The SH2 Domain-containing Inositol 5’-Phosphatase (SHIP)
Recruits the p85 Subunit of Phosphoinositide 3-Kinase during FcγRIIb1-mediated Inhibition of B Cell Receptor Signaling*

(Received for publication, August 19, 1998, and in revised form, December 14, 1998)

Neetu Gupta‡, Andrew M. Scharenberg§, David A. Fruman¶, Lewis C. Cantley‡, Jean-Pierre Kinet§, and Eric O. Long**

From the ‡Laboratory of Immunogenetics, NIAID, National Institutes of Health, Rockville, Maryland 20852-1727 and the Laboratories of §Allergy and Immunology and ¶Signal Transduction, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215

Coligation of FcγRIIb1 with the B cell receptor (BCR) or FcεRI on mast cells inhibits B cell or mast cell activation. Activity of the inositol phosphatase SHIP is required for this negative signal. In vitro, SHIP catalyzes the conversion of the phosphoinositide 3-kinase (PI3K) product phosphatidylinositol 3,4,5-trisphosphate (PIP3) into phosphatidylinositol 3,4-bisphosphate. Recent data demonstrate that coligation of FcγRIIb1 with BCR inhibits PIP3-dependent Btk (Bruton’s tyrosine kinase) activation and the Btk-dependent generation of inositol triphosphate that regulates sustained calcium influx. In this study, we provide evidence that coligation of FcγRIIb1 with BCR induces binding of P13K to SHIP. This interaction is mediated by the binding of the SH2 domains of the p85 subunit of P13K to a tyrosine-based motif in the C-terminal region of SHIP. Furthermore, the generation of phosphatidylinositol 3,4-bisphosphate was only partially reduced during coligation of BCR with FcγRIIb1 despite a drastic reduction in PIP3. In contrast to the complete inhibition of Tec kinase-dependent calcium signaling, activation of the serine/threonine kinase Akt was partially preserved during BCR and FcγRIIb1 coligation. The association of P13K with SHIP may serve to activate P13K and to regulate downstream events such as B cell activation-induced apoptosis.

Coengagement of FcγRIIb1 with the B cell receptor (BCR) by an immune complex consisting of antigen and a specific antibody provides a feedback mechanism for the down-regulation of B cell activation (1, 2). A distinct effect of BCR/FcγRIIb1 coligation is the loss of sustained calcium influx and a selective reduction in the tyrosine phosphorylation of certain proteins (3–7). The molecular events responsible for this phenotype are not clearly understood.

Coengagement of BCR with FcγRIIb1 results in recruitment of SHIP (SH2 domain-containing inositol-polyphosphate 5’-phosphatase) to the immunoreceptor tyrosine-based inhibition motif present in the cytoplasmic tail of FcγRIIb1 (8). Two approaches provided evidence for a functional requirement for SHIP during FcγRIIb1-mediated inhibitory signaling. Ectopic expression of a chimeric KIR/FcγRIIb1 protein, containing the extracellular and transmembrane regions of KIR and the cytoplasmic tail of FcγRIIb1, in natural killer cells inhibited the lysis of target cells bearing the HLA class I ligand for the extracellular KIR portion of the chimeric receptor (9). Coexpression of a dominant-negative mutant of SHIP, but not the tyrosine phosphatase Shp-1, reverted the inhibitory signal delivered by FcγRIIb1 in natural killer cells. Conversely, dominant-negative Shp-1, but not SHIP, reverted the negative signal mediated by KIR (9). The second approach made use of chicken DT40 B cells in which the SHIP or Shp-1 genes had been deleted by targeted homologous recombination (10). FcγRIIb1-dependent inhibition was lost in the absence of SHIP, but remained intact in cells lacking Shp-1 (10).

SHIP is a 145-kDa cytosolic protein that contains a single SH2 domain, a catalytic region that bears significant homology to inositol 5’-phosphatases, and several binding sites for other signaling proteins in its C-terminal region (11–13). SHIP interacts with Shc (14), which couples proximal signaling to the Grb2/Sos/Ras activation pathway. SHIP tyrosine phosphorylation and association with Shc increases upon BCR/FcγRIIb1 coligation (14). It was proposed that SHIP inhibits the BCR activation signal by competing with Grb2 for binding to Shc, thereby breaking the Ras signaling pathway (15).

BCR ligation leads to phosphorylation of the tyrosines at positions 484 and 515 in CD19, which then recruit and activate phosphoinositide 3-kinase (PI3K) (16, 17). Coligation of BCR with FcγRIIb1 leads to initial phosphorylation of CD19, followed by its rapid dephosphorylation (6, 7). One model proposes that a tyrosine phosphatase, such as Shp-1, dephosphorylates CD19, thereby blocking BCR-mediated activation by preventing PI3K activation (7). However, CD19 dephosphorylation cannot always account for FcγRIIb1-mediated inhibition (8–10, 18).

In vitro, SHIP cleaves the 5’-phosphate from phosphatidylinositol 3,4,5-trisphosphate (PIP3) and inositol 1,3,4,5-tetrakisphosphate to give rise to phosphatidylinositol 3,4-bisphosphate (PIP2) and inositol 1,3,4,5-tetrakisphosphate, respectively (12). Unlike other 5’-phosphatases, SHIP preferentially utilizes substrates that are phosphorylated on the D3 position of the inositol ring, thereby linking its activity to the PI3K pathway.
Coengagement of FcγRIIb with BCR leads to a drastic reduction of cellular PIP₂ at any time point of cross-linking as detected by thin-layer chromatography (19). PIP₂ may either not be produced because of inactivation of PI3K, as proposed in the CD19 dephosphorylation model, or be rapidly turned over. Recruitment of SHIP by FcγRIIb may serve to achieve a rapid conversion of PIP₂ to PIP₁. Therefore, the possibility of a physical association between SHIP and PI3K was investigated. Coengagement of BCR with FcγRIIb resulted in a tyrosine phosphorylation-dependent recruitment of the p85 subunit of PI3K to SHIP. This interaction is mediated by direct binding of the SH2 domain of PI3K to a signature motif in the C-terminal region of SHIP. In addition, production of PIP₂ and activation of Akt (also called protein kinase B) were observed during BCR/FcγRIIb coengagement.

EXPERIMENTAL PROCEDURES

Cells, Antibodies, and Other Reagents—The B cell line A20 was maintained in RPMI 1640 medium with 10% fetal bovine serum, 2 mM glutamine, and 50 µg/ml β-mercaptoethanol. NIH 3T3 cells were grown in Dulbecco’s modified Eagle’s medium with 10% calf serum and 2 mM glutamine. Fabγ₁,2, intact rabbit anti-mouse IgG, and peroxidase-conjugated goat anti-rabbit IgG were purchased from Jackson ImmuNoResearch Laboratories, Inc. (West Grove, PA). Anti-PI3K p85 and p110 subunit antibodies, unconjugated and biotin-conjugated anti-phosphotyrosine 4G10 antibodies, and a glutathione S-transferase (GST) fusion protein of the PI3K p85 C-terminal SH2 domain were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Recombinant GST protein and womannin were obtained from Sigma. Antibodies against Akt and phospho-Akt (specific for phosphoserine 473) were from New England Biolabs Inc. (Beverly, MA), and anti-Flag antibody (M2) was a gift of B. Moss), which contains a Kozak sequence and a Flag sequence (20). Not I site at the amino terminus, which adds amino acids EF arising from an N sequence VPACGVSSLNEMINP in the C-terminal region of SHIP was generated and amplified as described (21). Briefly, NIH 3T3 cells were infected with recombinant viruses encoding SHIPncat, SHIPnc, or SHIPcatc were used to infect NIH 3T3 cells as described (21). Briefly, NIH 3T3 cells (5 × 10⁶) were infected in suspension at 5 plaque-forming units/cell in 2 ml of infection medium consisting of Dulbecco’s modified Eagle’s medium, 2 mM glutamine, 10 mM HEPES, and 0.05% bovine serum albumin for 3 h at 37 °C. The cells were washed once with DPBS and incubated in 1 ml of DPBS or pervanadate solution (10 mM H₂O₂ + 0.1 mM sodium metavanadate in DPBS) for 15 min at 37 °C. Subsequently, the cells were washed with cold DPBS and lysed, and the lysates were used for immunoprecipitation as described above.

Synthetic Peptides and Agarose Beads—Synthetic peptides corresponding to amino acid sequences in the C-terminal region of SHIP and in the PI3K-binding motif in CD19 (SLGSQQ(pY)EDMRG) were purchased from Quality Controlled Biochemicals (Hopkinton, MA). The SHIP peptides used were EMINPNYGGMGP, EMNPNF(pY)GLGMP, and EMNPNF(pY)GRRGP. All peptides were synthesized with an N-terminal biotin tag for coupling with streptavidin-agarose beads. The peptides were dissolved at 0.1 mg/ml in PBS, pH 7.4, and incubated with streptavidin-agarose beads (1-ml packed volume) overnight at 4 °C. The beads were washed four times with PBS, pH 7.4, and suspended in 1 ml of PBS. Lysates of unstimulated A20 cells were prepared as described above and incubated with 100 µl of the above peptide-streptavidin-agarose conjugate overnight at 4°C. Beads were washed and boiled with SDS-PAGE sample buffer, and the bound material was separated by SDS-PAGE and subjected to silver staining or immunoblotsing.

Western and Far Western Blotting—Immunoprecipitates were separated on SDS-polyacrylamide gels and transferred to Immobilon P membranes. The blots were probed with the indicated antibodies and developed using the ECL detection reagents from Amersham Pharmacia Biotech. In the far Western blotting procedure, membranes were overlaid with 4 µg/ml recombinant GST protein or GST fused to the C-terminal SH2 domain of PI3K p85 in phosphate-buffered saline containing 0.1% Tween 20, and 1 mM dithiothreitol. The membranes were washed with buffer without dithiothreitol, reblocked, and incubated with rabbit polyclonal anti-GST antibodies. After washing, the membranes were incubated with peroxidase-conjugated goat anti-rabbit IgG and developed with ECL reagents.

Phosphoinositide Analysis—A20 cells were labeled with ³²P and stimulated as described above. This was followed by extraction and deacylation of lipids and high performance liquid chromatography (HPLC) analysis of the glycerophosphoinositol head groups (22, 23).

RESULTS

Coengagement of FcγRIIb-mediated Inhibition of B Cell Receptor Signaling

7490
with intact antibody (Fig. 2A, Expts. 1 and 2). Thus, p85 can bind directly to SHIP and to a tyrosine-phosphorylated protein that comigrated with SHIP on SDS-PAGE. GST alone did not bind SHIP under the same conditions (Fig. 2B), but it reacted with two nonspecific bands migrating at ~135 and 140 kDa in anti-SHIP immunoprecipitates of both unstimulated and F(ab)₂ and intact anti-Ig-stimulated cell lysates. The presence of SHIP in the anti-phosphotyrosine and anti-SHIP immunoprecipitates is shown in Fig. 2C. Increased tyrosine phosphorylation of SHIP under conditions of BCR and FcγRIIB1 coligation is evident. Direct binding of the PI3K SH2 domain to SHIP by far Western blotting was also greater after receptor coligation than after cross-linking BCR alone.

The SH2 Domain of PI3K Binds to the C-terminal Region of SHIP—The SHIP cDNA was broadly divided into three regions encoding the SH2 domain designated as n, the central catalytic region containing the sequences conserved in several 5'-phosphotyrosine binding domains of Src (12, 25) and multiple prolines that interact with Grb2 (11)). Deletion mutants containing different combinations of these three domains (Fig. 3), namely ncat (105 kDa), nc (110 kDa), and c (120 kDa), were inserted into recombinant vaccinia viruses and tested for their ability to bind PI3K.

The deletion mutants were expressed in NIH 3T3 fibroblasts, immunoprecipitated following a stimulation with pervanadate, and subjected to far Western blotting with the GST-p85 SH2 fusion protein. All three mutants were tyrosine-phosphorylated upon pervanadate treatment (Fig. 4A), but only the n and c cat mutants bound the SH2 domain of PI3K (Fig. 4B). As these two molecules share only the C-terminal sequence of SHIP, the binding site must be in that region. The level of expression of all three deletion mutants was comparable (Fig. 4C). The deletion mutants ncat and nc also coimmunoprecipitated a protein at ~52 kDa upon pervanadate stimulation (Fig. 4A), which could be the Shc adaptor protein associated with the N-terminal SH2 domain of SHIP.
with anti-PI3K antibodies revealed that these proteins comigrated with the p85 (Fig. 5B) and p110 (Fig. 5C) subunits of PI3K, respectively. Thus, the in vitro data shown in Figs. 4 and 5 suggest a possible mechanism by which SHIP binds PI3K upon B cell stimulation with intact anti-Ig antibodies or immune complexes.

Production of PIP₂ and Akt Activation during Coligation of BCR with FcγRIIb1—A potential outcome of the association of SHIP with PI3K in A20 cells stimulated with intact anti-Ig is the efficient production of PIP₂, provided that SHIP and PI3K retain their catalytic activities. To test this possibility, A20 cells were stimulated with F(ab')₂ or intact antibodies for different times, and the total cellular levels of PIP₂ and PIP₃ were determined using a sensitive HPLC assay. Production of PIP₂ upon FcγRIIb1 coligation was approximately two-thirds of that upon BCR stimulation alone (Fig. 6, upper panel). In contrast, there was a marked inhibition of the PI3K product PIP₃ at early time points and complete loss at sustained time points (Fig. 6, lower panel).

Activation of the serine/threonine kinase Akt requires binding of its pleckstrin homology domain to membrane-bound phosphatidylinositides (26, 27). In particular, binding to PIP₂ results in activation of Akt in vitro (28, 29). Full Akt activation requires sequential phosphorylation by two kinases, the second of which phosphorylates serine 473 in Akt after binding to PIP₃ (30). We used phosphorylation of serine 473 as an indicator of Akt activation after signaling via BCR. A20 cells were stimulated with F(ab')₂ or intact antibodies for 2, 5, or 10 min, and active Akt was immunoprecipitated and immunoblotted using antibodies specific for phosphoserine 473. Fig. 7A reveals a large increase in the activity of Akt, which was clearly diminished during coligation of BCR with FcγRIIb1. To test whether PI3K activity is required for Akt activation upon B cell stimulation, two inhibitors, wortmannin and LY294002, were used. At low concentrations, these inhibitors block PI3K activity without affecting phosphoinositide 4-kinases (31). A20 cells were pretreated with wortmannin (Fig. 7B) or LY294002 (data not shown) and then stimulated with F(ab')₂ or intact antibodies for 2 min. Both BCR- and BCR/FcγRIIb1-induced Akt activities were completely lost upon inhibition of PI3K (Fig. 7B). Thus, PI3K activity persists during BCR/FcγRIIb1 coligation and is required for Akt activation.

DISCUSSION

Coengagement of BCR with FcγRIIb1 results in a diminished transient calcium flux and a loss of sustained calcium flux (3–5). The sustained calcium flux in BCR-triggered B cells requires activation of Btk, a member of the Tec kinase family that, in turn, activates phospholipase Cγ (19, 32–34). Activation of Btk is dependent on the binding of its pleckstrin homology domain to PIP₃ (19). A noticeable effect of FcγRIIb1 coligation is a drastic reduction of PIP₂ otherwise produced very rapidly upon BCR triggering (19). The loss of PIP₂ could be due to a reduced PI3K activity and conversion to PIP₃ by a non-rate-limiting SHIP, to an increased SHIP activity, or to a complete loss of PI3K activity. However, the production of the SHIP metabolite PIP₂ suggests that PI3K remains active dur-
The inducible association of PI3K with tyrosine-phosphorylated SHIP described here is different from the constitutive association of PI3K with an unidentified PI3P 5-phosphatase activity in human platelets (35). The novel 5-phosphatase reported in that study is distinct from SHIP since its catalytic activity in vitro was limited to the substrate PIP2.

Production of PIP2 during BCR/FcγRIIb1 coligation was consistently less than during BCR-mediated activation. This is probably due, in part, to a lower activity of PI3K and hence lower production of the SHIP substrate PIP2. As CD19 is dephosphorylated rapidly after BCR/FcγRIIb1 coligation (6, 7), a major source of PI3K activation is lost. Recruitment of PI3K by tyrosine-phosphorylated SHIP may serve to compensate for this loss. However, SHIP is not absolutely required for PI3K activation in avian DT40 B cells because a sustained calcium signal was observed after BCR/FcγRIIb1 coengagement in a SHIP-negative DT40 mutant cell (10). It is also possible that the rapid conversion of PIP2 by SHIP affects PI3K activation directly, or indirectly through a diminished PIP3-dependent activation of Ras (via Sos) (36, 37). To clearly address whether the catalytic activity of SHIP and/or PI3K is responsible for the observed pattern of PIP3 and PIP2 production, an inhibitor of SHIP phosphatase activity would be necessary.

PI3P2 and PIP3 control the activation of Akt by recruiting the pleckstrin homology domains of Akt and of another serine/threonine kinase that phosphorylates Akt (26–30). Akt delivers an anti-apoptotic signal by phosphorylating the pro-apoptotic molecule BAD, a member of the Bcl-2 protein family (38, 39). Our data show residual activation of Akt during BCR/FcγRIIb1 coligation as measured by Akt phosphorylation on serine 473. This remaining Akt activation is in contrast to the complete loss of the sustained calcium flux mediated by the PIP3-dependent Tec kinase Btk during BCR/FcγRIIb1 coligation (19). The wortmannin sensitivity of Akt activation strongly suggests that PI3K activity is also retained. Although apoptosis of B cells after BCR/FcγRIIb1 coligation can occur and may even exceed that observed after BCR-mediated activation (40), the SHIP/PI3K/Akt pathway described here may lead to at least some anti-apoptotic signal. An anti-apoptotic effect of SHIP after BCR/FcγRIIb1 coligation has been suggested by the observation of increased apoptosis of DT40 cells deficient in SHIP and of DT40 cells expressing a mutant FcγRIIb1 that fails to bind SHIP (10). A pro-apoptotic mediator that binds to FcγRIIb1 was proposed to explain these observations (10). On the other hand, the reduced survival of DT40 cells expressing the mutated FcγRIIb1 that fails to recruit SHIP may have been caused by the lack of SHIP-mediated PI3P2 production and, in turn, by a reduced Akt-mediated survival signal.

In conclusion, this study demonstrates an association of the p85 subunit of PI3K with the inositol phosphatase SHIP in response to coligation of BCR with the inhibitory receptor FcγRIIb1. PI3K activity and PIP2 production were not abrogated by FcγRIIb1 ligation to BCR. We suggest that the physical association of SHIP and PI3K may provide a novel mode of PI3K activation and an enhanced conversion of PIP3 to PIP2.

Acknowledgments—We thank M. Lioubin and L. Rohrschneider for deletion mutants of SHIP, L. Samelson for helpful suggestions, and M. Weston for technical assistance.

REFERENCES
1. Chan, P. L., and Sinclair, N. R. (1971) *Immunology* 21, 967–981
2. Phillips, N. E., and Parker, D. C. (1983) *J. Immunol.* 130, 602–606
3. Bijsterbosch, M. K., and Klaus, G. G. (1985) *J. Immunol.* 134, 1712–1718
4. Wilson, H. A., Greenblatt, D., Taylor, C. W., Putney, J. W., Tsien, R. Y., Finkelman, F. D., and Chused, T. M. (1987) *J. Immunol.* 138, 1712–1718
5. Choquet, D., Partiseti, M., Amigorena, S., Bonnerot, C., Fridman, W. H., and Korn, H. (1993) *J. Cell Biol.* 121, 355–363
6. Kiener, P. A., Lioubin, M. N., Rohrschneider, L. R., Ledbetter, J. A., Nadler, S. G., and Diegel, M. L. (1997) *J. Biol. Chem.* **272**, 3838–3844
7. Hippen, K. L., Bubl, A. M., D'Ambrosio, D., Nakamura, K., Persin, C., and Gambier, J. C. (1997) *Immunity* **7**, 49–58
8. Ono, M., Bolland, S., Tempest, P., and Ravetch, J. V. (1996) *Nature* **383**, 263–266
9. Gupta, N., Scharenberg, A. M., Buhl, A. M., D'Ambrosio, D., Nakamura, K., Persin, C., and Gambier, J. C. (1997) *Immunity* **7**, 49–58
10. Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J. V. (1997) *Cell* **90**, 293–301
11. Lioubin, M. N., Algate, P. A., Tsai, S., Carlberg, K., Aebersold, R., and Rohrschneider, L. R. (1996) *Genes Dev.* **10**, 1084–1095
12. Damen, J. E., Liu, L., Rosten, P., Humphries, R. K., Jefferson, A. B., Majerus, P. W., and Krystal, G. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 1689–1693
13. Kavanaugh, W. M., Pot, D. A., Chin, S. M., Deuter-Reinhard, M., Jefferson, A. B., Norris, F. A., Masiarz, F. R., Cousens, L. S., Majerus, P. W., and Williams, L. T. (1996) *Curr. Biol.* **6**, 438–445
14. Chacko, G. W., Tridandapani, S., Damen, J. E., Liu, L., Krystal, G., and Coggeshall, K. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 293–301
15. Lioubin, M. N., Algate, P. A., Tsai, S., Carlberg, K., Aebersold, R., and Rohrschneider, L. R. (1996) *Genes Dev.* **10**, 1084–1095
16. Damen, J. E., Liu, L., Rosten, P., Humphries, R. K., Jefferson, A. B., Majerus, P. W., and Krystal, G. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 1689–1693
17. Kavanaugh, W. M., Pot, D. A., Chin, S. M., Deuter-Reinhard, M., Jefferson, A. B., Norris, F. A., Masiarz, F. R., Cousens, L. S., Majerus, P. W., and Williams, L. T. (1996) *Curr. Biol.* **6**, 438–445
18. Chacko, G. W., Tridandapani, S., Damen, J. E., Liu, L., Krystal, G., and Coggeshall, K. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 293–301
19. Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J. V. (1997) *Cell* **90**, 293–301
20. Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J. V. (1997) *Cell* **90**, 293–301
21. Scharenberg, A. M., Lin, S., Cuenod, B., Yamamura, H., and Kinet, J.-P. (1995) *EMBO J.* **14**, 3385–3394
22. Auger, K. R., Serunian, L. A., Shokelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratzoffsky, S., Lechleider, R. J., Neel, B. G., Birge, R. B., Fajardo, J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B., and Kinet, J.-P. (1996) *J. Biol. Chem.* **272**, 767–778
23. Liu, L., Green, E., Hughes, M. R., Babic, I., Jirik, F. R., and Krystal, G. (1997) *J. Biol. Chem.* **272**, 767–778
24. Hemmings, B. A. (1996) *Science* **275**, 628–630
25. Serunian, L. A., Auger, K. R., and Cantley, L. C. (1991) *Methods Enzymol.* **198**, 78–87
26. Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratzoffsky, S., Lechleider, R. J., Neel, B. G., Birge, R. B., Fajardo, J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B., and Kinet, J.-P. (1996) *EMBO J.* **15**, 3385–3394
27. Fluckiger, A.-C., Li, Z., Kato, R. M., Wahl, M. I., Ochs, H. D., Longnecker, R., Kinet, J.-P., Witte, O. N., Scharenberg, A. M., and Rawlings, D. J. (1998) *EMBO J.* **17**, 3563–3567
28. Tuveson, D. A., Carter, R. H., Soltoff, S. P., and Fearon, D. T. (1993) *Science* **260**, 886–899
29. Weng, W-K., Jarvis, L., and LeBien, T. W. (1994) *J. Biol. Chem.* **269**, 5241–5248
30. Yamauchi, Z., Sato, T., and Kadowaki, T. (1994) *J. Biol. Chem.* **269**, 5241–5248
31. PMID: 7494

**FcγRIIB1-mediated Inhibition of B Cell Receptor Signaling**