Critical Role of Protein Kinase C βII in Activation of Mast Cells by Monomeric IgE*

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Accumulating evidence suggests that IgE-mediated activation of mast cells occurs even in the absence of antigen, which is referred to as “monomeric IgE” responses. Although monomeric IgE was found to induce a wide variety of responses, such as up-regulation of the FcεRI, survival, cytokine production, histamine synthesis, and adhesion to fibronectin, it remains to be clarified how mast cells are activated in the absence of antigen. It has been controversial whether monomeric IgE responses are mediated by a similar signaling mechanism to antigen stimulation, although recent studies suggest that IgE can induce the FcεRI aggregation even in the absence of antigen. In this study, we focused on the role of conventional protein kinase C (cPKC), since this response is suppressed by a specific inhibitor for cPKC. Monomeric IgE-induced Ca²⁺ influx was not observed in a mouse mastocytoma cell line, which lacks the expression of PKCβII, although Ca²⁺ influx induced by cross-linking of the FcεRI was intact. Transfection of PKCβII cDNA was found to restore the Ca²⁺ influx induced by monomeric IgE in this cell line. Furthermore, the dominant negative form of PKCβII (PKCβII/T300V) significantly suppressed the Ca²⁺ influx, histamine synthesis, and interleukin-6 production in another mouse mast cell line, which is highly sensitive to monomeric IgE. Expression of PKCβII/T500V was found not to affect the antigen-induced responses. These results suggest that PKCβII plays a critical role in monomeric IgE responses, but not in antigen responses.

Activation of mast cells triggers allergic and inflammatory responses through the release of a wide variety of mediators, such as histamine, arachidonic acid metabolites, and neutral proteases, and modulates immune responses through the production of cytokines and chemokines (1, 2). One of the prominent mechanisms for activation of mast cells is cross-linking of the FcεRI, the high affinity receptor for IgE, by the multivalent antigen, and various signaling molecules have been identified in this pathway (3, 4). Furthermore, accumulating evidence has indicated that IgE-mediated activation of mast cells can occur even in the absence of the multivalent antigen (5). Sensitization of IL-3- and IL-2-deprived mouse bone marrow-derived mast cells (BMMCs) with IgE induces an array of events, such as up-regulation of the FcεRI (6, 7), resistance to apoptosis under IL-3 deprivation (8–11), histamine production (9, 10), histamine synthesis (12), and adhesion to fibronectin (13, 14). These results have clearly indicated that sensitization with IgE (monomeric IgE) is able to activate mast cells in the absence of antigen. However, it remains to be clarified as to how monomeric IgE activates mast cells and what kinds of signaling molecules are involved in this pathway.

Some preceding studies revealed that signaling molecules are shared between monomeric IgE responses and responses induced by cross-linking with multivalent antigen; weak but sustained tyrosine phosphorylation of several signaling components, which are intensively phosphorylated upon antigen stimulation, were observed in BMMCs stimulated with monomeric IgE (9, 14). High concentrations of monomeric IgE were found to have a potential to induce degranulation (10, 15, 16). Furthermore, monovalent haptens, for which the IgE is specific, were found to abrogate these responses (9, 10, 17). These results have raised the possibility that monomeric IgE can induce aggregation of the FcεRI via its antigen binding portion. Indeed, Kitaura et al. (10) demonstrated the increase in anisotropy of the surface FcεRI of a rat mast cell line stimulated with monomeric IgE. Very recently, Schweitzer-Stenner and Pecht (18) performed a simulation analysis for the distance between the surface IgE molecules bound to the FcεRI and proposed a hypothesis that monomeric IgE induces the receptor aggregation via low affinity interaction between the IgEs or between the IgE and another cell surface component.

We recently demonstrated that activation of BMMCs by monomeric IgE requires Ca²⁺ influx, which is mediated by pharmacologically distinct channels from those activated upon antigen stimulation (17). Based upon this result, we hypothesized that signal transduction activated by monomeric IgE is not only quantitatively but also qualitatively different from that upon antigen stimulation. Since monomeric IgE-induced responses, such as histamine synthesis and IL-6 production, were drastically suppressed by a conventional protein kinase C (cPKC) inhibitor, Go6976, we particularly focused on the role of cPKC and found the critical role of PKCβII in monomeric IgE-induced activation of mast cells.

**MATERIALS AND METHODS**

**Cell Culture**—A mouse mastocytoma cell line, P-815, and its transfectants were maintained in RPMI 1640 containing 10% heat-inactivated fetal calf serum. P-815/FcεRI (P-815/Fc), which is a stable cell line that expresses rat FcεRI α, β, and γ chain cDNAs, is a generous gift from Dr. H. Metzger (National Institutes of Health) (19). A mouse mast cell line, MC9, was maintained under the same conditions in the presence of 1 ng/ml IL-3 (R&D Systems, Minneapolis, MN).
Stable P-815 and MC9 Clones—Mouse PKCβII cDNA was amplified by reverse transcription-PCR using a pair of primers (forward: 5'-CCG GAT CCG CGC GCG CAA GAT GGC TGA CCC G-3'; reverse: 5'-GGC TCG AGA CGG ATC TAC TTA GCT CTT GAC TTC AGG TT-3'; underlines indicate the restriction sites for BamHI and XhoI) and cloned into the BamHI and XhoI sites of pcDNA3.1/Hygro (Invitrogen). P-815/Fc cells were transfected with pcDNA3.1/Hygro containing PKCβII cDNA (pcDNA3.1/Hygro/PKCβII) using GenePORTER transfection reagent (Gene Therapy Systems, San Diego, CA), and stable transfectants were selected in the presence of hygromycin (0.1 mg/ml). A dominant negative form of PKCβII (PKCβII/T500V) was constructed as described (20) and subcloned into a lentiviral vector, pLenti6/V5-D-TOPO (Invitrogen). MC9 cells were infected with the recombinant lentivirus prepared according to the manufacturer’s instruction, and stable transfectants were selected in the presence of blasticidin (6 μg/ml).

PKCβII Expression in COS-7 Expression System—COS-7 cells were transfected with pcDNA3.1/Hygro/βII and its mutant (pcDNA3.1/Hygro/βII-T500V), respectively, or simultaneously using GenePORTER transfection reagent. Expression of PKCβII was confirmed by immunoblot analysis as described below.

Preparation of IgEs—IgEs (an anti-DNP IgE, clone SPE-7, from Sigma, an anti-TNP IgE, clones IgE-3 and c38-2, from BD Biosciences, San Diego, CA) were purified before use by centrifugation at 100,000 × g at 4 °C for 1 h for removal of the aggregated form. No significant differences were observed between the IgE purified by centrifugation and the IgE purified by gel filtration in activation of mast cells as a monomeric IgE. We used the clone SPE-7 through this study otherwise stated.

Immunoblot Analysis—Cells were homogenized in 50 mM HEPES-NaOH, pH 7.3 containing 1 mM dithiothreitol, 0.5% Triton X-100, 0.5% deoxycholate, 0.05% SDS, and a protease inhibitor mixture (0.2 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 0.1 mM benzamidine, 1 μg/ml pepstatin A, and 10 μg/ml E-64) and were centrifuged at 15,000 × g for 20 min at 4 °C. The resultant supernatant was subjected to SDS-PAGE (10% slab gel). The separated proteins were electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA) and probed with primary antibodies against PKCβII (1:250, BD Biosciences), PKCβI (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), anti-PKCβIII antibody (1:200, Santa Cruz Biotechnology), anti-PKCγ antibody (1:200, BD Biosciences), anti-actin antibody (1:1000, Chemicon, Temecula, CA), horseradish peroxidase-conjugated anti-mouse IgG antibody (1:3000, Dako, Glostrup, Denmark), and horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:3000,
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Dako). The membrane was stained using the ECL kit (Amersham Biosciences) according to the manufacturer’s instruction.

Flow Cytometry—Cells were pretreated with 10 µg/ml anti-CD16/32 (2.4G2, BD Biosciences) at 4 °C for 10 min, then with 12.5 µg/ml each IgE at 4 °C for 50 min. Labeling of the cells was performed by incubation with an FITC-conjugated anti-mouse IgE (BD Biosciences) at 4 °C for 25 min. Flow cytometric analysis of the stained cells was performed with FACSCalibur (BD Biosciences) equipped with the CELLQUEST software.

IgE-mediated Activation of Mast Cells—Cells were treated with the indicated concentrations of an anti-DNP IgE. In case of antigen stimulation, cells were sensitized with 0.3 µg/ml anti-DNP IgE for 24 h, washed three times to remove unbound IgE, and then stimulated with the indicated concentrations of DNP-conjugated human serum albumin (Sigma).

Measurement of Cytosolic Ca2+ Concentrations—Cells were loaded with 2 µM Fura-2/AM in the modified Tyrode’s buffer (130 mM NaCl containing 5 mM KCl, 1.4 mM CaCl2, 1 mM MgCl2, 5.6 mM glucose, 10 mM HEPES-NaOH, pH 7.3, and 0.1% bovine serum albumin) for 45 min at room temperature and then washed in the modified Tyrode’s buffer. Fluorescent intensities were measured, at an excitation wavelength of 340 or 380 nm and an emission wavelength of 510 nm, with a fluorescein spectrometer (Jasco, CAF-100, Tokyo, Japan).

Membrane Translocation of cPKCs—MC9 Cells were treated with 1 µg/ml IgE for the indicated periods or with 100 mM O-tetradecanoylphorbol-13-acetate for 10 min. The cells were homogenized and lysed by sonication in 20 mM Tris-HCl, pH 7.5 containing 10 mM EGTA, 2 mM EDTA, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 20 µg/ml leupeptin. The resultant homogenate was centrifuged at 100,000 × g for 15 min at 4 °C to separate the cytosolic and membrane fractions. Each fraction was subjected to the immunoblot analysis using the specific PKC antibodies.

Measurement of l-Histidine Decarboxylase (HDC) Activity and IL-6 Release—Cells were rinsed with phosphate-buffered saline followed by centrifugation and the cell pellet was lysed (1 × 107 cells/ml) with 10 mM HEPES-NaOH, pH 7.3, containing 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100 and the protease inhibitor mixture on ice for 10 min. The cells were centrifuged at 10,000 × g for 30 min at 4 °C, and the supernatant was used for the measurement of HDC activity as described previously (21). IL-6 release was measured using mouse IL-6 BD OptEIA enzyme-linked immunosorbent assay system (BD Biosciences).

RESULTS

PKCβII Is Required for Monomeric IgE-induced Ca2+ Mobilization in a Mastocytoma Cell Line, P-815—Since activation of mast cells by monomeric IgE was found to be suppressed by a specific inhibitor for cPKC, Gö6976 (22), we investigated the expression of cPKC subtypes in mast cell lines, MC9 and P-815, by immunoblot analysis. PKCa, βI, and βII were expressed in MC9 cells, whereas PKCa and βI, but not βII, in P-815 cells (Fig. 1A). Expression of PKCy was not observed in P-815 and MC9 cells (data not shown). Since P-815 cells lack FcεRIα and β chains and the expression level of γ chain is low, we used a stable clone, P-815/Fc, which expresses sufficient amount of all three chains. IgE (clone SPE-7)-mediated Ca2+ mobilization was not observed in the parental P-815 cells due to the lack of FcεRI expression (Fig. 1, B and C). Although IgE-mediated antigen stimulation induced the intracellular Ca2+ mobilization in P-815/Fc cells, monomeric IgE failed to induce Ca2+ mobilization (Fig. 1C). We then prepared a stable clone, P-815/Fc/βII, which expresses PKCβII (Fig. 1A). In this clone, intracellular Ca2+ mobilization was observed not only upon antigen stimulation but also by monomeric IgE (Fig. 1C). The optimal condition of antigen stimulation was not changed in this clone (data not shown). Equal levels of surface expression of FcεRI were verified by flow cytometry in P-815/Fc and P-815/Fc/βII cells, indicating that PKCβII did not affect the surface expression of FcεRI (Fig. 1B). No or little changes of Ca2+ mobilization induced by thrombin, which evokes Ca2+ mobilization by acting on the heterotrimeric G protein-coupled receptor, were observed between P-815/Fc and P-815/Fc/βII cells. We investigated the monomeric IgE-induced Ca2+ mobilization using the other IgE clones, which exhibit the similar levels of surface binding to the clone, SPE-7 (Fig. 2A). An IgE clone, c38-2, was found to induce Ca2+ mobilization as well as SPE-7, whereas another clone, IgE-3, was not. These differential effects of the IgE clones were also observed in BMMCs (17). Monomeric IgE-induced Ca2+ mobilization in P-815/Fc/βII cells was suppressed by Gö6976, staurosporine, and a specific inhibitor of store-operated Ca2+ channels, SK&F96365, but not by La3+, Gd3+, which is consistent with our previous results using BMMCs (17) (Fig. 2B).
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Lentiviral Expression of the Dominant Negative Form of PKCβII in MC9 Cells—Since our preliminary study revealed that MC9 cells are highly sensitive to monomeric IgE, we first investigated the membrane translocation of cPKCs. A minor portion of cPKCs expressing in MC9 cells was found to rapidly translocate to the membrane fractions by monomeric IgE. Relatively prolonged translocation of PKCβII was observed compared with PKCa and βI (Fig. 3). Previous study demonstrated that Thr500, which is located in the activation loop of PKC and phosphorylated, is one of the critical residues for kinase activity of PKCβII and that the mutation of this residue to Val (PKCβII/T500V) resulted in the loss-of-function (20). We, therefore, prepared the stable MC9 clones, which express this mutant form of PKCβII, to evaluate its dominant negative effects on monomeric IgE-induced activation. Several clones that express unphosphorylated PKCβII were obtained. We selected two clones with high expression levels of the mutant PKCβII, MC9/DN1 and MC9/DN2. In these clones, high expression levels of the unphosphorylated PKCβII were observed, whereas little or no expression of the phosphorylated form was detected (Fig. 4A). It remains unknown why the phosphorylated form disappears in the presence of PKCβII/T500V. We investigated the expression of PKCβII and PKCβII/T500V in a mammalian expression system using COS-7 cells, which lack endogenous PKCβII. The phosphorylated form was also undetectable in the cells co-transfected with the wild and mutant PKCβII cDNA, although each form was detectable in the cells transfected with its cDNA alone (Fig. 4B). The expression of PKCβII/T500V did not affect the expression levels of the other cPKCs, PKCa and βI (Fig. 4A). Expression of PKCβII/T500V did not affect the surface expression of FcεRI in MC9 cells (Fig. 4C).

Suppression of Monomeric IgE-induced Activation in MC9 Cells Expressing the Dominant Negative Form of PKCβII—We then evaluated the dominant negative effects of PKCβII/T500V on monomeric IgE-induced activation. Ca2+ mobilization induced by monomeric IgE was partially suppressed in MC9/DN clones, whereas Ca2+ mobilization upon antigen stimulation was unchanged (Fig. 5). Since induction of HDC activity was observed in MC9 cells both by monomeric IgE and upon antigen stimulation (Fig. 6A, B), we then investigated the effects of PKCβII/T500V on induction of HDC. PKCβII/T500V was found to significantly suppress the induction of HDC by monomeric IgE but not upon antigen stimulation (Fig. 6C). No changes in the dose response curve were observed in between MC9 cells and each DN clone (data not shown). Similarly, monomeric IgE-induced IL-6 release was also significantly suppressed by PKCβII/T500V, whereas antigen-induced IL-6 release was not (Fig. 6D).

DISCUSSION

We focused on the role of cPKC in IgE-mediated activation of mast cells and found that PKCβII plays a critical role in monomeric IgE-induced responses but not in antigen responses. Previous studies have cast some doubt on the differences in signal transduction between monomeric IgE responses and antigen responses; they speculated that monomeric IgE can induce the FcεRI-aggregation and that a similar signaling pathway is activated upon both responses with different intensities (10, 18). Accumulating evidence has indicated that the FcεRI-mediated activation of mast cells is finely tuned. Gonzalez-Espinosa et al. (23) demonstrated that several chemokines, such as MCP-1, are released at low antigen concentrations or at low receptor occupancy with IgE compared with several cytokines such as IL-3 and IL-10. This
result indicated that antigen-induced IL-3 release requires a "strong" stimulation, in which linker for activation of T cells (LAT) and ERK are extensively phosphorylated. On the other hand, Kohno et al. (11) demonstrated that monomeric IgE-induced survival of BMMCs is mediated by a large amount of IL-3 autocrine production. In this study, IL-3 production by monomeric IgE was found to be much larger than that upon antigen stimulation. Since monomeric IgE was found to elicit a "weak and sustained" signal transduction including ERK phosphorylation (9), it is likely that IL-3 production by monomeric IgE is regulated by a different mechanism from that upon antigen stimulation. We also found that IL-6 production in MC9 cells stimulated with monomeric IgE was much larger than that upon antigen stimulation under the optimized condition. Our current results clearly demonstrated that PKCβII is a prerequisite component for monomeric IgE-induced activation of mast cells but not for antigen responses. We previously demonstrated that Ca^{2+} influx induced by monomeric IgE is mediated by channels different from Ca^{2+} release-activated Ca^{2+} channel, which is utilized upon antigen responses, using several channel blockers (17). We propose that the signaling cascades activated by monomeric IgE are distinguishable from those upon cross-linking of the FceRI by multivalent antigen.

A mouse mastocytoma cell line, P-815, is a suitable model for the analysis of FceRI-mediated activation, since it lacks the surface expression of FceRI (19). We can exhibit the direct evidence of involvement of the FceRI in monomeric IgE responses using this cell line, which is consistent with the recent study using BMMCs derived from the FcεRIα-deficient mice (24). Restored Ca^{2+} influx by PKCβII was observed only in the presence of certain IgE clones and exhibited a similar sensitivity to various kinase inhibitors and channel blockers to that observed in BMMCs (17), indicating that the responses in P-815 cells reproduce those observed in BMMCs. However, no or little induction of HDC and IL-6 was observed upon monomeric IgE treatment or antigen stimulation in P-815 and its transfectants. Since potent activators of mast cells, such as A23187 and thapsigargin, failed to induce histamine synthesis and IL-6 release in P-815 cells (maximum IL-6 release <0.1 pg/ml, data not shown), it is quite difficult to evaluate the effects of IgE-mediated responses. Although detectable levels of IL-6 were found in the concentrated medium of unstimulated P-815 cells, no increase of IL-6 was observed in the presence of A23187. P-815 cells may lack signaling molecules downstream of Ca^{2+} influx, which are required for detectable levels of induction of HDC and IL-6.

The suppressive effects of the mutant PKCβII/T500V on monomeric IgE-induced activation of MC9 cells were consistent with the results obtained in P-815 cells transfected with PKCβII. These suppressive effects were found exclusively in monomeric IgE responses, not in antigen stimulation. Since PKCβI and βII are generated by alternative splicing of the carboxyl-terminal exons and exhibit little differences in enzy-
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It remains unknown how PKC\(\beta\)II is activated by monomeric IgE. Kawakami et al. (28) demonstrated that 20–70% of PKC\(\beta\)II was localized in the membrane fraction of resting BMMC and only a part of it was tyrosine-phosphorylated upon antigen stimulation. We found that less than 1% of PKC\(\beta\)II is localized in the membrane fraction of MC9 cells and that a minor population of PKC\(\beta\)II was translocated to the membrane fraction by monomeric IgE. Antigen stimulation failed to induce detectable membrane translocation of PKC\(\beta\)II in MC9 cells (data not shown). We prepared another stable MC9 clone, in which an RNA interference sequence for the unique region of PKC\(\beta\)II is retrovirally transduced into BMMC derived from PKC\(\beta\)-deficient mice. Although antigen-induced histamine release was restored by either of PKC\(\beta\) isoform, antigen-induced IL-2 release was found to be completely dependent on PKC\(\beta\)II. This result clearly indicated the specific role of PKC\(\beta\)II in the signal transduction downstream of Fc\(\varepsilon\)RI. The PKC\(\beta\)II-Akt pathway may play an important role in monomeric IgE-induced activation of mast cells.

Although specific physiological functions of PKC\(\beta\)II remain to be figured out, several studies have suggested the critical role of PKC\(\beta\)II in vivo. Liu et al. demonstrated that azoxymethane-induced colon carcinogenesis was abolished in PKC\(\beta\) KO mice and that transduction of PKC\(\beta\)II can restore its susceptibility (32). Todt et al. (33) suggested that PKC\(\beta\)II is involved in phagocytosis of apoptic cells by murine peritoneal macrophages with pharmacological approaches. We revealed in this study that PKC\(\beta\)II isoforms are specifically exploited in the Fc\(\varepsilon\)RI-mediated signal transduction in mast cells.

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REFERENCES

1. Metcalfe, D. D., Baram, D., and Mekori, Y. A. (1997) Physiol. Rev. 77, 1033–1079
2. Galli, S. J., Nakae, S., and Tsai, M. (2005) Nat. Immunol. 6, 135–142
3. Siraganian, R. P. (2002) Curr. Opin. Immunol. 14, 728–733
4. Galli, S. J., Kalesnikoff, J., Grimbaldston, M. A., Piliponsky, A. M., Williams, C. M. M., and Tsai, M. (2005) Annu. Rev. Immunol. 23, 749–786
5. Kawakami, T., and Galli, S. J. (2002) Nat. Rev. Immunol. 2, 773–786
6. Hsu, C., and MacGlashan, D. J. D. (1996) Annu. Rev. Immunol. 14, 791–800
7. Yamaguchi, M., Lantz, C. S., Oettgen, H. C., Katona, I. M., Fleming, T., Yano, K., Miyajima, I., Kinet, J. P., and Galli, S. J. (1997) J. Exp. Med. 185, 663–672
8. Asai, K., Kitaura, J., Kawakami, Y., Yamagata, N., Tsai, M., Carbone, D. P., Liu, F., Galli, S. J., and Kawakami, T. (2001) Immunology 104, 290–300
9. Kalesnikoff, J., Huber, M., Lam, V., Damen, E. J., Zang, J., Shiragami, R. P., and Krystal, G. (2001) Immunology 104, 281–290
10. Kitaura, J., Song, J., Tsai, M., Asai, K., Maeda-Yamamoto, M., Mocsai, A., Kawakami, Y., Liu, F. T., Lowell, C. A., Barisas, B. G., Galli, S. J., and Kawakami, T. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 12911–12916
11. Kohtoh, M., Yamasaki, S., Tybulewicz, V. L. J., and Saito, T. (2005) Blood 105, 2059–2065
12. Tanaka, S., Takasu, Y., Mikura, S., Sato, N., and Ichikawa, A. (2002) J. Exp. Med. 196, 229–235
13. Lam, V., Kalesnikoff, J., Lee, C. W., Hernandez-Hanson, V., Wilson, B. S., Oliver, J. M., and Krystal, G. (2003) Blood 102, 1405–1413
14. Kitaura, J., Eto, K., Kimishita, T., Kawakami, Y., Leitges, M., Lowell, C. A., and Kawakami, T. (2005) J. Immunol. 174, 4495–4504
15. Oka, T., Hori, M., Tanaka, A., Matsuda, H., Karaki, H., and Ozaki, H. (2003) Am. J. Pathol. 286, C256–C263
16. Pandey, V., Mihara, S., Fensome-Green, A., Bosolver, S., and Cockcroft, S. (2004) J. Immunol. 172, 4048–4058
17. Tanaka, S., Mikura, S., Hashimoto, E., Sugimoto, Y., and Ichikawa, A. (2005) Eur. J. Immunol. 35, 460–468
18. Schweitzer-Stenner, R., and Pecht, I. (2005) J. Immunol. 174, 4461–4464
19. Miller, L., Alber, G., Varin-Blank, N., Ludowyke, R., and Metzger, H. (1990) J. Biol. Chem. 21, 12444–12453
20. Orr, J. W., and Newton, A. C. (1994) J. Biol. Chem. 269, 27715–27718
21. Safina, F., Tanaka, S., Inagaki, M., Tsuboi, K., Sugimoto, Y., and Ichikawa, A. (2002) J. Biol. Chem. 277, 14211–14215
22. Martin-Saiz, G., Kanzaniak, M., Gschick, H., Blumberg, P. M., Kochs, G., Hug, H., and Schächtele, C. (1993) J. Biol. Chem. 268, 9194–9197
23. Gonzalez-Espinosa, C., Odom, S., Oliveira, A., Hobson, J. P., Martinez, M. E., Oliveira-dos Santos, A., Barra, L., Spiegel, S., Penninger, J. M., and Rivera, J. (2003) J. Exp. Med. 197, 1453–1465
24. Kitaura, J., Kimishita, T., Matsumoto, M., Chung, S., Kawakami, Y., Leitges, M., Wu, D., Lowell, C. A., and Kawakami, T. (2005) Blood 105, 3222–3229
25. Ono, Y., Kikkawa, U., Ogita, K., Fujii, T., Kurokawa, T., Asaoka, Y., Seikuguchi, K., Ase, K., Igarashi, K., and Nishizuka, Y. (1987) Science 236, 1116–1120
26. Nechushhtan, H., Leitges, M., Cohen, C., Kay, G., and Razin, E. (2000) Blood 95, 1752–1757
27. Kawakami, Y., Kitaura, J., Hartman, S. E., Lowell, C. A., Siragianian, R. P., and Kawakami, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7423–7428
28. Kawakami, Y., Kitaura, J., Yao, L., Mehenry, R. W., Kawakami, Y., Newton, A. C., Kang, S., Kato, R. M., Leitges, M., Rawlings, D., and Kawakami, T. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 9470–9475
29. Kitaura, J., Asai, K., Maeda-Yamamoto, M., Kawakami, Y., Kikkawa, U., and Kawakami, T. (2000) J. Exp. Med. 192, 729–733
30. Kawakami, Y., Nishimoto, H., Kitaura, J., Maeda-Yamamoto, M., Kato, R. M., Littman, D. R., Rawlings, D. J., and Kawakami, T. (2004) J. Biol. Chem. 279, 47720–47725
31. Stehbens, E. G., and Mochly-Rosen, D. (2001) J. Biol. Chem. 276, 29644–29650
32. Liu, Y., So, W., Thompson, E. A., Leitges, M., Murray, N. R., and Fields, A. P. (2004) J. Biol. Chem. 279, 45556–45563
33. Todt, J. C., Hu, B., Punturieri, A., Sonstein, J., Polak, T., and Curtis, J. L. (2002) J. Biol. Chem. 277, 35906–35914