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CD72 Down-Modulates BCR-Induced Signal Transduction and Diminishes Survival in Primary Mature B Lymphocytes

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CD72, a 45-kDa type II transmembrane glycoprotein carrying an ITIM motif, is believed to be an inhibitory coreceptor of the BCR. Mature B cells lacking CD72 show enhanced Ca2+ mobilization and are hyperproliferative in response to BCR ligation. However, the signal transduction pathways downstream of BCR signaling that transmit the inhibitory effect of CD72 in mature B cells remain unknown. To address this question, we used hen egg lysozyme-specific BCR transgenic mice to elucidate the differential signaling between wild-type and CD72-deficient B cells in response to hen egg lysozyme Ag stimulation. Our results demonstrate that CD72 predominantly down-regulates the major signal transduction pathways downstream of the BCR, including NF-AT, NF-κB, ERK, JNK, p38-MAPK, and PI3K/Akt in mature B cells. CD72 ligation with anti-CD72 Ab (K10.6), which mimics the binding of CD100 (a natural ligand for CD72) to release the inhibitory function of CD72, augments cell proliferation, Ca2+ flux, IκBα activation, and ERK MAPK activity upon Ag stimulation in wild-type B cells. In addition, we show direct evidence that CD72 promotes cell cycle arrest and apoptosis after Ag stimulation in mature B cells. Taken together, our findings conclude that CD72 plays a dominant role as a negative regulator of BCR signaling in primary mature B lymphocytes. The Journal of Immunology, 2006, 176: 5321–5328.

The role of CD72 as a negative regulator of BCR activation is evidenced by its association with the protein tyrosine phosphatase Src homology protein tyrosine phosphatase-1 (SHP-1) through its ITIM after BCR stimulation (7–10). Activation of SHP-1 leads to dephosphorylation and deactivation of several signaling molecules that are activated by BCR ligation (11). Besides the ITIM, the cytoplasmic tail of CD72 also carries an ITIM-like motif that binds Grb-2 (7). The formation of the CD72/SHP-1/Grb-2 complex after BCR ligation is linked to a decrease in cellular proliferation in a retrovirus-transformed immature B cell line (12). The inhibitory effect of CD72 on BCR activation can also be seen in studies showing that both mature B cells and an immature B cell line lacking CD72 are hyperproliferative in response to anti-IgM stimulation (12, 13). Furthermore, the ligand for CD72, CD100, can turn off the negative signaling mediated by CD72 in both mature B cells and the immature B cell line WEHI231 (14, 15).

The signal transduction pathways downstream of BCR signaling that are regulated by CD72 are not well defined and are contradictory in several studies. Tsubata and colleagues (16) demonstrated that CD72 down-modulates ERK activation in the murine B lymphoma line K46 after BCR-specific Ag stimulation. In contrast, Ogimoto et al. (17) recently reported that CD72-deficient BAL-17 cells, a murine mature B lymphoma line, had impaired activation of ERK and JNK, but not p38, after anti-IgM stimulation, even though the CD72-deficient cells exhibited enhanced DNA synthesis. Similarly, Baba et al. (12) recently indicated that an immature B cell line lacking CD72, although hyperproliferative to BCR stimulation, had less NF-κB activity, less ERK activation, and no difference in p38 activation after BCR cross-linking with anti-IgM compared with its wild-type (WT) counterpart. These discrepancies may result from the different reagents (Ag vs anti-IgM) used to stimulate the BCR (10) or from the use of B cell lines at different developmental stages (immature vs mature) (18–20).

To clarify this uncertainty, we investigated the signal transduction pathways involved in the inhibition of B cell proliferation caused by CD72 and the role of CD72 in cell cycle arrest as well as cell death in primary B lymphocytes. For this purpose we used...
HEL-Ig transgenic (HEL-Tg) mice that allow Ag-specific B cell stimulation rather than surface IgM cross-linking (21). By comparing mature B cells from WT and CD72-deficient (CD72 knockout (KO)) HEL-Tg mice, we determined the effects of CD72 on modulating cell signaling in response to HEL Ag stimulation. Our findings demonstrate that CD72 primarily down-regulates the signal transduction pathways downstream of the BCR, including NF-AT, NF-κB, ERK, JNK, p38, MAPK, and Akt, thereby promoting cell cycle arrest and cell death in primary mature B cells.

Materials and Methods

Mice

CD72-deficient mice carrying the HEL-Ig transgene were generated by breeding C57BL/6J D4 Ig-/-Tg mice (expressing Ig heavy and light chains encoding anti-HEL IgM and IgD) with congenic C57BL/6J CD72-deficient mice. The HEL-Ig transgene and the endogenous Ig gene were screened by PCR using primers as follows: IgH-F1, 5′-GCCAGCTCCATCCAGCAGT-3′; IgH-F2, 5′-CTGGAGCTTACGCAAGGT-3′; and IgH-R1, 5′-ACAACAGAGCAGCAGCACA-3′. The IgH-F1/IgH-R1 pair generates a 430-bp HEL-Ig transgenic band, whereas the IgH-F2/IgH-R1 pair generates a 264-bp endogenous band. All mice in this study were 8–12 wk of age. Animals were used under approved animal protocols.

Antibodies

Phospho-ERK (Thr202/Tyr204), phospho-p38 (Thr180/Tyr182), phospho-IkBα (Ser32), phospho-Akt (Ser473), and p38 were purchased from Cell Signaling Technology. CD40 (HM40-3) was purchased from BD Pharmingen. ERK2 (C-14) and IkBα (C-21) were purchased from Santa Cruz Biotechnology. Anti-CD72 mAb K10.6 was purchased from Caltag Laboratories.

High-dimensional FACS analysis

Single-cell suspensions from adult spleens were stained with different mixtures of fluorochrome-conjugated Abs (prepared at Stanford or obtained from BD Pharmingen) containing IgM (DS-1), IgD (11-26), B220 (RA3-6B2), CD5 (53-7.3), CD21 (7G6), CD23 (B3B4), CD16/32 (2.4G2), CD72 (K10.6), CD22 (Cy34.1), and CD19 (1D3). These stain sets also contained Cascade Blue-conjugated DsRed (145-2C11), CD4 (GK1.5), CD8α (53-6.7), Gr-1 (RB6-8C5), and F4/80 Abs that are used to gate out irrelevant, non-B cells. Surface staining was performed as previously described (see http://herzenberg.stanford.edu/). Propidium iodide (PI) was added to all samples at a final concentration of 1 μg/ml before data collection to identify dead cells. Data from the stained cells were collected on a modified triple-laser FACS instrument at Stanford Shared FACS Facility. FlowJo (Tree Star) software was used for fluorescence compensation and analysis.

HEL binding assay

To identify HEL-binding B cells, splenic B cells were incubated with HEL (20 or 200 ng/ml; Sigma-Aldrich) on ice for 15 min and stained with biotinylated anti-HEL mAb 2D1 on ice for 15 min. Bound 2D1 anti-HEL Abs were detected with streptavidin-FTTC and flow cytometry.

B cell proliferation and BrdU incorporation assay

Naïve mature B cells (CD43−) were purified from spleens and lymph nodes by negative selection (CD43 microbeads) according to the manufacturer’s instructions (Miltenyi Biotec). The purified B cells (1 x 10⁶/well) were cultured in a 96-well plate and stimulated under various conditions for 48 h at 37°C. [3H]Thymidine (1 μCi/well) was added for the last 6 h. Cells were then harvested, and [3H]thymidine uptake was measured. To determine the frequency and nature of individual cells that synthesized DNA during culture, BrdU incorporation was performed using a BrdU flow kit (BD Pharmingen) as described previously (22).

Calcium flux detection

Splenocytes were suspended in RPMI 1640 (1 x 10⁶/ml) containing 3–5% FCS, loaded with indo-1/AM (1–2.5 μg/ml) (Molecular Probes) for 20 min, then stained with anti-B220-FITC and anti-CD43-PE for 10 min at 37°C. After washing, cells were stimulated with HEL Ag or anti-CD72 mAb. The ratio of indo-1 violet/blue of B220− and CD43− B cells was analyzed by flow cytometry.

ELISA for NF-ATc1, NF-κB p65, and JNK

The DNA binding activity of activated NF-AT was measured as previously described (23). Naïve mature B cells (3 x 10⁶) purified from lymph nodes were stimulated with HEL Ag (100 ng) for 30 or 60 s at 37°C. Nuclear protein was then extracted and assayed by ELISA for DNA-binding activity of NF-ATc1 (TransAm NF-ATc1; Active Motif). The DNA binding activity of activated NF-κB was measured as previously described (24). Naïve mature splenic B cells (2.5 x 10⁶) were stimulated with HEL Ag (250 ng) for 5 or 15 min at 37°C. Nuclear protein was then extracted and assayed by ELISA for DNA binding activity of NF-κB p65 (TransAm NF-κB Chemil; Active Motif). Briefly, 1–2 μg of nuclear extract was used per sample in duplicate in a 96-well plate precoated with consensus oligonucleotides for NF-ATc1 or NF-κB p65. The transcription factor proteins bound to the immobilized oligonucleotides were detected with specific Abs, followed by HRP-conjugated anti-Ig, according to the manufacturer’s instructions. To determine the activity of JNK1/2, naïve splenic mature B cells (2.5 x 10⁶) were stimulated with HEL Ag (1 μg) for 5, 10, or 15 min at 37°C and lysed with 6 M urea, 0.5% Triton X-100, and protease inhibitor mixture (Sigma-Aldrich). Lysate samples (equivalent to 5 x 10⁶ cells) were used for the JNK activity ELISA (JNK1/2 (pTyr183/185) assay kit; BioSource International) as previously described (25). A JNK1/2 (total) ELISA kit was also used for normalization of the JNK1/2 content of the samples.

Western blotting for ERK, p38 MAPK, and IκBα

To determine the activation levels of ERK and p38 MAPK, purified mature splenic B cells (3 x 10⁶) were stimulated with HEL Ag (0.5 μg) for various times at 37°C and lysed. Phosphorylation levels of ERK (Thr202/Tyr204) and p38 (Thr180/Tyr182) as well as their total protein levels were analyzed by standard Western blot and ECL (Amersham Biosciences). To determine the activation and degradation levels of IκBα, purified mature splenic B cells (3 x 10⁶) were stimulated with HEL Ag (0.5 μg) for various times at 37°C and lysed. Phosphorylated (Ser27) and total protein levels of IκBα were analyzed by standard Western blot and ECL.

Akt kinase assay

To determine Akt kinase activity, purified mature splenic B cells (1 x 10⁶) were stimulated with HEL Ag (1 μg) at 37°C for 5 min and lysed. Phosphorylated (activated) Akt was then immunoprecipitated with immobilized Akt Ab (Ser473) for 16 h at 4°C, followed by a kinase assay (Cell Signaling Technology) as described previously (24). Briefly, immobilized Akt was suspended in 50 μl of 1× kinase buffer supplemented with 1 μl of 10 nM ATP and 1 μg of the glycogen synthase kinase 3 (GSK-3) fusion protein substrate and incubated for 30 min at 30°C. The reaction was terminated with SDS sample buffer, and the protein samples were analyzed by Western blot.

Apoptosis detection

To examine apoptosis, purified splenic B cells (5 x 10⁵/well) were stimulated with HEL Ag with or without IL-4 for 24 h at 37°C and suspended in PI staining buffer (50 μg/ml PI, 0.1% Triton X-100, 1 mg/ml sodium citrate, and 1 mg/ml RNase A in ddH₂O) on ice for 30 min in the dark. The percentage of apoptotic cells was determined by cell cycle analysis and flow cytometry.

Results

Absence of CD72 has no or little effect on surface marker expression and the level of HEL binding on mature B-2 B lymphocytes

To address whether the presence or absence of CD72 has any influence on the phenotype of mature B cells, we analyzed the expression of characteristic surface markers, including the pan-B cell marker, B220; the markers of B cell maturity, CD21 and CD23; the positive regulator of BCR signaling, CD19; the negative regulators of BCR signaling, CD5, CD32 (FcγRII), and CD22; as well as surface IgM and IgD. For this purpose, multicolor flow cytometry was performed on total splenocytes collected from age-matched mice.

As shown in Fig. 1A, CD72 deficiency in HEL-Tg mice did not affect B-2 cell surface marker expression, except for a minor reduction of IgM expression. Importantly, there was no effect on the expression of other negative coreceptors (CD5, FcγRII), and
CD22). In addition, we also checked for potential differences in HEL Ag binding on mature B cells in the presence or the absence of CD72 expression. Incubation of WT and CD72KO B cells with increasing doses of HEL Ag resulted in comparable binding to the respective BCR (Fig. 1B).

**Effects of CD72 on mature B cell proliferation and cell cycle progression**

We next examined the effects of CD72 on B cell proliferation and cell cycle progression. Naive mature B-2 cells (CD43+/H11002) were purified by MACS from lymph nodes or spleens and cultured under various conditions. As shown in Fig. 2A, the presence of CD72 in WT naive mature B cells from lymph nodes resulted in reduced cellular proliferation after HEL Ag stimulation, and the addition of IL-4 or anti-CD40 partially relieved this inhibition. To determine whether the presence of CD72 had the same inhibitory effect in purified mature splenic B cells, we cultured and treated these cells under the same conditions as lymph node B cells. As shown in Fig. 2B, we found an identical pattern of B cell growth inhibition resulting from CD72 expression.

To confirm the inhibitory effect of CD72 on B cell proliferation, cell cycle progression in both WT and CD72KO B cells was analyzed using BrdU incorporation. Consistent with the hyperproliferative response to HEL Ag in the absence of CD72 in CD72KO B cells, WT B cells had a greater G1 phase arrest and less S phase progression compared with CD72-deficient B cells (G1 phase, 53 vs 28%; S phase, 44 vs 71%, Fig. 2C, second column).

**Effects of CD72 on activation of the transcription factors NF-ATc1 and NF-κB p65**

To examine the underlying biochemical mechanisms of the inhibitory effect of CD72 on B cell proliferation and cell cycling, we determined the activities of the transcription factors NF-ATc1 and NF-κB p65 (RelA), which are downstream of Ca2⁺ signaling and are known to play a crucial role in cellular proliferation after BCR ligation (2, 26, 27). We first compared calcium mobilization after HEL Ag stimulation in mature B cells with or without CD72 expression. After Ag stimulation, CD72-deficient B cells had a higher, more rapid, and sustained Ca2⁺ flux compared with that of WT B cells (Fig. 3A). Consistent with CD72-mediated suppression of Ca2⁺ mobilization, NF-ATc1 DNA binding activity was increased after HEL Ag stimulation in CD72-deficient mature B cells, which indicates increased activation (dephosphorylation) and nuclear translocation of NF-ATc1 in these cells (Fig. 3B).

We next investigated the role of CD72 in NF-κB activation. Western blot analysis showed that CD72 attenuated both phosphorylation and degradation of IκBα after Ag stimulation (Fig. 3C). In the NF-κB signaling pathway, phosphorylation and subsequent degradation of IκBα are the key steps for NF-κB activation and nuclear translocation. In accordance with the inhibition of IκBα degradation by CD72 in WT B cells, CD72 attenuated NF-κB p65 DNA binding activity between 5 and 15 min after HEL Ag stimulation (Fig. 3D).
Effects of CD72 on activation of MAPK and Akt kinase

We next examined the activation level of several MAPKs associated with BCR signaling, including ERK, JNK, and p38, in the presence or the absence of CD72, because these MAPKs transduce many extracellular signals to regulate diverse intracellular processes, including differentiation, survival, and growth (28). As shown in Fig. 4, A–C, Western blot analyses and ELISA-based phosphoprotein assays indicated that CD72 suppresses phosphorylation and, thus, activation of ERK1/2, JNK1/2, and p38 MAPK in primary mature B cells.

We also explored CD72 function in regulating the PI3K/Akt signaling pathway. Akt (also called protein kinase B), a key downstream signal effector of PI3K, is a serine/threonine kinase that promotes cell survival by regulating transcription factors (such as NF-κB) and proteins that control apoptosis (such as Bad) (29). As shown in Fig. 4D, CD72 attenuated both Akt phosphorylation (activation) and its kinase activity after HEL Ag stimulation in WT mature B cells vs CD72KO mature B cells. This indicates that CD72 may play a role in sensitizing mature B cells to apoptosis after Ag stimulation.

Effects of CD72 ligation on B cell proliferation, Ca\textsuperscript{2+} flux, IκBα activation, and ERK activity

To determine whether CD72 ligation could release the inhibitory effect of CD72, we examined B cell proliferation in the presence of anti-CD72 mAb (K10.6) and HEL Ag. It has been well documented that anti-CD72 mAb (K10.6) treatment can reduce phosphorylation of CD72 and its association with SHP-1 after Ag stimulation in primary B cells (10). We also examined HEL Ag-stimulated signal transduction with mature B cells that were
pretreated with anti-CD72 mAb. As shown in Fig. 5A, in the presence of anti-CD72 mAb and HEL Ag, WT mature B cell proliferation to a level similar to those observed in CD72KO mature B cells. Notably, incubation with anti-CD72 mAb alone did not induce either WT or CD72KO mature B cells to proliferate. Consistent with the above observation, addition of anti-CD72 mAb alone did not induce calcium flux in either WT or CD72KO splenic mature B cells (Fig. 5B). However, addition of HEL Ag to WT mature B cells preligated with K10.6 induced a remarkable calcium flux similar to that observed in CD72KO mature B cells pretreated with K10.6 (Fig. 5B). These findings confirm that CD72 suppresses cell cycle progression and Ca$^{2+}$ mobilization in B cells after Ag stimulation.

To clarify the role of CD72 in regulating NF-κB and MAPK signaling, phosphorylation of IκBα and ERK was examined under CD72 preligation conditions. As shown in Fig. 5, C and D, CD72 preligation augmented activation-induced phosphorylation of both IκBα and ERK in WT mature B cells to levels similar to those observed in CD72KO mature B cells upon Ag stimulation. To determine whether anti-CD72 mAb alone could induce any signals independent of the BCR, we examined ERK activation after stimulation with anti-CD72 mAb in mature B cells. As shown in Fig. 5E, stimulation with anti-CD72 mAb alone (CD72 ligation) did not induce ERK activation (phosphorylation) at either low (5 μg/ml) or high (30 μg/ml) doses of Ab at 2- or 9-min incubation intervals in either WT or CD72KO mature B cells. Together, these findings indicate that CD72 specifically inhibits Ag-stimulated signal transduction pathways, including NF-AT, NF-κB, and MAPK, and subsequently inhibits mature B cell proliferation. In addition, CD72 ligation uncouples the CD72 inhibitory function from BCR signaling without eliciting any detectable signals from the ligating CD72 itself.

Effects of CD72 on mature B cell apoptosis

Finally, we investigated the potential role of CD72 in mature B cell apoptosis after Ag stimulation. DNA fragmentation associated with apoptosis in Ag-stimulated WT vs CD72KO B cells was measured using PI staining and cell cycle analysis. As shown in Fig. 6, HEL Ag stimulation resulted in an increase in WT B cells with hypodiploid DNA content (apoptotic) compared with their control nonstimulated cells (18 vs 7%), whereas we observed no change in CD72KO B cells with hypodiploid DNA content (apoptotic) compared with their control nonstimulated cells (6 vs 5%). As expected, the addition of IL-4 rescued WT B cells from Ag-induced apoptosis (Fig. 6, bottom rows). This finding indicates that CD72 plays a role in BCR-mediated apoptosis in mature B cells.

Discussion

In this study, we investigated the effects of CD72 on the signal transduction pathways downstream of BCR signaling to clarify the role of CD72 as a negative regulator of BCR activation in primary mature B cells. To establish an experimental system that affords Ag-specific BCR activation, we used HEL-Tg mice that either expressed or lacked CD72. Extensive phenotyping confirmed that mature B cells isolated from WT or CD72KO mice did not differ in their HEL-binding capacity or their surface marker expression, except for a minor reduction of IgM expression on CD72KO B cells. In addition, functional comparison showed that naive mature B cells purified from either lymph nodes or spleen are sufficient to evaluate the function of CD72 in B cell signaling.

Using the HEL-Tg system we were able to demonstrate that CD72 plays a major role in negative regulation of BCR signaling based on several lines of evidence. We provide novel and direct
evidence that CD72 negatively regulates the major signal transduction pathways downstream of the BCR, including NF-ATc1, NF-κB p65, ERK1/2, JNK1/2, p38-MAPK, and PI3K/Akt, after Ag stimulation in primary mature B cells. These findings suggest that the CD72/SHP-1 complex targets a key signaling molecule(s) within the BCR complex or the signalosome complex directly downstream of the BCR, resulting in down-modulation of BCR signal transduction after Ag stimulation. In other words, in the absence of CD72 expression, SHP-1 is not fully activated (9), resulting in an enhanced tyrosine phosphorylation of a certain key signaling molecule(s), which, in turn, augments signal transduction downstream of the BCR. However, the early signaling target(s) of the CD72/SHP-1 complex after Ag engagement in primary B cells remains to be identified. Although Ig-α, Syk, and B cell linker protein (SLP-65) have been shown to be immediate targets of the CD72/SHP-1 complex after BCR stimulation in the myeloma cell line 558L.m3 (9), these findings have not been confirmed in either B cell lines or primary B cells. Moreover, other potential target(s) for the CD72/SHP-1 complex remains to be identified.

In collaboration with Baba et al. (12), we recently examined the effect of CD72 on NF-κB activity in an immortalized immature B cell line lacking CD72 and found that there was less NF-κB activity in the CD72-deficient line compared with its WT counterpart. In our present experiment (Fig. 3D), the presence of CD72 in WT mature B cells reduced NF-κB activity. This apparent discrepancy may reflect differences in responses after BCR stimulation between mature and immature B cells or between primary and immortalized B cells. It has been demonstrated that both cell signaling and cell fate after BCR stimulation are quite different in mature and immature B cells (18–20).

Our results regarding ERK activation in response to Ag stimulation are consistent with the results from a study by Adachi et al. (16) of B cell lymphoma lines. The (4-hydroxy-3-nitrophenoxy)-acetyl (NP)-BSA Ag stimulation of K46 mouse lymphoma cells that had been transduced to express CD72 resulted in inhibition of ERK activation compared with CD72-deficient K46 cells. In contrast, other studies using anti-IgM for BCR activation showed that CD72 expression results in increased activation of ERK and JNK, but has no effect on p38 (12, 17). Therefore, we hypothesize that the different modes of B cell activation, Ag vs anti-IgM, could account for the observed difference. This view is supported by the findings of Hokazono et al. (10), who demonstrated that Ag-induced BCR signaling differs from Ab-induced BCR signaling. In addition, the use of mature primary B cells in our study vs B cell lines at different stages of maturity may account in part for the observed difference.

CD72 ligation has been suggested to either release CD72 inhibitory function (10, 11, 30, 31) or induce a positive signal independent of the BCR (32–34) in primary B cells. Our findings support the view that CD72 ligation serves the role of releasing the inhibitory effect of CD72 on BCR signaling upon Ag stimulation (Fig. 5). Wu et al. (32) showed that CD72 ligation induces cell proliferation and activates ERK and JNK phosphorylation in total splenic B cells. In contrast, our findings demonstrate that CD72 ligation (incubation with anti-CD72 mAb alone) did not trigger B cell proliferation, Ca²⁺ mobilization, or ERK activation (Fig. 5, A, B, and E). However, anti-CD72 treatment or CD72 preligation enhanced cell proliferation, Ca²⁺ mobilization, IκBα activation, and ERK activity induced by Ag stimulation in WT mature B cells (Fig. 5, A–D). These discrepant observations of our group and Wu et al. (32) may reflect experimental differences, including differences in mouse strains (C57BL/6 vs DBA2), B cell populations (naive mature B cells vs total splenic B cells), and/or the concentration of anti-CD72 mAb (low dose vs high dose). For example, in our present study we used a low dose of anti-CD72 mAb (0.5 μg/
ml), which is sufficient for releasing the inhibitory effect of CD72 in B cell proliferation assays (Fig. 5A), whereas they used a much higher dose of anti-CD72 mAb (50 μg/ml) for the same assay.

Our data provide new direct evidence that CD72 enhances apoptosis (as shown by hypodiploid DNA content) after Ag-specific BCR stimulation in primary mature B cells (Fig. 6). This result is consistent with the function of CD72 in cell cycle arrest (Fig. 2C) and the reduction of Akt kinase activity, a key component capable of promoting cell survival (35), in WT mature B cells after Ag stimulation (Fig. 4D). Furthermore, these results are supported by reports demonstrating that anti-CD72 mAb (K10.6) treatment induces survival and cell cycle progression in NP-BSA-stimulated splenic B cells (10) and anti-IgM-stimulated splenic mature B cells (7). Because BCR-mediated apoptosis has been shown to play a critical role in the elimination of autoreactive B cells and consequent maintenance of B cell tolerance (35), our findings suggest that CD72 may play a role in controlling autoimmunity.

In summary, we demonstrate that CD72 exhibits an inhibitory effect on B cell proliferation through its attenuation of numerous signal transduction pathways downstream of BCR signaling. Moreover, enhanced Akt kinase activity and reduced apoptosis in CD72KO B cells confirm the role of CD72 in cell cycle arrest and apoptosis, thereby diminishing B cell proliferation after Ag stimulation in wild-type mice.
The percentage of apoptotic cells (hypodiploid) is noted in each B lymphocytes were stained in PI staining buffer, and analyzed by flow cytometry. The percentage of apoptotic cells (hypodiploid) is noted in each histogram.

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**Disclosures**

The authors have no financial conflict of interest.

**References**