Human Type II Fcγ Receptors Inhibit B Cell Activation by Interacting with the p21\(^{ras}\)-dependent Pathway*

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Co-ligation of antigen receptors and type II Fcγ receptors (FcγRIIb) on B cells interrupts signal transduction and ultimately inhibits antibody production. We have identified p52 Shc in the FcγRIIb1-specific immunoprecipitates isolated from the membrane fraction of BL41 Burkitt lymphoma cells following B cell receptor-FcγRIIb co-ligation. The insolubilized synthetic peptide representing the phosphorylated form of the tyrosine-based inhibitory motif of FcγRIIb also binds Shc from the lysates of activated but not from resting BL41 cells. This suggests that the binding does not depend on the interaction of FcγRIIb1-phosphotyrosine with the SH2 domain of Shc. Tyr phosphorylation of FcγRIIb1-associated Shc is low, indicating an impaired function. It is implicated in regulating p21\(^{ras}\) activity when cross-linked with antigen receptor, membrane-bound p52 Shc is low, indicating an impaired function. We have demonstrated that protein-tyrosine-phosphatase 1C with the 13-amino acid-containing phosphorylated immunoreceptor tyrosine-based inhibitory motif (P-ITIM) of FcγRIIb1 in murine B cells has been demonstrated recently. The authors suggest that protein-tyrosine-phosphatase 1C is an effector of BCR-FcγRIIb signal transduction (3). Others proposed that co-ligation of BCR and FcγRIIb may result in closing of a Ca\(^{2+}\) channel on the cell membrane, inhibiting thereby the Ca\(^{2+}\) influx (4). The exact mechanism of FcγRIIb-mediated inhibition of B cell function is not yet completely clarified.

On human B cells, both FcγRIIb1 and FcγRIIb2 isoforms are expressed. Neither FcγRIIb1 nor FcγRIIb2 transduce activation signals for human B cells; however, we have previously demonstrated that protein-tyrosine kinase Fyn and several unidentified Tyr-phosphorylated polypeptides as well as Ser/Thr kinase activities associate with FcγRIIb isolated from BCR cross-linked BL41 Burkitt lymphoma cells (6, 7). The vast majority of FcγRII expressed on BL41 cells represents FcγRIIb1 isoform (8). We have suggested that the association of certain signaling molecules with FcγRIIb1 may modify their function when BCR and FcγRb1 are co-ligated.

According to the early experiments of Klaus et al. (2), co-ligation of BCR and FcγRI uncouples BCR from the signal-transducing machinery upstream of G proteins. The best documented role for G proteins in B cells is the activation of p21\(^{ras}\), the low molecular mass G protein, since (i) co-localization of p21\(^{ras}\) and mlg was observed after mlg cross-linking (9), (ii) the p21\(^{ras}\) signaling pathway has been shown to be operative in both human and murine B cells connecting protein-tyrosine kinase activation and the regulation of gene transcription (10), and (iii) it has recently been reported that activation of the p21\(^{ras}\) pathway couples antigen receptor stimulation to the induction of the primary response gene egr-1 in B cells (11). The activity of p21\(^{ras}\) is regulated by guanine nucleotide exchange factors and GTPase-activating protein. m-Sos-1 augments the GDP-GTP exchange and thus activates, while GTPase-activating protein inactivates, p21\(^{ras}\) (12). It was recently demonstrated that a Tyr-containing motif of the adapter molecule Shc becomes phosphorylated by the Lyn-activated Syk protein-tyrosine kinase in B cells following mlg cross-linking (13). Via the Tyr(P) residue, Shc binds to the SH2 domain of Grb-2 in the Grb-2/m-Sos-1 complex and in turn the complex translocates to the cell membrane, where p21\(^{ras}\) is located then Sos activates ras (14). Downstream of p21\(^{ras}\), a cascade of kinases becomes activated. Raf activates MAP kinase, MAPK/ERK kinase, which in turn phosphorylates and activates mitogen-activated protein kinases, MAPK (or extracellular signal-regulated kinases, ERK). Activated MAPK then phosphorylates several intracellular substrates on Ser/Thr residues, such as other Ser/Thr kinases, cytoskeletal proteins, or transcription factors (15, 16).

The effect of BCR-FcγRII co-ligation on p21\(^{ras}\)/MAPK activation...
pathway has not been investigated yet. Co-cross-linking of mlg and FcγR1II by intact anti-lg molecules has been shown to inhibit the primary response genes egr-1 and egr-2 expression in the murine B cell line, BCL1 (17). The primary response gene egr-1 encodes a sequence-specific transcription factor, the expression of which is necessary for antigen receptor-stimulated activation of B cells, and it has been demonstrated that induction of egr-1 after BCR cross-linking is mediated by activation of the p21<sup>ras</sup>/MAPK-signaling pathway (11).

The aim of this work was to define signaling molecules, the activities of which might be regulated by FcγRII-BCR co-cross-linking and which have a definite role in the ras/MAPK activation pathway. We show here that when FcγRIIB1 and BCR are co-cross-linked on human B cells, a portion of the adapter molecule Shc associates with FcγRIIb. Furthermore, we demonstrated that the Tyr-phosphorylated inhibitory motif of FcγRIIb binds Shc from activated BL41 cell lysate. This association might alter the function of Shc or Shc-associated molecules. A partial inactivation of p21<sup>ras</sup> and a reduced activity of MAPK are also observed in FcγRII-BCR co-cross-linked samples. We suggest that FcγRIIB1 on human B cells may diminish antibody production by inhibiting the ras/MAPK activation pathway.

### EXPERIMENTAL PROCEDURES

**Reagents—**Polyclonal, affinity-purified human IgG and IgM-specific antibodies, anti-mouse IgG F(ab')<sub>2</sub> fragments, biotinylated anti-mouse IgG F(ab')<sub>2</sub>, anti-human IgG + IgM antibodies, and horseradish peroxidase (HRPO)-conjugated anti-mouse and anti-rabbit IgG F(ab')<sub>2</sub> antibodies (Axel); anti-phospho-p52<sub>Shc</sub> monoclonal antibody (mAb) (clone PT66) and anti-phosphotyrosine-coated agarose beads (Sigma); Shc and MAPK-specific antibodies (Transduction Laboratories); anti-erb-K1 polyclonal antibodies (UBI); rat anti-p21<sup>ras</sup> monoclonal antibody and Lyn-specific rabbit antibodies (Santa-Cruz); CD32, CD79a, and CD79b monoclonal antibodies (PharMingen). The monoclonal antibody specific for FcγRIIa used for Western blots was a kind gift from Dr. J. Frey, Bielefeld, Germany, and polyclonal antibody recognizing mb1/Ig was a kind gift from Dr. L. Smit, Amsterdam, The Netherlands. [γ<sup>32</sup>PI]ATP, [γ<sup>32</sup>P]<sub>γ</sub>ATP, enhanced chemiluminescence reagents, and nitrocellulose membranes were purchased from Amersham, Gamma-Bind G beads and Percoll from Pharmacia, and reagents and standards for electrophoresis from Bio-Rad. Synthetic peptides representing the ITIM (AENTITYS- LLMHP) of FcγRII and the EPTYIPT (AENTITYPOH-LMLMP) of human FcγRI were synthesized on a TentaGet-NH<sub>2</sub> resin by Dr. R. Reuscher, SFI, Vienna, Austria (18).

**Cells—**BL41 Burkitt lymphoma cells were maintained in stationary culture in RPMI 1640 medium containing 10% fetal calf serum; STα86 cells were grown in RPMI 1640 medium containing 20% fetal calf serum.

**Stimulation—**3 × 10<sup>7</sup> cells were activated with 20 μg of F(ab')<sub>2</sub> fragment or intact IgG of affinity-purified rabbit anti-IgM + IgG for 2 min at 37°C, the cells were pelleted for 20 s and immediately frozen in liquid nitrogen. To cross-link BCR and FcγRII, the cells were preincubated with different doses of heat-aggregated human IgG or 2 μg of Cd32 antibodies for 10 min. In the latter case, co-cross-linking was obtained by biotinylated goat anti-mouse IgG, followed by biotinylated anti-human Ig and the addition of avidin.

**Preparation of Membrane and Cytosolic Fractions—**Samples of cells were homogenized in a buffer containing 20 mM Tris-HCl, pH 7.5, 10 mM EGTA, 0.25 mM sucrose, 20 mg/ml leupeptin, 20 mg/ml soybean trypsin inhibitor, 20 mg/ml aprotinin, 10 mM phenylmethylsulfonyl fluoride, as described (19). The homogenized samples were homogenized sample was homogenized sample was homogenized into insoluble (membrane) fraction and soluble cytosolic fraction by ultracentrifugation (100,000 × g for 40 min, at 4°C). The membrane fractions were solubilized by sonication in 1 ml of lysis buffer containing 1% Triton X-100, 50 mM HEPES (pH7.4), 100 mM NaF, 10 mM EDTA, 2 mM sodium o-vanadate, 10 mM sodium pyrophosphate, 10% glycerol, 10 μg/ml aprotinin, 5 μg/ml leupeptin, and 0.2 mM phenylmethylsulfonyl fluoride. After 60 min of incubation on ice, cell lysates were centrifuged at 15,000 × g for 20 min, and the supernatants were used. The cytosolic fractions were concentrated five times with Amicon concentrators. The solubilized membrane fractions and the concentrated cytosolic fractions were used in the subsequent immunoprecipitation procedures.
Ont panel

Recognized a co-migrating 52-kDa band in the same samples (kDa adapter molecule Shc was co-isolated with Fc antibodies, similarly to the affinity-isolated samples, the 52-kDa Shc, and not the 47-kDa form, was found in CD32 immunoprecipitates (Fig. 3). Only the 52-kDa Shc, and not the 47-kDa form, was found in CD32 immunoprecipitates (Fig. 3a). This indicates that the main Tyr-phosphorylated component and the binding of Shc to FcγRIIb - was detected by enhanced chemiluminescence. The reaction was detected by enhanced chemiluminescence.

Shc can be observed. The association required an optimal ratio of anti-Ig and aggregated IgG for co-cross-linking. Comparing phosphotyrosine- and Shc-specific Western blots, a correlation between the association of Tyr-phosphorylated molecules and the binding of Shc to FcγRIIb was detected in CD32 and CD79a mAb negative control. No cross-contamination was detected in CD32 and CD79a precipitates isolated from resting, activated, or FcγRII-BCR co-ligated samples. The Western blots (WB) were exposed to nitrocellulose membrane and consecutively probed with anti-phosphotyrosine and anti-Shc antibodies. Arrowheads, position of Shc, agg, aggregated; WB, Western blot; H, heavy chain; L, light chain.

Correlation between the association of Tyr-phosphorylated components and the binding of Shc to FcγRIIb isolated by CD32 mAb. 5 × 10⁷ BL41 cells were stimulated with various doses of reagents as indicated. CD32 were immunoprecipitated (IPPT) using CD32 mAb, and the immunocomplexes were collected by Gamma Bind protein G beads. After SDS-PAGE, the proteins were blotted into nitrocellulose membrane and consecutively probed by anti-phosphotyrosine and anti-Shc antibodies. Arrowheads, position of Shc, agg, aggregated; WB, Western blot; H, heavy chain; L, light chain.

In the following experiments, FcγRII was isolated using CD32 monoclonal antibody followed by protein G-coated beads, and the precipitated components were analyzed by Western blotting using phosphotyrosine and Shc-specific antibodies, respectively. Fig. 2 shows that co-cross-linking of BCR and FcγRII with intact rabbit IgG anti-IgM antibodies dose-dependently induced the association of a 52-kDa Tyr-phosphorylated molecule with FcγRIIb, as detected just above the heavy chain of the anti-IgM antibody (first panel). Shc-specific antibodies recognized a co-migrating 52-kDa band in the same samples (second panel). When BCR and FcγRII were co-cross-linked using aggregated IgG and F(ab)₂ fragment of anti-IgM + IgG) antibodies, similarly to the affinity-isolated samples, the 52-kDa adapter molecule Shc was co-isolated with FcγRII. The association required an optimal ratio of anti-Ig and aggregated IgG for co-cross-linking. Comparing phosphotyrosine- and Shc-specific Western blots, a correlation between the association of the main Tyr-phosphorylated component and the binding of Shc can be observed.

Similar results were obtained using a different CD32 monoclonal antibody (A10) (Fig. 3b), while we could not detect Shc in CD19 and CD79a immunoprecipitates (Fig. 3a). Only the 52-kDa Shc, and not the 47-kDa form, was found in CD32 immunoprecipitates (Fig. 3b).

Shc was detected in CD32 immunoprecipitates of BCR-FcγRII-BCR co-cross-linked samples obtained from human tonsil B cells as well (data not shown).

Although under the conditions applied, in the presence of 1% Triton X-100 in the lysis buffer, no association of Shc with CD79a was observed (Fig. 3a), to exclude the possibility that the signal-transducing subunit of BCR, mI/ls/α (CD79a) contaminates the isolated FcγRII, immunoprecipitates obtained with CD32 and CD79a mAb were compared by probing the Western blots (WB) with the reagents indicated. Cell lysates were used as positive control for Shc (lys). H, heavy chain.

**FIG. 1.** Shc associates with FcγRIIb affinity-purified from the membrane fraction of FcγRII-BCR co-ligated BL41 cells. 5 × 10⁷ cells were stimulated with 30 µg of the reagents indicated for 2 min at 37 °C. The anti-IgM F(ab)₂ antibodies recognized the heavy (H) + light (L) chain and thus were cross-reactive with human IgG. The cells were pelleted and immediately frozen in liquid nitrogen. The samples were homogenized and then fractionated into cytosolic and membrane fractions, and the latter were lysed in lysis buffer containing 1% Triton X-100. Tyr-phosphorylated molecules were isolated by anti-phosphotyrosine-coated agarose beads, and FcγRII were affinity-purified by human IgG Fe fragments covalently coupled to Sepharose beads. The eluted samples were exposed to SDS-PAGE using 10–15% continuous gradient gel, and the proteins were blotted and probed with anti-phosphotyrosine antibody followed by anti-mouse IgG F(ab)₂-HRP. The reaction was detected by enhanced chemiluminescence. b, after the first antibodies were stripped, the same membranes were reprobed using Shc-specific rabbit antibodies followed by HRPO-conjugated anti-rabbit IgG F(ab)₂ agg, aggregated; IPPT, immunoprecipitation; WB, Western blot.

**FIG. 2.** Correlation between the association of Tyr-phosphorylated components and the binding of Shc to FcγRIIb isolated by CD32 mAb. 5 × 10⁷ BL41 cells were stimulated with various doses of reagents as indicated. CD32 were immunoprecipitated (IPPT) using CD32 mAb, and the immunocomplexes were collected by Gamma Bind protein G beads. After SDS-PAGE, the proteins were blotted into nitrocellulose membrane and consecutively probed by anti-phosphotyrosine and anti-Shc antibodies. Arrowheads, position of Shc, agg, aggregated; WB, Western blot; H, heavy chain; L, light chain.

**FIG. 3.** Specificity of the association of 52-kDa Shc with FcγRIIb in the membrane of FcγRIIb-BCR co-ligated BL41 cells. The cells were treated with 30 µg of anti-IgM F(ab)₂ fragment or anti-IgM intact IgG for 2 min at 37 °C. CD19, CD79a, CD32 (a and b), and Shc (b) were immunoprecipitated (IPPT) from the cell membrane fraction by the corresponding antibodies followed by the addition of Gamma Bind protein G beads. The Western blots (WB) were probed with the reagents indicated. Cell lysates were used as positive control for Shc (lys). H, heavy chain.
negative (Fig. 4, left). Reprobing the same blots with anti-phosphotyrosine antibodies showed that P-ITIM but not ITIM representing peptide bound a major 53-kDa molecule (just above Shc) and other minor Tyr-phosphorylated components (60, 75, and 110 kDa) from both resting and activated cell samples. The 75- and 110-kDa bands can be seen in nonactivated control cells only after longer exposure. Higher Tyr phosphorylation of the P-ITIM-associated components, in particular the 75-kDa and the 110-kDa ones, was observed in activated samples as compared with resting cells (Fig. 4, middle). Further reprobing of the blots with Lyn-specific antibody indicated that the 53-kDa Tyr-phosphorylated component was identical with Lyn protein-tyrosine kinases. P-ITIM bound Lyn from both resting and activated cells (Fig. 4, right).

Co-cross-linking of BCR and FcγRII Reduces the Activity of p21^{ras} in BL 41 Cells—Shc/Grb-2/Sos complexes are implicated in controlling p21^{ras} activity and thus in the regulation of the downstream serine/threonine kinase cascade (22). BL41 cells were metabolically labeled with 32P_I, and the cells were activated by cross-linking BCR or by co-cross-linking FcγRII and BCR as indicated. The p21^{ras}-bound GTP/GDP+GTP ratios were compared in the immunoprecipitated samples. Co-ligation of the receptors in three ways, with intact IgG anti-IgM, by aggregated IgG plus anti-Ig (Fig. 5a), or after pretreating the cells with CD32 antibody followed by biotinylated anti-mouse IgG and biotinylated anti-human Ig plus avidin (Fig. 5b), all reduced the ratio of GTP-bound p21^{ras}. This result indicates that the activity of p21^{ras} is lower in the BCR-FcγRII co-ligated samples than in those activated via BCR cross-linking.

Activity of MAPK Is Lower in BCR-FcγRII Co-ligated Samples Compared with the BCR-cross-linked Controls—MAPK were isolated from BL41 cells using ERK1 + 2-specific antibodies. The samples were precleared for CD32 to avoid CD32-associated kinases. The activities of MAPK were compared on the basis of MBP phosphorylation in the immunoprecipitated kinase assays, and the data were normalized for the quantities of MAPK present in the same samples. As shown in Fig. 6, co-cross-linking of FcγRII and BCR induced a lower MBP phosphorylation as compared to samples activated via BCR, suggesting that MAPK activity decreased in the co-ligated samples.

**DISCUSSION**

B cell activation by T-independent type 2 antigens is simulated by cross-linking BCR with F(ab)_2 fragments of anti-Ig. The immune response to such particular antigens as erythrocytes is suppressed when IgG-antigen complexes were administered for immunization (23). Co-cross-linking of BCR and FcγRII by intact IgG anti-Ig interrupts signal transduction in B cells as detected by the reduction of Ca^{2+} influx (2, 3). It has been described recently that FcγRIIb possesses an inhibitory motif containing 13 amino acid residues in the cytoplasmic tail, including a Tyr residue, which becomes phosphorylated after co-ligation, and more recent results (24) suggested that protein-tyrosine-phosphatase 1C is recruited after BCR-FcγRIIb co-ligation, and more recent results (24) suggested that protein-tyrosine-phosphatase 1C specifically induces dephosphorylation of a 35-kDa BCR-associated protein, probably representing Igα in murine B cells. However, this might not be the only way of the inhibition of B cell signaling since in human B cells, only phosphorylation of Tyr^{527} in FcγRIIb1 isosform is required for the inhibition of Ca^{2+} influx, while Tyr^{973} in FcγRIIb2 is not phosphorylated in the co-cross-linked samples, although Ca^{2+} influx is inhibited (25).

We have shown earlier that several Tyr-phosphorylated molecules associate with FcγRII in activated human B cells and have now tested which molecules associate when BCR and FcγRIIb were co-ligated. Under the conditions applied, in 1% Triton X-100 detergent, 200-, 130-, 110-, and 75-and 50–60-kDa Tyr-phosphorylated proteins were observed in FcγRII immunoprecipitates isolated from the membrane of BCR-FcγRII-
co-cross-linked samples, the 50–60-kDa protein being the most prominent one. This band seems to be composed of several Tyr-phosphorylated proteins, and a fraction of it is identical with Shc. Comparing the total amount of Shc present in the membrane of activated B cells, the FcRII-associated Shc represent only a portion of it. Shc-FcRIIα association in murine macrophages was described previously (26). BL41 cells express only FcγRIIb; we could not discern staining with the FcγRII-specific antibody, IV.3. More than 95% of FcγRIIb represents the b1 isoform (8); thus, our data indicate that Shc and unidentifed Tyr-phosphorylated proteins interact with human FcγRIIb1 in BCR-FcγRIIb1 co-cross-linked samples. Contamination of FcγRIIb1 immunoprecipitates with Igα is excluded, since we could not detect Igα in the isolated CD32 or FcγRIIb1 in the isolated Igα precipitates. Furthermore, in agreement with Smit et al. (27), we did not observe Igα coprecipitated with Shc from the Triton X-100-solubilized samples. Shc has a special adapter function coupling several Tyr-phosphorylated molecules together, since it possesses two Tyr(P)-reactive groups; one is an SH2 domain while the other is a phosphotyrosine-binding domain interacting with a consensus sequence, NPx-pY (28). Since this latter sequence cannot be seen in the intracellular tail of FcγRIIb1 and the SH2 domain of Shc was shown to interact with the motif P-Y-hydrophobic residue x-X-L/L (29), which is present in the ITIM motif of FcγRIIb1 (YSLL), we supposed that the SH2 domain of Shc binds to the Tyr-phosphorylated inhibitory motif of FcγRIIb1. To control this hypothesis, molecules absorbed by insolubilized ITIM and P-ITIM peptides were compared. ITIM did not bind any of the tested proteins, while P-ITIM bound Shc from activated but not from resting cells. In the contrary, the 53-kDa Tyr-phosphorylated components were associated with P-ITIM at a similar extent from both resting and activated cells, indicating that these are not identical with Shc. The same bands were recognized on the reprobed blots by Lyn-specific antibodies. These results suggest that the binding of Shc to FcγRIIb1 does not depend on the interaction of Shc SH2 domain with the phosphotyrosine within the ITIM motif of FcγRIIb1 but was probably mediated by the binding of Shc to some of the P-ITIM-associated, inducible Tyr-phosphorylated, unidentified molecules. It has been reported recently that Shc is able to interact with other molecules (adaptins) via its collagen-homologous domain (30); we cannot exclude the possibility of such an interaction.

Association with FcγRIIb1 might alter the function of Shc, a multifunctional signaling molecule. Since the P-ITIM-associated Tyr-phosphorylated 53-kDa molecules co-migrated with Lyn kinase but not with Shc, we assume that FcγRIIb1-bound Shc is not phosphorylated or dephosphorylated due to Shc-FcγRIIb1 interaction. Indeed, when Shc was isolated from the membrane fraction of BL41 cells and its Tyr phosphorylation was compared in BCR-cross-linked and BCR-FcγRII-co-cross-linked samples, a reduced Tyr phosphorylation was observed in the latter (data not shown). These results also suggest that Shc is not a direct substrate of Lyn. In accordance with this finding interaction of Shc with Syk but not with Lyn is described (13). Lyn might be responsible for phosphorylating FcγRIIb1 in the co-ligated samples. This latter suggestion is in agreement with recent finding of Bewarder et al. suggesting that Lyn is the most likely candidate for FcγRIIb1 phosphorylation in vivo (31).

Shc is implicated in the regulation of p21ras by forming Shc/Grb-2/Sos/p145 complexes after being Tyr phosphorylated and translocating these complexes to the cell membrane where p21ras is located (14). Monitoring p21ras activity we have found that various types of co-ligation of BCR and FcγRIIb1 reduce p21ras activity and this depends on the degree of co-cross-linking. The number of mlg molecules per cell is approximately 10 times higher than that of FcγRIIb1 molecules. Thus, a high concentration of intact IgG anti-IgM could not sufficiently induce inactivation of ras, probably since more BCR were cross-linked inducing cell activation than co-cross-linked with the inhibitory FcγRIIb1. The highest degree of inhibition was observed in samples when FcγRII and BCR were co-cross-linked with biotinylated antibodies followed by avidin. Thus, we suggest that FcγRIIb1 interrupts B cell signaling when co-ligated.
with BCR by inhibiting p21ras activation.
p21ras induced phosphorylation of the Ser/Thr kinase, raf, which phosphorylates MAPK kinase; in turn, this dual specificity kinase phosphorylates MAPK on Ser and Tyr residues. When MAPK activities were compared by detecting MBP phosphorylation in the MAPK immunocomplex kinase assays and the values were normalized for the amount of MAPK present, a partially reduced MAPK activity in FcγRIIb1-BCR co-cross-linked samples was observed when compared with that in BCR-cross-linked ones. These data suggest that BCR-FcγRIIb1 co-ligation may lower p21ras-dependent MAPK activity.

Taking these data together, we suggest the following model for the FcγRIIb1-mediated inhibition of B cell activation. Co-ligation of FcγRIIb1 and BCR induces the Tyr phosphorylation of the ITIM motif allowing the association of SH2 domain-containing molecules with FcγRIIb1. Among these, Shc and lyn were identified. Some of the FcγRIIb1-associated inducible Tyr-phosphorylated molecules bind Shc. Although we could not detect protein-tyrosine-phosphatase 1C in FcγRIIb1 precipitated from the human Burkitt lymphoma line, BL41, we cannot exclude that FcγRIIb1-bound Shc might be the target of phosphatases under certain conditions. Alternatively, Shc might just be sterically hindered to become a target of protein-tyrosine kinases. As a result, Tyr phosphorylation and thus the function of the FcγRIIb1-associated Shc are impaired. Since FcγRII and BCR are co-ligated, FcγRIIb1-bound Shc is transferred to the cell membrane where Shc cannot exert its proper function, the translocation of the Grb-2/Sos complexes to the vicinity of ras. Thus, p21ras is less active and has a reduced ability to activate the Ser/Thr kinase cascade leading to MAPK activation and egr-1 induction. This mechanism might be responsible for the inhibition of antibody synthesis by the FcγRIIb1-BCR co-ligated cells and in vitro may help to avoid production of autoantibodies.

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