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A Role for Lysosomal-Associated Protein Transmembrane 5 in the Negative Regulation of Surface B Cell Receptor Levels and B Cell Activation

Rika Ouchida,* Tomohiro Kurosaki,† and Ji-Yang Wang*

Mechanisms by which cell surface levels of the BCR are regulated remain largely unknown. We found that B cells lacking the lysosomal-associated protein transmembrane 5 (LAPTM5) expressed higher levels of cell surface BCR than did wild-type (WT) B cells after Ag stimulation in vitro and in vivo. In addition, LAPTM5-deficient mice contained an increased frequency of Ag-specific B cells and produced greater amounts of Abs than did WT mice after immunization with a T-dependent Ag. Adoptive transfer of LAPTM5-deficient B cells with WT T cells into RAG1-deficient mice revealed that the increased surface BCR levels and the enhanced B cell activation and Ab production were due to a B cell intrinsic defect. As they aged, the LAPTM5-deficient mice had increased titers of serum IgM and autoantibodies and immune complex deposition in the kidney. Immunofluorescent and biochemical analysis revealed that LAPTM5 physically interacted with the BCR complex and promoted its degradation in the lysosomal compartment in mouse B cells. These results demonstrate a role for LAPTM5 in the negative regulation of cell surface BCR levels and B cell activation. The Journal of Immunology, 2010, 185: 000–000.

The BCR is composed of Ag-binding membrane Ig non-covalently associated with the disulfide-linked signal-transducing Igα and Igβ subunits (1–3). BCR signaling is strictly regulated both positively and negatively to allow delivery of physiologically appropriate signals yet to prevent overstimulation that can lead to B cell hyperactivation (4–6). Deficiency of positive regulators of BCR signaling, such as the CD19 coreceptor (7–9) or the Bruton’s tyrosine kinase BTK (10, 11), can lead to impaired B cell responses and immunodeficiencies, whereas the absence of negative regulators, such as CD22 (12–14) or the Src homology region 2 domain-containing phosphatase 1 (15), can cause enhanced B cell activation and autoimmunity. Appropriate BCR signaling is thus crucial for the normal function of B cells and induction of an optimal humoral immune response.

Recent studies suggest that BCR signaling is also influenced by the levels of cell surface BCR. For example, B cells with a mutated Igα (16) or Igβ (17) ITAM had elevated BCR levels due to impaired BCR internalization. These B cells exhibited increased Ca2+ flux in response to Ag stimulation, suggesting that the proximal BCR signaling was enhanced. Conversely, B cells with two Igα cytoplasmic domains had reduced surface BCR levels due to enhanced BCR internalization (18). These B cells exhibited decreased Ca2+ flux and were hyporesponsive to Ag stimulation in vitro and in vivo. The Src-like adaptor protein (SLAP) (19) and the E3 ubiquitin ligase Cbl family proteins (20) have also been implicated in the regulation of BCR levels. SLAP and c-Cbl together were able to mediate BCR downmodulation (21), whereas Cbl-b and c-Cbl were required for efficient BCR internalization, although the functional relationship between SLAP and Cbl family proteins still remains obscure. Mice lacking c-Cbl and Cbl-b had elevated levels of serum IgM and anti-dsDNA Ab and developed autoimmunity as they aged (20). This phenotype was in part due to impaired B cell anergy to protein Ag, but the elevated BCR levels may also have contributed to the abnormal B cell response. Regulation of surface BCR levels is therefore an important mechanism to control B cell responses. It remains largely unclear how the internalized BCR is targeted to lysosomes for degradation and whether any abnormality in BCR degradation could affect BCR levels and B cell function. It is also unknown whether there are any specialized lysosomal proteins that mediate BCR degradation.

Lysosomal-associated protein transmembrane 5 (LAPTM5) is a 30-kDa lysosomal protein specifically expressed in the myeloid and lymphoid lineages (22–25). It contains five membrane-spanning segments, with its N terminus predicted to be located inside the lysosome and C terminus in the cytoplasm. It also contains three polyproline–tyrosine motifs (L/PPxY), which are known to bind WW domains involved in protein–protein interactions, and ubiquitin-interacting motif (LxxALxxSxxE) in the C terminus. We have recently found that LAPTM5 negatively regulates cell surface TCR expression by interacting with the CD3ε-chain and promoting its degradation (26). In the current study, we demonstrate that LAPTM5 interacts with the BCR complex and promotes its degradation in the lysosomal compartment. We also show that LAPTM5-mediated BCR downmodulation is an important mechanism to prevent B cell hyperactivation and autoantibody production.

Materials and Methods

Abs
Polyclonal rabbit Abs against mouse LAPTM5 were prepared as described. PE-conjugated 4-hydroxy-3-iodo-5-nitrophenylacetyl (NIP) was kindly
provided by Dr. Kohei Kometani (Laboratory for Lymphocyte Differentiation, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama, Japan). Commercial Abs from the following sources were used in these studies: BD Biosciences (San Jose, CA), FITC–, allophycocyanin–, and PerCP-anti-B220 (RA3-6B2), FITC– and PE–anti-mouse IgM (R6-60.2), PE–anti-mouse CD43, FITC–anti-mouse CD21, PE–anti-mouse CD23, anti–lyosomal-associated membrane protein 1 (LAMP1) (1D4B), streptavidin–allophycocyanin, FITC–anti-mouse CD40 (HM40-3), allophycocyanin–anti-mouse CD19 (1D5), allophycocyanin–anti-mouse MHC class II I-A/E; Southern Biotechnology Associates (Birmingham, AL), goat F(ab')2 anti-IgM, bio-
tin–goat F(ab')2 anti-IgM, biotin anti-rabbit IgG, and anti-mouse IgM (H chain [HC]-specific); Santa Cruz Biotechnology (Santa Cruz, CA), anti-CD79A (Ig, HM47), anti-CD79B (Ig, B29123), anti-CD79B (FL-229), Texas Red–anti-mouse IgG, Texas Red–anti-goat IgG, and FITC–anti-
mouse IgG; BioLegend (San Diego, CA), biotin–anti-IgM (AF6-78) and streptavidin–PE/Cy5; Sigma-Aldrich (St. Louis, MO), anti-actin; Molecular Clones (Eugene, OR), Alexa Fluor 555–anti-rat IgG and streptavidin–Alexa Fluor 647; eBioscience (San Diego, CA), PE–anti-mouse IgD and biotiny-
lated Y–Ae (anti-Eo52–68 peptide bound to the I-A(b)/MHC Class II).

Purification of spleen B cells and Ca2+ flux

Spleen B cells were purified by negative sorting with the IMag B cell purification kit (BD Biosciences). For Ca2+ flux, spleen B cells were first incubated with 1.2 M Indo 1-AM (Dojindo, Kumamoto, Japan) at 37˚C for 45 min in the dark. After being washed, the cells were stimulated with F(ab')2 anti-IgM Abs and immediately analyzed with an LSR flow cytometer (BD Biosciences).

BCR downmodulation in vitro and in vivo

To analyze BCR downmodulation in vitro, mature B cells were purified from spleen and cultured in the presence of plate-bound F(ab')2 anti-IgM Abs (coated at a concentration of 10 μg/ml). Cells were harvested at the indicated time points and stained for cell surface BCR expression. To analyze BCR downmodulation in vivo, mice (9- to 12-wk-old) were injected i.p. with 100 μg 4-hydroxy-3-nitrophenyl-acetyl coupled to chicken γ-globulin (NP-CGG) precipitated with alum. One week later, splenocytes were stained with allophycocyanin–anti-B220, FITC–peanut agglutinin (PNA), and PE–conjugated NIP, which binds to both high- and low-affinity NP-specific B cells.

Immune responses

Littermate wild-type (WT) and Laptm5−/− mice (11- to 12-wk-old), derived from breeding of Laptm5−/− mice, were injected i.p. with 100 μg NP-CGG precipitated with alum, 100 μg NP-LPS, or 100 μg NP-Ficoll. Mice were bled weekly, and serum titers of NP-specific IgM, IgG1, and IgG2 were measured by ELISA.

Adoptive transfer

Purified WT or Laptm5−/− spleen B cells (1 × 10^6) were mixed with purified WT spleen T cells (5 × 10^6) and injected i.v. into Rag1−/− mice. One week after the adoptive transfer, the recipient mice were immunized i.p. with 100 μg NP-CGG in alum.

Retrovirus transduction

Retrovirus transduction was performed essentially as described (26). Spleen B cells were stimulated with LPS (10 μg/ml) for 24 h before the transduction of retrovirus expressing GFP or LAPT5-internal ribosome entry site (IRES)-GFP.

Measurement of anti-dsDNA Ab and histochemical analysis

Poly-L-lysine (0.01%)-treated plates were coated with 2.5 μg/ml dsDNA from calf thymus DNA, blocked with 0.5% BSA in PBS, and incubated with serial serum dilutions. This was followed by incubation with HRP-
conjugated anti-mouse IgM and anti-mouse IgG. To detect immune complex deposition, the kidneys were fixed with 4% paraformaldehyde and embedded in OCT embedding medium. Kidney sections were blocked with 5% nonfat milk and stained with FITC–anti-mouse IgG to detect IgG-containing immune complex or with anti-mouse IgM followed by Texas Red anti-goat IgG to detect IgM-containing immune complex.

Immunofluorescent confocal microscopy and immunoprecipitation

WEHI231 cells transduced with LAPT5-IRES-GFP were treated with the lysosomal inhibitor NH4Cl (20 mM) for 2 h and then fixed and permeabilized with the BD Cytofix/Cytoperp solution (BD Biosciences). LAPT5, LAMP1, IgG, and IgG2 were each stained as in the following: LAPT5, anti-mouse LAPT5 followed by biotinylated anti-rabbit IgG, and then streptavidin–Alexa Fluor 647; LAMP1, anti-mouse LAMP1 followed by Alexa Fluor 555–anti-rat IgG; IgG, anti-mouse CD79A followed by Texas Red–anti-mouse IgG; IgG, anti-mouse CD79B (B29/123) followed by Texas Red–anti-mouse IgG. Immunoprecipitation was performed as de-
scribed previously (26). Briefly, cells were lysed on ice for 60 min in a buffer containing 1% digitonin and protease inhibitors, and the lysate was pre-
cleared with protein G-Sepharose beads and then immunoprecipitated with anti-LAPT5 (or rabbit IgG as a control) or anti-IgM (or goat IgG as a control). The immunoprecipitates were then blotted with anti-LAPT5, anti-μHC and anti-μβ Ab.

Ag presentation assay

Ag presentation assay was carried out as described previously (27, 28). B1-8 mice were immunized i.p. with 100 μg 4-hydroxy-3-nitrophenylacyl-
Ea-green fluorescent protein (NP-Ea-GFP) (kindly provided by Dr. Yuichi Aiba, Laboratory for Lymphocyte Differentiation, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama, Japan) in alum, and 6 h later splenocytes were stained with PE–anti-IgM, PerCP– anti-B220, and biotinylated Y–Ae (which recognizes the Eo52–68 peptide bound to the I-A(b)/MHC class II) followed by streptavidin–allophycoca-

Results

Laptm5−/− B cells express elevated cell surface BCR compared with normal B cells after in vitro Ag stimulation

B cell development and maturation appeared normal in Laptm5−/− mice as revealed by FACS analysis with various cell surface markers (Supplemental Fig. 1). However, Laptm5−/− B cells ex-
pressed a marginally increased level of the cell surface BCR compared with that in WT B cells (Fig. 1A, 1A, 0 h). Cross-linking of B cells with F(ab')2 anti-IgM Ab resulted in a transient down-
modulation of the cell surface BCR followed by a return to near pre-treatment levels after 24 h. Laptm5−/− B cells expressed higher levels of surface BCR than control B cells after BCR cross-

![FIGURE 1.](http://www.jimmunol.org/) Elevated cell surface BCR on Laptm5−/− mature B cells after in vitro Ag stimulation. A, BCR downmodulation in WT (red) and Laptm5−/− (blue) spleen B cells after BCR cross-linking. Spleen B cells were stimulated with plate-bound F(ab')2 anti-IgM Ab and analyzed for surface BCR expression at the indicated time points. Representative FACS profiles (left panel); average ± SD of three pairs of WT and Laptm5−/− mice (right panel). *p < 0.05 (unpaired t test). B, MHC class II and CD86 expression. C, CD19 and CD40 expression at 12 h after BCR cross-linking.
linking, in particular after 24 h of stimulation (Fig. 1A). The elevated BCR levels in Laptm5−/− B cells were associated with a moderate increase in the levels of MHC class II and the costimulatory molecule CD86 (Fig. 1B), indicating that Laptm5−/− B cells were more activated than WT B cells during this short-term culture. In contrast to the BCR levels, the levels of CD19 and CD40 (Fig. 1C) on WT and Laptm5−/− B cells were identical after BCR cross-linking, suggesting that LAPTM5 more specifically targeted the BCR. These observations suggest that LAPTM5 is involved in the negative regulation of cell surface BCR expression after Ag stimulation in vitro.

Increased levels of cell surface BCR on Ag-specific B cells generated in vivo and enhanced Ab production in Laptm5−/− mice

To further confirm a role for LAPTM5 in the downmodulation of cell surface BCR, we next analyzed the surface BCR levels on Ag-specific B cells generated in vivo. Mice were immunized with NP-CGG, and 1 wk later splenocytes were stained with NIP–PE to detect the NP-specific B cells (Fig. 2A). Remarkably, the mean fluorescence intensity (MFI) of the NIP binding was significantly increased in Laptm5−/− B cells compared with that in WT B cells (Fig. 2B, left panel). The frequency of NP-specific B220+ cells was also higher in the Laptm5−/− mice (Fig. 2B, middle panel). Similarly, Laptm5−/− germinal center B (GC B, B220+PNA+) cells exhibited elevated NIP binding compared with that in WT GC B cells (Fig. 2B, right panel). These observations suggest that LAPTM5 functions to downmodulate surface BCR expression in activated B cells in vivo during an immune response. Consistent with the increased frequency of NIP-binding B cells, Laptm5−/− mice produced more NP-specific IgM (Fig. 2C, upper panel) and IgG1 (Fig. 2C, lower panel) Ab than did WT mice. These results imply that the increased surface BCR in Laptm5−/− B cells leads to enhanced B cell activation and Ab production in response to the T-dependent (TD) Ag NP-CGG.

Increased surface BCR levels and enhanced B cell activation are due to an intrinsic defect in Laptm5−/− B cells

To determine whether the elevated BCR levels and enhanced B cell activation seen in Laptm5−/− mice were due to a defect in B cells, we purified B cells from WT or Laptm5−/− mice and then transferred them into Rag1−/− mice together with WT T cells. One week after the adoptive transfer, mice were immunized with NP-CGG and then analyzed for NIP-binding B cells in the spleen and NP-specific serum Ab. The MFI and frequency of NIP-binding B cells were significantly increased in recipients of Laptm5−/− B cells and WT T cells (Fig. 3A). Moreover, compared with Rag1−/− mice transferred with WT B and T cells, those transferred with Laptm5−/− B cells and WT T cells produced greater amounts of NP-specific IgM and IgG1 Ab (Fig. 3B), which was accompanied by an increased number of NP-specific Ab-forming cells in the spleen (Fig. 3C). These results collectively suggest that

**FIGURE 2.** Increased BCR expression by the NP-specific B cells generated in vivo and enhanced Ab production in Laptm5−/− mice. A, Representative FACS profiles of B220 versus NIP binding by spleen B cells from mice immunized with NP-CGG 1 wk previously. Nonimmunized mice were used as a control. B, MFI (left panel) and frequency (middle panel) of the NIP-binding cells in individual mice. MFI of NIP binding in gated GC B cells (right panel). C, Immune responses to a TD Ag. Mice (eight WT and eight Laptm5−/−) were immunized with NP-CGG in alum and analyzed for the production of NP-specific IgM (upper panel) and IgG1 (lower panel) Ab. Open and solid circles represent WT and Laptm5−/− mice, respectively. *p < 0.05; **p < 0.005; ***p < 0.001 (unpaired t test).

**FIGURE 3.** Increased surface BCR levels and enhanced B cell activation are due to an intrinsic defect in Laptm5−/− B cells. WT or Laptm5−/− B cells were mixed with WT T cells and transferred into Rag1−/− mice. One week later, the recipient mice were immunized with NP-CGG in alum, after which the recipient mice were bled weekly and the NP-specific IgM and IgG1 Ab were measured by ELISA. The mice were sacrificed 2 wk after the immunization, and the spleen cells were analyzed for the MFI and frequency of the NIP-binding B cells by FACS and the Ab-forming cells by ELISPOT assay. A, MFI (left panel) and frequency (right panel) of the NIP-binding cells. B, Titers of NP-specific Ab. IgM (left panel); total (high- and low-affinity) IgG1 (middle panel); high-affinity IgG1 (right panel). C, Frequency of the Ab-forming cells in the spleen. Open circles, WT B cells + WT T cells; closed circles, Laptm5−/− B cells + WT T cells. *p < 0.05; **pp < 0.001 (unpaired t test).
LAPTm5 deficiency in the B cells leads to elevated BCR levels and enhanced B cell activation in vivo.

**Increased levels of serum IgM and titers of anti-dsDNA Ab in Laptm5−/− mice**

Enhanced B cell activation can lead to autoantibody production, as has been observed in mice lacking FcγRIIB, Src homology region 2 domain-containing phosphatase 1, or other negative regulators of BCR signaling (15, 29, 30). We therefore measured the serum Ig levels as well as anti-dsDNA Ab titers in the knockout mice. We found a moderate but statistically significant increase in the Ig levels as well as anti-dsDNA Ab titers in the knockout mice. Figure 4A shows the increased levels of serum IgM in aged Laptm5−/− mice compared with those in WT littersmates (Fig. 4A, Supplemental Fig. 2). In addition, the titers of anti-dsDNA Ab, both IgM and IgG, were increased (Fig. 4B). Although we have not seen evidence of severe autoimmunity in Laptm5−/− mice, histological examination revealed the presence of immune complexes in the kidneys of aged Laptm5−/− but not WT mice (Fig. 4C). Furthermore, Laptm5−/− mice contained anti-nuclear Ab in the sera (Fig. 4D). These results collectively suggest that B cells in Laptm5−/− mice are hyperactivated and produce increased amounts of Ab, including autoantibodies.

**Normal BCR internalization and recycling in Laptm5−/− B cells**

We further investigated the mechanism by which LAPTm5 deficiency affected cell surface BCR levels. BCRs undergo constitutive and ligand-dependent internalization and recycling. Therefore, the increased cell surface BCR expression on Laptm5−/− B cells could be due to decreased internalization, enhanced recycling, or impaired degradation of the internalized BCR. As shown in Fig. 5, WT and Laptm5−/− B cells exhibited similar rates of ligand-independent (Fig. 5A) and ligand-dependent (Fig. 5B) BCR internalization. In addition, LAPTm5 deficiency did not affect BCR recycling (Fig. 5C). These results suggest that the elevated BCR expression in Laptm5−/− B cells could be due to impaired degradation of the internalized BCR.

**LAPTm5 downmodulates surface BCR expression by promoting the lysosomal degradation of the BCR complex**

To examine the role of LAPTm5 in BCR degradation, we next analyzed the effect of LAPTm5 overexpression in a mouse B lymphoma line, WEHI231. We transduced WEHI231 cells with retroviruses expressing LAPTm5-IRES-GFP or GFP alone as a control and analyzed the surface BCR expression. Although transduction of GFP had no effect on the cell surface BCR levels, transduction of LAPTm5-IRES-GFP downmodulated BCR expression (Fig. 6A). Moreover, the extent of BCR downmodulation correlated very well with the level of LAPTm5 expression, as assessed indirectly by GFP expression. In contrast, the levels of CD19 were not significantly affected by overexpression of LAPTm5 (Fig. 6B), indicating that LAPTm5 in B cells specifically targets the BCR. To determine whether LAPTm5-mediated BCR downmodulation requires the activity of lysosomes, we again transduced WEHI231 with retrovirus expressing LAPTm5-IRES-GFP or GFP alone and then treated the cells with NH4Cl, an inhibitor of lysosome activity, or with epoxomicin, a proteasome inhibitor. The BCR downmodulation was significantly inhibited by NH4Cl but not by epoxomicin (Fig. 6C). It should be noted that the treatment with epoxomicin completely inhibited proteasome activity, as assessed by using an artificial substrate succinyl-L-leucyl-L-leucyl-L-valyl-L-tyrosine 4-methyl-coumaryl-7-amide (Supplemental Fig. 3). These results indicate that lysosome but not proteasome function is required for BCR downmodulation. Intriguingly, treatment of control GFP-expressing WEHI231 cells with NH4Cl resulted in slightly increased BCR levels (Fig. 6C), suggesting that BCRs undergo constitutive lysosomal degradation in this B lymphoma line. Furthermore, LAPTm5 overexpression resulted in decreased protein levels of the μHC and Igβ subunits of the BCR (Fig. 6D), indicating that LAPTm5 downmodulated surface BCR levels by promoting the protein degradation of the BCR complex. LAPTm5 also downmodulated surface BCR levels in normal spleen B cells without affecting the levels of CD19 and CD40 (Fig. 6E).

To analyze the potential interaction between LAPTm5 and the BCR complex, we performed a confocal immunofluorescent staining analysis of WEHI231 cells. We transduced WEHI231 cells with retrovirus expressing LAPTm5-IRES-GFP because the level of endogenous LAPTm5 was low in these cells and difficult to be stained. LAPTm5 colocalized with LAMP1, a marker for late endosomes/lysosomes (Fig. 7A, upper panels). In GFP+ WEHI231 cells (i.e., cells not transduced with retrovirus expressing LAPTm5), Igα (Fig. 7A, middle panels) and Igβ (Fig. 7A, lower panels) were primarily localized on the plasma membrane. In contrast, in GFP+ cells (i.e., cells expressing exogenous LAPTm5), Igα and Igβ were internalized and colocalized with LAPTm5 (Fig. 7A). ImageJ analysis revealed that 61.8% of the Igα and 84.2% of the Igβ colocalized with LAPTm5 (Supplemental Fig. 4). WEHI231...
cells were not stimulated with anti-IgM Ab in these experiments, indicating that LAPTMs overexpression alone was sufficient to induce the translocation of Igα and Igβ to the LAPTMs compartment in this cell line. To investigate whether LAPTMs physically interacts with the BCR complex, we immunoprecipitated LAPTMs under mild detergent conditions. Immunoblot analysis of the immunoprecipitates reproducibly detected the μHC and Igβ subunits of the BCR complex (Fig. 7B, left panels). Conversely, immunoprecipitation with anti-μHC brought down both LAPTMs and Igβ (Fig. 7B, right panels). These results demonstrate that LAPTMs downmodulates cell surface BCR expression by interacting with the BCR complex and promoting its degradation in the lysosomal compartment.

**Normal Ag presentation by Laptm5<sup>−/−</sup> B cells**

Ag binding to the BCR not only initiates a cascade of signal transduction leading to B cell activation but also triggers rapid internalization of the Ag–BCR complex. The internalized Ag is processed for Ag presentation, which is essential for antigen-specific T cell transduction leading to B cell activation but also triggers rapid internalization of the Ag–BCR complex. The internalized Ag is sorted in the kidney. These results correspond well with recent findings that LAPTM5 downmodulates surface TCR expression and T cell activation, thus demonstrating that LAPTM5 is a common negative regulator of Ag receptor expression in both T and B cells.

**Laptm5<sup>−/−</sup> and WT B cells expressed similar levels of cell surface BCR before Ag stimulation, and after BCR cross-linking, both WT and Laptm5<sup>−/−</sup> B cells downmodulated surface BCR. However, the BCR levels on Laptm5<sup>−/−</sup> B cells gradually became higher than those on WT B cells, particularly after 24 and 48 h of stimulation when B cells were truly activated. These results indicate that LAPTM5 negatively regulates BCR levels in activated but not resting B cells. Consistently, Laptm5<sup>−/−</sup> GC B cells expressed elevated surface BCR compared with WT GC B cells. In contrast to GC B cells, the IgG<sup>+</sup>, IgM<sup>+</sup> B cells that are present in the spleen of naive Laptm5<sup>−/−</sup> mice did not express significantly higher levels of BCR compared with those in WT mice (Supplemental Fig. 5). These IgG<sup>+</sup> B cells might have undergone activation and Ig gene class switching at earlier time points and have terminated their activation and are now in a resting state. Together these observations suggest that LAPTM5 regulates BCR levels in a ligand-dependent manner. This is in contrast to the situation in the B cells expressing mutant Igα (IgαFF) or Igβ (IgβAA), in which the ITAM tyrosine residues were mutated to phenylalanines or alanines, respectively, and in Cbl<sup>−/−</sup>Cblb<sup>−/−</sup> B cells. IgαFF, IgβAA, and Cbl<sup>−/−</sup>Cblb<sup>−/−</sup> B cells expressed significantly higher levels of surface BCR than WT B cells before Ag stimulation. These mutant B cells also exhibited increased Ca<sup>2+</sup> flux in response to Ag stimulation, in part due to their elevated BCR levels. In contrast, Laptm5<sup>−/−</sup> and WT B cells exhibited a similar Ca<sup>2+</sup> flux after Ag stimulation (Supplemental Fig. 6), indicating that the absence of LAPTM5 had no demonstrable effect on BCR proximal signaling. Nevertheless, our findings indicate that the elevated BCR levels on Laptm5<sup>−/−</sup> B cells observed at the later stage after Ag stimulation can lead to enhanced B cell activation and Ab production.

IgαFF, IgβAA, and Cbl<sup>−/−</sup>Cblb<sup>−/−</sup> B cells are impaired in BCR internalization. In contrast, Laptm5<sup>−/−</sup> B cells exhibit normal BCR internalization and recycling but impaired degradation of the BCR complex in the lysosomal compartment. Therefore, LAPTM5 appears to function downstream of Igα, Igβ, and c-Cbl/Cbl-b. One possible mechanism is that LAPTM5 promotes BCR degradation by interacting with the internalized BCR and facilitating its sorting to the lysosomes. In agreement with this possibility, we found that LAPTM5 colocalized with Igα and Igβ in the lysosomes of mouse B cells. Moreover, LAPTM5 coprecipitated with both μHC and Igβ under mild detergent conditions, strongly
suggesting that LAPTM5 indeed physically interacts with the BCR complex. We were unable to detect Igα after immunoprecipitation with anti-LAPTM5 or anti-μHC Ab despite repeated attempts, likely due to the fact that the available Igα mAb is not suitable for Western blotting. Further studies are required to determine which component of the BCR complex mediates the interaction with LAPTM5, whether this interaction is regulated by phosphorylation, ubiquitination, or some other posttranslational modification of Igα or Igβ, and whether the interaction between LAPTM5 and the BCR is direct or mediated by another protein(s).

Consistent with the elevated BCR levels after Ag stimulation in vivo, the frequency of NIP-binding B cells was increased in Laptm5−/− mice, and they produced greater amounts of NP-specific Ab in response to the TD Ag NP-CGG. In contrast, IgαFF, IgβAA, and Cbl2/2Cblb2/2 mice all exhibited decreased Ab production against TD Ag despite the fact that their B cells expressed increased levels of surface BCR. One important difference between Laptm5−/− B cells and IgαFF, IgβAA, or Cbl2/2Cblb2/2 attempts, likely due to the fact that the available Igα mAb is not suitable for Western blotting. Further studies are required to determine which component of the BCR complex mediates the interaction with LAPTM5, whether this interaction is regulated by phosphorylation, ubiquitination, or some other posttranslational modification of Igα or Igβ, and whether the interaction between LAPTM5 and the BCR is direct or mediated by another protein(s).

Consistent with the elevated BCR levels after Ag stimulation in vivo, the frequency of NIP-binding B cells was increased in Laptm5−/− mice, and they produced greater amounts of NP-specific Ab in response to the TD Ag NP-CGG. In contrast, IgαFF, IgβAA, and Cbl2/2Cblb2/2 mice all exhibited decreased Ab production against TD Ag despite the fact that their B cells expressed increased levels of surface BCR. One important difference between Laptm5−/− B cells and IgαFF, IgβAA, or Cbl2/2Cblb2/2 mice was the increased BCR expression due to the absence of LAPTM5.
B cells is that the latter group is impaired in BCR internalization. BCR internalization and subsequent processing are essential for efficient Ag presentation and functional T–B interactions. Although it remains to be determined whether IgαF, IgβAA, or Cbl1+/−/Cblb−/− B cells are indeed impaired in Ag presentation, the decreased TD Ab responses despite the enhanced Ca2+ flux in these B cells following BCR cross-linking are consistent with such a possibility. Laptm5−/− B cells exhibited normal Ag internalization and presentation by MHC class II molecules. These observations suggest that the enhanced B cell expansion and Ab production in Laptm5−/− mice are attributable to the enhanced sustained BCR signaling caused by the elevated BCR levels. Intriguingly, Ab production against both type I (NP-LPS) and type II (NP-Ficoll) T-independent (TI) Ag appeared normal in Laptm5−/− mice (Supplemental Fig. 7). Likewise, IgαF and Cbl1+/−/Cblb−/− mice also exhibited relatively normal responses to TI Ag. At this point, it is unclear why LAPT5 deficiency affected B cell responses to TD but not TI Ag; however, a similar discrepancy between Ab responses to TD versus TI Ag has been observed previously. For example, CD22-deficient mice exhibited enhanced TD responses but normal or reduced TI responses (12–14), whereas CD19-deficient mice exhibited reduced TD but enhanced type II TI Ag responses (7–9). TI Ags are thought to directly trigger B cell activation and differentiation into IgM-producing plasma cells, whereas TD Ags need to be presented on the surface of follicular dendritic cells as membrane-bound immune complexes (36). BCR internalization and lysosomal degradation in B cells may be differentially regulated in response to TD versus TI Ag binding. Our results with the Laptm5−/− mice, together with previous studies of IgαF, IgβAA, and Cbl1+/−/Cblb−/− mice, suggest that B cell responses to TD Ags are more susceptible to abnormalities in BCR metabolism.

Although the increased BCR levels and the enhanced Ab production in response to a TD Ag appear to be due to a B cell intrinsic defect, the elevated levels of anti-dsDNA and anti-nuclear Ab observed in aged Laptm5−/− mice cannot be solely attributable to the abnormalities in B cells. We had previously shown that Laptm5−/− T cells were hyperresponsive to TCR cross-linking (26). Therefore, the elevated autoantibody production is likely caused by both B cell intrinsic and extrinsic mechanisms. How can LAPT5 target both TCR and BCR? In the case of TCR, we have shown that LAPT5 interacts with CD3ζ-chain and promotes its lysosomal degradation. By analogy with T cells, it is tempting to speculate that Igα and/or Igβ could be the target for LAPT5-mediated BCR degradation. Intriguingly, both CD3ζ and Igα undergo ubiquitination following Ag stimulation (20, 37). It is therefore possible that LAPT5 binds these molecules through its ubiquitin-interacting motif. Additionally, CD3ζ and Igα/β subunits share ITAM and LAPT5 and may directly or indirectly recognize these motifs. Although the precise mechanisms remain to be elucidated, our results suggest that LAPT5 is a common regulator of TCR and BCR. Notably, although overexpression of LAPT5 induced dramatic BCR downmodulation both in WEHI231 B cell line and in normal spleen B cells, LAPT5 deficiency only had a modest effect on BCR levels. These observations implicate functional redundancy in pathways leading to BCR downmodulation. In conclusion, our results suggest that LAPT5 functions to inhibit T and B cell activation as a feedback mechanism and to fine-tune the immune and allergic responses. To our knowledge, LAPT5 is the only lysosomal protein specifically expressed in myeloid and lymphoid lineage cells, and no paralogues with significant homology to LAPT5 have been identified in either mouse or human. These observations suggest that LAPT5-mediated receptor degradation is a mechanism unique to the immune system.

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Disclosures

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References


