**Insufficient Phosphorylation Prevents FcγRIIB from Recruiting the SH2 Domain-containing Protein-tyrosine Phosphatase SHP-1**

**Renaud Lesourne†, Pierre Bruhns, Wolf H. Fridman, and Marc Daéron‡**

From the Laboratoire d'Immunologie Cellulaire et Clinique, INSERM U.255, Institut Curie, 75005 Paris, France

FcγRIIB are IgG receptors that inhibit immunoreceptor tyrosine-based activation motif (ITAM)-dependent cell activation. Inhibition depends on an immunoreceptor tyrosine-based inhibition motif (ITIM) that is phosphorylated upon FcγRIIB coaggregation with ITAM-bearing receptors and recruits SH2 domain-containing phosphatases. Agarose bead-coated phosphorylated ITIM peptides (pITIMs) bind *in vitro* the single-SH2 inositol 5-phosphatases (SHIP1 and SHIP2) and the two-SH2 protein tyrosine phosphatases (SHP-1 and SHP-2). Phosphorylated FcγRIIB, however, recruit selectively SHIP1/2 *in vivo*. We aimed here at explaining this discordance. We found that beads coated with low amounts of pITIM bound *in vitro* SHP1, but not SHP-1, i.e. behaved as phosphorylated FcγRIIB *in vivo*. The reason is that SHP-1 requires its two SH2 domains to bind on adjacent pITIMs. Consequently, the binding of SHP-1, but not of SHIP1, increased with pITIM density on beads. When trying to increase FcγRIIB phosphorylation in B cells and mast cells, we found that concentrations of ligands optimal for FcγRIIB phosphorylation failed to induce SHP-1 recruitment. SHP-1 was, however, recruited by FcγRIIB when hyperphosphorylated following cell treatment with pervanadate. Our data suggest that FcγRIIB phosphorylation may not be sufficient *in vivo* to enable the recruitment of SHP-1 but that (pathological?) conditions that would hyperphosphorylate FcγRIIB might enable SHP-1 recruitment.

FcγRIIB are single-chain low-affinity receptors for the Fc portion of IgG antibodies that bind multivalent immune complexes. They exist as two (FcγRIIB1 and B2 in humans) or three (FcγRIIB1, B1*, and B2 in mice) alternatively spliced products of the *FcγR2b* gene (1). All murine and human FcγRIIB isoforms were shown to negatively regulate cell activation induced by all receptors bearing intracytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs)*1* (2).

---

*This work was supported in part by the Institut National de la Santé et de la Recherche Médicale, the Institut Curie, and the Association pour la Recherche sur le Cancer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†Recipient of a fellowship from the Ministère de l'Éducation Nationale, Développement de la Recherche et de la Technique.

‡To whom correspondence should be addressed: Laboratoire d'Immunologie Cellulaire et Clinique, INSERM U. 255, Institut Curie, 26 rue d'Ulm, 75005 Paris, France. Tel.: 33-1-4432-4223; Fax: 33-1-4051-0420; E-mail: Marc.Daeron@curie.fr.

*The abbreviations used are: ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; BCR, B cell receptor for antigen; BMMCs, bone marrow-derived mast cells; BSA, bovine serum albumin; DNP, dinitrophenyl; FcRI, high-affinity receptors for the Fc portion of IgE; FcγRIIB, low-affinity receptors for the Fc portion of IgG; GAM, goat anti-mouse Ig; GAR, goat anti-rabbit Ig; GST, glutathione S-transferase; HRP, horseradish peroxidase; MAR, mouse anti-rat Ig; pITIMs, phosphorylated ITIMs; NP, 3-nitro-4-hydroxyphenyl acetic acid; RAM, rabbit anti-mouse Ig; SH2, Src homology-2 domains; SHPs, SH2 domain-containing inositol 5-phosphatases; SHPs1, SH2 domain-containing protein-tyrosine phosphatases. TNP, trinitrophenyl; PAGE, polyacrylamide gel electrophoresis.

---

Received for publication, July 21, 2000, and in revised form, November 13, 2000. Published, JBC Papers in Press, November 30, 2000, DOI 10.1074/jbc.M006537200

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

The paper is available online at http://www.jbc.org.
Differential Phosphatase Binding and Recruitment by FcγRIIB

triphosphosphate which enables the membrane translocation of the Bruton’s tyrosine kinase via its pleckstrin homology do-
main (22). Bruton’s tyrosine kinase is mandatory for phospho-
lipase Cγ to be activated and to hydrolyze phosphatidylinositol (4,5)-bisphtosphatase into inositol (1,4,5)-trisphosphate, which in-
duces a Ca2+ response (23), and diacylglycerol, which activates protein kinase C. SHIP1 was recently shown to inhibit the Ras pathway by acting as an adapter molecule in B cells. When phosphorylated by Lyn, SHIP1 recruits Dok by its protein tyrosine-binding domain. Dok is phosphorylated and recruits RasGAP which inactivates Ras by exchanging GTP for GDP on the latter molecule (24). SHIP1 therefore arrests the propa-
gation of intracellular signals leading to the Ca2+ response and to the activation of the Ras pathway. The possible role of SHIP2 is not yet known.

The in vitro binding specificity of ITIMs was analyzed using phosphorylated synthetic peptides to precipitate phosphatases from cell lysates. Under these conditions, all known ITIMs, including the FcγRIIB ITIM, bound SHP-1 and SHP-2 (25). Remarkably, the FcγRIIB ITIM also bound SHIP1 (26) and SHIP2 (27). We previously identified two hydrophobic residues, at positions Y-2 and Y+2, that determine the binding of SHPs (28) and SHIPS (29), respectively. The in vivo recruitment of phosphatases by ITIM-bearing receptors was analyzed by co-
precipitation, following their tyrosyl phosphorylation upon co-
aggregation with ITAM-bearing receptors. Tyrosyl-phosphoryl-
ated FcγRIIB were initially reported to recruit SHP-1 in B cells following their coaggregation with B cell receptors (BCR) (30), (31). FcγRIIB, however, were found to recruit selectively SHIP1, when coaggregated with high-affinity IgE receptors (FcεRI) in mast cells (26, 32). SHIP1, but not SHP-1, was subsequently demonstrated to be necessary for FcγRIIB-dependent inhibi-
tion of cell activation in SHIP1-deficient DT40 B cells (33) and in SHP-1-deficient mast cells derived from motheaten mice (26, 32). These results altogether generated some confusion, and whether FcγRIIB indeed recruit SHP-1 in vivo remains un-
clear. Depending on the answer, the following two issues may be addressed: 1) if they do, do they recruit SHP-1 exclusively in B cells or also in other cell types, and 2) if they do not, how can one reconcile the apparent discordance between the in vitro binding of phosphatases to ITIM peptides and the in vivo recruitment of phosphatases by FcγRIIB.

In the present work, we aimed at clarifying these questions by analyzing the conditions required for SHP-1 to bind to phosphorylated ITIM-coated beads in vitro and to be recruited by phosphorylated FcγRIIB in mast cells and in B cells. We failed to detect SHP-1 recruitment by FcγRIIB in vivo when coaggregated either with FcεRI in mast cells, or with BCR in B cells. We found that the in vitro binding of SHP-1 required a higher level of FcγRIIB phosphorylation than SHIP1 binding. Indeed, the two SH2 domains of SHP-1 were required to bind phosphorylated ITIMs and, as a consequence, SHP-1 binding, but not SHIP1 binding, depended on the density of phosphory-
lated ITIMs. In vivo, SHP-1 recruitment also required a higher level of FcγRIIB phosphorylation than SHIP1 recruit-
ment. The level of FcγRIIB phosphorylation that enabled the recruitment of SHP-1 was reached after treating cells with pervanadate, but not following coaggregation of FcγRIIB with BCR or FcεRI, in B cells and mast cells, respectively.

EXPERIMENTAL PROCEDURES

Cells—The rat mast cells RBL-2H3 (34) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. FcγRIIB-deficient murine lymphoma B cells K46, expressing an an-
ti-μR BCR (35), and IIA1.6 (36) were cultured in RPMI supplemented with 10% fetal calf serum, 0.5 mM 2-mercaptoethanol, 2 mM sodium pyruvate, 100 IU/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, and 20 mM Hepes. Bone marrow-derived mast cells (BMMC) were obtained from BALB/c bone marrow cells as described (3). After 4 weeks, cultures contained more than 90% mast cells. Culture reagents were from Life Technologies, Inc. (Paisley, Scotland, United Kingdom).

Antigen-Precipitated Clones—Clones of RBL-2H3 cells, stably transfected with cDNA encoding murine FcγRIIB (37) or FcγRIIB1’ (38) and clones of IIA1.6 cells, stably transfected with cDNA encoding FcγRIIB1 (15) were described previously. cDNA encoding FcγRIIB1, inserted in a NT-neo vector (39), was stably transfected in K46a cells by electroporation. Transfectants were selected with neomycin (Cayla, Toulouse, France) and biotinylated using a polyclonal antibody to a biotinyl-ectein (Becton Dickinson, Mountain View, CA). FcγRIIB1-transfected K46a cells were cloned as described (37). The expression of recombinant receptors on clones remained stable over the duration of experiments.

Antibodies—The mouse IgG mAb 2682-1 was used as culture superna-
ant of a subclone of DNP-H1-e2-26 hybridomas (40). The rat anti-mouse FcγRIIB 2.4G2 mAb (41) was purified by affinity chromato-
tography on Protein G-Sepharose from ascitic fluid of nude mice inocu-
lated with 2.4G2 hybridoma cells intraperitoneally. F(ab’2)2 fragments were obtained by papain digestion for 48 h. The purity of IgG and F(ab’)2 fragments was assessed by SDS-PAGE analysis. The mouse IgG1 mAb anti-DNP was provided by Dr. Jacques Couderc (Institut Curie, Paris, France). The rabbit polyclonal IgG anti-DNP were pur-
chased from Sigma. F(ab’)2 fragments of polyclonal anti-DNP Ig (MAR) were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). MAR F(ab’)2 were trinitrophenylated by incubation for 1 h at room temperature with trinitrobenzenesulfonic acid (East-
man Kodak, Rochester, NY) in borate-buffered saline, pH 8.0. TNP-
MAR F(ab’)2 were obtained after purification on Sephadex G-25 (Am-
ersham Pharmacia Biochemicals, Uppsala, Sweden). Polyclonal rabbit anti-mouse immunoglobulins (RAM) IgG and F(ab’)2 were purchased from Amersham Pharmacia Biotech (Lake Placid, NY), and HRP-conjugated goat anti-rabbit and goat anti-mouse immunoglobulins from Santa Cruz Bio-
technology (Santa Cruz, CA). Rabbit polyclonal anti-SHP-1 antibodies were a gift from Dr. John Cambier (National Jewish Medical and Research Center, Denver, CO).

ITIM Peptides and in Vitro Binding of Phosphatases—Nonphosphory-
lated or tyrosyl-phosphorylated FcγRIIB ITIM-biotinylated peptides with the amino acid sequence KTAENTITYSLLK were synthesized by NeoSystems. Biotinylated ITIM peptides were coupled to streptavidin-
coated beads using as a template cDNA encoding murine FcγRIIB1, inserted in a NT-neo vector (39), was stably transfected in K46

m cells by electroporation. The following primers were used: sense 5’-CGTAGAGATTCAAGAAAGACGAGTTCCAA-3’; antisense 5’-TACGTCGACCTTAAATGGTGGTCA-3’. Nucleotides corresponding to the cDNA sequence encoding amino acids 273–285 of FcγRIIB1, i.e. the same 13 amino acids as synthetic ITIM peptides, were purchased from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire). cDNA encoding the SH2 domain of SHIP1 was amplified by polymerase chain reaction, using as a template a cDNA library generated from RNA extracted from RBL-2H3 cells. The following primers were used: sense 5’-CTGACCCAGTCTGAGGATCCATGCCT- GCTCTCAGG-3’; antisense 5’-GACACCTGACCTTCTGAGGACCCATGCTCA-3’. The sequence was checked on the two strands by dideoxynucleotide sequencing. ICIIIb1’ cDNA, ITIM-encoding nucleo-
tides, and SHIP1 SH2 domain cDNA were inserted into the pGEX-4T-2 vector (Amersham Pharmacia Biotech) and transfected into DH5α Escherichia coli. Bacteria producing SHP-1 SH2 domain-containing
Gust fusion proteins were a gift from Dr. Eric Vivier (Center d’Immunologie de Marseille-Luminy, Marseille, France). All GST fusion proteins were produced in DH5α E. coli following isopropyl-1-thio-
β-galactopyranoside induction, purified on glutathione-agarose (Sigma), and analyzed by SDS-PAGE. Soluble SH2 domain-containing GST fusion proteins were eluted from glutathione-agarose beads with a solution of 50 mM Tris and 25 mM glutathione, pH 8.0.

In Vitro Phosphorylation of GST-ITIM and GST-ICHI1 — and in Vivo Binding of Phosphatases—Ten μl of glutathione-agarose beads coated with GST-ITIM or GST-ICHI1 were washed in kinase buffer containing 100 mM Tris-HCl, pH 7.4, 125 mM MgCl2, 2 mM EDTA, 0.25 mM NaN3, 2 mM dithiothreitol, and incubated for the indicated periods at 30 °C with 20 μl of kinase buffer containing 2 units of the Src kinase Lyn (Chemicon) and 100 μM ATP. Kinase reaction was stopped on ice; beads were immediately washed in lysis buffer, and incubated for 2 h at 4 °C with lysates from 1 × 107 RBL-2H3 cells.

Cell Stimulation and Immunoprecipitation—RBL transfectants, resuspended at 5 × 106/ml, were incubated or not for 1 h at 37 °C with IgE anti-DNP (culture supernatant diluted 1/10) and washed at 1 × 107 cells/ml. Cells were challenged or not for 5 min or for the indicated periods of time at 37 °C with 10 μg/ml TNP-MAP F(ab’)2. Unless otherwise specified, IIA1.6 and K46α transfectants, resuspended at 5 × 106/ml, were stimulated at 37 °C for 5 min with 0.3 μM RAM IgG (45 μg/ml) or RAM F(ab’)2 (30 μg/μl). BMMCs were sensitized as RBL-2H3 cells with the indicated diluted anti-DNP, and resuspended at 1 × 107/ml. SHIP1 and SHP-1 phosphatases on the other membrane. Membranes were cut into two pieces. The upper part, containing molecules with a molecular mass higher than 100 kDa was hybridized with anti-SHIP1, and the lower part, containing molecules with a molecular mass lower than 100 kDa was hybridized with anti-SHP-1 antibodies after having been cut into two units of the Src kinase Lyn (Chemicon) and 100 μM ATP. Kinase reaction was stopped on ice; beads were immediately washed in lysis buffer, and incubated for 2 h at 4 °C with lysates from 1 × 107 RBL-2H3 cells.

Pervanadate was generated by mixing 1 ml of 20 mM Na3VO4 with 330 μl of 30% H2O2, followed by a 5-min incubation at room temperature, yielding a solution of 6 mM pervanadate. Unless otherwise specified, cells were incubated with a final concentration of 100 μM pervanadate for 15 min at 37 °C.

Following stimulation, cells were lysed as described above, and cell lysates were used for immunoprecipitation. Protein G-Sepharose (Amersham Pharmacia Biotech) was used to precipitate 2.4G2-bound FcγRIIB in lysates from RBL transfectants and 2.4G2-coated Sepharose beads were used to precipitate FcγRIIB in lysates from BMMCs or IIA1.6 and K46α transfectants.

Western Blot Analysis—Adsorbents were washed in lysis buffer and boiled for 5 min in sample buffer. Eluted material was fractionated by SDS-PAGE and transferred onto Immobilon-P membranes (Millipore, Bedford, MA). In most experiments, immunoprecipitates were fractionated and transferred onto two membranes. These were used to assess: 1) tyrosyl phosphorylation and FcγRIIB or GST on one membrane, and 2) SHP1 and SHP-1 phosphatases on the other membrane. Membranes were saturated with either 5% BSA (Sigma) or 5% skimmed milk (Béglais, Saint-Martin-Belle-Roche, France) diluted in 10 mM Tris buffer, pH 7.4, containing 0.5% Tween 20 (Merek, Schuchardt, Germany). Membranes Western blotted with HRP-conjugated anti-phosphotyrosine antibodies were stripped and reblotted with anti-FcγRIIB or anti-GST antibodies followed by HRP-conjugated GAR or GAM. In all experiments, the same membrane was used for blotting with anti-
SHP1 and anti-SHP-1 antibodies after having been cut into two pieces. The upper part, containing molecules with a molecular mass higher than 100 kDa was hybridized with anti-SHIP1, and the lower part, containing molecules with a molecular mass lower than 100 kDa was hybridized with anti-SHP-1 antibodies. mAb anti-SHP-1 was used for mast cells analysis while polyclonal antibodies was used for B cells

RESULTS

Tyrosyl-phosphorylated FcγRIIB Recruit SHIP1 but Not SHP-1 Both in Mast Cells and in B Cells—Because phosphatases were reported to be differentially recruited by FcγRIIB, when coaggregated with BCR in B cells or with FcεRI in mast cells, we compared the ability of FcγRIIB1 to recruit SHP-1 and SHP1 in a rat mast cell line, RBL-2H3, and in two FcγRIIB-deficient murine B cell lines, IIA1.6 cells and K46μ cells. The three cell lines were stably transfected with murine FcγRIIB1. FcγRIIB1 were coaggregated with FcεRI, that are constitutively expressed in RBL cells, by challenging transfectants, previously sensitized with mouse IgE anti-DNP and coated with F(ab’)2 fragments of the rat anti-FcγRIIB mAb 2.4G2, using TNP-MAR F(ab’)2. FcγRIIB1 were coaggregated with BCR, that are constitutively expressed in IIA1.6 cells and K46μ cells, using RAM IgG. FcγRIIB1 were immunoprecipitated, their phosphorylation was assessed by Western blotting with anti-phosphotyrosine antibodies, and phosphatases co-precipitated with FcγRIIB1 were examined by Western blotting with anti-SHP-1 and anti-SHIP1 antibodies. As previously observed, FcγRIIB1 became tyrosyl phosphorylated following co-aggregation with FcεRI or with BCR, in mast cells and in B cells, respectively. SHIP1, but not SHP-1, co-precipitated with tyrosyl-phosphorylated FcγRIIB1 in RBL cells, but also in IIA1.6 cells and K46μ cells (Fig. 1A). Because the recruitment of SHIP1 and SHP-1 may not have identical kinetics, we repeated the experiment at various time points in RBL transfectants. The induced phosphorylation of FcγRIIB1 was constant over the time range studied, and comparable amounts of SHIP1 co-precipitated with phosphorylated FcγRIIB1. No co-precipitation of SHP-1 was detected between 15 s and 15 min (Fig. 1B). SHIP1, but not SHP-1, was therefore detectably recruited by tyrosyl-phosphorylated FcγRIIB1, and we found no differential in vivo recruitment of phosphatases in a mast cell line and in two B cell lines.

In Vitro Binding of SHP-1 Requires a Higher Phosphorylation Level of FcγRIIB ITIM Than Binding of SHIP1—Such a selective in vivo recruitment of phosphatases by tyrosyl-phosphorylated FcγRIIB1 was in marked contrast with the ability of phosphorylated synthetic peptides corresponding to the FcγRIIB ITIM to bind in vitro not only to the two SHPs, but also to the two SHPs, when bound to agraso beads. In an attempt to understand this discrepancy, we reasoned that, among other differences between the two types of experiments, the proportion of FcγRIIB1 whose ITIM was phosphorylated in vivo was unknown, whereas all ITIM peptides used to coat agarose beads for in vitro experiments were phosphorylated. To evaluate the possibility that variations in the intensity of ITIM phosphorylation might differentially affect the affinity for SHPs and for SHPs, we coated beads with a constant amount of ITIM peptides, in which the proportion of phosphorylated peptides (pITIM) varied from 100 to 0%. These were incubated with cell lysate from RBL-2H3 cells, and the binding of SHP1 and SHIP-1 was examined by Western blotting. As previously observed, no phosphatase precipitated with beads coated with nonphosphorylated ITIM (0% pITIM) and both phosphatases precipitated with beads coated with 100% pITIM. SHP1 precipitation did not detectably decrease with the proportion of pITIM until beads were coated with less than 12% pITIM, and a detectable amount of phosphatase remained precipitated by beads coated with 6% pITIM. By contrast, SHP-1 precipitation progressively decreased with the proportion of pITIM coated to beads and it was not detected when beads were coated with less than 25% pITIM (Fig. 2A). Beads coated with ITIM peptides, a small proportion of which were phosphorylated, therefore bound in vitro SHP1 but not SHP-1, i.e. displayed the same selectivity for SHIP1 as tyrosyl-phosphorylated FcγRIIB in vivo.

Another difference between the two types of experiments is that intact receptors are used for in vivo recruitment whereas 13-amino acid peptides are used for in vitro binding. One must therefore consider the possibility that flanking sequences might affect the recruitment of phosphatases by the phosphorylated ITIM. To examine this possibility, we constructed GST
**Fig. 1.** Recruitment of SHIP1, but not SHP-1, by FcγRIIB in mast cells and in B cells after coaggregation with FcεRI or BCR, respectively. A, co-precipitation of phosphatases with FcγRIIB1 in RBL-2H3, IIA1.6 and K46μ transfectants. 6.5 × 10⁷ RBL-2H3 transfectants expressing FcγRIIB1 were incubated with 2.4G2 F(ab')², sensitized or not with IgE anti-DNP, and challenged or not with TNP-MAR F(ab')² for 5 min. 6.5 × 10⁷ IIA1.6 and K46μ transfectants expressing FcγRIIB1 were stimulated with 0.3 μg RAM F(ab')² or RAM IgG for 5 min. B, kinetics of phosphatase recruitment by FcγRIIB1 in RBL transfectants. 6.5 × 10⁷ RBL-2H3 transfectants expressing FcγRIIB1, incubated with 2.4G2 F(ab')², and sensitized or not with IgE anti-DNP, were challenged or not with TNP-MAR F(ab')² for the indicated periods of time. Cells were lysed and FcγRIIB were immunoprecipitated. Immunoprecipitated material was fractionated by SDS-PAGE, transferred onto Immobilon, and Western blotted with anti-FcγRIIB, anti-Tyr(P), anti-SHIP1, and anti-SHP-1 antibodies. Whole cell lysates (WCL) corresponding to 5 × 10⁷ cells were used as positive controls for Western blotting with anti-phosphatase antibodies.

**Fig. 2.** Differential in vitro binding of SHP-1 and SHIP1 to phosphorylated FcγRIIB ITIM. A, SHIP1 and SHP-1 binding to beads coated with FcγRIIB ITIM peptides in which the proportion of phosphorylated peptides (pITIM) varied. 12.5-μl agarose beads were coated with 3.2 nmol of a mixture of non-phosphorylated and phosphorylated ITIM peptides (pITIM), in variable proportions. Beads were incubated with RBL-2H3 cell lysate corresponding to 1 × 10⁷ cells. Precipitated material was fractionated by SDS-PAGE, transferred onto Immobilon, and Western blotted with anti-FcγRIIB, anti-Tyr(P), anti-SHIP1, and anti-SHP-1 antibodies. B, SHIP1 and SHP-1 binding to GST-ITIM and GST-ICIIB1 phosphorylated in vitro by Lyn for increasing periods of times. Agarose beads coated with GST fusion proteins containing either the same ITIM containing 13 amino acids as synthetic peptides (GST-ITIM) or the whole intracytoplasmic domain of the FcγRIIB1' isoform (GST-ICIIB1') were incubated for increasing periods of time with Lyn. Beads were incubated with RBL-2H3 cell lysate corresponding to 1 × 10⁷ cells. Precipitated material and whole cell lysate (WCL) were fractionated by SDS-PAGE, transferred onto Immobilon, and Western blotted with anti-GST, anti-Tyr(P), anti-SHIP1, and anti-SHP-1 antibodies.
Differential Phosphatase Binding and Recruitment by FcγRIIB

The Density of Phosphorylated ITIM Critically Determines the Cooperative Binding of the Two SH2 Domains—A major difference between SHIP1 and SHP-1 is that the phosphatidylinositol phosphatase contains one SH2 domain whereas the protein tyrosine phosphatase contains two tandem SH2 domains. We investigated the possible role of this difference in the differential binding of these phosphatases to pITIM-coated beads. Agarose beads were coated with increasing concentrations of FcγRIIB pITIM and incubated either with RBL cell lysates or with purified SH2 domain-containing GST fusion proteins. These were the SH2 domain of SHIP1 (GST-SH2 SHIP1), the two SH2 domains of SHP-1 (GST-SH2 (N+C) SHP-1), the N-terminal SH2 domain of SHP-1 (GST-SH2 (N) SHP-1), or the C-terminal SH2 domain of SHP-1 (GST-SH2 (C) SHP-1). Phosphatases precipitated from cell lysates were identified by Western blotting with anti-SHIP1 and anti-SHP-1 antibodies. GST fusion proteins precipitated were identified by Western blotting with anti-GST antibodies. SHP-1 precipitation became detectable with beads coated with as little as 0.2 nmol of pITIM and slowly increased with the amount of pITIM coated to beads. SHP-1 precipitation became detectable with beads coated with 0.4 nmol of pITIM and rapidly increased with the amount of pITIM. Parallel variations in binding were observed for GST-SH2 SHIP and GST-SH2 (N+C) SHP-1. No binding of GST-SH2 (N) SHP-1 was detectable, whatever the amount of pITIM coated to beads, and a faint binding of GST-SH2 (C) SHP-1 was observed for beads coated with high amounts of pITIM only (Fig. 3A). The differential binding of phosphatases, in a cell lysate, can therefore be reproduced using GST-SH2 fusion proteins. The single SHIP1 SH2 domain bound readily to ITIM-coated beads, but not isolated SHP-1 SH2 domains. The two SHP-1 SH2 domains, however, bound with a similar pattern as SHP-1 in a cell lysate, indicating the requirement for a cooperative binding between the two SH2 SH2 domains. When using increasing amounts of pITIM to coat beads, one increases not only the quantity of pITIM bound to beads, but also the density of pITIM on beads. To investigate whether pITIM density may determine the binding of the two SH2 domain-containing SHP-1, the same amounts of pITIM were used to coat different amounts of beads. These were incubated in RBL cell lysate, and phosphatase binding was then examined as above. For a given amount of pITIM, the binding of SHIP1 did not vary with the amount of beads. By contrast, for
Based on the above results, we wondered 1) whether the concentration of immune complexes does not enable SHP-1 to be recruited with FcRIIB, and 2) whether phosphorylation levels induced under these conditions might enable FcγRIIB to recruit SHP-1 in vivo.

We first tried to increase FcγRIIB phosphorylation by stimulating FcγRIIB1-transfected IIA1.6 cells with increasing concentrations of RAM IgG and, as negative controls, with the same molar concentrations of RAM F(ab')2. FcγRIIB1 were immunoprecipitated, their phosphorylation was assessed by Western blotting with anti-phosphotyrosine antibodies, and co-precipitated phosphatases were examined by Western blotting with anti-phosphatase antibodies. FcγRIIB1 phosphorylation induced by a RAM IgG concentration as high as 2.4 μM (360 μg/ml) was not higher than FcγRIIB1 phosphorylation induced by 0.3 μM (45 μg/ml) RAM IgG. Comparable amounts of SHP1 co-precipitated with phosphorylated FcγRIIB1, whatever the concentration of RAM IgG, but no detectable recruitment of SHP-1 (Fig. 4).

This negative result led us to use IgE immune complexes, which are the natural ligands of FcγRIIB. FcγRIIB1, to coaggregate these receptors with ITAM-bearing receptors in mast cells and B cells. FcεRI and FcγRIIB expressed constitutively in BMMCs (42) were coaggregated by challenging cells, sensitized with increasing concentrations of mouse anti-DNP IgE, with immune complexes made with increasing concentrations of DNP-BSA and of a monoclonal mouse IgG1 anti-DNP antibody. FcγRIIB1 phosphorylation increased with the concentration of IgE used for sensitization and with the concentration of IgG in immune complexes. It also varied with the concentration of DNP-BSA in immune complexes, and optimal concentrations depended on the concentration of IgG antibodies (Fig. 5A).

Likewise, FcγRIIB were coaggregated with BCR in FcγRIIB1-transfected K46μ B cells, which express an anti-NP BCR, by incubating cells with immune complexes made of increasing concentrations of polyclonal rabbit IgG anti-DNP and of NP-BSA-TNP. FcγRIIB1 phosphorylation varied with the concentration of IgG antibody and antigen: it peaked with higher concentrations of antigen as the antibody concentrations increased. Immune complexes that induced the highest FcγRIIB1 phosphorylation were made of 10 μg/ml IgG and 1 μg/ml NP-BSA-TNP (Fig. 5B).

The co-recruitment of SHP-1 and SHIP1 co-precipitated with phosphorylated FcγRIIB both in mast cells and in B cells, but not SHP-1 (Fig. 5C). The above results altogether indicate that even when using concentrations of ligands that were optimal for FcγRIIB phosphorylation, SHIP1 but not SHP-1 co-recruitment was detectable, both in mast cells and in B cells.

FcγRIIB Phosphorylation following Pervanadate Treatment Enables SHP-1 Recruitment — A possible reason explaining the absence of detectable recruitment of SHP-1 by FcγRIIB phosphorylated following stimulation with high concentrations of extracellular ligands was that FcγRIIB phosphorylation levels reached under these conditions were not high enough. We therefore compared the effect of coaggregating FcγRIIB with FcεRI, in RBL transfected cells, or with BCR, in IIA1.6 and K46μ transfectants, and of treating the same three cells with pervanadate.

FcγRIIB1-expressing RBL cells were sensitized with mouse IgE anti-DNP and incubated with 2.4G2 F(ab')2, treated or not with pervanadate and challenged or not with TNP-MAR-F(ab')2. Likewise, FcγRIIB1-expressing IIA1.6 and K46μ cells were treated or not with pervanadate and challenged with RAM F(ab')2 or IgG. FcγRIIB1 phosphorylation and phosphatase recruitment were assessed as in previous experiments. As expected, FcγRIIB1 phosphorylation was induced by coaggregating FcγRIIB1 with FcεRI in RBL transfected cells, and by coaggregating FcγRIIB1 with BCR in IIA1.6 and K46μ transfectants. In all three cells, pervanadate treatment alone induced a much higher level of FcγRIIB phosphorylation that did not further increase by coaggregating FcγRIIB with FcεRI or with BCR. SHIP1 co-precipitated also with FcγRIIB phosphorylated following their coaggregation with FcεRI or with BCR. SHIP1 co-precipitated also with FcγRIIB phosphorylated following treatment of cells with pervanadate (in higher amounts than following coaggregation of FcγRIIB with FcεRI, in RBL cells, or with BCR, in K46μ cells). SHP-1 did not co-precipitate with FcγRIIB phosphorylated following their coaggregation with

| RAM IgG or F(ab')2 concentrations (μM) | 0.3 | 0.6 | 1.2 | 2.4 |
|----------------------------------------|-----|-----|-----|-----|
| Blot a-FcγRIIB1                       |     |     |     |     |
| Blot a-pY                              |     |     |     |     |
| Blot a-SHIP1                           |     |     |     |     |
| Blot a-SHP-1                           |     |     |     |     |
| WCL                                    |     |     |     |     |

**Fig. 4. Absence of co-precipitation of SHP-1 with FcγRIIB1 in IIA1.6 transfectants stimulated with increasing concentrations of RAM IgG.**

**6.5 × 10⁶ IIA1.6-FcγRIIB1 cells were stimulated with increasing concentrations of RAM IgG. Cells were lysed and FcγRIIB were immunoprecipitated. Immunoprecipitated material and whole cell lysate (WCL) were fractionated by SDS-PAGE, transferred onto Immobilon, and Western blotted with anti-FcγRIIB, anti-Tyr(P), anti-SHIP1, and anti-SHP-1 antibodies.
FcRI or BCR. SHP-1, however, co-precipitated with FcγRIIB phosphorylated following pervanadate treatment in all three cells (Fig. 6A). Treating FcγRIIB1-expressing RBL transfec-ntants with decreasing concentrations of pervanadate induced a dose-dependent tyrosyl phosphorylation of FcγRIIB1. Interestingly, as FcγRIIB1 phosphorylation decreased, the co-precipitation of SHP-1 was lost before that of SHIP1 (Fig. 6B). Treating cells with pervanadate, but not coaggregating FcγRIIB with ITAM-bearing receptors, could therefore induce a phosphorylation of FcγRIIB that was high enough to enable the recruitment of SHP-1.

**DISCUSSION**

We show here that murine FcγRIIB recruit the inositol 5-phosphatase SHIP1, but not the protein-tyrosine phosphatase SHP-1 in vivo, although the FcγRIIB ITIM has an affinity for both phosphatases in vitro, because the binding of SHP-1 requires a higher degree of FcγRIIB phosphorylation than the binding of SHIP1. The same phosphorylation-dependent preference for SHIP1 was observed 1) in vitro using beads coated with suboptimal concentrations of pITIM, 2) in vitro using GST-ICIB11 phosphorylated by Lyn for short periods of time, 3) in vivo using phosphorylated FcγRIIB precipitated from cells treated with low concentrations of pervanadate, and 4) in vivo, when FcγRIIB was phosphorylated following coaggregation with BCR or FcεRI, in B cells and in mast cells, respectively. Our results suggest that, depending on their level of phosphorylation, FcγRIIB could potentially use the two phosphatases, with different consequences.

Evidence that, when tyrosyl phosphorylated, the FcγRIIB ITIM has an affinity for SH2 domain-containing phosphatases was first provided in 1995 by D’Ambrosio et al. (30) who dem-
onstrated that phosphorylated synthetic peptides containing the FcγRIIB ITIM precipitated several molecular species from [35S]methionine-labeled cell lysates, one of which was identi-

Fig. 5. Absence of co-precipitation of SHP-1 with FcγRIIB in cells stimulated with increasing concentrations of immune complexes. A, FcγRIIB phosphorylation in BMMCs stimulated with increasing concentrations of ligands. 1 × 10⁷ BMMCs were sensitized with a constant (1/10; left panel) or various dilutions of mouse IgE anti-DNP supernatant (right panel). Cells were then stimulated for 5 min with preformed immune complexes made with a constant (20 µg/ml; right panel) or increasing concentrations of DNP-BSA (left panel) and with increasing concentrations of monoclonal mouse IgG, anti-DNP antibody. B, FcγRIIB phosphorylation in K46μ-FcγRIIB1 stimulated with increasing concentrations of ligands. 1 × 10⁷ K46μ transfectants expressing both an anti-NP BCR and FcγRIIB1 (K46μ-FcγRIIB1) were stimulated for 5 min with preformed immune complexes made with increasing concentrations of NP-BSA-TNP and with increasing concentrations of rabbit polyclonal IgG anti-DNP. C, SHIP1 and SHP-1 co-precipitation when FcγRIIB is optimally phosphorylated by immune complexes. 6.5 × 10⁷ BMMCs sensitized with mouse IgE anti-DNP supernatant (diluted 1/10) were stimulated or not with immune complexes made with 5 µg/ml DNP-BSA and 50 µg/ml mouse IgG, anti-DNP (final concentrations). K46μ-FcγRIIB1 cells were stimulated for 5 min with 10 µg/ml rabbit IgG anti-DNP or with immune complexes made with 1 µg/ml NP-BSA-TNP and 10 µg/ml rabbit IgG anti-DNP (final concentrations). For comparison, K46μ transfectants were also stimulated with 0.3 µM RAM IgG or F(ab’)2. BMMCs and K46μ transfectants were lysed after stimulation, and FcγRIIB were immunoprecipitated. Immunoprecipitated materials and whole cell lysate from K46μ transfectants (WCL) were fractionated by SDS-PAGE, transferred onto Immobilon, and Western blotted with anti-FcγRIIB, anti-Tyr(P) (A, B, and C), anti-SHIP1 and anti-SHP-1 antibodies (C).
was recently found to bind also to phosphorylated FcγRIIB ITIM (27, 29). It follows that phosphorylated FcγRIIB ITIM peptides can bind all four known SH2 domain-containing phosphatases in vitro.

In the same 1995 paper, D’Ambrosio et al. (30) reported that SHP-1 co-precipitated with FcγRIIB bearing an intact ITIM, following coaggregation with BCR in A20 and in IIA1.6 B cells reconstituted with FcγRIIB, and that FcγRIIB-dependent inhibition of B cell proliferation was impaired in B cells from SHP-1-deficient motheaten mice. In 1996, Ono et al. (26) reported that SHIP1 co-precipitated with FcγRIIB following coaggregation with FceRI in BMMCs or with BCR in A20 cells, and that FcγRIIB-dependent inhibition of IgE-induced serotonin release was unaffected in BMMCs derived from moth-eaten mice. Fong et al. (32) reported that SHIP1, but not SHP-1 or SHP-2, co-precipitated with FcγRIIB following coaggregation with FceRI in BMMCs. In 1997, Ono et al. (33) showed that FcγRIIB-dependent inhibition of Ca2+ responses and of NF-AT activity was abolished in SHIP1-deficient, but not in SHP-1-deficient, DT40 chicken B cells, and that SHIP1, but not SHP-1, was detectably co-precipitated with FcγRIIB following coaggregation with BCR in A20 cells. In 1998, however, Sato et al. (31) observed the co-precipitation of both SHP1 and SHP-1 with FcγRIIB in A20 cells expressing an anti-TNP BCR following coaggregation with intact anti-idiotype antibodies. Contrasting with the consensus that FcγRIIB recruit SHIP1 both in B cells and in mast cells, their ability to recruit SHP-1 in vivo therefore remains controversial.

That SHP-1 was found by two groups to co-precipitate with FcγRIIB in B cells, but not in mast cells, suggested the possibility that some discrepancies might be accounted for by a cell type-specific differential in vivo recruitment. To address this issue, we examined the co-precipitation of SHP-1 with recombinant FcγRIIB1 stably expressed by transfecting the same cDNA into the rat mast cells RBL-2H3, and into the two FcγRIIB-deficient mouse lymphoma B cells IIA1.6 and K46μ. The coaggregation of FcγRIIB1 with FceRI or with BCR induced a comparable tyrosyl phosphorylation of FcγRIIB1 and the co-precipitation of SHIP1, but not of SHP-1, in all three cells. The same result, observed at 5 min in the three cell lines, was also observed between 15 s and 15 min in RBL cells. Failure to detect SHP-1 co-precipitation cannot be accounted for an insufficient sensitivity of Western blotting because traces of SHP-1 could be seen on overexposed films, but in equal amounts in unstimulated and in stimulated cells (data not shown). Due to experimental conditions inherent to the co-precipitation technique, however, we cannot exclude that SHP-1, possibly recruited by FcγRIIB in vivo was lost. Whatever the explanation, we observed no difference between the three cells examined in which FcγRIIB preferentially, if not exclusively, recruited SHIP1. There is therefore a discrepancy between the ability of SHP-1 and SHIP1 to bind in vitro to FcγRIIB pITIM peptides and to co-precipitate with phosphorylated FcγRIIB in vivo.

Due to the different experimental conditions used for the two assay systems many differences can possibly explain this discrepancy. One difference could bear on the level of ITIM phosphorylation. Indeed, all ITIMs are phosphorylated on beads used for in vitro binding assay whereas an unknown proportion of FcγRIIB are phosphorylated following coaggregation with ITAM-bearing receptors. To explore the possible role of quantitative differences in ITIM phosphorylation, we studied the binding of SHP1 and SHP-1 to beads coated with FcγRIIB ITIMs phosphorylated in varying proportions with GST fusion proteins containing the SH2 domain of SHIP1 or the two SH2 domains of SHP-1. The use of SH2 domains permitted comparison between the binding of the two molecules using the same anti-GST antibodies for blotting. It also excluded that phosphatase binding was mediated by unknown intermediates.
present in cell lysates. Supporting these results, the FcγRIIB ptTIM was reported to have a higher affinity for the SH2 domain of SHP1 than for the two SH2 domains of SHP-1, when measured by Biorex analysis (43). Results obtained in these two sets of experiments are reminiscent of the selective in vivo recruitment of SHP1 by FcγRIIB.

Based on data previously reported by others (43, 44), the binding of SHP-1 is likely to involve the two tandem SH2 domains of this phosphatase. Compared with GST fusion proteins containing the two SH2 domains of SHP-1, no GST fusion proteins containing the N-terminal domain of SHP-1 and minute amounts of GST fusion proteins containing the C-terminal SH2 domain of SHP-1 bound to ptTIM-coated beads, whatever the concentration of peptides on beads. This suggests that, since there is one tyrosine only in ptTIM, GST fusion proteins containing the two SHP-1 SH2 domains bound to adjacent ptTIMs on the same bead. The same holds for the binding of SHP-1 when incubating ptTIM-coated beads with a cell lysate. If so, variations in the affinity of SHP-1 to beads coated with increasing amounts of ptTIM might depend on the density of peptides coated to beads. To examine this possibility, we used a constant amount of ptTIM to coat variable numbers of beads that were incubated in a cell lysate, and we compared the ability of these beads to bind SHP1 and SHP-1. The binding of SHP1 was proportional to the amount of ptTIM on beads, and did not vary with the ptTIM density. By contrast, the binding of SHP-1 depended not only on the amount of ptTIM but also, critically, on the density of ptTIM bound to beads. The in vitro binding of SHP-1 therefore requires a cooperative binding of its two SH2 domains to two adjacent ptTIMs in trans, and this feature explains that ptTIMs need to be closer to each other for enabling the binding of SHP-1 than for enabling the binding of SHP1.

Another difference that might explain the discrepancy between the in vitro binding and the in vivo recruitment of SHP-1 is that isolated ITIMs are used in vitro whereas whole receptors are used in vivo. One cannot exclude that the recruitment of SHP-1 might be hampered by non-ITIM sequences or by molecules that could possibly bind to these sequences. Supporting this possibility, the N-terminal KIR2DL3 ITIM that could recruit SHP-2 in vivo, when kept in its original context, failed to recruit this phosphatase, when transposed in the intracytoplasmic domain of FcγRIIB1 (29). To answer this question, we compared the ability of GST fusion proteins containing the FcγRIIB ITIM only or the whole intracytoplasmic domain of FcγRIIB1 to bind SHP1 and SHP-1, when incubated with a cell lysate, following their phosphorylation with Lyn for various periods of time. No difference was observed between the two fusion proteins and, like the isolated ITIM, the intracytoplasmic domain of FcγRIIB could bind SHP-1 when high enough phosphorylated.

Based on the latter result, we searched for experimental conditions that would induce a FcγRIIB phosphorylation sufficient to enable them to recruit SHP-1 in vivo. To this aim, we used several extracellular ligands including IgG immune complexes that are the physiological ligands of FcyRRIIB, at various concentrations, in mast cells and in B cells. We found that indeed, the phosphorylation of FcγRIIB varied with the concentrations of antibody in immune complexes but that ligands which induced a maximal phosphorylation of FcγRIIB readily induced the recruitment of SHP1 but failed to induce a detectable recruitment of SHP-1. Based on our results of in vitro binding with ptTIM-coated beads, this suggests that a small proportion (less than 12%) of FcγRIIB become tyrosyl phosphorylated in vivo upon coaggregation with ITAM-bearing receptors by physiological ligands. If so, we wondered whether FcγRIIB phosphorylation would reach a level high enough to enable the recruitment of SHP-1 following treatment of cells with pervanadate.

In both mast cells and B cells, pervanadate treatment indeed induced a higher degree of FcγRIIB phosphorylation than co-aggregation with FcεRI or BCR, respectively, and under these conditions, not only SHP1 but also SHP-1 co-precipitated with phosphorylated FcγRIIB. This observation indicates that, in resting cells, FcγRIIB are tyrosyl phosphorylated but that protein-tyrosine phosphatases maintain this phosphorylation below the detection level. This implies that FcγRIIB are the substrates of both protein-tyrosine kinases and phosphatases and that, under resting conditions, the effect of phosphatases is dominant over that of kinases. FcγRIIB phosphorylation observed following their coaggregation with ITAM-bearing receptors results from the additional effect of a Src kinase, brought by activating receptors (13), leading to a displacement of the balance so that the effect of kinases becomes dominant over that of phosphatases. It should be emphasized that the higher intensity of FcγRIIB phosphorylation induced by pervanadate, compared with phosphorylation induced by coaggregation, may be due to the phosphorylation of a higher number of receptors and/or to the phosphorylation of a higher number of tyrosine residues in each receptor. The recruitment of SHP-1 by FcγRIIB phosphorylated after pervanadate treatment may indeed simply be explained by a quantitatively different phosphorylation, resulting in an increased density of phosphorylated ITIMs that might permit the binding in trans of the two SHP-1 SH2 domains. Supporting this interpretation, the recruitment of SHP-1 was lost before that of SHP1 when pervanadate-induced FcγRIIB phosphorylation decreased following treatment of cells with decreasing concentrations of pervanadate. Alternatively, the recruitment of SHP-1 after pervanadate treatment may be explained by a qualitatively different phosphorylation of FcγRIIB, enabling SHP-1 to be recruited through the binding in cis of its two SH2 domains to two tyrosines borne by the same receptor. The intracytoplasmic domain of FcγRIIB contains four tyrosine residues. Supporting this possibility, the recruitment of SHP-1 by KIR2DL3 was found to require the conservation of its two ITIMs (39) and all ITIM-bearing receptors that were shown to recruit SHPs in vivo bear more than one ITIM. Finally, SHP-1 may co-precipitate with FcγRIIB phosphorylated following pervanadate treatment because, under these conditions, the enzymatic activity of the phosphatase is inhibited. Several ITIM-bearing receptors were shown both to recruit and to be the substrates of SHP-1 (45) or SHP-2 (46, 47). If recruited by phosphorylated FcγRIIB under physiological conditions, SHP-1 might thus decrease FcγRIIB phosphorylation, thereby giving an advantage for the recruitment of SHP over that of SHP-1.

The latter interpretation of the effect of pervanadate may explain our failure to co-precipitate SHP-1 with FcεRIIB phosphorylated upon coaggregation with ITAM-bearing receptors. This hypothesis would also endow FcγRIIB with additional regulatory properties. These receptors could indeed transiently recruit SHP-1 which could dephosphorylate not only ITIMs, but also ITAMs and other signaling molecules whose phosphorylation is critical for positive signaling. This would have important consequences. By displacing the balance between kinases and phosphatases recruited to the receptor complex in favor of the latter, it would increase the signaling threshold and/or dampen activation signals. FcγRIIB might thus use two different mechanisms, i.e. SHIP-mediated and SHP-mediated, to adjust negative regulation to the intensity of extracellular signals. By decreasing positive signals, SHP-1 would in turn decrease ITIM phosphorylation bringing FcγRIIB back to con-
ditions under which they recruit SHIPs. It remains to be determined whether FcγRIIB could be phosphorylated enough to recruit SHP-1 under physiological or pathological situations such as diseases associated with exaggerated antibody responses or immune complexes.

Acknowledgments—We thank Dr. John C. Cambier for K46m cells expressing an anti-NP BCR, NP-BSA-TNF, and polyclonal anti-C-terminal sequences of SHP-1 antibodies, Prof. Catherine Sautès-Fridman for polyclonal antibodies to the extracellular domains of FcγRIIB, Odile Malbec (Institut Curie, Paris, France) for GST-ICIB1, Dr. Eric Vivier for SHP-1 SH2 domain-containing GST fusion proteins, and Dr. Jean-Luc Teillaud for monoclonal anti-GST antibodies. We acknowledge the expert assistance of Zosia Maciorowski for cells sorting.

REFERENCES

1. Daeón, M. (1997) Annu. Rev. Immunol. 15, 203–234
2. Daeón, M., Latour, S., Malbec, O., Espinosa, E., Fina, P., Pasmans, S., and Fridman, W. H. (1995) Immunity 2, 635–646
3. Malbec, O., Fridman, W. H., and Daeón, M. (1999) J. Immunol. 162, 4424–4429
4. Takai, T., Ono, M., Hikida, M., Ohmori, H., and Ravetch, J. V. (1996) Nature 379, 346–349
5. Ujike, A., Ishikawa, Y., Ono, M., Yuasa, T., Yoshino, T., Fukumoto, M., Ravetch, J., and Takai, T. (1999) J. Exp. Med. 189, 1573–1579
6. Yuasa, T., Kubo, S., Yoshino, T., Ujike, A., Matsumura, K., Ono, M., Ravetch, J. V., and Takai, T. (1999) J. Exp. Med. 189, 187–194
7. Clunes, R., Maizes, J. S., Guinamard, R., Ono, M., Takai, T., and Ravetch, J. V. (1999) J. Exp. Med. 188, 179–185
8. Nakamura, A., Yuasa, T., Ujike, A., Ono, M., Nukiwa, T., Ravetch, J. V., and Takai, T. (2000) J. Exp. Med. 191, 899–905
9. Clunes, R. A., Towers, L. T., Presta, L. G., and Ravetch, J. V. (2000) Nat. Med. 6, 443–446
10. Sinclair, N. R. S. C., and Chan, P. L. (1971) Adv. Exp. Med. Biol. 12, 609–615
11. Daeón, M., Latour, S., Malbec, O., Arock, M., and Fridman, W. H. (1995) J. Clin. Invest. 95, 577–585
12. Bely, M., Delon, J., Trautmann, A., Cambiaggi, A., Olcese, L., Biassoni, R., Moretta, L., Chavrier, P., Moretta, A., Daeón, M., and Ravetch, J. V. (1997) EMBO J. 16, 449–455
13. Binstadt, B. A., Billadeau, D. D., Jevremovic, D., Williams, B. L., Fang, N., Yi, T., Koretsky, G. A., Abraham, R. T., and Leibson, P. J. (1998) J. Biol. Chem. 273, 27518–27523
14. Roehrsmüller, L. R., Fuller, J. F., Wolf, I., Liu, Y., and Lucas, D. M. (2000) Immunity 12, 347–355
15. Binstadt, B. A., Billadeau, D. D., Jevremovic, D., Williams, B. L., Fang, N., Yi, T., Koretsky, G. A., Abraham, R. T., and Leibson, P. J. (1998) Immunity 8, 509–516
16. Scharenberg, A. M., El-Hillal, O., Fruman, D. A., Beitz, L. O., Li, Z., Lin, S., Gout, I., Cantley, L. C., Rawlings, D. J., and Kinet, J.-P. (1998) EMBO J. 17, 1961–1972
17. Tamir, I., Stolpa, J. C., Holgason, C. D., Nakamura, K., Bruhns, P., Daeón, M., and Cambier, J. C. (2000) Immunity 12, 347–355
18. D’Ambrosio, D., Hinnen, K., Shinoff, S. A., Munn, I., Pani, G., Siminovitch, K. A., and Cambier, J. C. (1995) Science 268, 293–296
19. Sato, K., and Ochi, A. (1998) J. Immunol. 161, 2716–2722
20. Fong, D. C., Malbec, O., Arock, M., Cambier, J. C., Fridman, W. H., and Daeón, M. (1996) Immunity 5, 83–91
21. Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J. V. (1997) Cell 89, 293–301
22. Barsumian, E. L., Isersky, C., Petrinio, M. G., and Siragian, R. P. (1981) Eur. J. Immunol. 11, 317
23. Jones, J., Hombach, J., Radbruch, A., Riesterer, C., and Reth, M. (1990) J. Immunol. 145, 1365–1373
24. Liu, T. T., Bohn, J. W., Ferry, E. L., Yamamoto, H., and Molinaro, C. A. (1980) J. Immunol. 124, 2728
25. Unkeless, J. C. (1979) J. Immunol. 123, 580–596
26. Benhamou, M., Bonnerot, C., Fridman, W. H., and Daeón, M. (1999) J. Immunol. 162, 3168–3175
27. Liu, T. T., Bohn, J. W., Ferry, E. L., Yamamoto, H., and Molinaro, C. A. (1980) J. Immunol. 124, 2728
28. Famiglietti, S., Nakamura, K., and Cambier, J. C. (1999) Immunity 68, 35–40
29. Pei, D., Wang, J., and Walsh, C. T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1141–1145
30. Adachi, T., Flaswinkel, H., Yakuha, H., Reth, M., and Tsuhta, T. (1998) J. Immunol. 160, 4662–4665
31. Kharitonenkov, A., Chen, Z., Sures, I., Wang, H., Schilling, J., and Ullrich, A. (1997) Nature 386, 181–186
32. Zhao, R., and Zhao, Z. J. (2000) J. Biol. Chem. 275, 5453–5459
Insufficient Phosphorylation Prevents FcγRIIB from Recruiting the SH2 Domain-containing Protein-tyrosine Phosphatase SHP-1

Renaud Lesourne, Pierre Bruhns, Wolf H. Fridman and Marc Daëron

J. Biol. Chem. 2001, 276:6327-6336.
doi: 10.1074/jbc.M006537200 originally published online November 30, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M006537200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 47 references, 22 of which can be accessed free at http://www.jbc.org/content/276/9/6327.full.html#ref-list-1