

Integration of activatory and inhibitory signals in human B-cells

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Abstract

Fc γ receptors type IIb1 (Fc γ RIIb1) inhibit B-cell activation when co-ligated with B-cell antigen receptors (BCR) by immune complexes. In murine B-cells the inhibition is mediated by the interaction of the phosphorylated immunoreceptor tyrosine-based inhibitory motif (P-ITIM) of Fc γ RIIb1 with the SH2 domain containing protein tyrosine phosphatase, SHP1. To clarify the mechanism of Fc γ RIIb mediated inhibition of human B-cells we have studied the association of signaling molecules with human Fc γ RIIb1 after co-ligating with BCR. Fc γ RIIb1 were affinity purified from the Burkitt lymphoma cell line, BL41. Several tyrosine phosphorylated proteins were co-isolated with Fc γ RIIb1 at 145, 110, and 50–60 kDa, which were not present in Fc γ RIIb1 free immune complexes. Among these molecules we have identified the p52 Shc adaptor protein. Furthermore, we have shown that the insolubilised synthetic peptide corresponding P-ITIM bound Shc, Lyn and the p75 and p110 unidentified tyrosine phosphorylated proteins. Here we describe that the cell membrane associated Shc is partially dephosphorylated in BCR-Fc γ RIIb1 co-ligated samples, suggesting that its function in regulating p21ras monomeric G protein is impaired. Indeed, we have detected a lower p21ras activity in BCR-Fc γ RIIb1 co-crosslinked samples. These data indicate that co-ligation of BCR and Fc γ RIIb1 interrupts signal transduction between protein tyrosine kinase activation and p21ras mediated activation pathway. Since in contrast to the mouse B-cells both Fc γ RIIb1 and Fc γ RIIb2 are expressed in human B-cells, we have investigated the inhibitory function of the two receptors in Fc γ RIIb negative Burkitt lymphoma cell line ST486 transfected with Fc γ RIIb1 and Fc γ RIIb2, respectively. Both Fc γ RIIb1 and Fc γ RIIb2 inhibited the rise of intracellular Ca²⁺ induced by the crosslinking of BCR. The rate of the inhibition depended on the ratio of the co-crosslinked receptors (BCR-Fc γ RIIb1) to the crosslinked BCR (BCR-BCR). Co-crosslinking of the two receptors inhibited not only the capacitive Ca²⁺ entry but rather the total Ca²⁺ response in both Fc γ RIIb1 and Fc γ RIIb2 transfected human B-cells. CD19 represents the signal transduction unit of complement receptor, CR2 (CD21), and is responsible for the complement activating IgM-immune complex induced enhancement of B-cell activation. Co-crosslinking of CD19 and BCR was shown to enhance B-cell activation due to the recruitment of further signaling molecules to the activator complex by the phosphorylated tyrosine residues of CD19. Here we show a novel finding that co-ligation of CD19 with Fc γ RIIb1 inhibits the CD19-induced upregulation of Ca²⁺ response. The results indicate that IgG plus complement containing immune complexes may inhibit B-cell activation *in vivo*, due to the Fc γ RIIb1-mediated interruption of signal transduction via both BCR and CD19. © 1996 Elsevier Science B.V. All rights reserved

Keywords: Fc γ RIIb; Human B-cells; Regulation; Signal transduction

1. Introduction

IgG containing immune complexes are negative regulators of B-cell activation due to their ability to co-crosslink B-cell antigen receptors (BCR) and Fc γ receptors (Fc γ RIIb) on the surface of the same cell. B-cells were shown to be arrested at the level of signal transduction, however the exact mechanism of the in-

hibitory process has not been clarified yet [2,3]. Phosphorylation of ITIM within BCR co-ligated Fc γ RIIb1 is a prerequisite of the inhibition, due to the affinity of P-ITIM to SH2 domain containing inhibitor molecules [4,5]. SHP1, a protein tyrosine phosphatase having an SH2 domain was shown to be recruited by murine Fc γ RIIb1 to the site of activation [1]. Human B-cells differ from the murine ones in expressing two isoforms of Fc γ RIIb, Fc γ RIIb1 and Fc γ RIIb2. Both contain ITIM but only the ITIM of Fc γ RIIb1 is detectably

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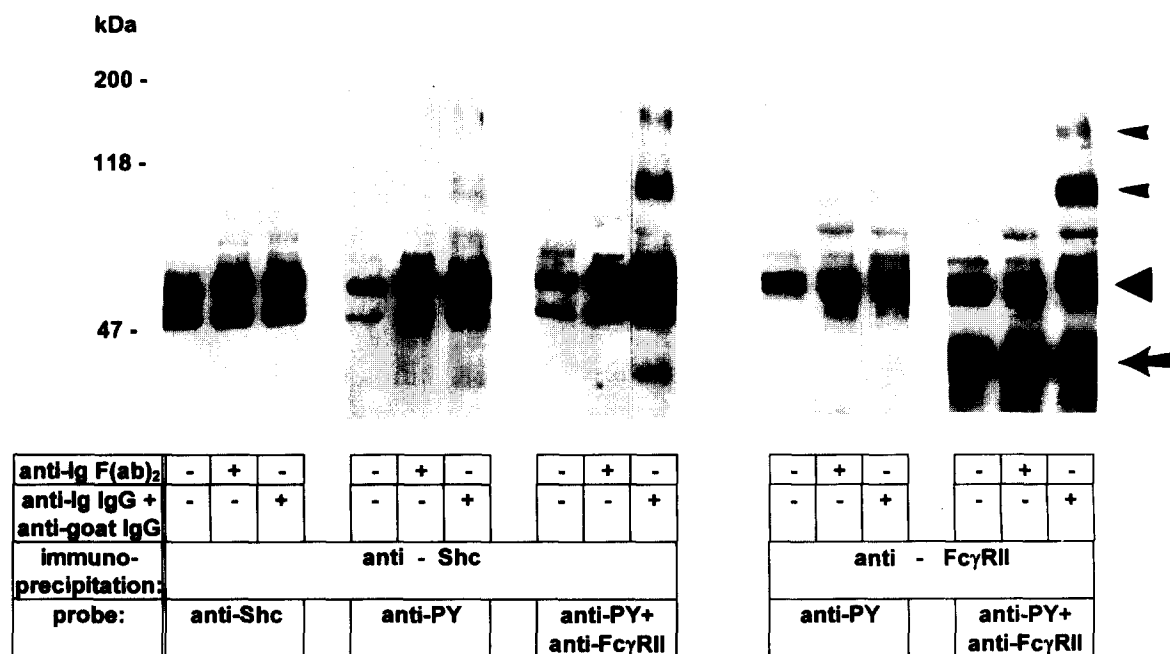


Fig. 1. Shc associated molecules co-precipitate with Fc γ RIIb1 and tyrosine phosphorylated Fc γ RIIb1 co-purify with Shc in BCR-Fc γ RIIb1 co-ligated samples. BL41 cells (5×10^7) were stimulated by 50 μ g/ml anti-Ig F(ab)₂ or anti-Ig intact IgG antibodies followed by the addition of 25 μ g/ml anti-goat IgG F(ab)₂ fragment for 2 min. The cells were immediately frozen in liquid nitrogen, then lysed in 1% Triton X-100 containing lysis buffer. Shc and Fc γ RIIb1 were isolated by the corresponding antibodies followed by the addition of Gamma Bind G beads from the postnuclear fraction. The blots were probed with anti-phosphotyrosine antibodies then re-probed with Fc γ RIIb specific mAb without stripping and developed by HRPO conjugated anti-mouse IgG (Fab)₂. Thin arrowheads show the position of Fc γ RIIb1 co-precipitated tyrosine phosphorylated components, the thick arrowhead indicates the position of Shc and finally the arrow shows the position of Fc γ RIIb1 on the corresponding immunoblot.

phosphorylated after BCR-Fc γ RIIb1 co-ligation [6]. When co-crosslinked with the antigen receptors both Fc γ RIIb1 and Fc γ RIIb2 are able to inhibit Ca²⁺ influx in murine B-cells transfected with the human isoforms of Fc γ RIIb [6]. As compared with Fc γ RIIb2, human Fc γ RIIb1 contains an additional 19 amino acid long stretch inserted into the cytoplasmic tail of the receptor, which includes a repetitive Pro-x-x-Pro sequence, a potential interacting group with an SH3 domain containing proteins.

We have demonstrated previously that the tyrosine kinase Fyn and protein kinase C were co-isolated with Fc γ RIIb from the human Burkitt lymphoma derived cell line, BL41, independently of cell activation [7,8]. Here we summarize our work on defining signaling molecules which associate with the P-ITIM of Fc γ RIIb1. Furthermore, we compare the requirements of the inhibition of the Ca²⁺ response in Burkitt lymphoma cells transfected with Fc γ RIIb1 and Fc γ RIIb2, respectively, and describe how Fc γ RIIb-BCR co-ligation may influence various activation pathways in human B-cells.

2. Results and discussion

We have shown earlier that several tyrosine phosphorylated molecules, co-purified with Fc γ RIIb1, isolated from the membrane fraction of BL41 Burkitt lymphoma cells after having co-ligated with BCR. The most significant bands migrated between 50 and 60 kDa. Re-probing the same blot with Shc specific antibodies, we detected Shc in the Fc γ RIIb precipitates obtained from the Fc γ RII-BCR co-crosslinked samples [9].

2.1. Fc γ RII associate with Shc and Shc associated tyrosine phosphorylated molecules upon BCR- Fc γ RII co-ligation

To investigate further the tyrosine phosphorylated molecules co-isolated with Fc γ RIIb1 after co-crosslinking with BCR, BL41 Burkitt lymphoma cells were treated with a F(ab)₂ fragment and with intact IgG of anti-human Ig antibodies, respectively, and a firm co-ligation of Fc γ RIIb1 and BCR was induced by adding the corresponding anti-IgG as a second antibody. Shc and Fc γ RIIb were isolated from the cell membrane

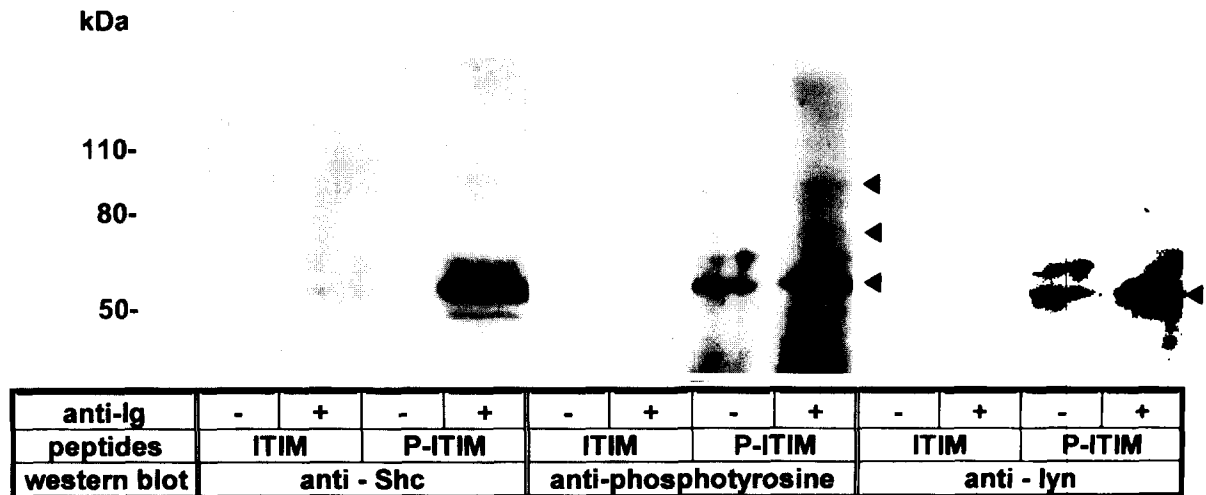


Fig. 2. Shc, Lyn and unidentified tyrosine phosphorylated proteins (p75 and p110) bind to the insolubilized synthetic peptide corresponding to the P-ITIM of $\text{Fc}\gamma\text{RIIb}$. BL41 cells (5×10^7) were treated with anti-Ig F(ab)_2 for 2 min. The cells were pelleted and lysed in 1% Triton X-100 containing lysis buffer. The postnuclear fractions of lysed samples were mixed with the Tentagel-bound peptides for 1 h. After several washings the samples were analyzed by SDS-PAGE followed by western blotting using anti-Shc, anti-phosphotyrosine and anti-Lyn antibodies, respectively. The membranes were probed with the corresponding antibodies, developed then stripped and re-probed with the next antibodies. The reactions were developed by using HRP conjugated species specific anti-Ig antibodies and enhanced chemiluminescence. The arrowheads indicate the position of tyrosine phosphorylated components and that of Lyn.

fraction by monoclonal antibodies and Protein G coated Sepharose beads. As shown in Fig. 1, tyrosine phosphorylated molecules with similar mobility (145, 110, 50–60 kDa and a faint band at 40 kDa) were present in both Shc and $\text{Fc}\gamma\text{RIIb}$ precipitates obtained from the BCR- $\text{Fc}\gamma\text{RIIb}$ co-ligated samples. Probing the same membranes with anti-CD32 mAb, the 40 kDa bands corresponding to $\text{Fc}\gamma\text{RIIb}$ were detected in all CD32 immunoprecipitates and as a co-isolated component in Shc immunoprecipitates obtained from BCR- $\text{Fc}\gamma\text{RIIb}$ co-ligated samples. A low degree of tyrosine phosphorylation of the same band was also observed. These data indicate that the tyrosine phosphorylated portion of $\text{Fc}\gamma\text{RIIb}$ co-purifies with Shc.

Several tyrosine phosphorylated components were described to co-precipitate with Shc in various cell types stimulated via multiple hemopoietic cell surface receptors, including T and B-cell antigen receptors [10]. However, among these only the 145 kDa phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase (SHIP) was identified [11]. The tyrosine phosphorylated SHIP is described to interact with the phosphotyrosine binding (PTB) domain of Shc [11]. SHIP was recently reported to associate with $\text{Fc}\gamma\text{RIIb}$ in bone marrow derived mast cells as well as in B-cells of mice [12]. Based on these findings we suggest that the 145 kDa tyrosine phosphorylated component co-isolated both with Shc and with $\text{Fc}\gamma\text{RIIb}$ from BCR- $\text{Fc}\gamma\text{RIIb}$ co-ligated samples of the human Burkitt lymphoma derived cell line, BL41 is identical with SHIP. We could not confirm in human B-cells the finding of D'Ambrosio et al., showing that SHP1 is recruited by the phosphorylated ITIM of

murine $\text{Fc}\gamma\text{RIIb}$ to the B-cell activator complex [1]. To explain the difference between these findings, in accordance with others results [12], we suggest that the $\text{Fc}\gamma\text{RIIb}$ may associate with SH2 domain containing molecules in a cell type-specific way.

Shc is a special adapter protein, containing a PTB domain at the N terminal part recognizing the NPxPY consensus motif, a gly-pro rich collagen-like middle part, and an SH2 domain, which interacts with pYxxL/M motif [13]. Shc itself becomes phosphorylated when

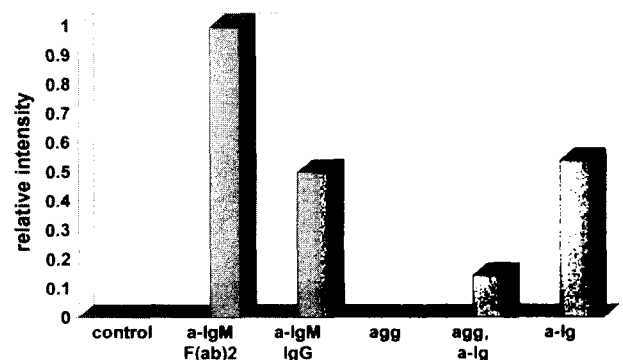


Fig. 3. Co-ligation of $\text{Fc}\gamma\text{RIIb}$ and BCR reduces the tyrosine phosphorylation of Shc. BL41 cells (5×10^7 sample) were treated with the F(ab)_2 fragment, and intact IgG of anti-Ig antibodies (30 $\mu\text{g/ml}$) or aggregated human IgG (200 $\mu\text{g/ml}$) followed by anti-Ig antibodies. The cells were lysed as described and the lysates were precleared with Gamma Bind G beads then Shc was precipitated. The membranes were subsequently probed by anti-phosphotyrosine antibodies and developed. The stripped membranes were re-probed by Shc specific antibodies and the data were analyzed by laser densitometer. The normalized data are shown, intensity values of the anti-P-Tyr blot are divided by the values of the second, anti-Shc blot.

Table 1

Crosslink	$\mu\text{g/ml}$	GDP	GTP	%
Control	—	99.1	2.41	2.37415
a-M1	28 (F(ab) ₂)	81.14	19.03	18.9977
a-M2	7	79.63	9.48	10.63854
IgG aM1	28 (IgG intact)	73.46	15.09	17.04122
IgG aM2	7	57.43	3.43	5.635886
a-Ig	30 (F(ab) ₂)	68.81	14.29	17.19615
agg + a-Ig	200 (aggr.IgG)	74.81	8.75	10.47152

the cells are stimulated via anti-Ig F(ab)₂ [10]. Thus Shc is potentially able to recruit various tyrosine phosphorylated and SH2 domain containing molecules to the site of activation, enhancing thereby the availability of signaling molecules responsible for triggering the p21ras activation pathway. Beside regulating p21ras activation, Shc is involved in the regulation of additional pathways as well, suggesting that Shc play a central role in coordinating multiple signals in stimulated cells [14].

Since the phosphorylated ITIM in Fc γ RIIb corresponds to the PY-hydrophobic-X-x-L motif which is suggested to interact with the SH2 domain of Shc, we have investigated whether Shc binds to P-ITIM. We have synthesized the peptide corresponding to the ITIM and P-ITIM sequences and identified the components associated with the insolubilized peptides from BL41 cell lysates. P-ITIM bound Shc from anti-Ig activated but not from resting B-cell lysates, while eluates of the non-phosphorylated ITIM were negative (Fig. 2, first panel). Re-probing the same blots with anti-phosphotyrosine antibodies showed that P-ITIM but not the ITIM peptide bound a major 53 kDa molecule, just above Shc and several minor tyrosine phosphorylated components (60, 75 and 110 kDa) from both resting and activated B-cell samples. A higher tyrosine phosphorylation of the P-ITIM associated components, in particular the 75 and 110 kDa ones and a faint diffuse band at 145 kDa was observed in activated samples as compared with resting ones (Fig. 2, second panel). The blots were subsequently reprobbed with Lyn specific antibodies. The 53 kDa tyrosine phosphorylated component appeared to be identical with Lyn PTK (Fig. 2, third panel). P-ITIM bound Lyn from both resting and activated B-cell lysates. Lyn was suggested to be responsible for both in vitro and in vivo phosphorylation of Fc γ RIIb1 [15], thus Lyn 1 may interact with Fc γ RIIb1 as with a substrate. This result is in accordance with a recent report showing that Lyn is involved in the Fc γ RIIb mediated negative regulation of B-cell activation [16].

Since P-ITIM binds Shc only from lysates of activated but not that of resting B-cells, these data suggest that the interaction of Shc with Fc γ RIIb1 in BCR/Fc γ RII co-ligated samples is probably indirect, medi-

ated through an unidentified SH2 domain containing molecule which directly binds to P-ITIM. This putative molecule becomes tyrosine phosphorylated in anti-Ig activated cells and consequently may interact with the SH2 or PTB domain of Shc. To our surprise, Shc associated to the P-ITIM bound complex is not detectably phosphorylated, thus we suppose that Shc is

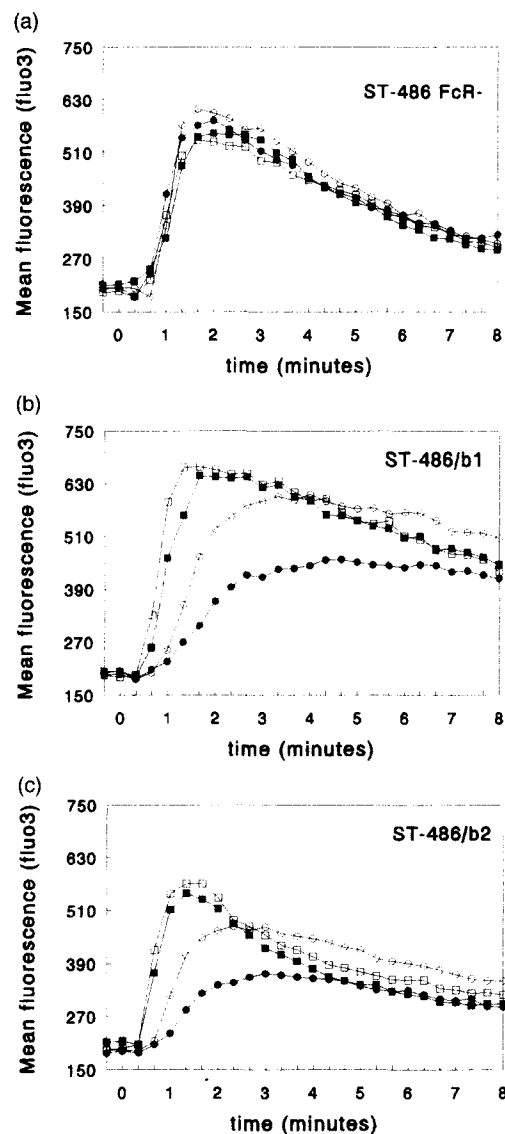


Fig. 4. Co-crosslinking of Fc γ RIIb1 or Fc γ RIIb2 with BCR dose dependently inhibits the rise of intracellular Ca⁺⁺ in ST486 cells transfected with Fc γ RIIb1 and Fc γ RIIb2, respectively. The cells were loaded with fluo 3, and after several washes, treated with CD32 specific monoclonal antibodies (2 $\mu\text{g/ml}$) for 5 min, followed by anti-mouse IgG F(ab)₂ and various doses of anti- κ chain mAb (open symbols: anti- κ , 0.1 $\mu\text{g/ml}$ (○), 1 $\mu\text{g/ml}$ (□); closed symbols: co-crosslinked samples, CD32 mAb 2 $\mu\text{g/ml}$, anti- κ , 0.1 $\mu\text{g/ml}$ (●), 1 $\mu\text{g/ml}$ (■)). Fc γ RII and BCR were co-crosslinked by 4 $\mu\text{g/ml}$ anti-mouse IgG F(ab)₂. Fluorescence was detected by a Beckton Dickinson flow cytometer. The mean fluorescence values were calculated at each time point.

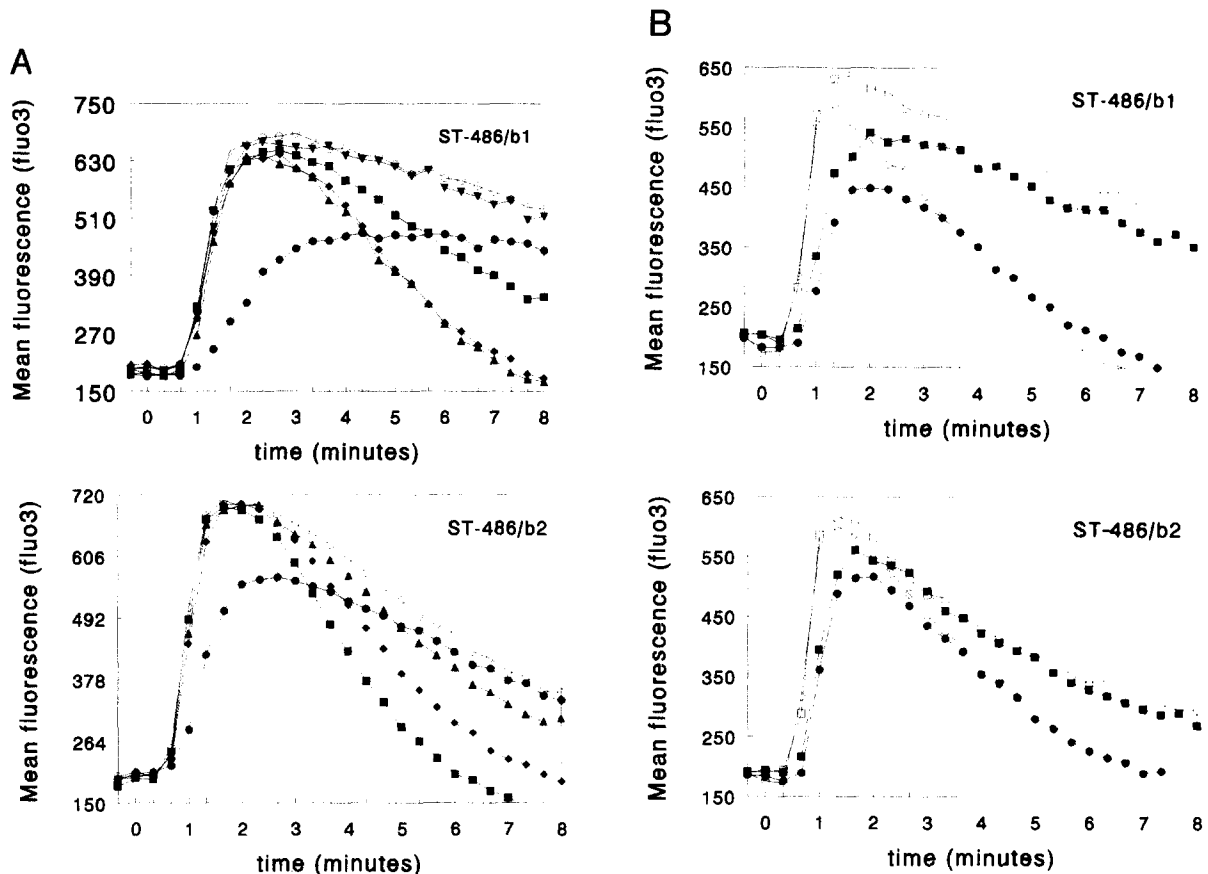


Fig. 5. Co-crosslinking of FcγRIIb and BCR reduces the release of Ca⁺⁺ from intracellular pools in ST486 cells transfected with FcγRIIb1 and FcγRIIb2, respectively. The fluo 3 loaded cells were stimulated by 0.1 μg/ml anti-κ antibodies and 2 μg/ml anti-mouse IgG F(ab)₂ (open circles) as described in Fig. 4. A, the cells were pretreated with various concentrations of EGTA (2 mM, triangles; 1 mM, diamonds; 0.6 mM, rectangles; 0.2 mM, inverse triangles) then stimulated; closed circles, FcγRIIb1 co-crosslinked samples. B, the cells were treated with 1 mM EGTA (circles) or left untreated (rectangles) immediately before BCR were crosslinked (open symbols) or FcγRIIb1 and BCR were co-crosslinked (closed symbols). The results were evaluated as described in Fig. 4.

dephosphorylated by one of the protein tyrosine phosphatases during the preparation.

To investigate tyrosine phosphorylation of Shc in detail, Shc was precipitated from the cell membrane fraction of resting, activated and BCR-FcγRII co-ligated cells. The results of the anti-phosphotyrosine Western blots indicate that tyrosine phosphorylation of Shc is lower in the BCR-FcγRII co-ligated samples as compared to the BCR activated ones, suggesting that a portion of Shc is indeed dephosphorylated when associated with FcγRIIb1 (Fig. 3). P-ITIM bound molecules were tested with PKCε and PKCβ and subsequently with MAPK and Fyn specific antibodies. All of these molecules which were found previously to associate with FcγRIIb [7,8] were not detectable in the P-ITIM precipitates, suggesting that they might interact with other parts of the whole receptor molecule independently of the P-Tyr-SH2 domain interaction.

2.2. Inhibition of p21ras activity by FcγRIIb:BCR co-ligation

Shc/Grb2/Sos complexes are implicated in controlling p21ras activity and thus in the regulation of the downstream serine/threonine cascade [17]. Activation of the p21ras pathway couples antigen receptor stimulation to the induction of primary response gene *egr1* [18]. *Egr1* and *egr2* were shown to be the targets of FcγRII mediated inhibition of anti-Ig induced B-cell activation [19]. Based on these earlier data, we have compared p21ras activities in resting, anti-Ig activated and BCR-FcγRIIb co-crosslinked cells. Co-ligation of BCR and FcγRII by either intact IgG anti-IgM or by using aggregated IgG plus anti-Ig decreased p21ras activation (Table 1). Significantly lower ras activity was observed in those samples where the rate of co-crosslinking was optimal, i.e. a limited number of BCR were co-crosslinked with FcγRIIb1. Increasing the number of crosslinked BCR by raising the amount of

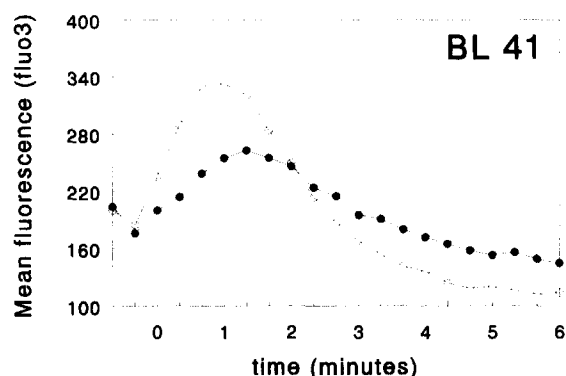


Fig. 6. Co-crosslinking of Fc γ RII and CD19 inhibits Ca⁺⁺ mobilization induced by anti-CD19 in BL41 Burkitt lymphoma cells. The fluo 3 loaded cells were pretreated with 2 μ g/ml CD32 mAb for 5 min then 4 μ g/ml anti-mouse IgG F(ab)₂ and 1 μ g/ml CD19 mAb were added (closed symbols); open symbols, anti-CD19 plus anti-mouse IgG.

intact IgG anti-Ig we can probably reach the point where all Fc γ RII are co-crosslinked, but since the B-cells express approximately ten times more BCR than Fc γ RIIb1, BCR-BCR complexes can be formed, inducing an activation signal. At this point BCR crosslinking overcomes the Fc γ RIIb1 mediated inhibition.

The data so far demonstrate, that Fc γ RIIb1 isolated from BCR-Fc γ RIIb1 co-ligated cells interact with tyrosine phosphorylated molecules at 145, 110, 70, and 50–60 kDa at the site of activation. We have identified Lyn and Shc among these molecules, furthermore, we suggest that the p145 band might be identical with the Shc associated tyrosine phosphorylated SHIP, while the

other proteins are not identified yet. The Fc γ RIIb1 associated Shc shows poor phosphorylation, thus the lower number of Shc molecules can form complexes with Grb2/SOS and be involved in the activation of p21ras, resulting in a decreased p21ras activity.

2.3. The effect of Fc γ RIIb-BCR co-ligation on the intracellular Ca²⁺ concentration in human B cells transfected with Fc γ RIIb1 or Fc γ RIIb2

Co-crosslinking of BCR and Fc γ RII is known to inhibit Ca²⁺ influx in murine B-cells [3], but less data are available on the human counterpart. In order to study the function of the two isoforms of Fc γ RIIb in a homologous system, an Fc γ RII negative Burkitt lymphoma cell line ST486 was transfected with Fc γ RIIb1 and Fc γ RIIb2 cDNA, respectively (provided by Jan van de Winkle). ST486 cells express sIgM with kappa chain. Previous experiments indicated that the Fc γ RII mediated negative regulation of B-cell activation depends on the ratio of Fc γ RII-co-crosslinked BCR compared with all the crosslinked BCR. In order to study the possible effect of immune complexes at various ratios of antigen and antibodies we set up a system in which various numbers of BCR and Fc γ RIIb were ligated. We used monoclonal antibodies specific for κ chains and CD32, respectively, then co-crosslinked the receptors by anti-mouse IgG F(ab)₂. Ca²⁺ response of the Fc γ RIIb negative parental cells was not influenced by the combined treatment of CD32 plus anti- κ mAb (Fig. 4(a)), while it was decreased both

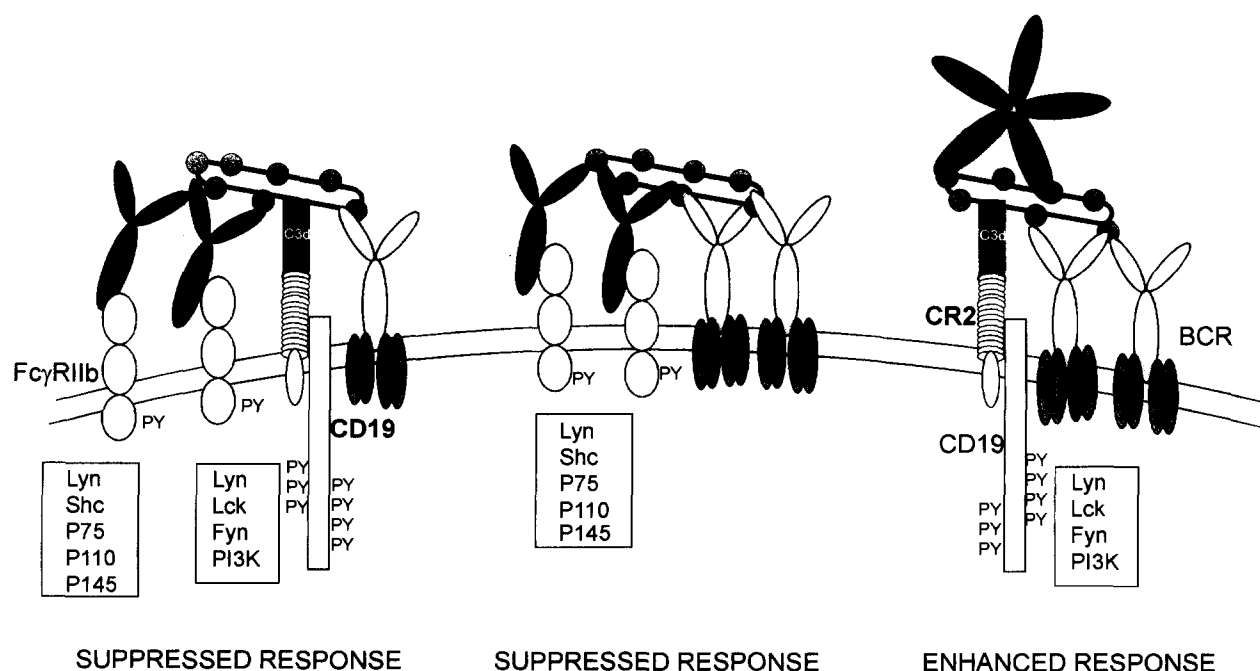


Fig. 7. Possible interactions of immune complexes with Fc γ RIIb1. Associated molecules which are involved in Fc γ RIIb1 mediated negative regulation or CR2 mediated positive regulation of B-cell activation are framed.

in Fc γ RIIb1 and in Fc γ RIIb2 transfected cells (Fig. 4(b), (c)). An optimal ratio of the two antibodies was required to inhibit Ca²⁺ mobilization. Crosslinking of too many BCR with the same number of Fc γ RIIb may overcome the inhibitory effect. These data indicate that immune complexes at a slight antigen excess are the best inhibitors of B-cell activation.

The characteristics of the curves suggested that the inhibitory effect of Fc γ RIIb is not restricted to the BCR induced Ca²⁺ influx in human cells but the overall Ca²⁺ response is affected. To control this, we compared Ca²⁺ signals in the presence of various concentrations of EGTA added just prior to stimulation. EGTA, depleting extracellular Ca²⁺, inhibited only the capacitive Ca²⁺ entry, while co-ligation of Fc γ RIIb1 and BCR inhibited the overall Ca²⁺ response (Fig. 5(a)). The degree of inhibition was always higher in cells transfected with Fc γ RIIb1 when compared with the ones transfected with Fc γ RIIb2, although the two cell types expressed the same level of Fc γ RII b1 or b2.

Experiments were performed in which the cells were treated first with EGTA and then with the combination of κ chain and CD32 specific antibodies. In the presence of EGTA, where Ca²⁺ was captured outside the cell, co-crosslinking of Fc γ RII and BCR inhibited the first phase of the Ca²⁺ response. The capacitive entry of Ca²⁺ was inhibited further by EGTA in the co-ligated samples (Fig. 5(b)). These results indicate that in contrast to previous results shown in murine B-cells [5], co-crosslinking of Fc γ RIIb1 or b2 with BCR in human B-cells partially inhibits the release of Ca²⁺ from the intracellular pool.

2.4. Fc γ RIIb-CD19 coligation inhibits Ca²⁺ mobilization

Immune complexes formed *in vivo* activate and bind complement receptors, on B-cells to CR2. CR2 has no signaling elements, but it associates with CD19-CD81-Leu 3 complex [20]. After BCR or CD19 crosslinking when CD19 becomes phosphorylated, it recruits Lyn, Fyn, and phosphatidylinositol 3 kinase (PI3K), synergistically enhancing thereby B-cell activation [21,22]. Ligation of CD19 or crosslinking of CD21 was shown to induce activation of PLC, PKC, and inositol(1,4,5)-trisphosphate which induces intracellular Ca²⁺ mobilization in mature B-cells [23]. This might explain how IgM containing complement activating immune complexes enhance antibody production. Therefore, it is difficult to envisage how IgG complexes, which also activate C3 may have a blocking effect on B-cells *in vivo*. We supposed that CD19 mediated signals might also be inhibited when CD32 and CD19 are co-ligated

by immune complexes. In order to control this, we co-crosslinked Fc γ RII and CD19 by using the corresponding mAbs followed by anti-mouse IgG. The results show that co-crosslinking of these two molecules inhibits Ca²⁺ mobilization triggered by anti-CD19 (Fig. 6). Since CD19 is known to activate PTK, furthermore, Lyn was reported to associate with CD19 [20,22], our results indicate that Lyn may phosphorylate Fc γ RIIb1 after CD19-CD32 co-crosslinking then Fc γ RIIb1 can exert its P-ITIM dependent inhibitory effect on CD19 triggered signal transduction.

Considering that activation of the PI3K pathway is common both in BCR and CD19 signaling and PI3K is involved in regulating Ca²⁺ mobilization, we suppose that the activity of PI3K is influenced by the co-crosslinking of Fc γ RIIb both with BCR and CD19. It was reported recently that p21ras is the main regulator of PI3K [24]. Since we detected a decreased p21ras activity after BCR-Fc γ RIIb co-ligation, our results indicate that the lower p21ras activity in the co-crosslinked samples might be responsible for the imperfect activation of PI3K, and consequently for the impaired Ca²⁺ signal. Fig. 7 summarizes the possible interactions between immune complexes and B-cells. While IgM plus complement containing immune complexes may enhance the immune response via CD19 dependent recruitment of signaling molecules to the activator complex, IgG and IgG plus complement containing complexes inhibit both BCR and CR2-CD19 signaling by inhibiting the function of crucial signaling molecules.

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