SHP-1 Requires Inhibitory Co-receptors to Down-modulate B Cell Antigen Receptor-mediated Phosphorylation of Cellular Substrates*

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Signaling through the B cell antigen receptor (BCR) is negatively regulated by the SH2 domain-containing protein-tyrosine phosphatase SHP-1, which requires association with tyrosine-phosphorylated proteins for activation. Upon BCR ligation, SHP-1 has been shown to associate with the BCR, the cytoplasmic protein-tyrosine kinases Lyn and Syk, and the inhibitory co-receptors CD22 and CD72. How SHP-1 is activated by BCR ligation and regulates BCR signaling is, however, not fully understood. Here we demonstrate that, in the BCR-expressing myeloma line J558Lμm3, CD72 expression reduces the BCR ligation-induced phosphorylation of the BCR component Igα/Igβ and its cytoplasmic effectors Syk and SLP-65. Substrate phosphorylation was restored by expression of dominant negative mutants of SHP-1, whereas the SHP-1 mutants failed to enhance phosphorylation of the cellular substrates in the absence of CD72. This indicates that SHP-1 is efficiently activated by CD72 but not by other pathways in J558Lμm3 cells and that inhibition of SHP-1 specifically activated by CD72 reverses CD72-induced dephosphorylation of cellular substrates in these cells. Taken together, BCR-induced SHP-1 activation is likely to require inhibitory co-receptors such as CD22, and SHP-1 appears to mediate the negative regulatory effect of CD72 on BCR signaling by dephosphorylating Igα/Igβ and its downstream signaling molecules Syk and SLP-65.

Cross-linking of the B cell antigen receptor (BCR) activates protein-tyrosine kinases (PTKs) and induces phosphorylation of the immunoreceptor tyrosine-based activation motifs in the cytoplasmic tails of the Igα/Igβ heterodimer, the signaling component of the BCR (1, 2). Phosphorylated Igα/Igβ can activate the cytoplasmic PTK Syk, which in turn phosphorylates the adapter protein called SLP-65 or B cell linker protein (BLNK) (1–4). Upon phosphorylation, SLP-65 initiates downstream signaling events by recruiting various signaling molecules such as phospholipase C-γ, Nck, and Btk (3–7). Studies on genetically manipulated mice or B cell lines have shown that Igα/Igβ, Syk, and SLP-65 are essential in downstream signaling such as Ca2+ mobilization and extracellular signal-regulated kinase activation (2, 3, 5, 8, 9). The downstream signaling events appear to lead ultimately to proliferation, functional inactivation, or death of B cells. BCR signaling is regulated either positively by co-receptors such as CD19 and negatively by other co-receptors such as CD22 and the low affinity receptor for IgG (FcγRII) (10–14). FcγRII modulates BCR signaling only when it interacts with the antigen-IgG complex and is involved in negative feedback regulation of IgG production. In contrast, both CD22 and CD72 appear to interact constitutively with BCR and regulate negatively both mitogen-activated protein kinase activation and Ca2+ mobilization induced by BCR ligation (12, 13, 15–17). By negatively regulating BCR signaling whenever BCR is ligated, both of these co-receptors are implicated in setting a signaling threshold for BCR ligation.

The Src homology 2 (SH2) domain-containing protein-tyrosine phosphatase SHP-1 is involved in negative regulation of several receptors (18). For SHP-1 activation, its tandem SH2 domains need to be associated with a tyrosine-phosphorylated peptide (19). In B cells, CD22 has been shown to activate SHP-1. CD22 contains the conserved immunoreceptor tyrosine-based inhibition motifs (ITIMs) in the cytoplasmic regions (20). Upon tyrosine phosphorylation, the ITIMs of CD22 associate with and activate SHP-1. PIR-B and CD72 also contain ITIMs in their cytoplasmic region and, upon phosphorylation, recruit SHP-1 (21–24), suggesting that these molecules may also activate SHP-1 in B cells. Indeed, SHP-1 is shown to be involved in negative regulation of BCR-mediated Ca2+ signaling by a chimeric FcγRII containing the cytoplasmic region of PIR-B in the chicken B cell line DT40 (22). It is not yet known whether intact PIR-B is involved in negative regulation of BCR signaling by SHP-1. In contrast, both CD22 and CD72 are phosphorylated upon BCR ligation probably because these co-receptors associate with BCR constitutively (23–26). Thus, CD22 and CD72 may activate SHP-1 upon BCR ligation, thereby negatively regulating BCR signaling. Whether SHP-1 mediates negative regulation of BCR signaling by CD22 and CD72, and how SHP-1 activated by these inhibitory co-receptors negatively

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† The abbreviations used are: BCR, B cell antigen receptor; PTK, protein-tyrosine kinase; FcγRII, low affinity receptor for IgG; SH2, Src homology 2; ITIM, immunoreceptor tyrosine-based inhibition motif; NP, (4-hydroxy-3-nitrophenyl)acetyl; BSA, bovine serum albumin; Ab, antibody; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein.

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regulates BCR signaling has not yet been fully elucidated.

BCR signaling involves various tyrosine-phosphorylated signaling molecules other than ITIM-containing inhibitory receptors. Indeed, activation receptors such as erythropoietin receptor and IL-3 receptor are shown to associate with and activate Shp-1 upon ligand-induced phosphorylation (27, 28). In B cells, Shp-1 is reported to co-precipitate with the BCR probably due to its association with Igo/Igb (29). Shp-1 is also activated by tyrosine-phosphorylated cytoplasmic signaling molecules such as PTK ZAP-70, the homolog of the PTK Syk expressed in B cells (30). It is not yet known whether Shp-1 is activated by Igo/Igb or Syk.

A dominant negative mutant of Shp-1 has been used to assess the role of Shp-1 in the regulation of BCR (24, 31, 32). This mutant Shp-1 blocks the effect of Shp-1 activated by various different molecules. To assess whether a certain molecule, for example CD72, activates Shp-1 and whether its function is mediated by Shp-1, the dominant negative mutant of Shp-1 needs to be expressed in the cells where Shp-1 is not activated by alternative pathways. Such a cellular system has not yet been established. J558Lm3 myeloma cells express surface IgM specific for the hapten (4-hydroxy-3-nitrophenyl) (NP) (33). J558Lm3 cells do not express most of the membrane molecules expressed in B cells (34) including the inhibitory co-receptor CD22 and CD72, but they express Shp-1 and various cytoplasmic signaling molecules involved in BCR signaling such as Syk and SLP-65, and BCR ligation activates these molecules (4, 34). Thus, J558Lm3 cells are a useful tool to analyze the molecular mechanisms of activation of Shp-1 and the function of inhibitory co-receptors. By using this cellular system, we demonstrate here that BCR-induced Shp-1 activation requires inhibitory co-receptors such as CD72 and that Shp-1 is a proximal effector molecule of CD72 to down-regulate BCR-mediated signal transduction.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Cells**—The expression plasmid pMKITSHP-1SH2 coding for a GFP fusion protein containing the tandem SH2 domains of SHP-1 was generated as follows. The NotI fragment containing the GFP cDNA and the EcoRI-PvuII fragment of pBlue-scriptSHP-1 (23) containing Shp-1SH2 were inserted into NotI and EcoRI of modified pMKITneo harboring NotI and EcoRI sites instead of EcoRI and NotI sites. The expression plasmid pMKITSHP-1C/S-Myc coding for a catalytically inactive mutant of Shp-1, in which cysteine residue at 453 was replaced by serine (Shp-1C/S), tagged by a c-Myc peptide (SMQKLISEEDLN) are generated as follows. pMKIT-neo containing the GFP cDNA and the EcoRI-PvuII fragment of pBlue-scriptSHP-1C/S containing the cDNA for Shp-1C/S was generated by site-directed mutagenesis using pBlue-scriptSHP-1 containing the mouse Shp-1 cDNA (23). The HindIII-SalI fragment of the DNA encoding the c-Myc sequence was generated by annealing the pair of synthetic oligonucleotides (5'-AGCTTGAAGGATCTGAATTAAG-3' and 5'-TCAGAAGAGGATCTGAATTAAG-3'). This cDNA fragment was inserted into the HindIII-SalI sites of pBlue-scriptSHP-1C/S and pBlue-scriptSHP-1 containing the GFP cDNA to generate the expression plasmid pMKITSHP-1C/S-Myc coding for a GFP fusion protein containing the tandem SH2 domains of Shp-1C/S containing the cDNA for Shp-1C/S was generated by site-directed mutagenesis using pBlue-scriptSHP-1 containing the mouse Shp-1 cDNA (23).

**Phosphorylation of Signaling Molecules**—Western blot analysis of total cell lysates and/or flow cytometry revealed that J558Lm3 does not express CD22, CD72, or FcγRIIa (data not shown). To address the regulatory effect of CD72 on BCR signaling, we transfected J558Lm3 cells with an expression plasmid for CD72. For further analysis, we chose two independent CD72 transfectants (J558Lm3CD72-1 and J558Lm3CD72-24) that express CD72 and the IgM-BCR on the cell surface (Fig. 1, A–F). Following BCR ligation of J558Lm3 cells and its CD72 transfectants with the specific antigen NP-BSA, we monitored PTK substrate phosphorylation by anti-phosphotyrosine mAb 4G10.

**RESULTS**

CD72 Negatively Regulates BCR-mediated Tyrosine Phosphorylation of Signaling Molecules—Western blot analysis of total cell lysates and/or flow cytometry revealed that J558Lm3 does not express CD22, CD72, or FcγRIIa (data not shown). To address the regulatory effect of CD72 on BCR signaling, we transfected J558Lm3 cells with an expression plasmid for CD72. For further analysis, we chose two independent CD72 transfectants (J558Lm3CD72-1 and J558Lm3CD72-24) that express CD72 and the IgM-BCR on the cell surface (Fig. 1, A–F). Following BCR ligation of J558Lm3 cells and its CD72 transfectants with the specific antigen NP-BSA, we monitored PTK substrate phosphorylation by anti-phosphotyrosine immunoblotting of total cell lysates. Tyrosine phosphorylation of various substrate molecules reached the maximum 1 min after exposure to NP-BSA and thereafter gradually declined (Fig. 1G). Substrate phosphorylation in J558Lm3CD72 transfectants was much weaker than in J558Lm3 cells, which indicates that CD72 expression inhibits BCR-induced substrate phosphorylation.

One of the predominantly phosphorylated PTK substrates in antigen-treated J558Lm3 was isolated and named SLP-65 (4). The antigen-induced phosphorylation of SLP-65 was much
negative regulation of BCR signaling mediated by SHP-1

Western blot analysis of total cell lysates revealed that CD72 ligation induced phosphorylation of CD72Y7F in J558Lm3CD72Y7F (Fig. 3D), indicating that tyrosine residues outside the ITIM are phosphorylated in CD72Y7F after BCR ligation. In contrast, the same analysis barely demonstrated phosphorylation of wild-type CD72 in J558Lm3CD72 (Fig. 1G), although more sensitive analysis using anti-CD72 immunoprecipitates showed phosphorylation of CD72 upon BCR ligation in these cells (data not shown). These results indicate that the ITIM negatively regulates tyrosine phosphorylation of CD72. Since the ITIM is essential for recruiting SHP-1, SHP-1 may dephosphorylate CD72. As reported previously (24).

CD72 Is Required for SHP-1-mediated Down-modulation of Tyrosine Phosphorylation of Signaling Molecules in BCR-ligated J558Lm3 Cells—To assess the role of SHP-1 in CD72-mediated dephosphorylation of cellular substrates such as Igα/ Igβ, Syk, and SLP-65, we constructed expression plasmids for two distinct dominant negative mutants of SHP-1, i.e. a catalytically inactive mutant of SHP-1 tagged with a c-Myc peptide (SHP-1C/S) and a GFP fusion protein containing the tandem SH2 domains but not the catalytic domain of SHP-1 (SHP-1SH2). We then transfected J558Lm3 and J558Lm3CD72 with these expression plasmids. Expression of surface IgM and CD72 in the transfectants was similar to that of the parent cells (data not shown). SHP-1C/S was dominantly co-precipitated with CD72 in the J558Lm3CD72 transfectants (Fig. 4D), indicating that SHP-1C/S out-competes endogenous SHP-1 for binding to CD72. The dominant negative SHP-1 thus appears to block efficiently recruitment and activation of endogenous SHP-1. The J558Lm3CD72 transfectants (J558Lm3CD72/C/S and J558Lm3CD72SH2) and the J558Lm3 transfectants (J558Lm3C/S and J558Lm3SH2) expressed similar levels of SHP-1C/S or SHP-1SH2 (Fig. 4, A–C). However, antigen-induced phosphorylation of various substrates in both J558Lm3CD72/C/S and J558Lm3CD72SH2 transfectants was markedly enhanced compared with the parent cells.

Next, we examined the phosphorylation of other BCR signaling substrates in both J558Lm3 cells (Fig. 3A) and J558Lm3CD72 (Fig. 3B and D), whereas antigen-induced phosphorylation of Syk (Fig. 2B) and Igα/Igβ (Fig. 2C) was reduced in the CD72 transfectants compared with parent J558Lm3 cells. In consistent with this finding, the kinase activity of Syk was reduced in the CD72 transfectant (Fig. 2E). However, both phosphorylation and the kinase activity of Lyn were not up-regulated by BCR ligation in J558Lm3, as described previously (39), nor altered by CD72 expression (Fig. 2, D and F). Taken together, CD72 negatively regulates BCR-mediated phosphorylation of signaling molecules such as Igα/Igβ, Syk, and SLP-65 but not Lyn.

CD72 Carrying a Mutation in ITIM Does Not Down-modulate BCR-mediated Phosphorylation of Cellular Substrates—To assess whether CD72 requires the ITIM for down-modulation of BCR signaling, we transfected J558Lm3 cells with an expression plasmid encoding the mutated form of CD72 in which tyrosine in the ITIM was replaced by phenylalanine (CD72Y7F). The CD72Y7F transfectants (J558Lm3CD72Y7F-3 and J558Lm3CD72Y7F-5) expressed a higher level of CD72 on the surface (Fig. 3, B and C) than J558Lm3CD72 which expresses wild-type CD72 (Fig. 3A). However, tyrosine phosphorylation of cellular substrates was not reduced in J558Lm3CD72Y7F-5 compared with the parent J558Lm3 cells (Fig. 3D). Essentially the same result was obtained with J558Lm3CD72Y7F-3 (data not shown). These results show that the CD72 ITIM mutant fails to down-modulate BCR-mediated phosphorylation of cellular substrates. The ITIM in CD72 is thus essential for down-modulating BCR signaling probably by recruiting and activating SHP-1.

Western blot analysis of total cell lysates revealed that the CD72 transfectants (J558Lm3CD72-1 and CD72-24) were stimulated with NP-BSA for indicated times. Cell lysates were immunoblotted with anti-phosphotyrosine mAb 4G10 (upper panel). The same blot was reprobed with goat anti-mouse Igα chain Ab to ensure the equal loading (lower panel). Representative data of three experiments are shown.
compared with the parent J558Lm3 cells (Fig. 4, E and G), whereas J558Lm3C/S and J558Lm3SH2 transfectants showed a level of substrate phosphorylation similar to that seen in J558Lm3 (Fig. 4, F and H). The dominant negative mutants of SHP-1 thus enhanced phosphorylation of cellular substrates in the presence of CD72 but not in its absence.

Moreover, expression of SHP-1C/S enhanced phosphorylation of Igα/Igβ, Syk, and SLP-65 in the J558Lm3CD72 transfectant (Fig. 5, A–C), whereas the phosphorylation of Igα/Igβ and Syk was not altered by SHP-1C/S in the absence of CD72 (Fig. 5, D and E), supporting the notion that CD72 is required for enhancement of substrate phosphorylation by SHP-1.
mutants. These results suggested that SHP-1 is efficiently activated by CD72 but no other pathways in antigen-treated J558Lm3 cells. To confirm this notion, we assessed the tyrosine-phosphorylated proteins associated with the SH2 domains of SHP-1. Activation of SHP-1 requires association of its SH2 domains with a tyrosine-phosphorylated peptide (19). When we analyzed the anti-SHP-1 immunoprecipitates from J558Lm3CD72C/S and J558Lm3CD72SH2 cells treated with NP-BSA, CD72 was co-precipitated with SHP-1C/S (Fig. 6A) or SHP-1SH2 (Fig. 6B). However, no other tyrosine-phosphorylated proteins were detected. Taken together, SHP-1 appears to be activated exclusively by CD72 in antigen-stimulated J558Lm3 cells. This observation is not restricted to J558Lm3 cells. Indeed, the inhibitory co-receptors CD22 and CD72 are the only major phosphoproteins co-precipitated with SHP-1 in BCR-ligated B cell lines K46mCD72 and WEHI-231 (Fig. 7). CD22 and CD72 may thus play a major role in SHP-1 activation upon BCR ligation.

DISCUSSION

The myeloma line J558Lm3 expresses BCR, SHP-1, and various molecules involved in BCR signaling such as Lyn, Syk, and SLP-65, whereas it does not express co-receptors such as CD22 and CD72. Thus J558Lm3 is a useful tool to analyze the role of SHP-1 and co-receptors in BCR signaling regulation. By using J558Lm3 cells, we demonstrate that CD72 negatively regulates BCR-mediated phosphorylation of cellular substrates such as Igα/Igβ, Syk, and SLP-65. The substrate phosphorylation was not altered by the mutant CD72 lacking the tyrosine residue within the ITIM required for SHP-1 activation. Moreover, BCR-mediated substrate phosphorylation was restored by inhibiting SHP-1 specifically activated by CD72 using dominant negative mutants of SHP-1. Thus, SHP-1 is a specific intracellular effector of CD72 to negatively regulate BCR-mediated phosphorylation of cellular substrates. In contrast, dominant negative mutants did not enhance BCR-induced substrate phosphorylation in J558Lm3 cells in the absence of...
CD72 suggesting that SHP-1 is activated upon BCR ligation in the presence of CD72 but in its absence in these cells. This notion is further supported by our result that among the proteins tyrosine-phosphorylated upon BCR ligation in J558Lμm3 cells, CD72 is the only protein detected to associate with the SH2 domains of SHP-1. This indicates that SHP-1 is efficiently activated by CD72 but not by other pathways because SHP-1 activation requires its association with a tyrosine-phosphorylated peptide. In addition, CD22 and CD72 are the only major phosphoproteins associated with SHP-1 in BCR-ligated mouse

**Fig. 5.** SHP-1C/S enhances BCR-mediated phosphorylation of Igα/Igβ, Syk, and SLP-65 in J558Lμm3 in the presence of CD72. Cells (2 × 10^7) of indicated J558Lμm3 transfectants were incubated with or without NP-BSA for 3 min. Cell lysates were immunoprecipitated with anti-Igβ mAb (A and D), anti-Syk Ab (B and E), or anti-SLP-65 Ab (C). Immunoprecipitates (IP) were analyzed by immunoblotting with anti-phosphotyrosine mAb 4G10 (upper panels). The same blots were reprobed with indicated Abs (lower panels). Representative data of three experiments are shown.

**Fig. 6.** The SH2 domains of SHP-1 associate with CD72 but no other tyrosine-phosphorylated proteins in BCR-ligated J558Lμm3 CD72 transfectants. Cells (1 × 10^7) of J558Lμm3CD72C/S-14 (A) and J558Lμm3CD72SH2–11 (B) were treated with or without NP-BSA for 3 min. Cell lysates were immunoprecipitated (IP) with anti-SHP-1 Ab-coupled Sepharose beads (A) or anti-GFP Ab together with protein G-Sepharose beads (B). Immunoprecipitates were analyzed by immunoblotting with anti-phosphotyrosine mAb 4G10 (upper panel). The same blots were reprobed with anti-CD72 Ab for confirming co-precipitation of CD72 with SHP-1 mutants (middle panel). The blots were also reprobed with anti-SHP-1 Ab (A) or anti-GFP Ab (B) (lower panel). Representative data of three experiments are shown.
Fig. 7. CD22 and CD72 are the major phosphoproteins associated with SHP-1 in BCR-ligated K46μmLCD72 and WEHI-231 cells. A, cells (1 x 10^6) of K46μmL and K46μmLCD72 were treated with or without NP-BSA for 3 min. Cell lysates were immunoprecipitated (IP) with anti-SHP-1 Ab together with protein G-Sepharose beads. Immunoprecipitates were analyzed by immunoblotting with anti-phosphotyrosine mAb 4G10 (upper panel). Positions for CD22, SHP-1, and CD72 are indicated by arrows. The same blot was reprobed with anti-SHP-1 Ab (lower panel). B, cells (1 x 10^6) of WEHI-231 were treated with or without anti-IgM Ab for 3 min. Cell lysates were immunoprecipitated with anti-SHP-1 Ab together with protein G-Sepharose beads. Immunoprecipitates were analyzed by immunoblotting with anti-phosphotyrosine mAb 4G10 (upper panel). Positions for CD22, SHP-1, and CD72 are indicated by arrows. The same blot was reprobed with anti-CD72 Ab to confirm presence of CD72 in the SHP-1 immunoprecipitates (middle panel). The blot was also reprobed with anti-SHP-1 Ab (lower panel). Representative data of three experiments are shown.

B lymphoma lines WEHI-231 and K46μmL. Taken together, BCR-mediated SHP-1 activation requires inhibitory co-receptors such as CD72 in both J558Lμm3 and B cell lines.

SHP-1 has been shown to be associated with and activated by various tyrosine-phosphorylated molecules independently of inhibitory receptors. These SHP-1-activating molecules include stimulatory receptors such as the erythropoietin receptor (27, 28) or cytoplasmic signaling molecules such as ZAP-70 (30). BCR ligation results in the phosphorylation of various molecules such as the BCR component Igα/Igβ and the cytoplasmic signaling molecules Syk and SLP-65 in J558Lμm3 cells as well as in B cells (4, 34). Moreover, J558Lμm3 cells express active Lyn, because specific substrates of Lyn such as CD22 and CD19 (40–43) are phosphorylated upon BCR ligation in J558Lμm3 transfectants expressing CD22 and CD19.² However, our results indicate that Igα/Igβ, Syk, SLP-65, and Lyn are unable to activate SHP-1. Previously, SHP-1 was reported to associate with BCR, Lyn, and Syk (29, 32, 44, 45). These molecules may associate with SHP-1 without activating SHP-1 probably by interacting at the site outside the SH2 domains. The association with these molecules appears to be weaker than that with SHP-1-activating molecules because we detected association of SHP-1 with CD72 but not Igα/Igβ, Lyn, or Syk in both J558Lμm3 and B cell lines (Figs. 6 and 7). Recently, Igα was shown to have a negative signaling function in B cells (46, 47). Igα may carry the negative signaling function either independently of SHP-1 or by activating SHP-1 through a yet unknown molecule that is not expressed in J558Lμm3 cells.

Previously, SHP-1 was shown to regulate negatively both phosphorylation and kinase activity of Lyn in B cells and myeloid cells (44, 45). However, Lyn is not regulated by BCR nor CD72 in J558Lμm3 cells (Fig. 2). In these cells, Lyn may not be regulatory due to lack of CD45 as introduction of CD45 restores BCR ligation-induced enhancement of Lyn activity (39). In contrast, SHP-1 activated by CD72 down-modulates phosphorylation of Igα/Igβ, Syk, and SLP-65 in J558Lμm3 cells (Fig. 2). Thus, modulation of Lyn activity is not required for SHP-1-mediated negative regulation of Igα/Igβ, Syk, and SLP-65, although dephosphorylation of Lyn may play a role in down-modulation of BCR signaling by SHP-1 in normal B cells.

Previously, Dustin et al. (32) demonstrated that co-expression of wild-type SHP-1 together with Syk reduces phosphorylation of Syk in insect cells, suggesting that Syk is a substrate of SHP-1. Because Syk phosphorylates Igα/Igβ in vitro (48), inhibition of Syk activity may reduce phosphorylation of Igα/Igβ. Alternatively but not mutually exclusively, SHP-1 may directly dephosphorylate Igα/Igβ. Since Syk is phosphorylated by association with phosphorylated Igα/Igβ (49, 50), dephosphorylation of Igα/Igβ may reduce activation and phosphorylation of Syk even in the absence of direct dephosphorylation of Syk by SHP-1. Thus, SHP-1 appears to dephosphorylate Igα/Igβ, Syk, or both in B cells. Since SLP-65 is a substrate of Syk (3–5), SHP-1 may reduce phosphorylation of SLP-65 by inactivating Syk. Yet SLP-65 may also be a substrate of SHP-1 as suggested by Mizuno et al. (51). Both Igα/Igβ and Syk have been shown to play a crucial role in BCR signaling (1, 2). Recent studies have demonstrated that SLP-65 is essential for Ca²⁺ mobilization and extracellular signal-regulated kinase activation by BCR ligation (3, 5, 8, 9). Dephosphorylation of these proximal signaling molecules may be involved in CD72-induced down-modulation of BCR signaling events such as Ca²⁺ mobilization and extracellular signal-regulated kinase activation (16, 17). These distal signaling events are not induced in J558Lμm3 and cannot be assessed in these cells. It is also possible that down-modulation of BCR signaling by CD72 involves other SHP-1 substrates. Moreover, other BCR co-receptors such as CD5 may also activate SHP-1 to coordinately control BCR signaling (52). The J558Lμm3 cells may provide a useful tool to address these questions in the future.

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