Altered Antigen Receptor Signaling and Impaired Fas-mediated Apoptosis of B Cells in Lyn-deficient Mice

By Jiyang Wang, Toshiki Koizumi, and Takeshi Watanabe

From the Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-82, Japan

Summary

Mice deficient in the src-related protein tyrosine kinase, Lyn, exhibit splenomegaly and accumulate lymphoblast-like and plasma cells in spleen as they age, resulting in elevated levels of serum IgM (10–20-fold of control) and glomerulonephritis due to the presence of immune complexes containing auto-reactive antibodies. It remains unclear, however, how antibody-producing cells are accumulated in the lymphoid tissues of Lyn−/− mice. To elucidate the role of Lyn in B cell function, we have studied the proliferative responses to various stimuli and Fas-mediated apoptosis in B cells from young Lyn−/− mice which do not yet show apparent abnormality such as splenomegaly. Compared with control B cells, Lyn−/− B cells were hyper responsive to anti-IgM-induced proliferation and defective in FcγRIIB-mediated suppression of B cell antigen receptor (BCR) signaling, indicating that Lyn is involved in the negative regulation of BCR signaling. In addition, the BCR-mediated signal in Lyn−/− B cells, unlike that in control B cells, failed to act in synergy with either CD40- or IL-4 receptor-triggered signal in inducing a strong proliferative response, suggesting that the BCR signaling pathway in Lyn−/− B cells is altered from that in control B cells. Furthermore, Lyn−/− B cells were found to be impaired in the induction of Fas expression after CD40 ligation and exhibited a reduced susceptibility to Fas-mediated apoptosis. Moreover, BCR cross-linking in Lyn−/− B cells suppressed Fas expression induced by costimulation with CD40 ligand and IL-4. Collectively, these results suggest that the accumulation of lymphoblast-like and plasma cells in Lyn−/− mice may be caused, in part, by the accelerated activation of B cells in the absence of Lyn, as well as the impaired Fas-mediated apoptosis after the activation.

The B cell antigen receptor (BCR)1 consists of membrane immunoglobulin M (IgM) non-covalently associated with disulfide-linked heterodimers of Igα and Igβ subunits which couple mig to several protein tyrosine kinases (PTKs) (1). Cross-linking of the BCR rapidly activates two classes of PTKs: the src family kinases (Lyn, Fyn, Blk, Hck, and Lck) (2-5) and the Syk kinase (6, 7), resulting in the phosphorylation of several proteins (8, 9), including PTKs themselves, Igα and Igβ (10), phospholipase C-γ (11), phosphatidylinositol (PI)-3 kinase (12, 13), the protooncogene product Vav (14-16), Ras GTPase-activating protein (17), Shc (18), and HS1 (19).

The src-related kinase, Lyn, is expressed preferentially in hematopoietic cells and in cells of neural tissues (20, 21). Lyn is physically associated with the BCR in B cells and is rapidly phosphorylated upon receptor cross-linking (2-5).

1Abbreviations used in this paper: BCR, B cell antigen receptor; CD40L, CD40 ligand; FcγRIIB, B cell receptor for IgG Fe region; IgM, membrane immunoglobulin M; NMS, normal mouse serum; PI, propidium iodide; PTK, protein tyrosine kinase; SHP-1, hematopoietic cell phosphatase; smlg, cell surface IgM.
Daudi cell lines (32). These observations suggest a role for Lyn in the negative regulation of BCR signaling.

In addition to a role of Lyn in regulating BCR signaling, biochemical analysis has suggested its involvement in the signaling pathway through CD40 (33, 34), a member of the TNF receptor family that plays a critical role in the survival, growth, differentiation and apoptosis of B lymphocytes (35-37). Lyn thus appears to be involved in the signal transduction from multiple receptors on B cells.

Lyn-/- mice, generated recently by gene targeting, exhibited splenomegaly with age and accumulated the lymphoblast-like and plasma cells in spleen (38, 39). There was a high concentration of serum IgM in Lyn-/- mice, and a significant number of Lyn-/- mice showed glomerulonephritis due to the presence of immune complex containing auto-reactive antibodies. It remains unclear, however, how large numbers of antibody producing cells accumulate in lymphoid tissues of Lyn-/- mice. Recent studies revealed a critical role for the CD40-mediated signal in upregulating Fas expression and inducing susceptibility to Fas-mediated apoptosis in B cells (40-42). By contrast, BCR engagement was shown to protect B cells from Fas-mediated apoptosis (43), indicating that Fas susceptibility is positively and negatively regulated by CD40- and BCR-mediated signals, respectively. It has been suggested that activated bystander B cells, which may produce various Abs including autoantibodies, are eliminated by Fas-dependent apoptosis (43). Since Lyn was implicated in the signal transduction pathways of both CD40 and BCR, we examined whether Lyn-/- B cells have defects in Fas-mediated apoptosis. We demonstrate in the present study that Lyn-/- B cells are impaired in Fas-mediated apoptosis due to both an impaired induction of Fas expression and a reduced susceptibility to Fas-mediated apoptosis. In addition, B cells from young Lyn-/- mice are hyper responsive to anti-IgM-induced proliferation and defective in FcyRll-mediated suppression of signals generated by BCR engagement. These results suggest that Lyn-/- B cells have a reduced threshold for antigen-triggered activation, and may survive for prolonged period after the activation due to impaired Fas-mediated apoptosis, resulting in the accumulation of antibody-secretory cells.

Materials and Methods

Reagents. Goat F(ab')2 and intact anti-mouse IgM Abs purchased from Southern Biotechnology Associates, Inc. (SBA, Birmingham, AL) were extensively dialyzed in PBS. The Jo2 anti-mouse Fas Ab and anti-mouse CD8 Ab were purchased from Pharmingen (San Diego, CA). The IC10 rat anti-mouse CD40 Ab and the biotinylated Jo2 anti-mouse Fas Ab were kindly provided by Dr. M. Howard (DNAX, Palo Alto, CA) and Dr. S. Nagata (Osaka Bioscience Institute), respectively. Culture supernatants from a myeloma cell line producing soluble CD40 ligand CD8 fusion protein (CD40L or L), or CD40L plus an anti-CD8 Ab (L + anti-CD8), were considerably reduced compared with control B cells (Fig. 1 A). The reduced response to CD40 ligation is unlikely to reflect a low level of CD40 expression as the level of CD40 on Lyn-/- B cells was found to be similar as that on control B cells (Fig. 2 A). The data demonstrate that Lyn, which was reportedly activated by CD40 ligation (33, 34), is indeed critical for CD40-mediated proliferative response in B cells. Since Lyn-/- B cells could still respond to CD40 ligation to some extent, it is likely that other PTKs, such as Fyn and Syk which were also activated by CD40 ligation (33, 34), may be involved in the observed proliferative response. These PTKs, however, could not compensate for the activity of Lyn to restore a normal proliferative response induced by CD40 ligation.

Cells and Cell Culture. Cells were cultured at 37°C with 5% CO2 in a humidified atmosphere, in RPMI 1640 containing 5 x 10^{-5} M 2-mercaptoethanol, 25 μg/ml of Gentamycin, supplemented with 10% heat-inactivated fetal calf serum (GIBCO BRL, Gaithersburg, MD). Splenic B cells were isolated from 4-8-wk-old mice after T cell depletion by treatment with anti-mouse Thy1.2 mAb for 30 min on ice and then with complement for 30 min at 37°C, followed by Ficoll-Hypaque density centrifugation and incubation at 37°C for 1 h to remove adherent cells. Approximately 90-95% of the isolated cells were found to express the B cell marker B220.

Proliferation Assay. Splenic B cells (5 x 10^5/ml, 100 μl) were placed in 96-well flat-bottom plates and cultured in the presence of various stimuli. The cultures were pulsed for the last 6 h with [3H]thymidine (1 μCi/ml) and the mean [3H]thymidine incorporation and standard deviation were calculated for triplicate cultures.

Immunofluorescence Analysis. For CD40 staining, cells (10^5) were incubated in 50 μl of 10 μg/ml of the 1C10 antibody on ice for 20 min, followed by a phycoerythrin (PE) conjugated goat anti-rat Ig Ab (SBA). For Fas staining, cells were incubated with normal mouse serum (NMS) to block Fc receptors and then stained with the biotinylated Jo2 anti-mouse Fas Ab, followed by staining with streptavidin conjugated PE (SAPE; SBA). Cells stained with NMS and then SAPE served as controls. Cells were fixed in 1% paraformaldehyde before analyzing with a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA).

Analysis for Cell Death. Cells were stained with 1 μg/ml of propidium iodide (PI) in PBS containing 2% FCS and 0.02% NaN3 for 5 min on ice and the percentage of PI positive (dead) and negative (live) cells were determined with a FACScan® flow cytometer.

Results

Proliferative Responses to Various Stimuli in Lyn-/- B Cells. In the previous study, we have demonstrated that lymphoblast-like cells carrying Mac-1 antigen and cytoplasmic IgM accumulated in spleen of Lyn-/- mice (39). To find out the intrinsic defects of Lyn-/- B cells, we have investigated the function of B cells from young (4-8-wk-old) mice which do not yet show apparent abnormality such as splenomegaly and the accumulation of lymphoblast-like cells. In accordance with earlier observations (39), the proliferative responses of Lyn-/- B cells to either soluble CD40 ligand-CD8 fusion protein (CD40L or L), or CD40L plus an anti-CD8 Ab (L + anti-CD8), were considerably reduced compared with control B cells (Fig. 1 A). The reduced response to CD40 ligation is unlikely to reflect a low level of CD40 expression as the level of CD40 on Lyn-/- B cells was found to be similar as that on control B cells (Fig. 2 A). The data demonstrate that Lyn, which was reportedly activated by CD40 ligation (33, 34), is indeed critical for CD40-mediated proliferative response in B cells. Since Lyn-/- B cells could still respond to CD40 ligation to some extent, it is likely that other PTKs, such as Fyn and Syk which were also activated by CD40 ligation (33, 34), may be involved in the observed proliferative response. These PTKs, however, could not compensate for the activity of Lyn to restore a normal proliferative response induced by CD40 ligation.
Much to our surprise, Lyn−/− B cells from young mice were hyper responsive to anti-IgM-induced proliferation compared with control B cells. As indicated in the dose-response profiles (Fig. 1 B), Lyn−/− B cells responded greatly to a low dose of F(ab′)2 anti-IgM (1 μg/ml) within 1 d of culture whereas control B cells responded poorly even after 2 d. At a higher dose of F(ab′)2 anti-IgM (20 μg/ml), however, the proliferative response was similar in Lyn−/− and control B cells (Fig. 1 B, compare [3H]thymidine incorporation of Lyn−/− vs. control B cells stimulated with 20 μg/ml of F(ab′)2 anti-IgM for 2 d). This is likely because the response of Lyn−/− B cells reached near plateau at relatively high concentrations of anti-IgM Abs whereas the response of Lyn+/+ B cells continued to increase. The hyper responsiveness of Lyn−/− B cells to antigen stimulation does not reflect an increased expression of cell surface IgM (slgM) as the level of slgM is similar in control and Lyn−/− B cells (Fig. 2 C). These results demonstrate that Lyn−/− B cells have a reduced threshold for anti-IgM induced proliferation with an accelerated time course than do control B cells.

Another interesting finding is that in Lyn−/− B cells, the whole anti-IgM Abs induced a similar, if slightly reduced, proliferative response as did F(ab′)2 anti-IgM Abs (Fig. 1 B), whereas in control B cells, the proliferative responses were significantly reduced when stimulated with whole anti-IgM Abs which have been shown to cross-link BCR with FcyRIIB, a predominant form of Fcy receptors expressed on B cells (45–47). The results demonstrate that FcyRIIB-mediated downregulation of BCR signaling is not properly functional in the absence of Lyn, although it has not yet been studied whether Lyn is activated by cross-linking FcyRIIB.

Response to IL-4 does not seem to be impaired in Lyn−/− B cells as indicated by both the proliferation (Fig. 1 A) and upregulation of CD40 expression in response to IL-4 (Fig. 2 B). Notably, stimulation with CD40L in the presence of IL-4 (L + IL-4) for one or 2 d induced a normal or somewhat higher proliferative response in Lyn−/− B cells relative to control B cells, suggesting a possibility that IL-4 receptor-mediated signals acted in synergy with and restored the CD40-mediated proliferative signal which was otherwise reduced in Lyn−/− B cells (Fig. 1 A). Stimulation with CD40L plus both IL-4 and anti-IgM also induced a normal proliferative response in Lyn−/− B cells on day 1, but unlike the combination of CD40L plus IL-4, the response was significantly reduced in Lyn−/− B cells, as compared with control B cells, on day 2. It appears that the restored proliferative response of Lyn−/− B cells induced by CD40L plus IL-4, when combined with the hyper responsiveness to anti-IgM stimulation, was accelerated and decayed more rapidly than that of control B cells. Alternatively, the BCR-mediated abnormal signal in Lyn−/− B cells may have suppressed the restored proliferative response induced via CD40L plus IL-4.

The data shown in Fig. 1 A also suggested that, in the absence of Lyn, the BCR-mediated signal failed to act in synergy with CD40- or IL-4 receptor-mediated signals in inducing strong proliferative responses. In control B cells, the combinations of anti-IgM and CD40L or anti-IgM and IL-4 induced a strong proliferative response that was more than the sum of that induced by each stimulation alone.
whereas in \textit{Lyn}^-/- B cells, these combinations only acted additively, suggesting that the BCR-mediated signal transduction pathway in the absence of Lyn is clearly distinguished from that in control B cells.

\textbf{Reduced Fas Induction After Various Stimuli in \textit{Lyn}^-/- B Cells.} We and others have recently found that CD40 ligation upregulated Fas expression and enhanced susceptibility to Fas-mediated apoptosis in B cells (40-43). Since \textit{Lyn}^-/- B cells exhibited a reduced proliferative response to CD40 ligation, Fas expression (Fig. 3) and Fas-mediated apoptosis (Fig. 4) after various stimuli were investigated. In both control and \textit{Lyn}^-/- mice, fresh splenic B cells (Fig. 3 A) or B cells stimulated with anti-IgM, IL-4 or anti-IgM plus IL-4 (data not shown), expressed only a low level of Fas antigen, confirming our earlier finding that Fas is not induced by BCR-cross-linking or IL-4 stimulation (42). Stimulation with CD40L upregulated Fas expression in control but not \textit{Lyn}^-/- B cells (Fig. 3 B). CD40L plus an anti-CD8 Ab which delivered a stronger signal as judged by the proliferative response (Fig. 1 A), however, induced Fas expression in \textit{Lyn}^-/- B cells to a similar level of that in control B cells (Fig. 3 C), suggesting that Fas induction in \textit{Lyn}^-/- B cells required a stronger CD40-mediated signal. CD40-mediated Fas induction could be restored by IL-4 (Fig. 3 E), in accordance with the observation that CD40L plus IL-4 restored the proliferative response (Fig. 1 A). The reduced Fas induction by CD40L in \textit{Lyn}^-/- B cells could not be restored by co-stimulation with anti-IgM (Fig. 3 D), in keeping with the observation that anti-IgM and CD40L did not act in synergy to restore a normal proliferative response in \textit{Lyn}^-/- B cells. Moreover, anti-IgM stimulation downregulated Fas expression of \textit{Lyn}^-/- B cells induced by CD40L plus IL-4 (compare Fig. 3, C and D). Interestingly, while a normal proliferative response was induced on day 1 by stimulation with either CD40L plus IL-4 or CD40L plus IL-4 and anti-IgM (Fig. 1 A, day 1), Fas expression was induced on day 1 by treatment with CD40L plus IL-4 but not CD40L plus IL-4 in the presence of anti-IgM (data not shown). Collectively, these results revealed two abnormal aspects of \textit{Lyn}^-/- B cells in the induction of Fas expression: one is that Fas induction in \textit{Lyn}^-/- B cells required a stronger CD40-mediated signal, and the other is that the abnormal BCR-triggered signal suppressed Fas induction.

\textbf{Impaired Fas-mediated Apoptosis in \textit{Lyn}^-/- B Cells.} Having shown the abnormality of Fas induction in \textit{Lyn}^-/- B cells, we next examined the susceptibility to Fas-mediated apoptosis in these B cells. In accordance with our prior finding, both control and \textit{Lyn}^-/- B cells activated for 1 d were resistant to Fas-triggered apoptosis (data not shown). B cells activated for 2 d were therefore examined for Fas susceptibility. Consistent with the reduced Fas expression, \textit{Lyn}^-/- B cells stimulated by CD40L alone, CD40L plus anti-IgM or CD40L plus anti-IgM and IL-4, all exhibited reduced susceptibility to Fas-mediated apoptosis after treatment with the Jo2 anti-Fas Ab for 4 h (43) or 16 h (Fig. 4 F). In keeping with the observation that IL-4 acted in synergy with CD40-mediated signals to restore a normal proliferative response (Fig. 1 A) and upregulated Fas expression (Fig. 3 E), \textit{Lyn}^-/- B cells stimulated with CD40L plus IL-4 underwent Fas-triggered apoptosis in a similar dose dependent manner as did control B cells after treatment with increasing concentrations of the Jo2 anti-Fas Ab (Fig. 4 A), or after incubation with the anti-Fas Ab for 16 h (Fig. 4 B).

Although a similar level of Fas expression was observed in control and \textit{Lyn}^-/- B cells stimulated with CD40L plus the anti-CD8 Ab, \textit{Lyn}^-/- B cells exhibited a reduced susceptibility to the anti-Fas Ab-induced apoptosis relative to control B cells (44). The data suggest that a stronger CD40-mediated signal (CD40L plus anti-CD8) upregulated Fas expression but did not completely restore the susceptibility to Fas-mediated apoptosis in \textit{Lyn}^-/- B cells. CD40 ligation has recently been shown to not only induce cell surface Fas expression, but also enhance Fas susceptibility in B cells (40-42). It is thus likely that Fas susceptibility was more affected by the Lyn-dependent, CD40-mediated signal, whereas Fas induction could be restored by a stronger CD40 ligation in the absence of Lyn. Taken together, these results demonstrate that \textit{Lyn}^-/- B cells are impaired in the induction of both Fas expression and Fas susceptibility induced by CD40L. Furthermore, the BCR-mediated sig-
Figure 4. Fas-mediated apoptosis of activated B cells. Splenic B cells stimulated for 2 d as in Fig. 3 were treated with indicated amounts of the Jo2 anti-Fas Ab for 4 h (A) or treated with 1 µg/ml of the anti-Fas Ab for 16 h (B). Percent cell death was calculated by the formula: % experimental cell death - % spontaneous cell death/100 - spontaneous cell death. Shown in A is one of two experiments which gave similar results. Shown in B are the average and standard deviation of five independent experiments. Unpaired student's t test was calculated in B and the P values indicated by * were <0.05.

Discussion

To begin to elucidate the role of Lyn in B cell function, we have investigated in the present study the function of B cells from young Lyn-/- mice. We reasoned that while the aged Lyn-/- mice, which have accumulated abnormal B cells, are suitable for studying the consequence of B cell defects, young mice are better models to investigate the cause of the B cell abnormality. The results presented here demonstrate that Lyn-/- B cells are hyper responsive to antigen stimulation and have defects in Fas-mediated apoptosis, suggesting that Lyn-/- B cells are likely to be activated by low doses of antigen stimulation and may not be efficiently eliminated after the activation due to impaired Fas-mediated apoptosis, which has been implicated in the elimination of activated bystander B cells (43).

In contrast to the finding here that Lyn-/- B cells are hyper responsive to BCR-cross-linking, earlier studies have suggested a severe reduction in anti-IgM-induced proliferation in Lyn-/- B cells (38, 39). Several possibilities could be considered for the observed discrepancy. IgM+ B cells in young mice may be characteristically distinguished from those in aged mice, for example BCR-associated signal transduction molecules including the src family protein tyrosine kinases or phosphatases may be differentially expressed. It is also possible that the reduced proliferative response of Lyn-/- B cells observed in earlier studies may reflect a decreased percentage of antigen-responsive B cells due to the accumulation of abnormal B cells which carry Mac-1 antigen and cytoplasmic IgM and therefore do not respond to BCR cross-linking (39). The hyper responsiveness to anti-Ig-induced proliferation observed in Lyn-/- B cells is in accordance with the previous findings that Lyn-/- DT40 cells exhibited enhanced IP-3 generation (31) and that Lyn is involved in the cell cycle arrest induced by anti-IgM stimulation in B lymphoma cell lines (32). It is notable that B cells from young Lyn-/- and control mice are phenotypically similar and express a similar level of surface IgM (Fig. 2 B). The hyper responsiveness observed in young Lyn-/- B cells thus indicates an intrinsic defect in B cell function, but not a consequence of abnormalities in other cell types.

The whole anti-IgM Ab is known to cross-link the BCR with FcyRIIB, a predominant form of the Fcy receptors expressed on B cells, resulting in the suppression of BCR-mediated signaling (45-47). As shown in Fig. 1 B, Lyn-/- B cells apparently have defects in FcγRII-mediated suppression. It is intriguing to note that recently generated FcγRII-/- mice have been shown to have enhanced immune responses to both T-dependent and -independent antigens (48), a phenomenon also observed in Lyn-/- mice (39). These results suggest a critical role for Lyn in FcγRII-mediated downregulation of BCR-mediated proliferative
response although it remains to be examined how Lyn is involved in FcγR-mediated suppression of BCR signaling.

The hyper responsiveness to anti-IgM-induced proliferation and lack of FcγRIIB-mediated suppression of BCR signaling observed in Lyn\(^{-/-}\) mice are similar to that observed in B cells from moth-eaten (me) and viable moth-eaten (me\(^{v}\)) mice (49, 50), which express no or aberrantly spliced mutant SHP-1 protein (also called PTP1C or HCP), respectively (51-53). In addition, both me and Lyn\(^{-/-}\) mice accumulate plasma cells in their lymphoid tissues (38, 39, 54, 55). Both SHP-1 and Lyn thus negatively regulate BCR signaling for proliferation. It is conceivable that Lyn may directly or indirectly interact with SHP-1 in BCR signaling, a hypothesis that can be proved by detailed biochemical analysis. Unlike me mice which die shortly after birth and predominantly contain B-1 (CD5\(^{+}\)) type peripheral B cells (55, 56), Lyn\(^{-/-}\) mice are viable for at least one year and contain a largely conventional B cell population with increased number of abnormal CD5\(^{-}\), B220\(^{-}\), Mac-1\(^{-}\) and cytoplasmic µ\(^{+}\) lymphoblast-like cells as they age (39). The less severe phenotype in Lyn\(^{-/-}\) mice compared with me mice may suggest that Lyn and SHP-1 may regulate overlapping, but distinct B cell signaling pathways. It is also conceivable that SHP-1, which is expressed in various tissues and cell types, may regulate additional signaling pathways (55).

Stimulation with CD40L induced Fas expression in control but not Lyn\(^{-/-}\) B cells, demonstrating that Lyn is involved in the CD40-mediated induction of Fas. A stronger CD40 ligation, generated by cross-linking CD40L with an anti-CD8 Ab, almost completely restored Fas induction but only partially restored the susceptibility to Fas-mediated apoptosis. The data suggest that Lyn is also involved in the enhancement of Fas susceptibility. IL-4-mediated signal could compensate for the impaired CD40 signaling in Lyn\(^{-/-}\) B cells as CD40L plus IL-4 restored the proliferative response, the induction of Fas expression and the susceptibility to Fas-mediated apoptosis. B cells are activated by stimulation through multiple receptors (37). Signals through BCR, IL-4R, and CD40 apparently act in synergy in normal B cells to generate strong proliferative responses since the combination of any two of the above three signals generated a much stronger growth signal that is more than the sum of that induced by each stimulation alone (Fig. 1A). In the absence of Lyn, the enhanced BCR-mediated growth signal failed to cooperate with either CD40- or IL-4R-mediated signals to provide a strong synergy in inducing B cell growth. Although further studies are required to clarify at the molecular level how the abnormal signals through BCR and CD40 result in the dysfunction of B cells, the results presented here provided evidence of a critical role for Lyn in regulating not only the signal transduction through BCR, and CD40 but also the interactions between these signals and with other signals.

Engagement of BCR has been shown to rescue Fas-mediated apoptosis in B cells (43). The mechanism for this protection is unclear, but our preliminary data suggest a possible role of bcl-X\(_{L}\) in regulating the Fas susceptibility of B cells. It is thus intriguing to examine whether the enhanced BCR signaling in Lyn\(^{-/-}\) B cells would result in a rapid and sustained induction of the anti-apoptotic gene such as bcl-X\(_{L}\). As is the case for B cells in me or me\(^{v}\) mice, the enhanced BCR-mediated signal in Lyn\(^{-/-}\) B cells is likely to lead to accelerated activation and differentiation of B cells in response to even low doses of antigen stimulation. The impaired Fas induction and Fas-mediated apoptosis after B cell activation in Lyn\(^{-/-}\) B cells, along with the observation that the abnormal BCR signaling suppressed Fas expression, indicate that activated Lyn\(^{-/-}\) B cells may not be efficiently eliminated by Fas-mediated apoptosis. The accumulation of lymphoblast-like and plasma cells in Lyn\(^{-/-}\) mice may be consequent to the combined effects of an accelerated activation/differentiation of B cells and an impaired Fas-dependent elimination of activated B cells.

Although apoptosis of B cells may be mediated through multiple pathways, Fas-mediated apoptosis plays an essential role in maintaining peripheral B and T cell tolerance. Mutations in gene Fas or its ligand result in autoimmune disorders of lpr and gld mice, respectively (57). While Fas is important for both T and B cell function, Lyn is not expressed in T cells and correspondingly no obvious defects have been observed in Lyn\(^{-/-}\)T cells (38, 39). Lyn\(^{-/-}\) mice thus provide a unique model of autoimmune disorders that are largely due to the impaired function of B cells. It remains to be investigated whether there is a human counterpart of a similar autoimmune disorder arising from the dysfunction of Lyn or other src family tyrosine kinases.

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Address correspondence to Takeshi Watanabe, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-82, Japan.

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