Inhibition of the B Cell by CD22: A Requirement for Lyn

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Summary

Mice in which the Lyn, Cd22, or Shp-1 gene has been disrupted have hyperactive B cells and autoantibodies. We find that in the absence of Lyn, the ability of CD22 to become tyrosine phosphorylated after ligation of mlg, to recruit SHP-1, and to suppress mlg-induced elevation of intracellular [Ca²⁺] is lost. Therefore, Lyn is required for the SHP-1–mediated B cell suppressive function of CD22, accounting for similarities in the phenotypes of these mice.

A regulated immune response requires that lymphocytes not only can be stimulated by antigen, but also that they receive signals that suppress spontaneous and antigen-dependent activation. The components of a cell involved in such a “suppressive” signaling pathway have been suggested by the phenotype of hyperactive B cells in mice having mutations affecting each of three genes the naturally occurring mutations of the Shp-1 gene in the mothete and mothete viable (mε) mouse strains (1, 2), and the targeted interruptions of the Lyn (3–5) and Cd22 (6–9) genes.

SHP-1 was the most obvious of these three to have a role in the downregulation of signal transduction because its protein tyrosine phosphatase function can potentially reverse cellular activation induced by protein tyrosine kinases. The two SH2-domains of SHP-1 directly couple its inhibitory function to the activation of tyrosine kinases by localizing and activating the phosphatase at the site of the active kinase (10). The biological importance of SHP-1 for B cell responses is exemplified by mε mice, in which diminished levels of SHP-1 cause expansion of the B-1 subset of B cells, elevated levels of serum IgM, and a low threshold of membrane immunoglobulin (mlg) signaling (11, 12).

CD22 is a member of the immunoglobulin superfamily that is expressed only on B cells, and early studies had suggested it to be a positive regulator of cellular activation (13). However, the findings that (a) tyrosine phosphorylated CD22 recruits SHP-1 (14, 15), (b) coligating CD22 to mlg suppresses activation of mitogen-activated protein (MAP) kinases (16), and (c) sequestering CD22 from mlg enhances B cell activation (15) have indicated that CD22 serves primarily as an inhibitor. Although the precise role of CD22 in the biology of the B cell is not yet understood in relation to its natural ligand (sialic acid in the structure, Sia₂-6Galβ1-4GlcNAc; reference 17), the association of CD22 with mlg in resting B cells (18, 19) suggests that it may constitutively suppress signaling by the antigen receptor. The absence of this function may account for the spontaneous, antigen-independent activation of CD22-null B cells in vivo, and for their enhanced activation in vitro when mlg is ligated (6–9).

Lyn is an src-related nonreceptor tyrosine kinase that associates with the Igα–Igβ heterodimer (20), contributing to the activation of Syk (21) and regulation of intracellular Ca²⁺ concentration ([Ca²⁺]; reference 22), functions that suggest an important role in the stimulation of the B cell. Therefore, the finding that the targeted disruption of the Lyn gene in mice caused elevated levels of IgM, production of autoantibodies, and accentuated signaling through mlg was unanticipated (3–5, 23). One must conclude that Lyn also has inhibitory functions that are not duplicated by other kinases, whereas its activating role may be at least partially redundant and shared by other src-type kinases of the B cell, such as Fyn and Blk (24).

Although inhibition of B cell activation by FcγRIIB1 is impaired in Lyn−/− mice (23), the phenotype of the Lyn−/− B cell more closely resembles that of the Cd22−/− B cell than that of the FcγRIIB−/− cell (25). A role for Lyn in CD22 function is also suggested by the physical association of the two proteins (26). In this study, we find that Lyn has an essential, nonredundant role in regulating the ability of CD22 to recruit SHP-1 for the suppression of signaling by mlg.

Materials and Methods

Animals and Cells. Lyn-deficient mice were generated as previously described (3) and used at 6–8 wk of age. Mice were genotyped by PCR amplification of the wild-type and/or targeted Lyn allele from tail DNA (3). Splenic and lymph node B cells were purified by
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To detect tyrosine phosphorylation, nitrocellulose membranes were incubated with 4G10 (0.1 mg/ml), radio labeled as previously described (29), washed, and exposed to XOMAT film (Eastman Kodak Co., Rochester, N.Y.) at -80°C using an intensifying screen. CD22 and SHP-1 were detected using rabbit anti-human CD22 antibody and mouse anti-human SHP-1 mAb (both shown to cross-react with the relevant mouse protein), followed by horseradish peroxidase-conjugated mouse anti-rabbit and rabbit anti-mouse Ig, respectively. After washing an enhanced chemiluminescence detection system (ECL; Supersignal; Pierce Chemical Co., Rockford, IL) was used for visualization.

Results

B cells lacking CD22, Lyn, or SHP-1 more readily elevate [Ca^{2+}]i in response to ligation of mlg than do normal B cells (5–9, 12, 23), indicating that these proteins may regulate B cell activation by a common mechanism. As cross-linking CD22 to mlg provides a means by which its inhibitory function can be assessed (16), we determined whether coligating CD22 to mlg diminishes the [Ca^{2+}]i response induced by the antigen receptor. B cells were loaded with the Ca^{2+}-sensitive fluorescent indicator, indo-1, and were preincubated with biotinylated Fab fragment of anti-body to k light chain in combination with either biotinylated Fab anti-CD22 or control irrelevant Fab. The cells were activated by addition of avidin, and [Ca^{2+}]i was monitored by flow cytometry. In B cells from Lyn+/+ mice, cross-linking of anti-κ alone induced a prompt rise in [Ca^{2+}]i that was followed by a gradual decline after 320 s to a level that was 33% of the maximal increment (Fig. 1). Coligating CD22 to k suppressed the initial response by 40%, and maintained this inhibitory effect until the [Ca^{2+}]i had returned to that of unstimulated cells (Fig. 1). In contrast to these findings in wild-type B cells, CD22 coligation did not inhibit the rise in [Ca^{2+}]i induced by mlg in Lyn−/− B cells (Fig. 1). These results were not caused by absent expression of CD22 on B cells from Lyn−/− mice, as expression was similar to that of their +/+ littermates (Fig. 1). Similar results were obtained in four additional experiments in which B cells were identified either by gating out all non-B cells that had been stained by a cocktail of monoclonal antibodies, or, as was done in this experiment, by their staining with antibody to B220. Therefore, juxtaposition of CD22 to mlg diminishes the capacity of the antigen receptor to elevate [Ca^{2+}]i, and this function requires Lyn.

To determine whether Lyn mediates the mlg-induced tyrosine phosphorylation of CD22, purified splenic B cells from Lyn−/− and Lyn+/+ mice were held in buffer or were stimulated with the F(ab')2 fragment of polyclonal anti-μ, for 1 and 5 min, the cells were lysed, and CD22 was immunoprecipitated. The precipitated proteins were assessed both by immunoblotting with antibody to phosphotyrosine and antibody to CD22. CD22 in Lyn+/+ B cells demonstrated constitutive tyrosine phosphorylation, and liglation of mlg increased this modification (Fig. 2 A). This change in CD22 was especially apparent when, by scanning densitometry, the ratio of the antiphosphotyrosine signal to the anti-CD22 signal which adjusts for minor changes in

Antibodies used in this study were Lyb 8.2 allo-
typic mouse anti-mouse CD22 (PharMingen, San Diego, CA); biotinylated control mouse IgG1-κ anti-trinitrophophenyl (Phar-
Mingen); rabbit anti-human CD22 antisera raised to a CD22-
glutathione S transferase fusion protein; mouse anti-human SHP-1 (which cross-reacts with mouse SHP-1; Transduction Laborato-
ries, Lexington, KY); control mouse IgG1-κ (PharMingen); per-
oxidase-coupled mouse anti-rabbit IgG (Jackson Immuno-
researc Labs., West Grove, PA); peroxidase-coupled rabbit anti-
mouse IgG (Jackson Immuno-research Labs.); mouse 4G10 mAb
to phosphotyrosine (UBI, Lake Placid, N.Y.); RA3-6B2 anti-
CD45/R/B220; Thy 1.2 mAb (Sigma Chemical Co., Poole, U.K);
F(ab')2 goat anti-mouse IgM (Jackson Immuno-research Labs.,
LO-M K-1 rat IgG2a anti-mouse κ (Zymed, South San Francisco,
CA); BC5 anti-Gr-1 (gift of Dr. R. Coffman, DNAX, Stanford,
CA); M 1/70 anti-CD11b; 2.4G2 anti-FCyRII and II; F4/80 anti-
mouse monocyte/macrophage mAb; and Ter119 anti-terythrocyte precursor mAb.

Flow Cytometric Analysis. Single cell suspensions were pre-
pared and cell staining was performed as previously described
(28). Cells were analyzed and sorted using an argon laser, and
[Ca^{2+}]i was measured using a U V laser of a M oflow flow cytometer (Cytometry Inc., Fort Collins, CO).

[Ca^{2+}]i Measurement. Splenocytes were stained with a cock-
tail of FITC-conjugated antibodies (Thy 1.2, 8C5, M 1/70, F4/80,
and Ter119) with B cells left unstained (confirmed by coun-
tries, Lexington, KY); control mouse IgG1-
[k light chain in combination with either biotiny-
ated Fab anti-CD22 or control irrelevant Fab. The cells
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the loading of CD22, was determined (Fig. 2 B). CD22 was also constitutively phosphorylated in B cells from Lyn−/− mice to almost the same level as in wild type B cells, but the ratio of the antiphosphotyrosine signal to the anti-CD22 signal was not increased by ligation of mIg (Fig. 2). Therefore, Lyn selectively mediates mIg-induced tyrosine phosphorylation of CD22.

The recruitment of SHP-1 is considered to mediate the inhibitory effects of CD22. Therefore, we examined the interaction of SHP-1 with CD22 in Lyn+/+ and Lyn−/− B cells before and after cross-linking mlgM by analyzing immunoprecipitates of SHP-1 for the presence of tyrosine phosphorylated CD22. In resting wild-type B cells, SHP-1 was associated with a tyrosine phosphorylated protein having the molecular weight of CD22, and activation of these B cells through mlgM increased this complex by almost threefold (Fig. 3). In contrast, in Lyn−/− B cells SHP-1 was not constitutively nor inducibly associated with CD22, the amount of the complex being at least an order of magnitude less than in Lyn+/+ cells (Fig. 3). Thus, in Lyn−/− B cells, CD22 is not phosphorylated at tyrosines appropriate for the recruitment of SHP-1, accounting for its inability to inhibit mlg signaling.

Discussion

The mlg-induced tyrosine phosphorylation of CD22 (Fig. 2) leading to the recruitment of SHP-1 (Fig. 3) and inhibition of mlg signaling, as exemplified by an impaired [Ca^2+]i response (Fig. 1), requires the presence of Lyn. Loss of this function of Lyn may be the basis for the hyperactive B cell response of Lyn-deficient B cells in vitro (24), and may contribute to the production of autoantibodies in vivo. The loss of FcγRIIB1 inhibitory function, which is also a characteristic of the Lyn-deficient B cell (5, 23), is less likely to contribute to the phenotype of Lyn−/− mice, as mice lacking FcγRIIB1 have more modest changes in B cell function in vivo and in vitro (25), reflecting the negative regulatory role of this receptor in maintenance rather than in the initiation of the B cell response to antigen.

This study demonstrates that Lyn, although largely redundant for positive regulation of mlg signaling, is irreplaceable for suppressive signaling via CD22. The former observation could have been anticipated based on studies of the activation of other src-related kinases (such as Blk and Fyn), by mlg in mammalian B cells (24), and from the finding of Syk-dependent activation of phospholipase C-γ in chicken DT 40 cells lacking Lyn (22). Thus, the association of Lyn with CD22 (26) may have the unique functional consequence of mediating the phosphorylation of specific tyrosines which recruit SHP-1. Consistent with this possibility is the preference of Lyn for phosphopeptides having the consensus sequence shown in Fig. 4 (30). The three phosphopeptides of CD22 previously shown to bind Lyn+/+ and Lyn−/− mice.
Presumably another tyrosine kinase(s) is responsible for the constitutive phosphorylation of the tyrosines of CD22 in the Lyn−/− B cells that do not mediate the binding of SHP-1 (Fig. 2). Although this study has not addressed the role of these other phosphorysines, the absence of any effect on Ca²⁺ signaling when CD22 is cross-linked to mIg on Lyn-deficient B cells indicates that they do not engage intracellular proteins that augment this early cellular response. Thus, these studies fail to support a role for CD22 in the positive regulation of B cell activation, even when interaction with SHP-1 does not occur.

In the B cell, tyrosine kinases have both positive and negative effects on cellular activation through the phosphorylation of membrane proteins with distinct regulatory functions. The phosphorylation of the Ig-α–Ig-β heterodimer of the antigen receptor complex and of CD19 promotes the activation of the B cell by recruiting enzymes such as Syk, Vav, and phosphatidylinositol 3-kinase. Conversely, the phosphorylation of the FcγRIIB1 and CD22 recruits the phosphatases, SHIP and SHP-1, for suppression of cellular stimulation. Thus, ligation of the antigen receptor, even when sufficient stimulus is provided for activation of a tyrosine kinase, does not necessarily lead to cellular activation. Rather, this stimulus creates the potential for either activation or suppression, with the outcome determined by presence or absence of the ligands for the regulatory membrane proteins, CD19, CD22, and FcγRIIB1.

The autoimmunity that occurs when either Lyn or SHP-1 is deleted is more striking than the modest occurrence of autoantibodies that is observed when CD22 expression by B cells is ablated. Although the absence of any one of these three molecules would have a similar effect on the B cell by promoting signaling through mIg, Lyn and SHP-1 are expressed in other hematopoietic cells in which they have important functions. Therefore, for the production of pathogenic autoantibodies, impaired regulation of B cells alone (as occurs in Cd22−/− mice) may not be sufficient, and significant autoimmunity may require dysregulated signaling in the several cell types that cooperate in the immune response.

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