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VprBP is required for efficient editing and selection of Igκ+ B cells, but is dispensable for Igλ+ and marginal zone B cell maturation and selection

Victoria L. Palmer[*], **Razia Aziz-Seible**[*], **Michele D. Kassmeier**[*,1], **Mary Rothermund**[*], **Greg A. Perry**[*], and **Patrick C. Swanson**[*]

[*]Department of Medical Microbiology and Immunology, Creighton University, 2500 California Plaza, Omaha, NE, USA 68178

Abstract

B cell development past the pro-B cell stage in mice requires the Cul4-DDB1-Roc1 E3 ubiquitin ligase substrate recognition subunit VprBP. Enforced *Bcl2* expression overcomes defects in distal V_H-DJ_H and secondary Vκ-Jκ rearrangement associated with VprBP-insufficiency in B cells, and substantially rescues maturation of marginal zone and Igλ+ B cells, but not Igκ+ B cells. In this background, expression of a site-directed Igκ light chain transgene increases Igκ+ B cell frequency, suggesting VprBP does not regulate light chain expression from a productively rearranged *Igk* allele. In site-directed anti-dsDNA heavy chain transgenic mice, loss of VprBP function in B cells impairs selection of Igκ "editor" light chains typically arising through secondary *Igk* rearrangement, but not selection of Igλ editor light chains. Both heavy and light chain site-directed transgenic mice show increased B cell anergy when VprBP is inactivated in B cells. Taken together, these data argue that VprBP is required for the efficient receptor editing and selection of Igκ+ B cells, but is largely dispensable for Igλ+ B cell development and selection, and that VprBP is necessary to rescue autoreactive B cells from anergy induction.

Keywords

VprBP; DCAF1; receptor editing; B cell development; Ig kappa; Ig lambda

Introduction

During B cell development, the Ig gene loci undergo an ordered series of DNA rearrangements to assemble functional Ig heavy and light chain genes. This process, called V(D)J recombination, is initiated by proteins encoded by RAG1 and RAG2, which introduce DNA double strand breaks in these loci that are subsequently repaired via non-homologous end-joining. Structure-function studies have established that the amino-terminal third of RAG1 is dispensable for the catalytic activity of the RAG protein complex, yet this region is evolutionarily highly conserved, and is required for efficient and high-fidelity V(D)J

Correspondence: Patrick C. Swanson, Ph.D., Department of Medical Microbiology and Immunology, Creighton University, 2500 California Plaza, Omaha, NE, USA 68178, Phone: (402)-280-2716, FAX: (402)-280-1875, pswanson@creighton.edu.
[1]Current address: Physician Assistant Program, Doisy College of Health Sciences, St. Louis University.

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recombination (1). How the RAG1 amino-terminus promotes this outcome remains unclear, but evidence from our laboratory and others suggest it functions at least in part as a protein interaction domain to recruit factors to facilitate chromosomal V(D)J recombination. We recently identified that Vpr binding protein (VprBP; DCAF1), a substrate adaptor molecule for the Cul4-Roc1-DDB1 (CRL4) and EDD/UBR5 E3 ubiquitin ligase complexes (2), associates with the amino-terminal region of RAG1, and that VprBP is required for B cell development and plays a role in V(D)J recombination (3). Specifically, we found that conditional disruption of *Vprbp* early in B cell development arrests B cell maturation at the pro B-to-pre-B cell transition, but this developmental block is partially rescued by expressing functionally rearranged Ig transgenes. Loss of VprBP expression in B cells is associated with impaired V_H-DJ_H gene rearrangement, reduced fidelity of V_H-DJ_H joining, defects in cell cycle progression, and increased apoptosis (3).

Given the elevated levels of apoptosis observed in VprBP-deficient B cells, here we investigated whether enforced expression of the pro-survival factor Bcl2 can compensate for the loss of VprBP during B cell development, as has been observed in other cases of genetic insufficiency manifesting impaired B cell development (4–7). As in those cases, we find that *Bcl2* expression partially rescues B cell development, substantially reconstituting marginal zone, but not follicular, B cell populations. Unexpectedly, however, most B cells maturing under this program express Igλ rather than Igκ. The loss of $Ig\kappa^+$ B cells in this context can be partially rescued in mice bearing a site-directed Igκ light chain transgene, suggesting VprBP does not regulate light chain expression from a productively rearranged *Igk* allele. More detailed analysis of V(D)J rearrangement patterns in pre-B cells and rare $Ig\kappa^+$ B cells isolated from VprBP-deficient mice provides evidence for inefficient distal V_H-DJ_H gene rearrangement and secondary *Igk* rearrangements associated with receptor editing in these animals. However, the apparent V(D)J recombination defects are substantially rescued by enforced Bcl2 expression, ruling out a direct role for VprBP in mediating the V(D)J rearrangement process itself.

As an alternative, we speculated that VprBP functions indirectly to regulate the efficiency of B cell receptor editing and selection of $Ig\kappa^+$ B cells. To test this possibility, we analyzed how the loss of VprBP function affects B cell development and selection in mice harboring the site-directed V_H3H9/56R (56R) anti-DNA heavy chain transgene, which is used as a model of V_H gene replacement as well as light chain receptor editing and selection (8). Our results suggest that VprBP insufficiency impairs V_H gene replacement and selection of Igκ editor light chains, but does not interfere with the selection of Igλ editor light chains. Interestingly, both heavy and light chain site-directed transgenic mice show an increased frequency of phenotypically anergic B cells when VprBP is inactivated. Taken together, these data argue that VprBP is required for the efficient editing and selection of $Ig\kappa^+$ B cells, but is largely dispensable for $Ig\lambda^+$ B cell development and selection, and is necessary to salvage B cells from potential anergy induction.

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Materials and Methods

Mice

Mice with the following conditional alleles or transgenes have been previously described: *Vprbp*^{fl} (9), mb1-Cre (10), Eμ-2-22 Bcl2 (11), site-directed V_H3H9/56R heavy chain (56R) (8), and site-directed 3-83 kappa chain lacking all 3’ Jκ segments (3-83κiJκ$^-$) (12). The latter mouse strain was derived by breeding mice harboring both site-directed 3-83 heavy and light chains (Jackson Laboratory strain C.129P2(B6)-*Igk*^{tm2Rsky} *Igh*^{tm2Rsky}/J) to C57Bl/6 mice and screening for offspring heterozygous for only the 3-83κiJκ$^-$ allele. All mice were bred on a C57Bl/6 background and maintained in individually ventilated microisolator cages in an AAALAC certified animal facility at Creighton University. All experimental procedures were reviewed and approved by the Creighton University Institutional Animal Care and Use Committee.

Flow cytometry

For detecting surface antigens, single cell suspensions were prepared for bone marrow and spleen and stained with cocktails of biotin- or fluorochrome-conjugated antibodies as described previously (Fusby et al., 2010). The following antibodies and clones were used: BD Biosciences (San Jose, CA) anti-B220-PE, –PE-Texas Red, or –CF594 (all clone RA3-6B2), anti-CD19-APC-Cy7 (1D3), anti-CD23-biotin (B3B4), anti-CD43-APC (S7), anti-CD43-biotin (S7), anti-CD93-APC (AA4.1), anti-IgMa-biotin (DS-1), anti-IgMb-PE (AF6-78), anti-kappa-PerCP-Cy5.5 (187.1), anti-lambda-FITC (R26-46), anti-Ly-51-PE (BP.1) and anti-Ly6C-PerCP-Cy5.5 (AL-21), and Biolegend (San Diego, CA) anti-CD21/CD35-PE-Cy7 (7E9) and anti-IgD-APC-Cy7 (11-26c), and eBioscience (San Diego, CA) anti-CD19-A700 (1D3), anti-CD4-A700 (GK1.5), anti-CD49b-PE-Cy7 (DX5), anti-CD93-PE (AA4.1), anti-CD93-PE-Cy7 (AA4.1), anti-IgM-FITC (II/41), anti-IgM-APC (II/41), anti-IgM-PE-Cy5 (II/41), anti-IgD-FITC (11-26c), and Southern Biotech (Birmingham, AL) anti-CD24/HSA-Spectral Red (30-F1). Samples stained with biotinylated antibodies were detected using streptavidin-Qdot585 (Invitrogen, Carlsbad, CA) or streptavidin-BUV737 (BD Biosciences). To detect Vλx by flow cytometry, a protein G-purified Vλx-specific monoclonal antibody, called clone 10C5 (13) (kind gift from E.L. Prak), was conjugated to Alexa Fluor647 using a kit according to manufacturer’s instructions (Life Technologies) and stained as described above, except that Fc blocking reagent (anti-mouse CD16/32 antibody; BD Biosciences) was added prior to and during the staining procedure.

To detect intracellular proteins, cells were Fc blocked and stained first with antibodies to detect surface antigens, washed, and then incubated with streptavidin Q585 or BUV737 to detect biotinylated antibodies. A second round of surface staining with 10-fold excess anti-IgM-FITC (II/41) and unconjugated anti-kappa antibodies was done to block all surface IgM and Igκ. Next, cells were fixed and permeabilized for 20min on ice (Cytofix/CytoPerm kit; BD Biosciences). After washing, cells were stained with eBioscience anti-Pax5-APC (1H9) and anti-Irf4-PerCP-eFluor710 (3H4), or eBioscience anti-IgM-APC (II/41) and BD Bioscience anti-kappa-PerCP-Cy5.5 (187.1), or with control antibodies conjugated to APC (eBR2a; eBioscience), PerCP-eFluor710 (eBRG1; eBioscience); or PerCP-Cy5.5 (R3-34; BD Bioscience).

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Data collection and cell sorting was performed using a FACSAria flow cytometer (BD Biosciences). Data was analyzed using the FlowJo software (Tree Star, Inc. Ashland, OR).

V(D)J and IRS-RS rearrangement assays

Genomic DNA was prepared from sorted bone marrow pre-B cells ($Ly6C^{-}CD4^{-}DX5^{-}B220^{+}CD43^{-}IgM^{-}$) and splenic $CD19^{+}$ $Ig\kappa^{+}$ B cells as described (14). Briefly, 10^6 cells were lysed in 200 μL PCR lysis buffer (10mM Tris, pH 8.4, 50 mM KCl, 2mM $MgCl_2$, 0.45% NP-40, 0.45% Tween-20, 60 μg/ml proteinase K), incubated at 55°C for 1 h, and then heated to 95°C for 10 min to inactivate proteinase K. This procedure yielded DNA template equivalent to 5000 genomes/μl that was used directly for PCR analysis. *Igh, Igk, Igl,* and IRS-RS rearrangements were amplified by PCR from template DNA (10000, 2500 and 625 genome-equivalents). Briefly, PCR reactions (50 μl) containing template DNA and 0.5 μM of each primer (see Table 1) in sample buffer (0.2 mM of dNTPs, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5mM MgCl2 and 2.5 units Taq polymerase [Promega, Madison, WI]) were subjected to initial denaturation (*Igh, Igk*, IgλR1 and IRS-RS rearrangements: 94°C for 1 min; Igλx rearrangements: 94°C for 4 min), 30–40 cycles of amplification (*Igh* and IRS-RS rearrangements: 94°C for 30 sec, 59°C for 1 min, 72°C for 2 min; IgVλx rearrangements: 94°C for 20 sec, 60°C for 30 sec, 72°C for 1.5 min; IgλR1 rearrangements: 94°C for 30 sec, 48°C for 1 min, 72°C for 2 min; Vκ1 rearrangements: 94°C for 30 sec, 60°C for 1 min, 72°C for 2 min; Vκ21 rearrangements: 94°C for 30 sec, 55°C for 1 min, 72°C for 2 min), and then a final extension (*Igh, Igk*, IgλR1 and IRS-RS rearrangements: 72°C for 4 min; IgVλx rearrangements: 72°C for 5 min). Primers flanking a fragment from the CD14 locus were used as a loading control. PCR products were separated by agarose gel electrophoresis, and visualized by ethidium bromide staining or by Southern blotting using nested ^{32}P-labeled oligonucleotide probes (see Table 1) and autoradiographic imaging with a Typhoon 9410 Variable Mode Imager.

Statistics

Data are presented as mean values ± standard error of the means. Collected data were subjected to analysis of variance and post hoc testing using the PASW Statistics 22.0 software package (SPSS Inc., Chicago, IL). Differences with a *p*-value of 0.05 are considered statistically significant.

Results

Enforced Bcl2 expression partially rescues B cell development in Vprbp$^{fl/fl}$ Cre^{+} mice, but most B cells developing in this background are Igλ^{+}

We previously used a Cre-*loxP* approach to conditionally disrupt *Vprbp* expression in the B lineage by breeding the mb1-Cre transgene onto a strain background in which both *Vprbp* alleles contain *lox*P sites flanking exons 7 and 8 (*Vprbp*$^{fl/fl}$-Cre^{+}) (see Fig. 1A), and showed that B cell development was arrested at the pro B-to-pre B cell transition in these animals (3). Concomitant expression of functionally rearranged anti-HEL Ig heavy and light chain transgenes could partially bypass the developmental block, arguing that VprBP plays a role in V(D)J recombination, but evidence for defects in cell cycle progression and increased apoptosis in pre-B cells from these animals pointed to potential additional roles for VprBP

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in cell proliferation and survival (3). In several other genetic models of B cell developmental arrest, enforced Bcl2 expression has been shown to enable some maturing B cells to bypass the developmental block (4–7). To determine whether Bcl2 expression can similarly rescue B cell development in *Vprbp*$^{fl/fl}$ Cre^{+} mice, we bred the Eμ-2-22 Bcl2 transgene (Bcl2^{+}) (11) onto this strain background and analyzed the frequency of developing and mature B cell subsets in the bone marrow and spleen using flow cytometry.

Consistent with our previous work, total bone marrow and spleen cellularities, and the absolute number of lymphocytes in these organs in wild-type and *Vprbp*$^{fl/fl}$ mice were not significantly different, but *Vprbp*$^{fl/fl}$ Cre^{+} mice showed a significant decrease in total and lymphocyte cellularities in the spleen, but not bone marrow (Fig. 1B) and a strong developmental arrest at the pro-B cell stage (B220^{+}CD43^{+}IgM^{-}), with numbers of pre-B cells (B220^{+}CD43^{-}IgM^{-}) reduced about 80% in bone marrow, and B cells at later developmental stages reduced to less than 1% of normal levels in bone marrow and spleen (Fig. 2A–C, Supplemental Table 1). For this analysis, cell populations found to contaminate the gates used to identify the early B cell subsets in the bone marrow were specifically excluded, including DX5^{+} NK cells, CD4^{+} dendritic cell progenitors, and Ly6C^{+} myeloid and plasma cells (15). As expected from earlier studies (4, 11), *Vprbp*$^{fl/fl}$ Bcl2^{+} mice showed significantly higher spleen cellularities and increased absolute numbers of total lymphocytes and all B cell developmental subsets in the spleen compared to their *Vprbp*$^{fl/fl}$ counterparts (Fig. 1B, Fig. 2A–C, Supplemental Table 1). We also detected a small splenic pro-B cell-like population (B220^{lo}CD43^{+}AA4.1^{+}) that has been observed in Bcl2-transgenic mice by others (16) (Fig. 2A). Notably, when Bcl2 was expressed in the *Vprbp*$^{fl/fl}$ Cre^{+} background, pre-B cell numbers were restored to levels similar to *Vprbp*$^{fl/fl}$ mice, but still remained well below levels observed in *Vprbp*$^{fl/fl}$ Bcl2^{+} mice, whereas the abundance of later B cell developmental subsets were increased 10–100 fold compared to *Vprbp*$^{fl/fl}$ Cre^{+} mice, but still remained well below levels found in *Vprbp*$^{fl/fl}$ mice (Fig. 2B–C, Supplemental Table 1). Unexpectedly, when B cells were interrogated for kappa and lambda light chain expression, we found that about 60–75% of the light chain-expressing CD19^{+} B cells in the bone marrow and spleen of *Vprbp*$^{fl/fl}$ Cre^{+} Bcl2^{+} mice were Igλ^{+}, whereas the proportion was closer to 10–20% in wild-type, *Vprbp*$^{fl/fl}$, and *Vprbp*$^{fl/fl}$ Bcl2^{+} mice (Fig. 3A–B). This outcome is primarily due to a significant reduction of Igκ^{+} B cells, rather than an increase in absolute numbers of Igλ^{+} B cells in *Vprbp*$^{fl/fl}$ Cre^{+} Bcl2^{+} mice compared to *Vprbp*$^{fl/fl}$ Bcl2^{+} mice (Fig. 3C). One additional noteworthy observation is that Bcl2 expression in *Vprbp*$^{fl/fl}$ Cre^{+} mice restored marginal zone (B220^{+}AA4.1^{-}CD21^{+}CD23^{-}) B cell numbers to levels observed in *Vprbp*$^{fl/fl}$ Bcl2^{+} mice, whereas transitional and follicular mature populations only recovered to about 10% of the levels detected in *Vprbp*$^{fl/fl}$ Bcl2^{+} mice (Fig. 2C, Supplemental Table 1). Taken together these results show that enforced Bcl2 expression in *Vprbp*$^{fl/fl}$ Cre^{+} mice partially rescues B cell development, enabling substantial reconstitution of Igλ^{+}, but not Igκ^{+} B cells, and marginal zone, but not follicular, B cells.

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Loss of VprBP function in B cells is associated with impaired distal V_H-DJ_H rearrangement and a bias toward proximal Jκ rearrangements, but both are normalized by enforced Bcl2 expression

Using a PCR-Southern blotting approach to analyze V(D)J rearrangement patterns in total bone marrow of *Vprbp*$^{fl/fl}$ Cre$^+$ mice, we previously showed that V_H-DJ_H and Vκ-Jκ rearrangement is impaired in these animals (3). To explain the selective deficit in Igκ$^+$ B cells in *Vprbp*$^{fl/fl}$ Cre$^+$ Bcl2$^+$ mice, we considered the fact that the *Igl* locus is about 1/10th the size of the *Igh* and *Igk* loci in mice, and therefore hypothesized that VprBP is required for efficient V(D)J recombination of the large *Igh* and *Igk* loci, but is dispensable for V(D)J rearrangement involving the smaller *Igl* locus. To test this hypothesis, we extended our previous studies of V(D)J rearrangement patterns in *Vprbp*$^{fl/fl}$ Cre$^+$ mice to *Vprbp*$^{fl/fl}$ Cre$^+$ Bcl2$^+$ mice and included additional primer sets to evaluate V(D)J rearrangements involving *Igh* and *Igk* variable (V) genes that are proximal or distal to the joining (J) segments, those occurring in the *Igl* locus, and those involving IRS-RS recombination (17), a form of secondary V(D)J rearrangement that generally occurs after exhaustive Vκ-Jκ rearrangement and results in the excision of Cκ from the *Igk* locus (kappa deletion).

Because the B cell developmental block in *Vprbp*$^{fl/fl}$ Cre$^+$ mice occurs at the pro-B to pre-B cell transition, we focused our analysis on the pre-B cell subset. Among sorted pre-B cells (B220$^+$CD43$^-$IgM$^-$), the pattern of D-J_H rearrangement or V_H-DJ_H rearrangement involving proximal (V_HQ52 and V_H7183) or distal (V_HJ558) gene clusters was observed to be quite similar between *Vprbp*$^{fl/fl}$, *Vprbp*$^{fl/fl}$ Bcl2$^+$, and *Vprbp*$^{fl/fl}$ Cre$^+$Bcl2$^+$ mice, but in *Vprbp*$^{fl/fl}$ Cre$^+$ mice, levels of D-J_H rearrangement were consistently enriched and rearrangements to the distal V_HJ558 gene cluster were consistently diminished (Fig. 4A). By contrast, V_H-DJ_H rearrangements involving the proximal gene V_H clusters (V_HQ52 and V_H7183) were reduced only modestly if at all in *Vprbp*$^{fl/fl}$ Cre$^+$ mice. Elevated D-J_H rearrangements detected in *Vprbp*$^{fl/fl}$ Cre$^+$ mice most likely reflects poor ongoing V_H gene rearrangement that would normally diminish the pool of template DNA that had not yet undergone V_H-DJ_H rearrangement.

When *Igk* and *Igl* rearrangements in sorted pre-B cells were analyzed, we found these levels to be slightly reduced in *Vprbp*$^{fl/fl}$ Cre$^+$ mice compared to *Vprbp*$^{fl/fl}$ mice (Fig. 4A). By contrast, however, both *Vprbp*$^{fl/fl}$ Bcl2$^+$ and *Vprbp*$^{fl/fl}$ Cre$^+$ Bcl2$^+$ mice showed levels of these rearrangement products that were similar to each other, but were consistently elevated compared to *Vprbp*$^{fl/fl}$ and *Vprbp*$^{fl/fl}$ Cre$^+$ mice, particularly for IRS-RS and Vλ1-Jλ1 rearrangements, suggesting this effect is attributed mainly to enforced Bcl2 expression.

To determine whether the distribution of *Igk* and *Igl* rearrangements observed in pre-B cells differs from those having undergone selection and migration out of the bone marrow, we analyzed the pattern of these rearrangements in splenic Igκ$^+$ B cells sorted from the four mouse genotypes (Fig. 4B). Interestingly, the rare Igκ$^+$ B cells isolated from *Vprbp*$^{fl/fl}$ Cre$^+$ mice show predominant rearrangement to Jκ2 by the proximal Vκ21 segment, whereas rearrangements involving the distal Vκ1 segment, IRS-RS, and Vλ1-Jλ1 are at or below levels detected in *Vprbp*$^{fl/fl}$ mice. However, when Bcl2 is expressed in the *Vprbp*$^{fl/fl}$ Cre$^+$ background, proximal and distal Vκ rearrangement patterns are normalized to those detected

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in *Vprbp*$^{fl/fl}$ Bcl2$^+$ mice, whereas IRS-RS and Vλ1-Jλ1 rearrangement levels are slightly higher than those observed in *Vprbp*$^{fl/fl}$ Bcl2$^+$ mice.

VprBP does not regulate Pax5 and IRF4 expression

The strong bias of rearrangements toward Jκ2 observed in Igκ$^+$ B cells from *Vprbp*$^{fl/fl}$ Cre$^+$ mice, taken together with the apparent defect in distal V_H-DJ_H rearrangement in these animals and the striking reduction of Igκ$^+$ B cells *Vprbp*$^{fl/fl}$ Cre$^+$ Bcl2$^+$ mice compared to *Vprbp*$^{fl/fl}$ Bcl2$^+$ mice, raised the possibility that loss of VprBP function adversely affects factors or processes that regulate distal V_H-DJ_H rearrangement and/or secondary V(D)J rearrangements initiated in response to self-reactivity (receptor editing). The transcription factors Pax5 and Irf4 have been previously implicated in regulating distal V_H-DJ_H rearrangement and light chain receptor editing, respectively (18, 19). To determine whether loss of VprBP function affects Pax5 and Irf4 expression, we compared the intracellular levels of these proteins between *Vprbp*$^{fl/fl}$, *Vprbp*$^{fl/fl}$ Cre$^+$, *Vprbp*$^{fl/fl}$ Bcl2$^+$, and *Vprbp*$^{fl/fl}$ Cre$^+$Bcl2$^+$ mice by flow cytometry (Fig. 5A). We found that the levels of these proteins in pro-B and pre-B cells were not significantly different between the four mouse genotypes, suggesting VprBP does not directly regulate the expression of these proteins.

Absence of surface Igκ$^+$ B cells in Vprbp$^{fl/fl}$ Cre$^+$Bcl2$^+$mice is not due to cytoplasmic sequestration of Igk chain

If loss of VprBP function impairs V(D)J recombination efficiency, one might expect that intracellular levels of Igμ and Igκ chains may be reduced in early stages of B cell development. Alternatively, if loss of VprBP function impairs surface expression of these molecules, they may accumulate inside the cell. To address these possibilities, we compared the intracellular levels of Igμ and Igκ chains in pro-B and pre-B cells between *Vprbp*$^{fl/fl}$, *Vprbp*$^{fl/fl}$ Cre$^+$, *Vprbp*$^{fl/fl}$ Bcl2$^+$, and *Vprbp*$^{fl/fl}$ Cre$^+$Bcl2$^+$ mice by flow cytometry (Fig. 5B). Consistent with the former possibility, levels of cytoplasmic Igμ and Igκ (cμ and cκ) chains were significantly reduced in both pro-B and pre-B cell subsets in *Vprbp*$^{fl/fl}$ Cre$^+$ mice compared to *Vprbp*$^{fl/fl}$ mice. Enforced Bcl2 expression in *Vprbp*$^{fl/fl}$ Cre$^+$ mice substantially rescued cμ levels in pro-B cells, but a substantial fraction of pre-B cells lacked cμ expression. Notably, cκ levels were not rescued by enforced Bcl2 expression in pre-B cells in *Vprbp*$^{fl/fl}$ Cre$^+$ mice, despite the apparent ability of these cells to undergo *Igk* rearrangement (see Fig. 4).

Loss of VprBP function in anti-dsDNA transgenic mice impairs receptor editing and Igκ chain selection, but not Igλ chain selection

To more specifically test whether loss of VprBP function impairs receptor editing in B cells, we used a well-established mouse model of receptor editing in which a transgene expressing an Ig heavy chain specific for dsDNA, called V_H3H9/56R (hereafter called 56R), is knocked into the endogenous locus (8). In 56R mice, most developing B cells undergo receptor editing to remove or silence dsDNA autoreactivity. Two major mechanisms of receptor editing have been described in the 56R model: (i) heavy chain gene replacement, in which an endogenous upstream V_H and/or D_H gene segment replaces most of the 56R transgene via rearrangement with a cryptic recombination signal sequence at the 3’ end of the

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transgene (20); and (ii) successive light chain rearrangement, which enables selection of one of a restricted set of "editor" light chains that neutralize the dsDNA binding activity of the 56R heavy chain (8). Certain editor light chains paired with 56R, notably Vκ38c, also confer reactivity toward a Golgi-associated antigen, causing the B cell to adopt a marginal zone phenotype and allowing it to escape negative selection (21).

In the experiments described here, we bred the 56R transgene onto the *Vprbp*$^{fl/fl}$, *Vprbp*$^{fl/fl}$ Cre^{+}, *Vprbp*$^{fl/fl}$ Bcl2^{+}, *Vprbp*$^{fl/fl}$ Cre^{+} Bcl2^{+} strain backgrounds and compared the frequency of B cells that express μ heavy chain from the 56R transgene or the non-targeted allele (IgM^{a} or IgM^{b}, respectively), or that express the Igκ or Igλ light chain. The abundance of other B cell developmental subsets was also analyzed. One important initial finding from these studies was that, compared to *Vprbp*$^{fl/fl}$ Cre^{+} mice, *Vprbp*$^{fl/fl}$ Cre^{+}56R^{+} mice had over twice the number of bone marrow pre-B cells, and 10–20-fold more splenic transitional (B220^{+}AA4.1^{+}) and mature (B220^{+}AA4.1^{-}) B cells (Supplemental Table 1). Thus, the developmental block observed in *Vprbp*$^{fl/fl}$ Cre^{+} mice is partially overcome by 56R transgene expression. Consistent with this finding, cμ was detected at similar levels in bone marrow pro-B and pre-B cells from *Vprbp*$^{fl/fl}$ 56R^{+} mice as compared to *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} mice (Fig. 6A). This result suggests that reduced cμ expression detected in pro-B and pre-B cells in *Vprbp*$^{fl/fl}$ Cre^{+} mice is unlikely to be attributed to impaired expression from a functionally rearranged allele.

When splenic B cells expressing IgM^{a} were analyzed in the different 56R transgenic mouse strains, we found that the proportion of total CD19^{+}IgM^{+} B cells expressing IgM^{a} increased from about 89–90% in *Vprbp*$^{fl/fl}$ 56R^{+} and *Vprbp*$^{fl/fl}$ 56R^{+} Bcl2^{+} mice, to 94% in *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} and *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} Bcl2^{+} mice (Fig. 7A-B). These data suggest the efficiency of 56R V_H gene replacement is impaired by loss of VprBP function. When the proportion of Igλ-expressing B cells was analyzed in the spleens from the different 56R transgenic mouse strains, we found that about 10% of the light chain-expressing cells in *Vprbp*$^{fl/fl}$ 56R^{+} mice were Igλ^{+}, a value which is close to that observed in *Vprbp*$^{fl/fl}$ mice. This proportion increased to about 25% in *Vprbp*$^{fl/fl}$ 56R^{+} Bcl2^{+} mice, and was further elevated to 45% and 75% in *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} and *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} Bcl2^{+} mice, respectively (Fig. 7A-B). Like the comparison between *Vprbp*$^{fl/fl}$ Bcl2^{+} and *Vprbp*$^{fl/fl}$ Cre^{+} Bcl2^{+} mice, the basis for the skewing toward Igλ-expressing B cells in *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} Bcl2^{+} mice relative to *Vprbp*$^{fl/fl}$ 56R^{+} Bcl2^{+} mice is attributed to a >10-fold reduction in the absolute number of Igκ^{+} cells in the former strain, whereas the abundance of Igλ^{+} cells differs between these strains by only about 2-fold (Supplemental Fig. 1A, Supplemental Table 1). We also detected low cκ levels in developing and mature B cell subsets from *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} and *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} Bcl2^{+} mice (Fig. 6B, Supplemental Fig. 1C), suggesting that loss of Igκ^{+} cells in these animals is not caused by intracellular retention of the kappa chain. Interestingly, we note that the mean fluorescence intensity of Igλ staining is markedly reduced in *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} Bcl2^{+} mice (as well as other 56R-transgenic strains) compared to *Vprbp*$^{fl/fl}$ Cre^{+} Bcl2^{+} mice (Fig. 7A; Supplemental Fig. 2A). We speculated that this phenomenon is due to the prevalent pairing of the 56R heavy chain with Vλx as an editor light chain, which is stained poorly if at all by commercially available Igλ-specific antibodies which detect Vλ1/2 (22). Consistent with this possibility, among the Igλ^{+}

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cells in all the $56R^+$ strains, about 90% were stained a Vλx-specific antibody (13) (Figs 7A-B). Few $V\lambda x^+$ B cells were co-stained with the Vλ1/2-specific antibody, but most were $sIg\kappa^{dim}$ (Fig. 7A). Dual κ/λ expression may be a means to dilute the density of autoreactive B cell receptors expressing Igκ (23). Further characterization of the splenic $CD19^+$ $V\lambda x^+$ B cells in *Vprbp*$^{fl/fl}$ Cre^+ $56R^+$ $Bcl2^+$ mice shows that most of these cells have a $B220^{hi}sIgM^{int}sIgD^+CD21^+CD23^+AA4.1^-$ phenotype (data not shown).

Loss of VprBP function in anti-dsDNA transgenic mice increases the frequency of phenotypically anergic B cells

Analysis of other splenic B cell populations also revealed several interesting effects of 56R transgene expression on Bcl2 transgenic and/or *Vprbp*$^{fl/fl}$ Cre^+ backgrounds. First, the splenic pro-B cell-like ($B220^{lo}AA4.1^+$) population observed in *Vprbp*$^{fl/fl}$ $Bcl2^+$ mice is not detected when the 56R transgene is concomitantly expressed (Fig. 8A). Second, among the three transitional ($CD19^+B220^+AA4.1^+$) B populations that have been defined (24), the T3 ($sIgM^{lo/-}$ $CD23^+$) subset, which is thought to be functionally anergic (25), represents the vast majority of transitional B cells in both *Vprbp*$^{fl/fl}$ Cre^+ $56R^+$ mice and *Vprbp*$^{fl/fl}$ Cre^+ $56R^+$ $Bcl2^+$ mice, whereas this population is much less predominant relative to T1 and T2 populations in the other mouse strains tested (Fig. 8A; Supplemental Table 1). An expanded population of $sIgM^{lo/-}$ $CD23^-$ cells is also detected in these animals (Fig. 8A). Further phenotypic characterization of the T3 subset and the $sIgM^{lo/-}CD23^-$ population reveals they share a similar phenotype aside from differential CD23 expression: mostly $sIgD^{dim}CD21^{dim}sIg\kappa^-sIg\lambda^-$, but $c\mu^+$ and $c\kappa^-$ (Fig. 8B, and data not shown). Third, in the mature ($CD19^+B220^+AA4.1^-$) B cell compartment, *Vprbp*$^{fl/fl}$ Cre^+56R^+ mice and *Vprbp*$^{fl/fl}$ Cre^+ $56R^+$ $Bcl2^+$ mice consistently show a smaller proportion of marginal zone B cells relative to follicular B cells compared to their counterparts lacking the Cre transgene (Fig. 8C). *Vprbp*$^{fl/fl}$ $56R^+$ $Bcl2^+$ (−/+ Cre) mice also show an expanded population of mature B cells that are $CD21^-CD23^+$ (Fig. 8A). Further characterization of these cells indicates that they phenotypically resemble transitional T3 B cells: mostly $sIgM^{dim}sIgD^{dim}Ig\kappa^-Ig\lambda^-$ and $c\mu^+$, but whereas this population contains a large fraction of $c\kappa^+$ cells in *Vprbp*$^{fl/fl}$ $56R^+$ $Bcl2^+$ mice, this population is uniformly $c\kappa^-$ in *Vprbp*$^{fl/fl}$ Cre^+ $56R^+$ $Bcl2^+$ (Fig. 8B, and data not shown). Taken together, these data suggest that loss of VprBP function in 56R mice reduces the efficiency of receptor editing by both heavy chain gene replacement and sequential light chain gene rearrangement, and impairs selection of $56R^+$ B cells into the marginal zone compartment. As a result, most B cells developing in 56R mice in the absence of functional VprBP undergo rearrangement and selection of lambda light chain expressing Vλx and/or acquire an anergic phenotype.

VprBP does not regulate Igκ expression from a functionally rearranged allele

Although the data presented to this point suggest VprBP is required for efficient receptor editing, we wished to exclude the possibility that Igκ expression, rather than *Igk* locus recombination, is adversely affected by loss of VprBP function. One piece of evidence arguing against this possibility is that the mean fluorescence intensity of Igκ staining among $CD19^+Ig\kappa^+$ B cells in *Vprbp*$^{fl/fl}$ Cre^+ mice is at or above that observed in *Vprbp*$^{fl/fl}$ mice (Supplemental Fig. 2A), suggesting that, once rearranged, the *Igk* locus can be expressed at normal levels. If the frequency of $Ig\kappa^+$ B cells emerging in *Vprbp*$^{fl/fl}$ Cre^+ mice is limited by

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Igk rearrangement efficiency, then enforced expression of a functionally rearranged light chain transgene in *Vprbp*$^{fl/fl}$ Cre$^+$ mice should increase the number of Igκ$^+$ B cells in these animals. To test this possibility, we obtained mice harboring a site-directed Igκ-expressing transgene, called 3-83κiJκ$^-$ (hereafter termed 3-83κ), which lacks endogenous 3' Jκ segments to prevent transgene excision by secondary Vκ-Jκ rearrangement (12). We then bred the 3-83κ transgene onto the *Vprbp*$^{fl/fl}$, *Vprbp*$^{fl/fl}$ Cre$^+$, *Vprbp*$^{fl/fl}$ Bcl2$^+$, and *Vprbp*$^{fl/fl}$ Cre$^+$ Bcl2$^+$ strain backgrounds, and compared the abundance and distribution of B cells expressing the Igκ or Igλ light chain, as well as other B cell developmental subsets (Fig. 9A; Supplemental Table 2). We find that the number of splenic Igκ$^+$ B cells in *Vprbp*$^{fl/fl}$ Cre$^+$3-83κ$^+$ Bcl2$^+$ mice is about 3-fold higher than in their *Vprbp*$^{fl/fl}$ Cre$^+$ Bcl2$^+$ counterparts, with the proportion of Igκ$^+$ cells increased by about 20% (Fig 9B; Supplemental Table 2). Moreover, surface Igκ expression in B cells from *Vprbp*$^{fl/fl}$ 3-83κ$^+$ mice (−/+ Bcl2) is similar to their *Vprbp*$^{fl/fl}$ Cre$^+$3-83κ$^+$ (−/+ Bcl2) counterparts as judged by comparing mean fluorescence intensity (Supplemental Fig. 2A), and levels of cκ staining are significantly higher in all splenic transitional and mature B cell subsets in *Vprbp*$^{fl/fl}$ Cre$^+$3-83κ$^+$ (−/+ Bcl2) mice compared to their *Vprbp*$^{fl/fl}$ Cre$^+$ (−/+ Bcl2) counterparts (Fig. 10B-C, Supplemental Fig. 2E). Taken together, these data suggest that a functionally rearranged kappa light chain gene can be expressed at normal levels from the endogenous locus in the absence of functional VprBP, and that inefficient primary and secondary rearrangement of the *Igk* locus is the most likely contributing factor for the inability of Igκ$^+$ B cells to develop under these conditions. Nevertheless, it is somewhat surprising that such a large proportion of light chain-expressing B cells in *Vprbp*$^{fl/fl}$ Cre$^+$3-83κ$^+$ Bcl2$^+$ mice are Igλ$^+$ considering these cells encode a functionally rearranged kappa light chain transgene. One potential explanation is that the site-directed 3-83κ allele is still susceptible to IRS-RS rearrangement that could delete Cκ and trigger endogenous *Igl* rearrangement. In support of this possibility, PCR-southern blotting experiments show higher levels of IRS-RS rearrangement in the bone marrow of *Vprbp*$^{fl/fl}$ Cre$^+$3-83κ$^+$Bcl2$^+$ mice compared to *Vprbp*$^{fl/fl}$ Cre$^+$3-83κ$^+$ mice (Fig. 9C), and concomitantly lower levels of cκ staining in all splenic transitional and mature B cell subsets (Supplemental Fig. 2E).

Two other interesting observations were also gleaned from the flow cytometric analysis of 3-83κ transgenic mice. First, we find that 3-83κ transgene expression in *Vprbp*$^{fl/fl}$ Bcl2$^+$ mice does not perturb the population of splenic pro-B-like cells detected in these animals, which stands in contrast to the effect of 56R expression in the same background (compare Figs. 8A and 10A). Second, whereas 3-83κ expression is less efficient than 56R expression in rescuing B cell development in *Vprbp*$^{fl/fl}$ Cre$^+$ mice (as assessed by comparing the absolute numbers of cells in each developmental subset in bone marrow and spleen), the opposite is true when the transgenes are expressed in *Vprbp*$^{fl/fl}$ Cre$^+$ Bcl2$^+$ mice (compare Supplemental Tables 1 and 2). Nevertheless, for both *Vprbp*$^{fl/fl}$ Cre$^+$ 3-83κ and *Vprbp*$^{fl/fl}$ Cre$^+$ 56R mice, enforced Bcl2 expression further restores B cell development (Supplemental Tables 1 and 2).

Discussion

How the amino-terminal third of RAG1 helps promote efficient and high-fidelity antigen receptor gene rearrangement remains ambiguous. At least part of its role appears likely to

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involve recruiting other cellular factors to regulate or direct the V(D)J recombination machinery or facilitate the repair of RAG-induced DNA double-strand breaks, but few reported RAG1-interacting factors have been tested to formally establish their requirement and role in V(D)J recombination. Previously, we discovered that VprBP and its associated CRL4 E3 ubiquitin ligase complex associates with the RAG1 amino-terminus. Using conditional gene disruption and complementation strategies in mice, we further established that VprBP is required for normal B cell maturation, and that the developmental arrest induced by loss of VprBP function can be partially rescued by the expression of functionally rearranged Ig transgenes (3) . Further insight into how VprBP promotes V(D)J rearrangement of endogenous Ig loci is limited by the fact that the cells undergo apoptosis at the pro-B to pre-B cell transition. However, the events leading to apoptosis upon loss of VprBP function may be separable from VprBP's potential role in V(D)J recombination, because conditional disruption of VprBP expression also causes apoptosis in non-lymphoid cells (9). Therefore, we tested whether enforced expression of the pro-survival gene *Bcl2* might suppress apoptosis triggered by factors extrinsic to V(D)J recombination, and allow B cells to develop beyond the pro-B to pre-B cell transition, where the requirement of VprBP in processes occurring at later stages of B cell development could be evaluated.

Our finding that loss of VprBP function is associated with impaired distal V_H and V_κ rearrangement indicates that VprBP function is not required for V(D)J recombination *per se*, but is rather required to support long-distance V(D)J recombination events. However, this defect can be overcome by enforced *Bcl2* expression, arguing that VprBP is not directly involved in rendering the distal regions of the *Igh* and *Igk* loci accessible to V(D)J recombination. Rather, the observation that enforced *Bcl2* expression partially restores B cell development in *Vprbp*$^{fl/fl}$ Cre$^+$ mice, yet only selectively reconstitutes maturation of Igλ$^+$ B cells, but not Igκ$^+$ B cells, argues that VprBP plays a role in supporting rearrangement and/or selection processes that operate intrinsically at the *Igk* locus. This contention is made plausible by previous studies showing that rearrangement and selection processes at the *Igk* and *Igl* loci are independently regulated, with the latter relying heavily on NF-κB-dependent signaling that can be functionally substituted by transgenic expression of Bcl2 (26). One attractive possibility is that VprBP supports receptor editing events initiated in the *Igk* locus to rescue autoreactivity. In this scenario, loss of VprBP function would inhibit secondary V(D)J rearrangements in the *Igk* locus. This possibility is supported by the finding that rare Igκ$^+$ B cells recovered from *Vprbp*$^{fl/fl}$ Cre$^+$ mice mostly harbor an *Igk* rearrangement involving Vκ and Jκ segments that lie in close proximity to one another (Vκ21-Jκ2), which would likely represent a primary Vκ-Jκ rearrangement in this locus (Fig. 4B). Further evidence for this contention is that rearrangements involving Vκ1, though only detected at low levels in these Igκ$^+$ B cells, are also skewed toward rearrangement with Jκ1 (Fig. 4B).

A plausible alternative explanation for the presence of Igλ$^+$ B cells in *Vprbp*$^{fl/fl}$ Cre$^+$ mice is that VprBP normally functions to *suppress* receptor editing events in the *Igk* locus. In this scenario, loss of VprBP function might then enable exhaustive rearrangement of the *Igk* locus, activation of *Igl* rearrangement, and selection of Igλ$^+$ B cells. However, if this were the case, one might have expected the pattern of Vκ rearrangement in Igκ$^+$ B cells from

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Vprbp$^{fl/fl}$ Cre^{+} mice to be heavily skewed toward usage of Jκ5 as an indicator of exhaustive *Igk* rearrangement, which is not observed. One might argue, however, that because the Vκ21-Jκ2 light chain is effective at vetoing heavy chain-associated anti-DNA autoreactivity (27), and because most of the rare Igκ^{+} B cells from *Vprbp*$^{fl/fl}$ Cre^{+} mice harbor rearrangements involving 3' V_H7183 and V_HQ52 gene family members which tend to be expressed early in ontogeny (28) and may be prone to autoreactivity (29), the over-representation of the Vκ21-Jκ2 light chain gene rearrangement may simply reflect a selection bias. Although we cannot entirely exclude this possibility, two lines of evidence argue that this is not the case. First, V(D)J rearrangements in the rare Igκ^{+} B cells from *Vprbp*$^{fl/fl}$ Cre^{+} mice involving the distal Vκ1 segment, though reduced compared to *Vprbp*$^{fl/fl}$ mice, are also mostly rearranged to the proximal Jκ1 segment (Fig. 4B). Second, one might predict that if heavy chain autoreactivity were further enforced, and *Igk* rearrangement was not affected by loss of VprBP function, selection of specific Igκ editors should be increased. However, this outcome is not observed in our experiments with the 56R mice, because *Vprbp*$^{fl/fl}$ Cre^{+} 56R^{+} mice have a larger proportion of B cells expressing heavy chain from the site-directed 56R allele than *Vprbp*$^{fl/fl}$ 56R^{+} mice, yet the proportion of Igκ^{+} cells is nevertheless diminished in these animals (Fig. 7). Concomitantly, selection of B cells into the marginal zone compartment, which is driven by 56R heavy chain pairing with the Vκ38c-bearing editor light chain (21), is also diminished in 56R mice lacking VprBP (regardless of Bcl2 transgene status) (Fig. 8C). Taken together, these data suggest that in 56R mice, loss of VprBP function limits the ability of B cells to undergo sequential V(D)J rearrangements in the *Igk* locus to obtain an editor light chain capable of neutralizing the anti-DNA reactivity of the 56R heavy chain.

One consequence of a failure to effectively edit the Igκ chain when heavy chain anti-dsDNA autoreactivity is enforced by transgene expression is an increase in B cells with anergic phenotypes. For example, in transgenic mice that cannot rescue anti-dsDNA B cell specificity by receptor editing due to concomitant RAG2-deficiency, most developing B cells acquire an antigen-experienced IgM^{dim}CD21^{-}CD23^{+} phenotype (30), which is similar to the phenotype of the transitional T3 B cell population reported to be functionally anergic (25). Our finding that B cells with a transitional T3-like phenotype represented the largest subset of transitional B cells in *Vprbp*$^{fl/fl}$ Cre^{+}56R^{+} mice (regardless of Bcl2 expression), and were also enriched in the mature (AA4.1^{-}) B cell compartment, suggests that loss of VprBP impairs the ability of autoreactive B cells to be rescued by receptor editing, leading them to acquire an anergic phenotype.

Taken together, the data suggest VprBP plays a key role in the editing and selection of Igκ light chains in response to B cell autoreactivity. Loss of VprBP function impairs receptor editing, which may trigger apoptosis of self-reactive B cells. This would be the fate for most developing B cells, since estimates suggest 25–75% of B cells in a primary repertoire are autoreactive (31). In *Vprbp*$^{fl/fl}$ Cre^{+} mice, this frequency may be higher due to impaired distal V_H gene rearrangement and skewing of the primary heavy chain repertoire toward 3' V_H gene families enriched in autoreactive specificities. The rare Igκ^{+} B cells detected in *Vprbp*$^{fl/fl}$ Cre^{+} mice may represent examples of B cells in which the primary Vκ rearrangement successfully neutralized the autoreactivity of the heavy chain. Enforced Bcl2

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expression allows developing B cells in *Vprbp*$^{fl/fl}$ Cre^{+} mice to bypass the restriction on the usage of distal V_H and Vκ gene segments, supports cellular signals that promote the generation of Igλ^{+} B cells, and also promotes survival of autoreactive B cells that would otherwise undergo apoptosis due to a failure to successfully edit BCR specificity away from autoreactivity. Under these conditions, cells may acquire an Igλ^{+} or an anergic phenotype, thereby avoiding clonal deletion. This possibility could explain the overrepresentation of B cells with these phenotypes in *Vprbp*$^{fl/fl}$Cre^{+}Bcl2^{+} mice compared to *Vprbp*$^{fl/fl}$ Bcl2^{+} mice, particularly when 56R is concomitantly expressed. We note however, that whereas loss of VprBP function impairs selection of Igκ editor light chains in 56R mice, it does not appear to affect Igλ light chain selection, as most B cells developing in *Vprbp*$^{fl/fl}$Cre^{+}Bcl2^{+}56R^{+} mice express the effective Vλx editor light chain, rather than the poor Vλ1/2 light chain editors that predominate in *Vprbp*$^{fl/fl}$Cre^{+}Bcl2^{+} mice. Tethering VprBP to the V(D)J recombinase through its association with full-length RAG1 provides an attractive and convenient means to rapidly communicate signals to reinitiate V(D)J recombination in the *Igk* locus when the B cell receptor is subjected to autoantigenic stimulation. The underlying mechanism(s) by which VprBP regulates this process remains to be determined, but could involve positive or negative feedback through its function as a substrate receptor for the CRL4 E3 ubiquitin ligase, through the actions of one of its associating factors, or through its recently described activity as serine/threonine kinase (2, 32).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

VprBP	Viral protein r binding protein
DCAF1	DDB1- and CUL4-associated factor homolog 1
Cul4	Cullin 4
DDB1	DNA damage-binding protein 1
CRL4	Cul4-DDB1-Roc1 E3 ubiquitin ligase
T1-T3	transitional 1–3
MZ	marginal zone
FM	follicular mature

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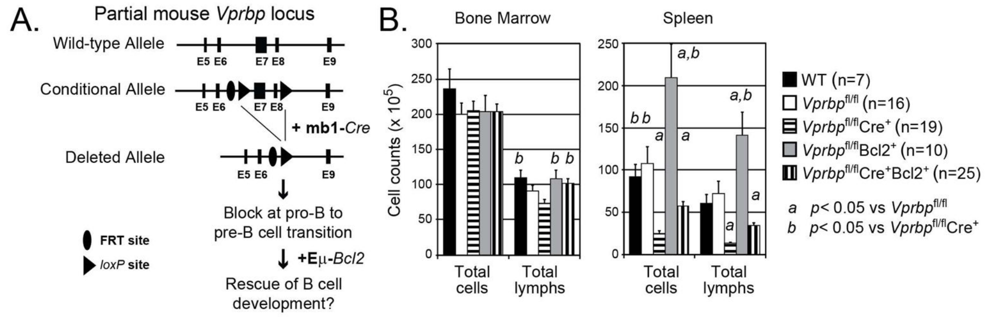

Figure 1.

Enforced Bcl2 expression partially rescues lymphocyte cellularity in *Vprbp*$^{fl/fl}$ Cre^{+} mice. (A) Diagram of wild-type and conditional *Vprbp* alleles; mb1-Cre expression deletes exons 7–8 in mice homozygous for the conditional *Vprbp* alleles (*Vprbp*$^{fl/fl}$) and causes B cell developmental arrest at the pro-B-to-pre-B cell transition. (B) Analysis of wild-type (WT) mice and *Vprbp*$^{fl/fl}$ mice lacking or containing the mb1-Cre (Cre^{+}) and/or Eμ-2-22 Bcl2 (Bcl2^{+}) transgenes. For each genotype, the total number of cells and lymphocytes was determined in the bone marrow (BM) and spleen (SPL). Data are represented as mean +−- SEM. Statistically significant differences are indicated for selected group comparisons.

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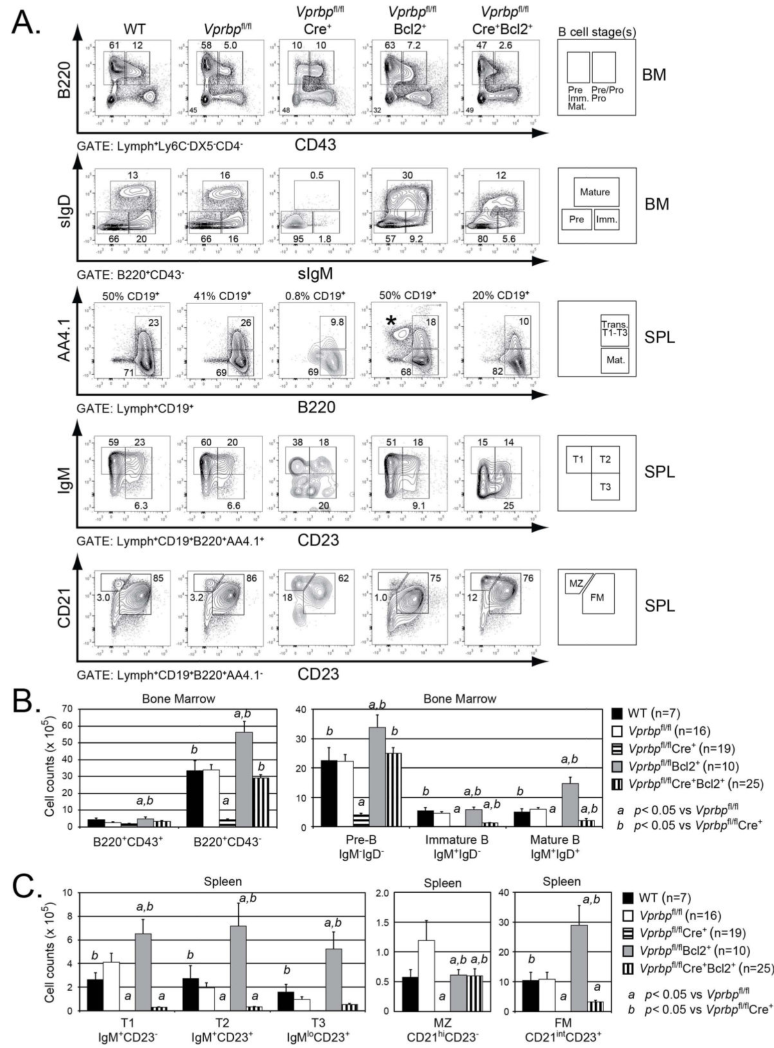

Figure 2.

Enforced Bcl2 expression partially rescues B cell development in *Vprbp*$^{fl/fl}$ Cre^{+} mice. (A) Lymphocytes from mice with the indicated genotype were analyzed by flow cytometry for the expression of different surface markers using gating strategies defined under each row. Developmental subsets identified by the staining pattern are shown at right with corresponding gates. The percentage of cells in each gate is shown for representative animals. A splenic pro-B cell-like population detected in *Vprbp*$^{fl/fl}$ Bcl2^{+} mice is identified with an asterisk. (B-C) The absolute number of cells in various B cell developmental subsets in the bone marrow (B) or spleen (C) defined by flow cytometry in panel A was determined for each of the indicated mouse genotypes. Data are represented as mean +/− SEM and are summarized in Supplemental Table 1). Statistically significant differences are indicated for selected group comparisons.

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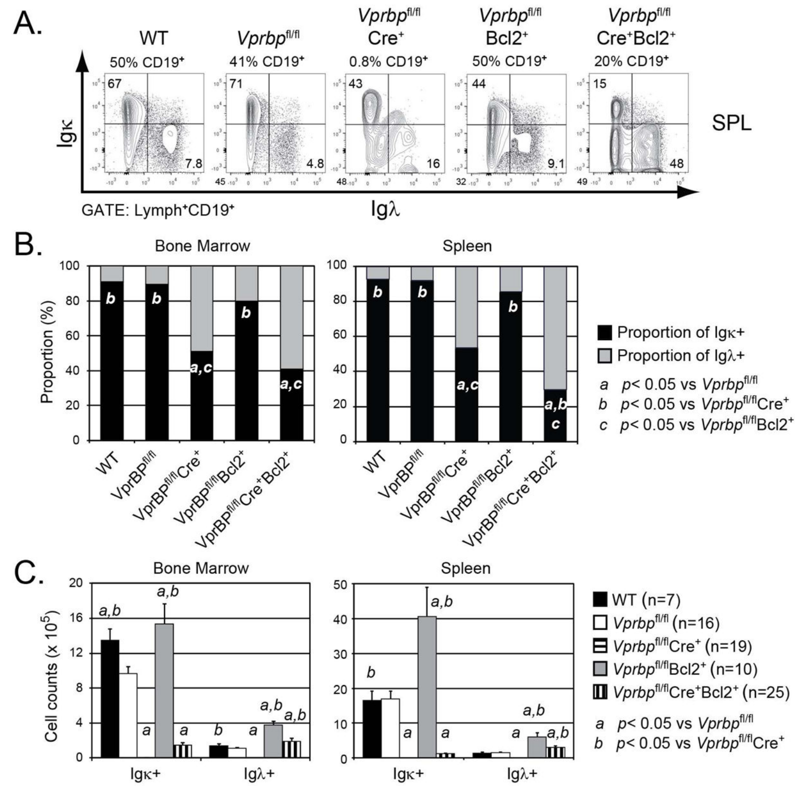

Figure 3.

B cells developing in *Vprbp* $^{fl/fl}$ Cre$^+$ Bcl2$^+$ mice are mostly Igλ$^+$. (A) Gated splenic CD19$^+$ lymphocytes from mice with the genotypes shown in Fig. 2 were analyzed for Igκ and Igλ expression by flow cytometry. (B-C) The proportion (B) and absolute number (C) of Igκ$^+$ and Igλ$^+$ cells in the bone marrow and spleen for each of the indicated mouse genotypes was determined using flow cytometric data shown in panel (A). Proportions were calculated as the percentage of Igκ$^+$ cells (black bar) or Igλ$^+$ cells (gray bar) among total CD19$^+$ light chain-expressing lymphocytes (i.e., Igκ$^+$ + Igλ$^+$). Data in panel C are represented as mean +/− SEM (see also Supplemental Table 1). Statistically significant differences are indicated for selected group comparisons.

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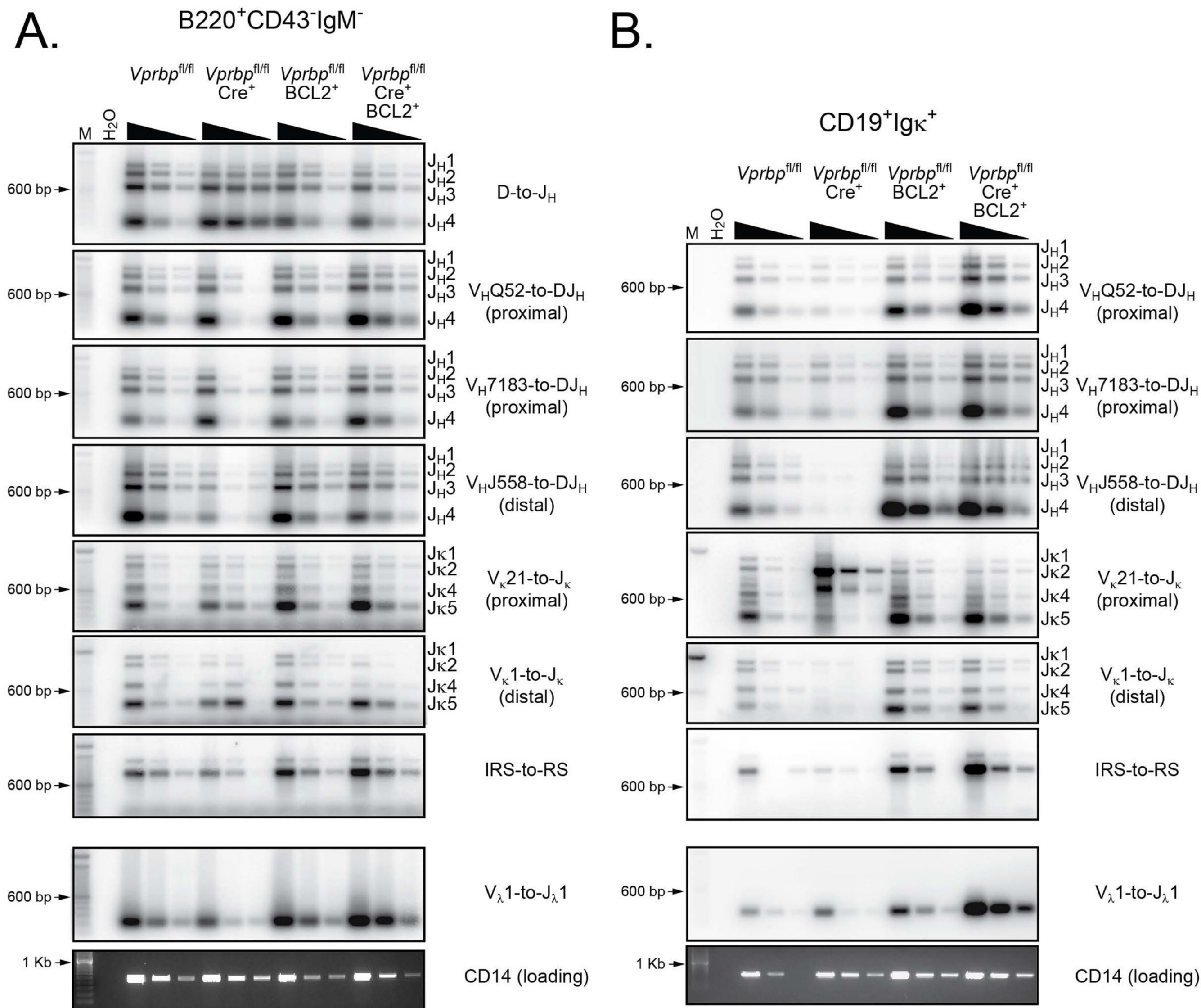

Figure 4.

Vprbp fl/fl Cre+ mice show inefficient distal V(D)J rearrangement in the Ig heavy and kappa light chain loci which is rescued by enforced Bcl2 expression. Genomic DNA prepared from bone marrow pre-B cells (B220+CD43−IgM−) or splenic Igκ+ B cells (10000, 2500, or 625 cell equivalents) sorted from mice with the indicated genotypes was subjected to PCR and Southern hybridization to detect the V(D)J rearrangements shown at right. Amplification of the non-rearranging CD14 locus was performed as a loading control.

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Figure 5.

Loss of VprBP function does not alter intracellular Pax5 or Irf4 levels, but reduces cytoplasmic mu and kappa chain levels. (A-B) Gated bone marrow pro-B ($B220^{+}CD43^{+}sIgM^{-}$) and pre-B cells ($B220^{+}CD43^{-}sIgM^{-}$) from mice with the genotypes shown in Fig. 2 were analyzed for the expression of (A) intracellular Pax5 and Irf4 or (B) cytoplasmic mu (cμ) and kappa (cκ) chains by flow cytometry. Representative staining by antigen-specific and isotype control antibodies (solid and dashed lines, respectively) is shown for animals of each genotype. In panel B, the additional shaded histogram represents isotype-specific staining of cells that were not permeabilized. Note that pro-B cells with a $Pax5^{lo}$ phenotype are $CD19^{-}$ whereas the $Pax5^{hi}$ cells are $CD19^{+}$. The mean fluorescence intensity was calculated for n=5 animals/genotype and the data presented as the mean +/– SEM.

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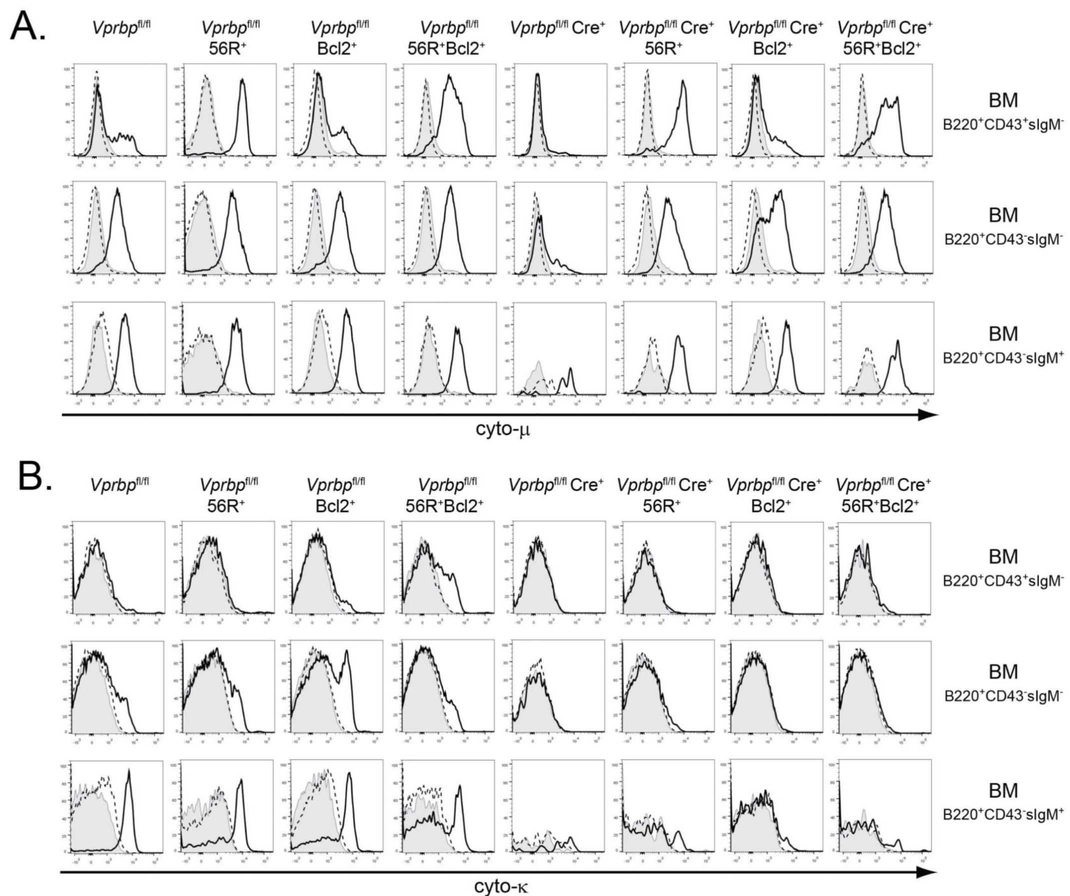

Figure 6.

56R mice lacking functional VprBP express Ig heavy chain, but not Ig kappa chain, through early B cell developmental stages. (A-B) Bone marrow (BM) B cell developmental subsets identified by the staining pattern at right were analyzed for the expression of cytoplasmic mu heavy chain (cyto-μ; panel A) or kappa light chain (cyto-κ; panel B) and presented as in Fig. 5B. (C) The mean fluorescence intensity (MFI) of antigen-specific staining is summarized in Supplemental Fig. 1C.

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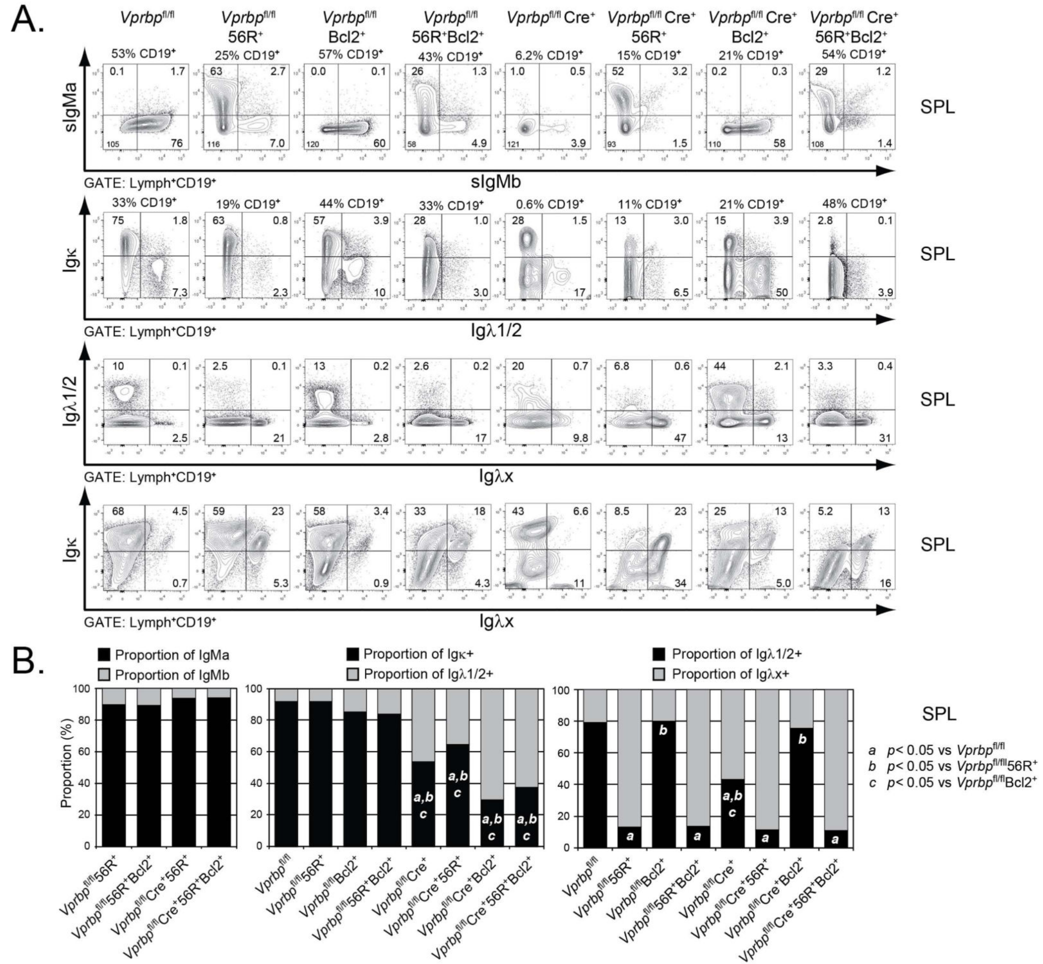

Figure 7.

Loss of VprBP function in 56R anti-dsDNA transgenic mice impairs receptor editing and selection of Ig kappa chains, but is dispensable for Ig lambda chain selection. (A) Splenocytes isolated from *Vprbp*$^{fl/fl}$ mice lacking or carrying the 56R, Cre, and/or Bcl2 transgenes in various combinations were analyzed for the expression of sIgMa (expressed from the 56R transgene) and sIgMb (expressed from the endogenous allele), Igκ and Igλ1/2 Igλx and Igλ1/2, or Igκ and Igλx on CD19^{+} lymphocytes using flow cytometry. The absolute numbers of CD19^{+} B cells expressing these markers are shown for each genotype in Supplemental Fig. 1A and Supplemental Table 1. (B) The proportion of CD19^{+} B cells expressing the markers examined in panel (A) were determined and presented as in Fig. 3B. Statistically significant differences are indicated for selected group comparisons.

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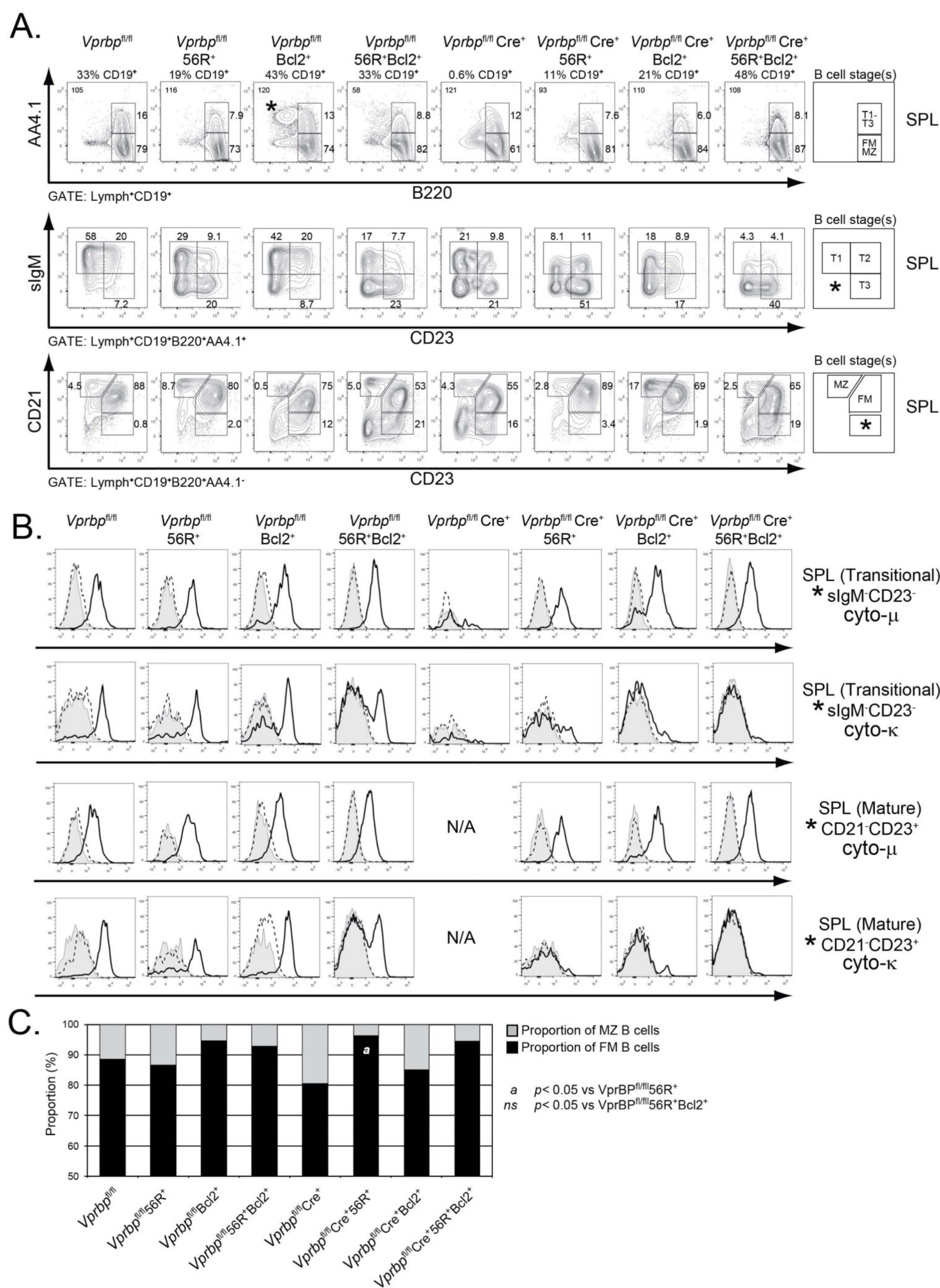

Figure 8.

Loss of VprBP function in 56R mice increases anergic B cell populations and impairs B cell selection into the marginal zone compartment. (A) Flow cytometry was used to identify various splenic transitional and mature B cell subsets as in Fig. 2A; the absolute numbers of cells in each subset are summarized in Supplemental Fig. 1B and Supplemental Table 1. A splenic pro-B cell-like population detected in *Vprbp*$^{fl/fl}$ Bcl2^{+} mice (identified with an asterisk) is notably absent in *Vprbp*$^{fl/fl}$ 56R^{+}Bcl2^{+} mice. (B) Cytoplasmic mu heavy chain (cyto-μ) or kappa light chain (cyto-κ) was analyzed by flow cytometry in the populations

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identified in panel A and presented as in Fig. 5B. Examples of cyto-μ and cyto-κ staining profiles are shown for non-conventional B cell populations identified by an asterisk in panel A (transitional sIgM^{-}CD23^{-} and mature CD21^{-}CD23^{+}, respectively). The mean fluorescence intensity (MFI) of antigen-specific staining is summarized in Supplemental Fig. 1C. (C) The proportion of marginal zone (MZ) and follicular mature (FM) B cells in the spleen for each of the indicated mouse genotypes was determined using flow cytometric data shown in panel (A). Proportions were calculated as the percentage of FM cells (black bar) or MZ cells (gray bar) among total gated FM and MZ cells (i.e., FM + MZ). Statistically significant differences are indicated for selected groups expressing the 56R transgene.

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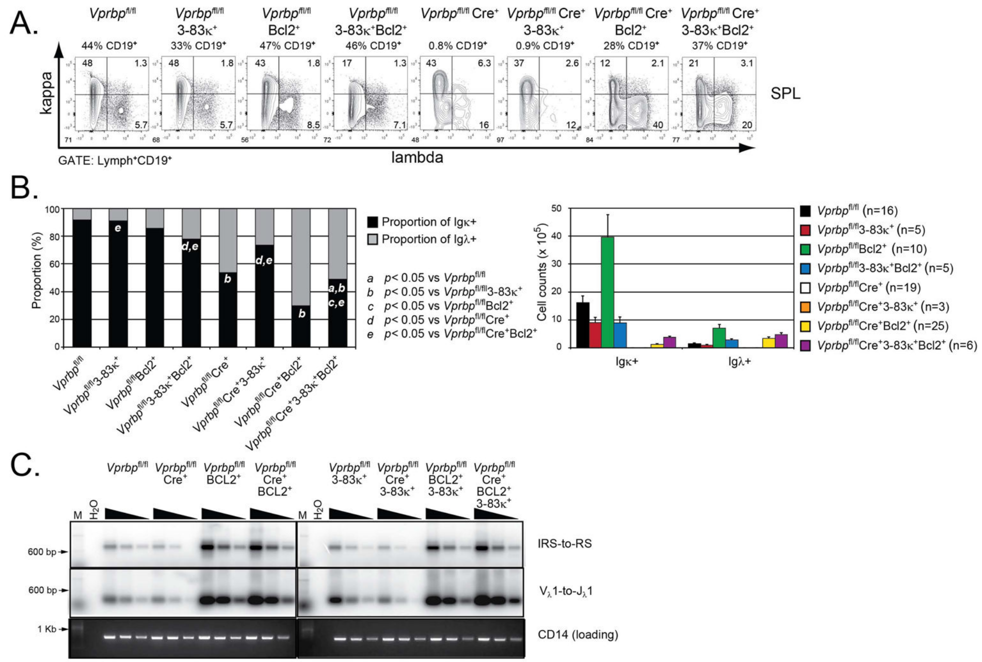

Figure 9.

Enforced 3-83κ light chain expression promotes development of Igκ^{+} B cells in *Vprbp*$^{fl/fl}$ Cre^{+} Bcl2^{+} mice. (A) Splenocytes isolated from *Vprbp* $^{fl/fl}$ mice lacking or carrying the 3-83κ, Cre, and/or Bcl2 transgenes in various combinations were analyzed for the expression of Igκ and Igλ on CD19^{+} lymphocytes using flow cytometry. (B) The proportion and absolute number of CD19^{+} B cells expressing Igκ or Igλ was determined and presented as in Fig. 3B-C. Statistically significant differences are indicated for selected groups expressing the 3-83κ transgene. (C) Genomic DNA prepared from total bone marrow (10000, 2500, or 625 cell equivalents) with the indicated genotypes was subjected to PCR and Southern hybridization to detect IRS-RS and Vλ1-to-Jλ1 rearrangements. Amplification of the non-rearranging CD14 locus was performed as a loading control.

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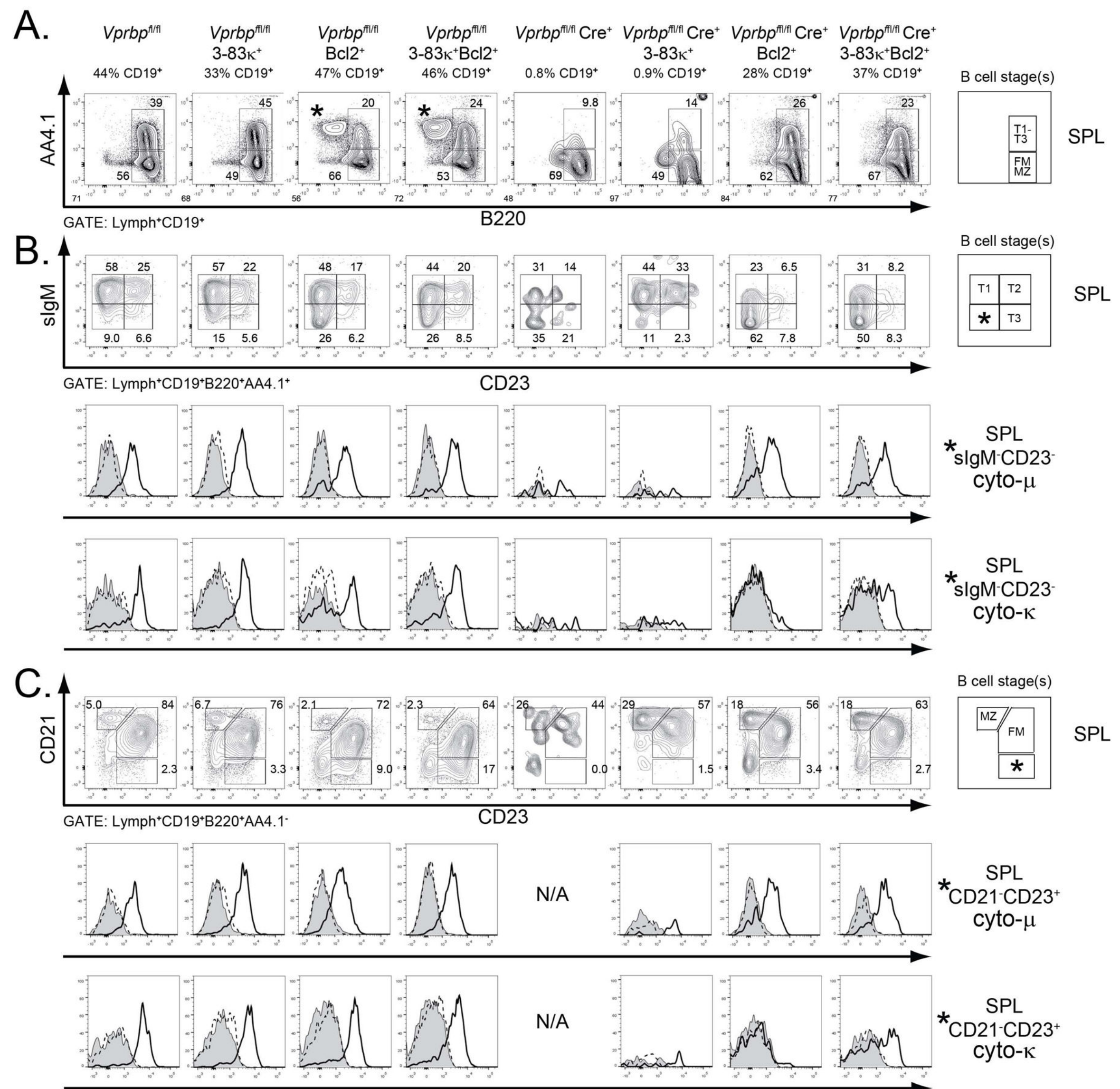

Figure 10.

Enforced 3-83κ light chain expression promotes development of Igκ+ B cells in *Vprbp*$^{fl/fl}$ Cre+ Bcl2+ mice. (A-C) Flow cytometry was used to identify various splenic transitional (panels A and B) and mature (panels A and C) B cell subsets as in Fig. 2A; the absolute numbers of cells in each subset are summarized in Supplemental Fig. 2C and Supplemental Table 2. A splenic pro-B cell-like population detected in *Vprbp*$^{fl/fl}$ Bcl2+ mice (identified with an asterisk) is notably present in *Vprbp*$^{fl/fl}$ 3-83κ+Bcl2+ mice. Conventional and non-conventional splenic transitional (panel B) and mature (panel C) B cell subsets were analyzed for cytoplasmic mu heavy chain (cyto-μ) or kappa light chain (cyto-κ) using flow cytometry as in Fig. 5B.

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Table 1

Primer and probes sequences used for PCR-Southern blot analysis of V(D)J rearrangements

Designation	Sequence 5'-3'	Reference
Normalization		
5'CD14L	GCTCAAACTTTCAGAATCTACCGAC	(33)
5'CD14R	AGTCAGTTCGTGGAGGCCGGAAATC	(33)
IgH		
5' DHL deg	GGAATTCGMTTTTTGTSAAGGGATCTACTACTGTG	(14)
5' VHJ558	CGAGCTCTCCARCACAGCCTWCATGCARCTCARC	(14)
5' VHQ52	CGGTACCAGACTGARCATCASCAAGGACAAYTCC	(14)
5' VH7183	CGGTACCAAGAASAMCCTGTWCCTGCAAATGASC	(14)
3' JH4	GGGGAATTCCTGAGGAGACGGTGACT	(34)
JH4 Probe	ACCCCAGTAGTCCATAGCATAGTAAT	(34)
Igκ		
5'Vκ21Proximal	YBWGCTSACYCARTCTCCWRC	(35)
5'Vκ1Distal	CARACTCCACTCTCCCTGCC	(35)
5'IRS (κ deletion)	CTGACTGCAGGTAGCGTGGTCTTCTAG	(36)
3'RS (κ deletion)	CTCAAATCTGAGCTCAACTGC	(12)
3'Jκ5	CCAAGCTTGTACTTACGTTTCAGCT	(37)
Jκ5 Probe	GCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGTAAGTAC	(38)
IRS-RS Probe	CTAGTGGCAGCCCAGGGTGGATCTCCCTAGGACTGCAGTTGAGCTC	This study
Igλ		
5'VλR1	ATGAATTCACTGGTCTAATAGGTGGTACCA	(39)
3'JλR1	TAGAATTCACTYACCTAGGACAG	(39)
VλR Probe	CTGTGCTCTATGGTACAGCACCC	(39)
5'Vλx	GAGCTTAAGAAAGATGGAAGCCA	(40)
3'VλxR2	GTTCCACCGCCGAAAACATA	(41)
Vλx Probe	TGCTGATCGCTACCTTAGCATTTCCAACATCCAGCCTGAAGATGAAGCAATATACATCTG	This study