatase inhibitors. Postnuclear supernatants were diluted with 5× SDS sample buffer, separated by SDS/PAGE in 7.5% gels, and analyzed on an immunoblot with anti-Tyr(*P*) antibodies. Immunoblots were exposed to XAR-5 film for 3 days (−70°C).

novo induction of tyrosine phosphorylation of others (Fig. 4), in agreement with other studies (8, 30, 31). Peak phosphorylation of distinct substrates was seen at different times and declined, for all substrates, by 15 min. The tyrosine-phosphorylated proteins included PTK substrates at 36, 42, 56–60 (two bands), 72, 100, 110, 130, and 150 kDa. Tyrosine phosphorylation of the minor ≈150-kDa substrate was detectable within 30 sec of stimulation and was maintained for at least 15 min. Since the size of this substrate is reminiscent of PLC-γ, it may represent one of the PLC-γ isoforms.

This possibility was directly examined by testing anti-Tyr(*P*) immunoprecipitates from resting or activated murine splenic B cells for the presence of PLC-γ1 or PLC-γ2 isoforms by immunoblot analyses with the respective, isoform-specific affinity-purified antibodies. The results (Fig. 5) indicate that both antibodies reacted with Tyr(*P*)-containing proteins of the expected size of ≈150 kDa. More important, the anti-Tyr(*P*) immunoprecipitates from activated, but not resting, B cells (Fig. 5 *B* and *D*) reacted predominantly with the PLC-γ2-specific antibodies (although some reactivity was also detected with the anti-PLC-γ1 antibodies), a finding consistent with the cellular distribution of these isoforms (Figs. 2 and 3). This result shows that the 150-kDa PTK substrate detected in activated B cells (Fig. 4) represents primarily, if not exclusively, tyrosine-phosphorylated PLC-γ2. Conversely, the Tyr(*P*)-containing protein fraction from anti-CD3-activated, but not unstimulated, murine splenic T cells reacted strongly with the anti-PLC-γ1 antibody (Fig. 5 *A* and *C*), confirming reports (16–19) that document the phosphorylation of the PLC-γ1 isoform on tyrosine after ligation of the T-cell receptor–CD3 complex (16–19). The specificity of the anti-Tyr(*P*) immunoprecipitation is indicated by the finding that immunoprecipitation was blocked by a competing hapten, pNPP (Fig. 5, lanes 3).

To confirm this finding, a reverse protocol was used in which anti-PLC-γ2 immunoprecipitates were prepared from extracts of resting or anti-immunoglobulin-stimulated B cells and, after SDS/PAGE separation, the precipitates were analyzed on an immunoblot with anti-Tyr(*P*) antibodies. The results demonstrated that only the activated cells displayed tyrosine-phosphorylated PLC-γ2 (Fig. 6*A*). Parallel immunoblots with anti-PLC-γ2 antibodies confirmed that immunoprecipitates from resting and stimulated B cells contained similar amounts of PLC-γ2 (Fig. 6*B*).

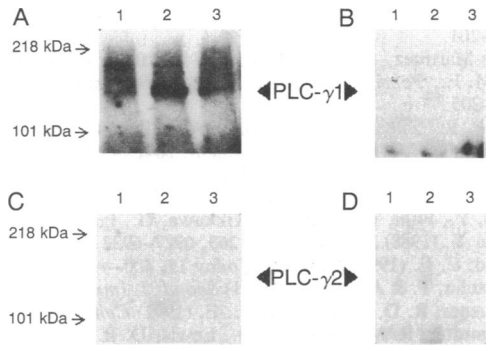

FIG. 5. Activation-induced tyrosine phosphorylation of PLC-γ1 and PLC-γ2 in murine T (*A* and *C*) and B (*B* and *D*) cells. T or B cells (40×10^6) were incubated (2 min, 37°C) with no stimulus (lane 1) or with anti-receptor antibodies (10 μg/ml; lanes 2 and 3). The cells were lysed and immunoprecipitated with an anti-Tyr(*P*) monoclonal antibody (4G10; 5 μg) in the absence (lanes 1 and 2) or presence (lane 3) of the competing hapten pNPP (10 mM). Immunoprecipitated material was eluted with pNPP, separated by SDS/PAGE in a 7.5% gel, and analyzed on an immunoblot with affinity-purified anti-PLC-γ1 (*A* and *B*) or anti-PLC-γ2 (*C* and *D*) antibodies. The T- or B-cell immunoblots were exposed to XAR-5 film at −70°C for 18 or 6 h, respectively.

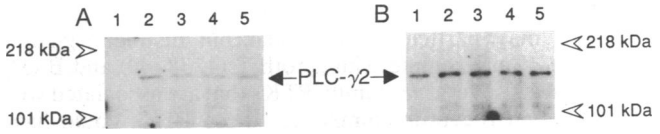

FIG. 6. Tyrosine phosphorylation of PLC-γ2 in receptor-stimulated B cells. PLC-γ2 immunoprecipitates were prepared from extracts (25×10^6 cell equivalents per group) of untreated B cells (lanes 1) or cells stimulated with anti-immunoglobulin (40 μg/ml) for 0.5 (lane 2), 1 (lane 3), 2.5 (lane 4), or 5 (lane 5) min. Electrophoresed immunoprecipitates were transferred to nitrocellulose membranes and analyzed on an immunoblot with anti-Tyr(*P*) (*A*) or anti-PLC-γ2 (*B*) antibodies. Immunoblots were exposed to XAR-5 film for 18 h. Tyrosine phosphorylation of PLC-γ2 in resting B cells (*A*) (lane 1) was not detected even after a 3-day exposure (data not shown).

DISCUSSION

Similar to what is observed in T cells (5, 32), in B cells sIg triggering induces rapid tyrosine phosphorylation of distinct substrates, including two proteins that are physically associated with sIg and are thought to serve as primary signal transducers, namely, MB-1 or Ig-α and B29 or Ig-β (ref. 30; for review, see refs. 33 and 34). Furthermore, as with the signaling cascades initiated by tyrosine kinase receptor (12, 35) or T-cell receptor–CD3 complex (4–6), in B cells PLC stimulation also depends on the prior activation of an unknown PTK, since it can be blocked by several PTK inhibitors (7–10). A recent report indicated that PLC-γ1, which becomes phosphorylated on tyrosine in response to PTK receptor (11–14) or T-cell receptor–CD3 (16–19) crosslinking, is also an early PTK substrate in a B-lymphoblastoid cell line activated by sIg triggering (9). This finding implicates the phosphorylation of this particular PLC isoform as a primary signaling event leading to its activation and thereby promoting the production of inositol phospholipid-derived second messengers in B cells.

The results presented herein identify PLC-γ2 as another early PTK substrate in freshly isolated splenic B cells and, moreover, indicate that, although PLC-γ1 is expressed in B cells, PLC-γ2 is usually the predominant isoform in these cells. PLC-γ2 transcripts were reported to have a ubiquitous distribution (23), with high levels found in spleen (23) and B-lymphoblastoid cells (22). These findings strongly suggest that the PTK-mediated phosphorylation of PLC-γ2 may constitute a critical and early event in the stimulation of downstream signaling processes during B-cell activation.

The effects of tyrosine phosphorylation on the enzymatic activity of PLC-γ1 or -γ2 in lymphocytes is unknown. In this regard, two mechanisms have been proposed for the EGF receptor-coupled PLC-γ1. One study (15) provided evidence for increased PLC enzymatic activity as a direct consequence of its *in vivo* or *in vitro* tyrosine phosphorylation by the activated EGF receptor kinase. Others (36) reported that profilin, a cytoskeletal actin- and PIP_2-binding protein, inhibits the hydrolysis of PIP_2 by unphosphorylated, but not by tyrosine-phosphorylated, PLC-γ1. In preliminary studies, we have not been able to detect a difference in enzymatic activity between unphosphorylated or tyrosine phosphorylated (either *in vivo* by anti-CD3 stimulation or *in vitro* by coincubation with immunoprecipitated $p56^{lck}$) PLC-γ1 isolated from Jurkat cells.

The expression of two PLC-γ isoforms in B (as well as T) cells and our finding that both isoforms become phosphorylated on tyrosine in response to antigen receptor triggering raise an intriguing question—what, if any, is the relative contribution of each PLC-γ isoform to signaling events in lymphocytes. The 50.2% amino acid homology between these two isoforms (23) is comparable to, or lower than, those among members of other families of signaling proteins [e.g.,

the protein kinase C family (37)]. Thus, the γ1 and γ2 isoforms may participate in similar but distinct signaling pathways. For example, since both T (4, 38–40) and B (31) cells express several *src* family PTKs that are associated with their antigen receptor complexes, these PTKs could have differential affinities for the two PLC-γ isoforms. If so, the PLC-γ isoform recruited by the cellular signaling machinery could be determined by the type of receptor-coupled tyrosine kinase that becomes activated [e.g., the *lck*- or *fyn*-encoded PTKs in T cells (38–40) and the *lyn*-, *blk*-, *fyn*-, or *lck*-encoded kinases in B cells (31, 41–43)]. The differential PTK activation in B vs. T cells is consistent with the finding that these two cell types display distinct Tyr(*P*)-containing proteins in *in vitro* PTK assays (44).

One of the consequences of the activation of receptor, and nonreceptor, tyrosine kinases is the formation of complexes that include, in addition to the receptor and the associated PTK, other tyrosine phosphorylated components, usually enzymes that regulate signal transduction events—i.e., PLC-γ, the GTPase-activating protein (GAP), phosphatidylinositol 3-kinase (PI3-K), and perhaps others (for review, see ref. 45). These interactions are thought to be mediated by the binding of the regulatory SH2 domains (24), shared by *src* family PTKs, PLC-γ, GAP, and PI3-K, to Tyr(*P*)-containing proteins (45). SH2 domains are known to specifically recognize and bind to short sequence motifs surrounding Tyr(*P*) (24, 25). Sequence differences in SH2 domains were found to have a profound influence on the range of affinities of these domains for distinct Tyr(*P*)-containing proteins (24). Since the amino acid homology between the respective SH2 domains of PLC-γ1 and PLC-γ2 is ≈60% (23, 24), differences in this domain may impart different binding specificities for other components of the signaling complex. This, in turn, could provide a potential mechanism for fine-tuning the signaling cascade and possibly account for subtle but critical differences in signaling events between B cells in distinct maturation stages or in response to triggering of different B-cell activation receptors. Additional studies are necessary to determine whether, indeed, the activation of each PLC-γ isoform is associated with a distinct PTK and/or maturational stage and whether activation of both PLC-γ1 and PLC-γ2 is required for the complete spectrum of lymphocyte activation.

Note. Findings similar to those reported here were recently obtained by DeFranco and colleagues (46) and S. G. Rhee and colleagues (personal communication).

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