

Fc γ RIIB as a potential molecular target for intravenous gamma globulin therapy

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The ability of the immune system to respond appropriately to foreign antigen is dependent on a delicate balance of activating and inhibitory signals. Recently, the role of cell surface inhibitory receptors in attenuating immune responses, thereby preventing pathologic conditions including autoimmunity and atopy, has been recognized. It is postulated that the beneficial effects of intravenous gamma globulin in the treatment of immune disorders may be attributable, at least in part, to engagement of Fc γ RIIB, a member of the recently described family of immune inhibitory receptors. Recent genetic and biochemical studies have identified the SH2 domain-containing inositol 5-phosphatase (SHIP) as a critical effector in Fc γ RIIB inhibitory signaling. This review summarizes recent work from our laboratory and others aimed to define the mechanism(s) by which Fc γ RIIB and its effector, SHIP, inhibit immune responses. Elucidation of these mechanisms may lead to the development of therapeutic strategies for the treatment of autoimmune and inflammatory pathologies that specifically target Fc γ RIIB or its effector(s). (*J Allergy Clin Immunol* 2001;108:S95-8.)

Key words: IVIG, Fc γ RIIB, SHIP, inhibitory receptors, signal transduction

The immune system is tightly regulated by both activating signals, which trigger appropriate immune responses, and inhibitory signals, which terminate these responses when they are no longer needed. In the absence of inhibitory signals, immune responses can proceed unchecked, resulting in pathologic conditions such as autoimmunity and atopy. A growing family of structurally related receptors that function to inhibit immune responses has recently been described.¹ Members of this family of inhibitory receptors are single-pass transmembrane receptors that contain either C-type lectin (eg, CD22, Ly49, NKG2, MAFA) or immunoglobulin-like (eg, Fc γ RIIB, KIR, gp49B1, ILT/LIR/MIR, LAIR, PIR-B, SIRP α) extracellular domains. The most characterized

Abbreviations used

BCR:	B-cell receptor
Btk:	Bruton's tyrosine kinase
Ig:	Immunoglobulin
Ins:	Inositol
ITIM:	Immunoreceptor tyrosine-based inhibition motif
IVIG:	Intravenous gamma globulin
PI3-K:	Phosphatidylinositol 3-kinase
PLC γ :	Phospholipase C γ
PtdIns:	phosphatidylinositol
SHIP:	SH2 domain-containing inositol 5-phosphatase
SHP:	SH2 domain-containing protein tyrosine phosphatase
TCR:	T-cell receptor

member of this family is Fc γ RIIB, a low-affinity receptor for immunoglobulin G (IgG). Fc γ RIIB is expressed in numerous hematopoietic cell types and inhibits cellular activation when coaggregated with B-cell receptor (BCR), T-cell receptor (TCR), "activating" Fc receptors (eg, Fc γ RIII, Fc γ RIIA, Fc ϵ RI), and cytokine receptors (eg, c-kit).^{2,3} The inhibitory function of Fc γ RIIB has been best described in B cells. Here, coaggregation of Fc γ RIIB and the BCR, which occurs in vivo when B cells encounter antigen complexed with antigen-specific IgG, leads to inhibition of antigen-induced blastogenesis and proliferation and the induction of apoptosis. The importance of Fc γ RIIB inhibitory signaling in regulating immune responses is evident in Fc γ RIIB-deficient mice, which exhibit enhanced antibody production, autoimmunity, anaphylactic responses,³ and antibody-dependent clearance of tumor metastases.⁴ It is probable that some of the immunomodulatory effects of intravenous gamma globulin (IVIG) are mediated by this receptor. In this paper we describe the results of work performed in our laboratory and others aimed to define the mechanism(s) by which Fc γ RIIB inhibits immune responses. Understanding these mechanisms may lead to the development of novel therapeutic strategies for the treatment of immune disorders.

MECHANISMS OF Fc γ RIIB INHIBITORY SIGNALING FUNCTION

All members of the inhibitory receptor family contain at least 1 copy of a conserved sequence (I/VxYxxL) in their cytoplasmic domains, which is termed the "immunoreceptor tyrosine-based inhibition motif" (ITIM).³ Coaggregation with activating receptors (eg, Fc ϵ RI, BCR) results in the phosphorylation of the ITIM tyrosine by receptor-associated kinases and the subsequent recruitment of the SH2 domain-containing protein tyrosine phosphatases

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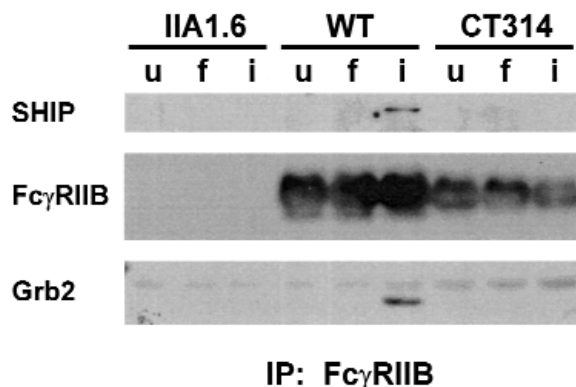


FIG 1. Coaggregation of FcγRIIB and the BCR results in FcγRIIB recruitment of SHIP and Grb2. The FcγRIIB-deficient B-cell line IIA1.6, either untransfected (IIA1.6), transfected with wild-type FcγRIIB (WT), or transfected with FcγRIIB that lacks the C-terminal 16 amino acids (CT314) were used in this experiment. Cells were either left untreated (u) or stimulated with equimolar amounts of either rabbit anti-mouse F(ab')₂ (to aggregate the BCR) or rabbit anti-mouse IgG (to coaggregate the BCR and FcγRIIB) for 5 minutes at 37°C. Cells were lysed in 1% NP-40 lysis buffer for 10 minutes on ice, followed by centrifugation to remove the insoluble material. FcγRIIB was isolated from cleared lysates by immunoprecipitation with an anti-FcγRIIB antibody and protein A-conjugated Sepharose beads. Immune complexes were resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membrane. FcγRIIB, SHIP, and Grb2 were detected by immunoblotting with specific antibodies, followed by horseradish peroxidase (HRP)-conjugated secondary antibodies and detection with enhanced chemiluminescence (ECL) and autoradiography.

SHP-1 (65 kDa) and SHP-2 (70 kDa) and the SH2 domain-containing inositol 5-phosphatases SHIP1 (145 kDa) and SHIP2 (160 kDa).^{3,5} Tyrosine-phosphorylated peptides corresponding to the FcγRIIB ITIM bind to both SHP and SHIP phosphatases *in vitro*; however, FcγRIIB preferentially recruits SHIP1 and SHIP2 *in vivo*. Recent studies suggest that FcγRIIB recruitment of SHIPs versus SHPs may be a function of coaggregate size. For example, coaggregation of FcγRIIB and the BCR *in vivo* leads to the recruitment and activation of SHIP; however, “superclustering” of the 2 receptors results in SHP-1 activation.⁶ Further supporting this hypothesis, results from *in vitro* studies indicate that SHIP binds to phosphorylated ITIM peptides immobilized to beads over a range of peptide densities, whereas SHP-1 binds only when the density of ITIM peptides is high.⁷ Therefore, immune complexes containing varying amounts of FcγRIIB ligand (ie, IgG) may trigger distinct cellular responses via FcγRIIB recruitment of different effector molecules.

Binding of SHPs and SHIPs to FcγRIIB peptides is dependent on the phosphorylation of the ITIM tyrosine; however, additional residues within the ITIM determine effector specificity. Recent studies have demonstrated that the Y+2 leucine within the ITIM is critical for FcγRIIB binding to SHIP1 and SHIP2, but not SHP-1 or SHP-2.⁸ Conversely, mutation of the Y-2 isoleucine abrogates FcγRIIB binding to SHP-1 and SHP-2 *in vitro* but has no effect on FcγRIIB binding to SHIP1 or SHIP2 *in vitro* or *in vivo*.^{8,9}

Although the ITIM is essential for FcγRIIB inhibitory activity and recruitment of SHIP, additional residues within the FcγRIIB cytoplasmic domain have been implicated in the recruitment and activation of SHIP.¹⁰

Specifically, mutational analysis of the FcγRIIB cytoplasmic domain reveals that the receptor's C-terminal 16 amino acids are required for detectable FcγRIIB association with SHIP *in vivo* and for FcγRIIB-mediated phosphatidylinositol (PtdIns)(3,4,5)P₃ hydrolysis by SHIP (see following section). This region contains a tyrosine (Y₃₂₆) that becomes phosphorylated after FcγRIIB coaggregation with the BCR. This tyrosine is located within the sequence YQNH, which is similar to the Grb2 SH2 binding motif, YQNY, and retains the Y+2 asparagine residue that is critical for Grb2 binding.¹¹ These observations suggest that FcγRIIB may recruit Grb2, in addition to SHIP, after coaggregation with the BCR. This hypothesis is supported by recent data demonstrating that Grb2, in addition to SHIP, co-immunoprecipitates with FcγRIIB following FcγRIIB coaggregation with the BCR (Fig 1). As predicted, Grb2 does not associate with FcγRIIB that lacks the C-terminal 16 amino acids.

In addition to its proposed interaction with FcγRIIB, Grb2 reportedly binds directly to SHIP via an interaction between the Grb2 SH3 domain and proline-rich regions within the C-terminus of SHIP.¹² These interactions suggest the formation of a ternary complex between FcγRIIB, SHIP, and Grb2 that may be required for stable association of SHIP and Grb2 with FcγRIIB. Supporting this hypothesis, SHIP is not detectably associated with FcγRIIB that lacks the C-terminal 16 amino acids; however, a mild increase in SHIP phosphorylation is detected.¹⁰ These data suggest that in the absence of Grb2, SHIP associates only transiently with FcγRIIB.

MECHANISMS OF SHIP FUNCTION

Recent studies from our laboratory and others have provided insight into the mechanism(s) through which SHIP mediates FcγRIIB-inhibitory signaling.³ In B cells, coaggregation of FcγRIIB with the BCR inhibits BCR-mediated Ca²⁺ mobilization, Ins(1,4,5)P₃ production, and p21^{ras}/Erk activation. One pathway through which these biologic responses may be regulated is via the B-cell surface glycoprotein CD19. After BCR engagement, CD19 becomes phosphorylated on tyrosine and recruits phosphatidylinositol 3-kinase (PI3-K) (Fig 2). In response to FcγRIIB-BCR coaggregation, CD19 is dephosphorylated, resulting in failed recruitment and activation of PI3-K. This leads to decreased production of PtdIns(3,4,5)P₃, which is required for the recruitment and activation of PLCγ and Btk and subsequent BCR-induced Ins(1,4,5)P₃ production and Ca²⁺ mobilization. The mechanism by which FcγRIIB induces CD19 dephosphorylation is not presently known, but recent data indicate that this occurs independent of the FcγRIIB ITIM. In addition, SHIP has been implicated in the direct cleavage of PtdIns(3,4,5)P₃ to PtdIns(3,4)P₂ via its 5-phosphatase activity, which

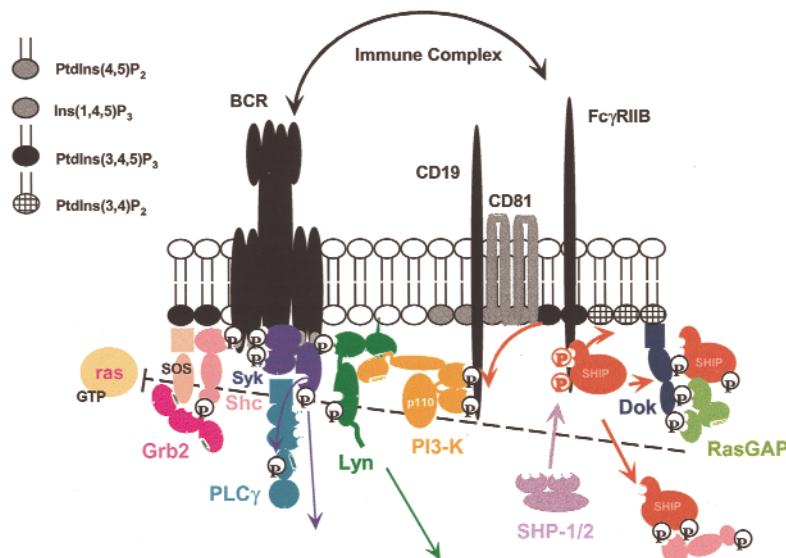


FIG 2. Schematic of BCR signal transduction and inhibition by FcγRIIB. *PtdIns*, phosphatidylinositol; *Ins*, inositol; *BCR*, B-cell receptor; *PLCγ*, phospholipase Cγ; *PI3-K*, phosphatidylinositol 3-kinase; *SHIP*, SH2 domain-containing tyrosine phosphatase; *SHP*, SH2 domain-containing inositol 5-phosphatase.

would inhibit BCR-induced activation of PLCγ and Btk. PtdIns(3,4,5)P₃ levels are reduced in B cell blasts derived from SHIP1-deficient mice after coaggregation of FcγRIIB and the BCR.¹³ This inhibition may be a result of the inhibitory function of CD19, or alternatively, it could reflect the activity of SHIP2 that is detected at low levels in these cells.

In addition to its enzymatic activity, SHIP contains 2 C-terminal NPXY motifs and several proline-rich regions that can potentially recruit signaling molecules containing phosphotyrosine-binding and SH3 domains, respectively. In B cells, coaggregation of FcγRIIB and the BCR induces the tyrosine phosphorylation of SHIP and its association with the adapter molecule Shc and the RasGAP-binding protein p62^{dok}.³ These interactions suggest 2 mechanisms by which FcγRIIB inhibits BCR-induced p21^{ras} activation (see Fig 2). By binding to Shc, SHIP is postulated to recruit Shc away from the Grb2-Sos complex, thereby inhibiting p21^{ras} activation.¹⁴ Alternatively, or in addition, SHIP may mediate FcγRIIB inhibition of p21^{ras} activation via its association with p62^{dok}.¹⁵ Coaggregation of the BCR and FcγRIIB also stimulates the tyrosine phosphorylation of p62^{dok} and its association with RasGAP, which inhibits p21^{ras} by enhancing its intrinsic GTPase activity. Supporting a role for p62^{dok} in FcγRIIB inhibitory signaling, coaggregation of the BCR with a chimeric receptor in which the cytoplasmic domain of FcγRIIB is replaced with the RasGAP-binding region of p62^{dok} inhibits antigen-induced p21^{ras}/Erk activation. In addition, FcγRIIB fails to inhibit BCR-induced Erk activation and cell proliferation in B cells from p62^{dok}-deficient mice.¹⁶ However, p62^{dok} is dispensable for FcγRIIB-mediated inhibition of

calcium mobilization, suggesting that FcγRIIB mediates this effect via hydrolysis of PtdIns(3,4,5)P₃.

SUMMARY

Elucidation of the role of FcγRIIB in negative regulation of immune responses has led investigators to hypothesize that the lack of FcγRIIB function may contribute to pathologic conditions including autoimmunity and atopic disease.² For example, engagement of FcγRIIB may somehow explain the beneficial effects of intravenous administration of Ig pooled from healthy individuals in the treatment of autoimmune disease. Coaggregation of FcγRIIB with the high-affinity IgE receptor FcεRI expressed on mast cells may also explain the beneficial effects of specific immunotherapy in the treatment of allergic disease.¹⁷ Here, increasing doses of allergen administered to atopic patients stimulates an allergen-specific IgG response, leading to the formation of allergen-IgG complexes. It is hypothesized that these complexes inhibit IgE-mediated mast cell activation by coaggregating FcγRIIB and FcεRI. IVIG may contain IgG auto-allergens that mimic the effect of repeated allergen immunization.

Based on these observations, the development of therapeutics that specifically target FcγRIIB or its effector, SHIP, may be effective in the treatment of a variety of immunologic disorders. These could be biologics, such as bispecific antibodies that constitutively tether activating and inhibitory receptors so that normally activating stimuli trigger the inhibitory loop. Small-molecule agonists of SHIP should act to inhibit all signaling responses that are dependent on PtdIns(3,4,5)P₃. This would include all inflammatory responses.

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