One of the earliest detectable events following engagement of lymphocyte antigen and Fc receptors is activation of the phospholipase C isozyme γ (PLCγ) (reviewed in Refs. 1–5). Activated PLCγ acts to hydrolyze the membrane lipid phosphatidylinositol 4,5-biphosphate, resulting in the generation of the second messengers diacylglycerol (DAG) and inositol 3,4,5-trisphosphate. Soluble inositol 3,4,5-trisphosphate diffuses through the cytoplasm to bind to and gate inositol 3,4,5-trisphosphate receptor ion channels expressed on intracellular calcium store membranes, thereby initiating a general increase in cytosolic Ca2+⁴, which is a critical component of antigen and Fc receptor cell activation signals (reviewed in Refs. 6–9 and by others). In contrast, DAG remains associated with cellular membranes and serves as an essential cofactor in the assembly of a functional “signalseme” in the subplasmalemmal region beneath engaged receptors. Whereas a large body of evidence from studies of PLCγ and PKC signaling indicates that the DAG-dependent component of antigen and Fc receptor signals influence diverse aspects of immune cell biology (2, 10–16), understanding the molecular mechanisms through which DAG acts requires a detailed knowledge of the direct targets of DAG and how they are influenced by the production of DAG following receptor engagement.

A novel serine-threonine kinase with two potential DAG-binding C1 domains has recently been cloned and designated PKCγ. But its activation mechanism and the identity of cell surface receptors that utilize its signaling capacity remain uncharacterized. As our initial analyses indicated that PKCγ is abundantly expressed in human B-cells, we investigated whether PKCγ was involved in signals mediated by the B-cell antigen receptor (BCR). Utilizing a combination of biochemical, genetic, and pharmacologic approaches, here we show that PKCγ is a downstream effector for BCR-mediated DAG production and that its activation mechanism probably involves DAG-mediated membrane trans-location followed by trans-phosphorylation of two conserved residues within its “activation loop” by novel PKC enzymes.

**MATERIALS AND METHODS**

**Reagents**—Constitutively active PKC mutants were obtained from David Rawlings (Department of Pediatrics, University of Washington) and Peter Parker (Protein Phosphorylation Laboratory, Cancer Research UK). An M2 FLAG monocalonal antibody covalently coupled to agarose beads was from Sigma. A polyclonal antibody recognizing the carboxyl-terminal 16 residues of human and chicken PKCγ (HIFMAP-NPDDMEEDP) was generated by standard immunological techniques and affinity-purified against the immunizing peptide. Monoclonal PKC antibodies and a V5 epitope antibody were from Transduction Laboratories and Invergent, respectively. A polyclonal antibody that specifically recognizes a phosphorylated serine 735 residue in the activation loop of PKCγ was obtained from Doreen Cantrell (Lymphocyte Activation Laboratory, Cancer Research UK). This antibody is directed against a phosphorylated epitope that is conserved in all three members of the PKD kinase family. Fnabγ⁵ fragments of anti-human IgM were from Jackson Laboratories. A monoclonal-stimulating antibody recognizing the chicken BCR (M4) was purified from hybridoma supernatant using standard procedures. The classical/novel PKC inhibitor Ro-31-8205 was from Calbiochem. All of the other reagents were from standard suppliers or as indicated in the text.

**Cell Culture and Transient Transfections**—Human Raji and Ramos and mouse A20 B-cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 units/ml penicillin/streptomycin, 2 mM glutamine, and 50 μg 2-mercaptoethanol. Chicken DT40 cells were cultured in RPMI 1640 medium in the presence of 10% fetal bovine serum, 1% chicken serum, 10 units/ml penicillin/streptomycin, and 2 mM glutamine. HEK 293 endothelial cells expressing the TET Repressor protein were maintained in DMEM supplemented with 10% fetal bovine serum, 10 units/ml penicillin/streptomycin, 2 mM glutamine, and 5 μg/ml blasticidin.

Transient transfection of HEK 293 cells was carried out using a
Beckman Gene-Pulser electroporation apparatus. 1 x 10^7 cells/0.5 ml serum-free media were pulsed in 0.4-cm cuvettes with 10 μl of plasmid DNA at 330 volts and 1000 microfarads before diluting with 10 ml of complete medium. Cells were allowed to recover overnight before experimental use. For transfection of A20 B-cells, the electroporation conditions used were 250 volts and 950 microfarads.

cDNA Cloning and Mutagenesis—The PKCα coding sequence was PCR-amplified from a human brain cDNA library (Clontech) using 5′-ACGTGGCGGCGCGCTTGCTGGAAATATCCCTCCCTACGCCCC-3′ forward and 5′-ACGTCTAGATTTAGATCTTCTCATTATCATTGGATTAG-3′ reverse primers. The PCR fragment was subcloned NotI/XhoI sites (underlined) into a modified pcDNA4/TO/TOX doxycycline-inducible mammalian expression vector. This modified vector contains an in-frame FLAG epitope coding sequence, resulting in the expression of an amino-terminally tagged FLAG-PKCα protein. A similar method was used to construct the pcDNA4/TO FLAG-PKD vector. To generate the pcDNA4/TO GFP-PKCα construct, the coding sequence for GFP was PCR-amplified with 5′-HindIII and 3′-NotI restriction sites. This PCR fragment was then cloned into the pcDNA4/TO vector, and the PKCα coding sequence was cloned in-frame COOH-terminal to the GFP sequence using NotI and XhoI restriction sites. PKCα, PKCε, and PKCo mutants were cloned into a modified pcDNA5/TO vector with an in-frame amino-terminal V5 epitope tag.

Site-specific mutations within the catalytic domain of PKCα, resulting in single or double amino acid substitutions, were generated by overlap PCR using wild-type PKCα as the template. Mutants were generated using the above primers together with internal forward and reverse primers complementary to each other and containing specific nucleotide substitutions as required. Primers (forward sequence only shown) containing the desired mutation(s) (underlined) were as follows: PKCα-R685N, 5′-GGGAGGATGTTGATTAGTAAGTATTAGATAG-3′; PKCα-S731A/S735A, 5′-CATGGTTGGAAAGACATCAGGAGGACGCTGTGTTAGAAGACTCCGACGC-3′; and PKCα-S731ES735F, 5′-CATTGTGGAAAGAGATCCAGGAGGACGTGGTTAGAAGACTCCAGC-3′.

Following the second PCR reaction, the amplified cDNAs were subcloned (NotI/XhoI) into the modified pcDNA4/TO expression vector. Constructs were sequenced using an Applied Biosystems automated DNA sequencer before they were used in transient expression experiments. Protein expression was induced by treating cells with 5 μg/ml doxycycline for 24 h.

Cell Lysis and Immunoprecipitation—Cells were lysed in a buffer containing 50 mM Tris-HCl, pH 7.4, 2 mM EDTA, 2 mM E-DTA, 1 mM dithiothreitol, protease inhibitors, 1 mM AEBSF, and 1% Triton X-100. Exogenously expressed PKCα was immunoprecipitated with either a FLAG monoclonal antibody or with an affinity-purified PKCα antibody recovered with protein G-Sepharose beads and resuspended in 2X SDS-PAGE reducing sample buffer (1 x Tris-HCl, pH 6.8, 0.1 mM Na3VO4, 6% SDS, 0.5% EDTA, 4% 2-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue). Immunoprecipitates were separated by 8% SDS-PAGE, transferred to polyvinylidene difluoride membrane, and Western blotted with appropriate antibodies.

Cell Fractionation—DT40 B-cells were washed in ice-cold phosphate-buffered saline and resuspended in 1 ml of ice-cold fractionation buffer (10 mM Tris, pH 7.4, 2 mM EDTA, 2 mM E-DTA, 1 mM dithiothreitol, protease inhibitors, 1 mM AEBSF). Cells were lysed by homogenization, and unbroken cells/nuclear debris were removed by centrifugation at 800 x g for 10 min at 4°C. The supernatant was subjected to high speed ultracentrifugation at 100,000 x g for 30 min at 4°C, resulting in a soluble cytosolic fraction and an insoluble membrane pellet. The membrane pellet was solubilized in 1 ml of fractionation buffer containing 1% Triton-X for 20 min at 4°C before insoluble material was removed by centrifugation at 20,000 x g for 10 min at 4°C. PKCα was then immunoprecipitated from both cytosolic and membrane fractions and analyzed by Western blotting.

In Vitro Kinase Assays—Immunocomplexes were washed twice in lysis buffer (described above) and once in kinase buffer (50 mM Tris-HCl, pH 7.4, 10 mM MgCl2). PKCα autophosphorylation was determined by incubating immunocomplexes with 20 μl of kinase buffer containing 100 μM [γ-32P]ATP at 30°C for 10 min. Reactions were terminated by the addition of 2X SDS-PAGE sample buffer, and the samples were analyzed by 8% SDS-PAGE and autoradiography.

Microscopy—For immunofluorescent localization of endogenous PKCα, DT40 B-cells were resuspended in phosphate-buffered saline and allowed to attach to polylysine-coated glass bottom dishes (MatTek Inc.). The cells were then left untreated or treated with 50 ng/ml PMA for 10 min before fixing with 4% paraformaldehyde for 15 min at room temperature. The cells were then permeabilized with a 0.5% saponin buffer and sequential incubation with primary (anti-PKCα, 1 μg/ml) and secondary antibodies (anti-rabbit Alexa Fluor 488, 1:3000 dilution) for 20 min. After each step, the cells were washed three times in phosphate-buffered saline containing 1% bovine serum albumin. For GFP visualization, A20 B-cells transiently expressing GFP-PKCα were
plated on polylysine-coated glass bottom dishes in phosphate-buffered saline and allowed to adhere before stimulation with 50 ng/ml PMA. The cells were excited with a 495-nm wavelength light, and emitted fluorescence was measured by an in vitro kinase assay and anti-FLAG immunoblotting. SS/EE, S731E/S735E; SS/AA, S731A/S735A.

**RESULTS**

Although the PKC\(\beta\) gene and transcripts have been described previously (17), there is no present literature regarding its regulation or receptor systems that utilize PKC\(\beta\) as a signaling mechanism. However, PKC\(\beta\) has two putative C1 domains. These domains (see schematic in Fig. 1A) of \(-50\) residues are thought to bind the lipid second messenger diacylglycerol, suggesting that PKC\(\beta\) might participate in a diacylglycerol-mediated signaling pathway. As the tumor-promoting phorbol esters serve as pharmacological substitutes for DAG and mimic many aspects of the biological activity of DAG, we used one of them, PMA, to evaluate the potential involvement of PKC\(\beta\) in DAG signaling by imaging the subcellular localization of PKC\(\beta\) in cells that had been left untreated or treated with PMA (Fig. 1B). As can be seen, both endogenous PKC\(\beta\) (imaged in fixed and antibody-stained cells, *top panels*) and GFP-tagged PKC\(\beta\) (imaged in live cells, *bottom panels*) are substantially redistributed from the cytosol to the plasma membrane in response to PMA treatment, consistent with DAG serving as a membrane recruitment signal for PKC\(\beta\).

Recruitment to the plasma membrane often serves as a means for activation of protein kinases, and PMA has previously been shown to induce both membrane recruitment and activation of PKD1, one of the closest homologues of PKC\(\beta\) (18, 19). To understand how membrane recruitment affects PKC\(\beta\) function, we produced a FLAG-tagged PKC\(\beta\) construct as a backbone for mutational analysis of PKC\(\beta\), confirming its expression after transfection of HEK 293 cells (Fig. 1C, *left panel*). The treatment of cells expressing FLAG-PKC\(\beta\) with PMA induced easily detectable enzymatic activation as measured by an in vitro kinase assay (Fig. 1C, *top panel*). For many serine-threonine kinases, phosphorylation within the activation loop serves as a marker for enzymatic activation. The putative activation loop residues of PKC\(\beta\) were mutated to alanine (PKC\(\beta\)-AA), and analyzed by anti-PKC\(\beta\) immunoblotting.

In addition, tyrosine phosphorylation at serine 735 is necessary and sufficient for PKC\(\beta\) activation. HEK 293 cells were transiently transfected with pcDNA4/TO vectors driving the expression of WT or PKC\(\beta\)-AA mutant and analyzed by anti-FLAG immunoprecipitation followed by either an in vitro kinases assay (measuring PKC\(\beta\) autophosphorylation) or by anti-pS735 and anti-FLAG immunoblotting. Note that the anti-pS735 immunoreactivity of stimulated PKC\(\beta\)-AA is because of a trans-phosphorylation event. PKC\(\beta\)-AA mutant demonstrates that a significant fraction of anti-PKC\(\beta\) immunoreactivity of stimulated PKC\(\beta\) is because of a trans-phosphorylation event.

**Fig. 2.** Activation of PKC\(\beta\) is via trans-phosphorylation of serines 731 and 735. A, PMA induces trans-phosphorylation of serine 735 of PKC\(\beta\). HEK 293 cells were transiently transfected with pcDNA4/TO vectors driving the expression of WT or PKC\(\beta\)-AA mutant. The cells were left untreated or treated with PMA, and expressed proteins were analyzed by anti-FLAG immunoprecipitation followed by either in vitro kinases assays (measuring PKC\(\beta\) autophosphorylation) or by anti-pS735 and anti-FLAG immunoblotting. B, phosphorylation of activation loop serines is necessary and sufficient for PKC\(\beta\) activation. HEK 293 cells were transiently transfected with pcDNA4/TO vectors driving the expression of the indicated constructs, treated with doxycycline to induce protein expression, and treated or not treated with PMA. Cells were lysed, and expressed proteins were analyzed by anti-FLAG immunoprecipitation followed by in vitro kinase assay and anti-FLAG immunoblotting. SS/EE, S731E/S735E; SS/AA, S731A/S735A.

**Fig. 3.** B-cell receptor engagement induces activation of endogenous PKC\(\beta\) in chicken and human B-cell lines. A, chicken DT40 B-cells were treated or not treated with anti-chicken IgM, lysed at the indicated times, immunoprecipitated with anti-PKC\(\beta\), and analyzed by anti-pS735 and PKC\(\beta\) immunoblotting. B, human Raji and Ramos B-cell lines were treated or not treated with Fab\(\lambda\)\(\gamma\) fragments of anti-human IgG, lysed at the indicated times, immunoprecipitated with anti-PKC\(\beta\), and analyzed by anti-pS735 and PKC\(\beta\) immunoblotting. C, chicken DT40 B-cells were left untreated (\(-\)) or were treated with either anti-chicken IgM (BCR) or with 50 ng/ml PMA for 3 min as indicated. Cytosolic and membrane fractions were prepared as described under “Experimental Procedures,” and PKC\(\beta\) activity was analyzed by anti-pS735 and PKC\(\beta\) immunoblotting.
Fig. 4. BCR-activation of PKC\(\beta\) is dependent on PLC\(\gamma\) and DAG. A, all panels, wild-type and mutant DT40 B-cell lines lacking the indicated signaling molecules were analyzed for BCR-mediated activation of PKC\(\beta\). Cells were left unstimulated (–) or were stimulated with either 50 ng/ml PMA for 10 min (P) or with 10 \(\mu\)g/ml anti-chicken IgM (B) for the indicated times. Cells were lysed, and the endogenous PKC\(\beta\) was immunoprecipitated (IP). Samples were analyzed by SDS-PAGE and Western blotting using the indicated antibodies. B, wild-type DT40 B-cells were left untreated or were treated with 3.5 \(\mu\)M of the competitive DAG antagonist calphostin C (Cal.C) prior to PMA (P) or BCR stimulation (times are indicated) and analysis of PKC\(\beta\) activity as in A. DMSO, Me\(\text{SO}\).

right panel, middle blot). We further utilized anti-pS735 to examine at the activation of endogenous PKC\(\beta\). As our initial analyses (data not shown) had indicated the presence of abundant PKC\(\beta\) in several B-cell lines, we evaluated whether PMA treatment could activate endogenous PKC\(\beta\) in chicken DT40 B-cells and the Raji and Ramos human B-cell lines (Fig. 1D). As can be seen, PMA treatment strongly induced anti-pS735 immunoreactivity of anti-PKC\(\beta\) immunoprecipitates, indicating that endogenous PKC\(\beta\) is activated by PMA in the same manner as the recombinant FLAG-PKC\(\beta\).

To further investigate the role of pS735 phosphorylation in PKC\(\beta\) activation, we constructed a kinase-deficient mutant of PKC\(\beta\) on the FLAG-PKC\(\beta\) backbone via the mutation of a conserved lysine residue within the putative ATP-binding cassette of the kinase domain (FLAG-PKC\(\beta\)-KN). This mutant had no detectable kinase activity as assessed by an \textit{in vitro} kinase assay (Fig. 2A, top panel). However, a comparison of the anti-pS735 immunoreactivity induced by PMA treatment of wild-type FLAG-PKC\(\beta\) with that of the FLAG-PKC\(\beta\)-KN mutant demonstrated essentially intact phosphorylation of this site (Fig. 2A), indicating that this site is trans-phosphorylated by an upstream kinase in intact cells. In some proteins whose function is modulated by phosphorylation at serine or threonine residues, the replacement of the regulatory serine or threonine residues with negatively charged glutamate or aspartate residues induces the protein to act as if it is constitutively phosphorylated at the mutated sites. Conversely, the replacement with alanine produces a protein whose function can no longer be modulated by phosphorylation. Therefore, we further analyzed the activation mechanism PKC\(\beta\) by producing mutants on the FLAG-PKC\(\beta\) backbone with potentially activating mutations at positions 731 and 735 (serine to glutamate, S731E/S735E) or deactivating mutations (serine to alanine at the same positions, S731A/S735A) and analyzing their responses to PMA treatment (Fig. 2B). Although the S731A/S735A mutant is no longer activated by PMA, the S731E/S735E mutant shows high constitutive activity that is PMA-independent, indicating that phosphorylation at serines 731 and 735 is both necessary and sufficient for PKC\(\beta\) activation. When viewed in conjunction with the redistribution to the plasma membrane induced by PMA treatment, a compelling model for PKC\(\beta\) activation can be constructed in which its activation occurs as the result of its membrane trans-location and subsequent trans-phosphorylation by an upstream PMA-regulated protein kinase on serine 731/735.

Based on its abundance in B-cells, its activation by the DAG mimic PMA and the well established role of PLC\(\gamma\) in B-cell antigen receptor signal transduction, we next investigated whether PKC\(\beta\) was activated after B-cell antigen receptor engagement. The engagement of the BCR on either chicken DT40 B-cells (Fig. 3A) or human Raji and Ramos human B-cells (Fig. 3B) induced strong and rapid activation of PKC\(\beta\) as assessed by the induction of anti-pS735 immunoreactivity. To address the question of where activated PKC\(\beta\) is localized within the cell, fractionation experiments were preformed in both PMA and BCR-stimulated B cells. As illustrated in Fig. 3C, PKC\(\beta\) translocates from the cytosol to the membrane fraction in response to PMA treatment (Fig. 3C, lower panels), consistent with the observation that PMA induces the trans-location of GFP-PKC\(\beta\) from the cytosol to the plasma membrane (see Fig. 1B). In addition, pS735 immunoblotting reveals that activated PKC\(\beta\) is restricted to the membrane fraction of PMA-treated B-cells (Fig. 3C, upper panels). In contrast, a portion of PKC\(\beta\) translocates to the membrane fraction of BCR-stimulated B-cells, and activated PKC\(\beta\) is detectable in both the cytosolic and membrane fractions (Fig. 3C). Kinetic analysis indicates that PKC\(\beta\) rapidly redistributes from the cytosol to the membrane compartment of B-cells in response to BCR ligation (within \(\leq 30\) s) and that activated PKC\(\beta\) is found in cytosolic and membrane compartments both at early (\(\leq 30\) s) and late (\(\geq 10\) min) time points (data not shown).

That the activation of PKC\(\beta\) by BCR ligation is entirely dependent on PLC\(\gamma\) activation was demonstrated through the use of DT40 B-cell lines engineered to be deficient in individual components of the signaling cascade, leading to PLC\(\gamma\) activation (Fig. 4A). Lyn-deficient DT40 cells have intact but delayed PLC\(\gamma\) activation (20). They also exhibit relatively intact but delayed activation of PKC\(\beta\) (peak activation occurs at \(>10\) min as opposed to \(-1\) min in wild-type DT40 cells). This closely tracks the published time course of PLC\(\gamma\) activation as measured by inositol phosphate turnover (20). In contrast, DT40 cell lines deficient in BLNK, BTK, and PLC\(\gamma\) each of which have completely abrogated PLC\(\gamma\) activation (reviewed in Ref. 21), show completely abrogated PKC\(\beta\) activation. Note that in addition PMA-mediated PKC\(\beta\) activation is intact in all of the DT40 cell lines tested, eliminating the possibility that direct effects of the deficiency of these proteins might be affecting PKC\(\beta\) activation. Consistent with these results (Fig. 4B), the treatment of cells with the putative DAG antagonist calphostin C also abrogated BCR-mediated PKC\(\beta\) activation in chicken and human B-cells.

The above results demonstrate that PLC\(\gamma\) is a probable...
source of DAG for BCR-mediated PKCγ activation. Because DAG would plausibly serve to membrane target and activate both classical and novel PKC enzymes in the same general microdomain area(s) as PKCγ would be localized, we investigated whether either of these classes of enzymes might serve as an upstream activating kinase for PKCγ. Consistent with this possibility, the treatment of B-cells with the classical/novel PKC inhibitor Ro-31-8245 completely blocked BCR-mediated PKCγ activation (Fig. 5A). Whereas this inhibitor is thought to be relatively specific for the classical and novel classes of PKC enzymes relative to other serine/threonine kinases (including PKD1, the closest homologue of PKCγ (22)), the use of inhibitors is always open to questions regarding specificity within the cellular environment. Therefore, to further evaluate the role of PKC-dependent trans-phosphorylation as an activation mechanism for PKCγ, we tested the ability of activated mutants of various PKC subtypes to induce PKCγ phosphorylation (and thus activation) in a heterologous expression assay. The expression of constitutively activated mutants of novel PKC isozymes (η, ε, and θ) produced robust constitutive activation of PKCγ in the absence of PMA stimulation (Fig. 5B). In contrast, the co-expression of kinase-deficient or wild-type PKCε or PKCθ had little or no effect on basal or PMA-induced activation of PKCγ. Interestingly, the expression of a constitutively activated classical PKC enzyme, PKCα, produced no detectable change in either constitutive or PMA-induced PKCγ activation (Fig. 5C), suggesting that PKCγ is a poor substrate for PKCα and potentially other classical PKC isoforms.

**DISCUSSION**

We have analyzed the activation mechanism of the novel serine-threonine kinase PKCγ and show that PKCγ is activated by PMA and BCR-mediated DAG production via the trans-phosphorylation of two serine residues (Ser 731 and Ser 735) within its activation loop. The ability of activated mutants of novel PKC isozymes but not the classical PKC enzyme, PKCα, to induce constitutive PKCγ activation suggests that this trans-phosphorylation event may be mediated primarily by novel PKC enzymes. As PKCγ exhibits robust activation in response to BCR engagement, our results suggest that PKCγ is an important downstream target of activated novel PKC enzymes during BCR signaling.

The closest homologues of PKCγ are PKD1/PKCμ and PKD2, and together these three kinases form a distinct protein kinase subfamily. They share a predicted tertiary structure that includes two C1 domains contained in their amino-terminal halves, a single central pH domain, and closely homologous kinase domains in their COOH-terminal halves. Consistent with their structural similarity to PKCγ, PKD1 and PKD2 (similar to PKCγ) appear to act downstream from both DAG and protein kinase C enzymes. Although the data in this paper suggest that PKCγ appears to relatively specifically targeted by novel PKC isoforms, PKD1 and PKD2 are activated by both classical and novel PKCs (19, 23–25). From the standpoint of their catalytic domains, these three kinases are only distantly related to the ACG kinases (consisting of the PKA, PKC, and PKG protein kinase families). Instead, their kinase domains exhibit the closest sequence similarity to those of calcium-regulated kinases (25). Consistent with this finding, small peptides phosphorylated by PKD1 in vitro do not appear to significantly overlap with those phosphorylated by classical/novel PKC enzymes (23, 26), suggesting that PKCγ/PKD1/PKD2 substrates represent distinct signaling pathways downstream from DAG and PKCs.

Whether PKD1, PKD2, and PKCγ share similar substrate ranges or downstream biological effector functions remains to be demonstrated. However, their position downstream from novel PKCs suggests that one or more of them is involved in linking novel PKC activation with effector responses downstream from the BCR in B-cells. In this regard, a recent report (14) has implicated the novel PKC isoform PKCδ in controlling the mechanisms of anergy and tolerance in B-cells. Because PKD1 and PKCγ are both expressed in B-cells and appear to be targets of novel PKC enzymes, either one or both could plausi-
bly function as a link between PKCδ (and possibly other novel PKC enzymes) and downstream effectors and mechanisms involved in the creation of B-cell energy and tolerance. Determining whether PKCδ and/or its homologues operate in this pathway or an alternative signaling pathway will depend on the future development of genetic or pharmacologic tools for the manipulation of their signaling function.

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Regulation of Protein Kinase Cγ by the B-cell Antigen Receptor
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