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The Adaptor Protein Sh2d3c Is Critical for Marginal Zone B Cell Development and Function

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Sh2d3c is an adaptor protein that has been implicated in T cell activation and shown to associate with different components of the integrin signaling pathway ex vivo. However, the in vivo significance of Sh2d3c expression in the regulation of the immune response and/or hematopoietic cell lineage development is not known. In this study, we show that expression of Sh2d3c is more critical for development and function of marginal zone B (MZB) cells than for T cell maturation. Mice deficient in Sh2d3c expression (Sh2d3c−/−) had a reduced number of MZB cells, and the residual MZB cells failed to properly capture polysaccharide Ags. Activation-induced proliferation, cytokine production, and migration of Sh2d3c−/− splenic B cells were also significantly reduced in vitro compared with wild-type (Sh2d3c+/+) cells. In contrast, T cell development and function were largely normal in Sh2d3c−/− mice. The thymi of Sh2d3c−/− mice showed no maturational abnormalities, the number of splenic T cells was only modestly reduced, and the T cells responded normally to in vitro polyclonal activation. The observed B cell deficiency in the Sh2d3c−/− mice led to diminished humoral immune response against thymus-independent type 2, but not thymus-dependent Ags, which highlights the primary role in vivo of Sh2d3c in regulating B cell development and function. The Journal of Immunology, 2010, 185: 327–334.

Materials and Methods

Generation of Sh2d3c−/− mice

The Sh2d3c−/− line was generated by homologous recombination as part of Lexicon and Genentech’s collaboration to knock out and analyze the function of 500 secreted and transmembrane proteins. The targeting vector

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Abbreviations used in this paper: BM, bone marrow; Cas, Crk-associated substrate; ES cell, embryonic stem cell; FOB, follicular B; KO, knockout; MFI, mean fluorescence intensity; MZB, marginal zone B; NSP, novel Src homology 2-containing protein; SDF, stromal cell-derived factor; TI-2, thymus-independent type 2; TNP, trinitrophenyl; WT, wild type.
was derived using the λ KOS system (21). The λ KOS phage library was screened by a PCR primer pair overlapping exon 4 of the murine Sh2d3c gene (GenBank accession number NM-013781 [www.ncbi.nlm.nih.gov/Genbank]). Sh2d3c+1 [5'-CTT ACT TGG CCT TGT AAG AAC C] and Sh2d3c-2 [5'-GCC AGC TGA GCA GCA ATG CCC]). One genomic clone, pKOS-82, was isolated from the library and confirmed by sequence analysis. To generate the target vector, first gene-specific arms (5'-GACC AAT CTC GCT CTC CCC GTG CAG TTC TCC AAG GA-3') and 5'-AAA CAG C) and Sh2d3c arm of homology using PCR primers (Lex-1) ES cells. G418/FIAU-resistant ES cell clones were isolated, and vector was linearized by NotI digest and electroporated into 129/SvEvBrd embryonic stem (ES) cell selection cassette containing lacZ, the targeting selectable marker were isolated. After replacing the yeast cassette with an appropriate cassette containing the URA3 gene (GenBank accession number NM-013781 [www.ncbi.nlm.nih.gov/Genbank]), 357-bp 5'-CCC CTA GTG GAC and 5'-3' 9'-TAG GCT GGT GAG TGA GTC TCG G-3') were added by PCR to a yeast selection cassette containing the URA3 marker. The yeast selection cassette and pKOS-82 were cotransformed into yeast, and clones that had undergone homologous recombination to replace the 2395-bp genomic region containing exons 4–6 were selected. After replacing the yeast cassette with an embryonic stem (ES) cell selection cassette containing lacZ, the targeting vector was linearized by NotI digest and electroporated into 129/SvEvBrd (Lex-1) ES cells. G418/FIAU-resistant ES cell clones were isolated, and correctly targeted clones were identified and confirmed by Southern blot analysis. The 330-bp 5' external probe was generated outside the pKOS-82 arm of homology using PCR primers Sh2d3c-27 (5'-CCC CTA GTG GAC AAC AAG C) and Sh2d3c-28 (5'-CCC ATA GGT GTG C AT GTT AAA). A 357-bp 5' internal probe was generated using PCR primers Sh2d3c-22 (5'-AGA TTT CTG GCC ATT GAC TCT AGT) and Sh2d3c-23 (5'-TAG GCC AGC TCA GTA ATG CT). Southern blot analysis using the 27 + 28 3' probe detected a 14-kb wild-type (WT) band and a 5.3-kb targeted band in SphI-digested genomic DNA, whereas the 22 + 23 5' probe detected a 14-kb wild-type band and a 5.3-kb targeted band in SphI-digested genomic DNA. Three targeted ES cell clones were microinjected into C57BL/6 (albino) blastocysts. Resulting chimeras were mated to C57BL/6 (albino) females to generate mice that were heterozygous (Sh2d3c+/-) for the exon 4-6 deletion mutation. Sh2d3c+/- mice were intercrossed, yielding Sh2d3c+/- mice.

The animals were maintained at the American Association for the Accreditation of Laboratory Animal Care-accredited animal facility at Lexicon Pharmaceuticals. All experiments were carried out on 6- to 16-wk-old mice of mixed genetic background (129/SvEvBrd and C57BL/6J) representing both sexes of littermate Sh2d3c+/- and Sh2d3c+/- animals. Procedures involving animals were conducted in conformity with Lexicon’s Institutional Animal Care and Use Committee guidelines that are in compliance with the state and federal laws and the standards outlined in the Guide for the Care and Use of Laboratory Animals.

Expression analysis of Sh2d3c by RT-PCR

To confirm disruption of the Sh2d3c gene in the knockout (KO) mice, total RNA were collected from lung, spleen, and thymus from three WT and three homozygous mice using a bead homogenizer (BioSpec Products, Bartlesville, OK) and TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions. Reverse transcription was performed to produce cDNA using 5 μg total RNA with SuperScript II (Invitrogen) and random hexamer primers, according to the manufacturer’s instructions. PCR amplification was performed using 1 μl of the RT product at initial denaturing step of 95°C for 2.5 min, followed by 35 cycles of 95°C (30 s), 59°C, (30 s), and 70°C (1 min) with oligonucleotide primers complementary to exons 4 and 5 of Sh2d3c (5'AGG AAG TGA AGC TCA GTA TTA ATG CT and 5'-TAG GCT GGT GAG TGA GTC TCG G-3'). Primers to the mouse β actin gene (GenBank accession number M12481 [www.ncbi.nlm.nih.gov/Genbank]) 5'-GCG TGG CCG GGA CCT GCA GTA CCT CAT-C and 5'-GCC TAG AAG CAT TGC CGG TGC ACC ATG GAG-3') were also used as internal control for sample handling.

Immunizations, Ig, and trinitrophenyl measurements

Basal, anti-trinitrophenyl (TNP), and anti-OVA Ig concentrations in serum were measured by ELISA using Abs from Southern Biotechnology Associates (Birmingham, AL). Mice were immunized by i.p. injection of 100 μg TNP-Ficoll, TNP-LPS, or OVA in CFA, and Ag-specific Abs were captured on plates coated with TNP or OVA (BD Biosciences, San Jose, CA). TNP retention on MZB cells was assessed, as described previously (22),
with the exception of using PE-labeled streptavidin (BD Biosciences) for detection.

Immunofluorescent histochemistry
Spleens were rapidly frozen in Tissue-Tek OCT compound (Sakura Fine-technical, Torrance, CA) and sectioned. Sections were stained with Abs to Moma-1 (biotinylated; Abcam, Cambridge, MA), MARCO (unconjugated; AbD Serotec, Raleigh, NC), B220 (PE), IgM (FITC), or IgD (PE), and with secondary anti-rat Abs or streptavidin (FITC; all from BD Biosciences), as required. Slides were examined using Axiovert inverted fluorescent microscope (Zeiss, Thornwood, NY).

BM transplantation
Recipient mice were irradiated, as described before (23), with the following exception: 2 \times 10^6 donor BM cells were injected 8 h after the second dose of irradiation. The average percentage of Neo\(^+\) cells derived from donor Sh2d3c\(^{+/+}\) BM was 93% in the reconstituted animals.

FACS analysis
These procedures were performed, as described previously (23); all Abs were from BD Biosciences. Indicated B cell subsets were sorted to 99% purity by using a FACSAria (BD Immunocytometry Systems, San Jose, CA).

Cell migration, proliferation, and cytokine production assays
Migration of splenocyte subsets was assessed after a 4-h incubation in 5-\(\mu\)m pore-size 96-well plates (NeuroProbe, Gaithersburg, MD), with 1 ng/ml murine stromal cell-derived factor (SDF)-1\(\alpha\) or 100 ng/ml CXCL13 (PeproTech, Rocky Hill, NJ) in the bottom chamber. Cell proliferation was assessed, as described before (24), using mAbs to CD3 and CD28 at equal concentrations (as indicated), LPS (1 \(\mu\)g/ml), \(\alpha\)IgM mAb (5 \(\mu\)g/ml), or a mixture of CD40 mAb and IL-4 (2.5 \(\mu\)g/ml and 5 ng/ml, respectively). B cell subsets were purified with an enrichment kit (StemCell Technologies, Vancouver, BC, Canada). The concentration of cytokines in cell culture supernatants was measured 48 h after activation using CBA mouse inflammation kits (BD Biosciences).

Ca\(^{2+}\) mobilization assay
Measurements were made by using a Fluo-4 NW Ca\(^{2+}\) assay kit (Invitrogen). B cell-enriched splenocytes (1 \times 10^7) were activated with 10 \(\mu\)g/ml \(\alpha\)IgM mAb, and maximal fluorescence was assessed by addition of 100 ng/ml ionomycin. Basal fluorescence measured at the first time point was subtracted from each subsequent measurement.

Western blot analysis
Enriched B cells from spleen (4 \times 10^7 cells/ml) were stimulated with 10 \(\mu\)g/ml LPS, and Western blotting of lysed samples was performed using polyclonal Abs to Pyk2 and phosphorylated Pyk2 (Cell Signaling Technology, Beverly, MA), HRP-conjugated goat anti-rabbit Ab (Bethyl Laboratories, Montgomery, TX), and ECL detection system (GE Life Sciences, Pittsburgh, PA). Spleen lysates from Sh2d3c\(^{+/+}\) and Sh2d3c\(^{-/-}\) mice were tested by anti-Sh2d3c (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-GAPDH (Bethyl Laboratories) Abs.

Statistical analyses
Statistical significance of group differences was evaluated by unpaired, two-tailed, Student t test. A p value of <0.05 was considered significant.
Results

**Sh2d3c**−/− mice do not show gross abnormalities

We generated **Sh2d3c**−/− mice by targeted disruption of exons 4–6 of the murine homolog of the **Sh2d3c** gene (GenBank accession number NM_013781 [www.ncbi.nlm.nih.gov/Genbank/]) by homologous recombination in ES cells (21), followed by germline transmission of the mutant gene into chimeric, **Sh2d3c**+/−, and **Sh2d3c**−/− mice (Fig. 1A). Successful targeting of the **Sh2d3c** gene in the KO mice was confirmed by Southern hybridization analysis of ES cell (Fig. 1B), expression analysis of the gene transcript (Fig. 1C), and Western blot analysis of the **Sh2d3c** protein (Fig. 1D). Mating of **Sh2d3c**+/− mice generated pups of the three possible genotypes with ratios that fit well with normal Mendelian frequencies. **Sh2d3c**−/− mice exhibited no substantial difference in growth rate and size. Likewise, comprehensive clinical diagnostic and pathologic analysis (described in detail in Ref. 25), including tests of inflammation, behavior, obesity, diabetes, bone, cell proliferation, and cardiovascular function, did not demonstrate significant differences in **Sh2d3c**−/− mice relative to their WT littermates (data not shown).

Absence of **Sh2d3c** leads to impaired MZB cell development

Hematologic and FACS analysis of lymphoid organs revealed decreased cellularity and markedly reduced representation of B220+**CD21**^high**CD23**^med/low MZB cells in the spleen of **Sh2d3c**−/− mice compared with WT littermates (Fig. 2). A decrease in the proportion of the **IgM**^high**CD21**^high T2/MZB cell population, which includes MZ precursor cells in addition to MZB cells in the spleen, was also readily apparent in the **Sh2d3c**−/− mice. Although the absolute number of FOB cells also appeared to be reduced, the difference did not reach statistical significance. Furthermore, no differences were observed in the immature T1 or mature B cell populations (Fig. 2B). Immunohistological examination of the spleen showed that the region of **IgM**^high**IgD**low MZB cells that form a rim around the follicles was greatly diminished in the absence of **Sh2d3c** (Fig. 2C), which correlates with the flow cytometric analysis data. In contrast, the resident metallocophilic macrophages that are important components of the marginal zone (26), identified by the marker Moma-1, were correctly located at the border between the marginal and follicular zones in both WT and mutant mice (Fig. 2C). Similarly, the distinct MARCO+ marginal zone macrophages that are required for retaining MZB cells (27) were also detected at comparable levels in WT and **Sh2d3c**−/− deficient spleens (Fig. 2C). Of note, the blood, BM, and lymph nodes of **Sh2d3c**−/− mice did not demonstrate obvious B cell deficiencies, and the peritoneal cavity also contained normal number of cells of the B1 B cell phenotype (data not shown).
findings indicate that Sh2d3c is critical for MZB cell development, but less so for the homeostasis of other B cell subsets. Transplantation of BM cells from Sh2d3c<sup>−/−</sup> mice to irradiated WT recipients recapitulated the MZB cell phenotype of the complete KOs, with the additional observation that the KO chimeras showed a modest reduction in the fraction of B220<sup>+</sup> cells in the BM (Supplemental Fig. 1). These findings demonstrate that the defect in MZB cell formation in Sh2d3c-deficient mice is intrinsic to hematopoietic cells.

Sh2d3c<sup>−/−</sup> mice exhibit deficient humoral immune response to TI-2 Ags

MZB cells have been associated with the TI-2 Ab response (18); therefore, we immunized Sh2d3c<sup>−/−</sup> and Sh2d3c<sup>+/+</sup> mice with TI-2 Ag TNP-Ficoll and measured the levels of TNP-specific Abs in the serum 7, 14, and 28 d after immunization. The basal serum concentrations of Ig subclasses were similar in unchallenged Sh2d3c<sup>−/−</sup> and Sh2d3c<sup>+/+</sup> cells (Fig. 3A). On days 7 and 14 after immunization with TNP-Ficoll, there was a clear trend of reduced TNP-specific Ig in Sh2d3c<sup>−/−</sup> mice that was statistically significant for IgG1, IgG2b, and IgG3 on day 7 (Fig. 3B). By day 28 after immunization, the levels of TNP-specific IgM, IgG1, IgG2b, and IgG3 Abs all were significantly lower in Sh2d3c<sup>−/−</sup> mice compared with their Sh2d3c<sup>+/+</sup> littermates. The Ag-specific IgG2a response was also reduced in the KO mice, but the decrease did not achieve statistical significance. Importantly, the humoral response to OVA, a thymus-dependent Ag, and to TNP-LPS, a thymus-independent type 1 Ag, showed no significant differences at either...
time points (Supplemental Fig. 2), which demonstrates the TI-2-specific nature of the immunodeficiency in the Sh2d3c−/− mice.

**The Ag-retention capacity of MZB cells is decreased in the absence of Sh2d3c**

Shortly after injection, TI-2 polysaccharide Ags are taken up by MZ macrophages and B cells, and retained on the cell surface to facilitate the generation of a robust Ab response (22). To compare the capacity of Sh2d3c−/− and WT MZB cells to retain Ag in vivo, mice were injected with TNP-Ficoll i.v., and their splenocytes were examined for the presence of TNP on the cell surface. Staining with anti-TNP Ab indicated that MZB cells in Sh2d3c−/− mice were less efficient at retaining the Ag than their WT counterparts (Fig. 3C). Furthermore, immunofluorescent histochemistry revealed that in WT spleens TNP-Ficoll was localized exclusively in the marginal zone area (Fig. 3D), whereas Sh2d3c−/− spleens showed weak and thin rim of positive staining that sometimes appeared to be in a dotted pattern, most likely reflecting the TNP-Ficoll bound to MZ macrophages.

**Sh2d3c expression is essential for the robust LPS response of MZB cells**

We explored the response of B cells from Sh2d3c−/− and Sh2d3c+/+ mice to in vitro stimulation by using a variety of polyclonal stimuli, including LPS. When treated with LPS, cell proliferation (Fig. 4A) as well as the production of the inflammatory cytokine IL-6 (Fig. 4B) were greatly reduced in splenic B cell cultures of Sh2d3c-deficient mice compared with WT littermates. In contrast, no differences were observed in the proliferation rate when the cells were stimulated with a mixture of CD40 mAb and IL-4 or with anti-IgM mAb (Fig. 4A). We also examined whether the absence of Sh2d3c would affect early signaling events, such as calcium mobilization via the BCR. Enriched B cells from Sh2d3c+/+ and Sh2d3c−/− mice did not differ in their kinetic response to anti-IgM–elicited calcium mobilization (Supplemental Fig. 3).

To test whether MZB cells were responsible for the observed deficiency in the LPS response of Sh2d3c−/− B cells, we repeated the LPS activation study with purified MZB and FOB cells. Consistent with previous reports (28), WT MZB cells proliferated more to LPS than WT FOB cells. However, Sh2d3c-deficient MZB cells proliferated poorly after LPS activation compared with WT MZB cells. The cell proliferation defect was restricted to MZB cells because purified FOB cells from Sh2d3c−/− and Sh2d3c+/+ mice proliferated similarly when activated with LPS (Fig. 4C). Of note, the deficient LPS response of Sh2d3c−/− MZB cells was not due to differences in TLR4 expression, as confirmed by FACS analysis (data not shown).

Sh2d3c−/− B cells are refractory to CXCL13-induced chemotactic response in vitro

Proper migratory response of B cell precursors is a prerequisite for normal MZB cell development (14, 18). Furthermore, Sh2d3c has been implicated in cell migration in COS cells (5). Therefore, we examined the ability of Sh2d3c−/− splenocytes to migrate in response to the chemokines SDF-1α and CXCL13 in vitro. SDF-1α is produced in the red pulp of the spleen and is involved in migration of B cells (29, 30), whereas CXCL13 is required for the localization of B cells in the spleen (31, 32). The motility of Sh2d3c-deficient splenocytes to both SDF-1α and CXCL13 was significantly reduced when compared with WT cells (Fig. 5). FACS analysis revealed that the deficiency in the migratory response was not limited to MZB cells. Both FOB and MZB cells were largely refractory to the CXCL13-induced migratory signal in the absence of Sh2d3c. In contrast, only Sh2d3c−/− FOB cells appeared to show deficient migratory response to SDF-1α, although the response of MZB cells was generally poor to this chemokine irrespective of Sh2d3c expression (Fig. 5). The surface density of CXCR4 and CXCR5, the receptors for SDF-1α and CXCL13, respectively, was comparable between WT and Sh2d3c-deficient cells (data not shown), ruling out a role for Sh2d3c in regulating the expression of these receptors.

**Absence of Sh2d3c alters the kinetics of Pyk2 phosphorylation**

Pyk2 is a signaling component of the integrin pathway that is involved in regulating cell morphology and cell migration to chemotactic signals (33). Deficiency in Pyk2 has been shown to result in a diminished number of MZB cells (22). Because Sh2d3c reportedly interacts with Pyk2 (6), it seemed plausible that the defect in MZB cell development in Sh2d3c−/− mice might be associated with an alteration in Pyk2 signaling. To test this hypothesis, and to provide a validation in primary B cells for the reported links Sh2d3c and Pyk2 (6), we examined the phosphorylation of Pyk2 in B cell-enriched splenocytes of Sh2d3c−/− and Sh2d3c+/+ mice (Fig. 6). In WT B cells, LPS induced a time-dependent phosphorylation of Pyk2 that was observed as early as 1 min after activation and was maintained throughout the test period (60 min). In contrast, a weaker phospho-Pyk2 signal was detected in Sh2d3c−/− B cells, which was apparent only 5 min after LPS stimulation and continued to strengthen over the entire 60-min time course.

**Sh2d3c is dispensable for normal T cell development**

Previous reports have shown that overexpression of Sh2d3c increases TCR-induced IL-2 production in Jurkat T cells (6), thus implicating this adaptor protein in T cell function. However, the distribution of the major thymocyte subpopulations was normal in the Sh2d3c−/− mice (Fig. 7), and there were no substantial differences in the naive and memory T cell numbers in the blood or distribution of the same cell subsets in the spleen of Sh2d3c−/− versus Sh2d3c+/+ mice (data not shown). Furthermore, despite a modest reduction in numbers (Fig. 7), splenic T cells from
Sh2d3c<sup>-/-</sup> mice proliferated normally to activation via the CD3/CD28 pathway (Fig. 7) and produced similar levels of IL-2 (data not shown). Together, our data suggest that Sh2d3c plays a non-critical role in T cell development or function.

Discussion
Comprehensive physiological analysis of Sh2d3c-deficient mice demonstrated that Sh2d3c has an essential and relatively specific role in MZB cell development. The residual MZB cells in the KO mice also had impaired function, as demonstrated by decreased ability to retain and get stimulated by polysaccharide Ags, and to respond to CXCL13-mediated chemotactic signals.

The pathway leading to the generation of MZB cells proceeds through a series of checkpoints and is regulated by multiple factors, including BCR signal strength, Notch signaling, and localization of precursors (reviewed in Refs. 14, 18, 34). For example, weak BCR signaling favors MZB cell formation (35, 36), whereas strong BCR signals promote the generation of FOB cells while curtailing MZB cell development (36–38). Our data indicate that neither the early steps (Ca<sup>2+</sup> mobilization) nor the later stages (e.g., proliferation) of BCR signaling via anti-IgM are dependent on Sh2d3c expression, arguing against defective BCR signaling as the primary contributor to the MZB cell deficiency in the KO mice. Furthermore, the macrophage populations that are known to play a role in generating MZB cells (26, 27) are unlikely to be responsible for the defect observed in Sh2d3c<sup>-/-</sup> mice because they appeared to have normal distribution in the marginal zone area of the spleen.

The deficiency of MZB cells in Sh2d3c<sup>-/-</sup> mice is more likely related to the role of Sh2d3c in regulating cell migration (10). The importance of motility in the generation of MZB cells was demonstrated by the observation that treatment of WT mice with antibody to VLA-4, which are also hallmarks of the B cell responses, and defective migration of splenic B cells (22, 40), reduced MZB cell formation, significantly blunted TI-2 immune responses, and impaired function, as demonstrated by decreased ability to retain and get stimulated by polysaccharide Ags, and to respond to CXCL13-mediated chemotactic signals.

Recently, this proposed mechanism. Each of these three KO lines showed reduced MZB cell formation, significantly blunted TI-2 immune responses, and defective migration of splenic B cells (22, 40), which are also hallmarks of the Sh2d3c<sup>-/-</sup> mice phenotype. Furthermore, overexpression of Sh2d3c enhanced the phosphorylation of Pyk2. It is important to note that the surface density of integrin receptors CD11a, CD18, CD49d, and CD29, which are involved in regulating B cell homing (40), was normal on Sh2d3c<sup>-/-</sup> splenocyte subsets (unpublished results), which rules out integrin expression as a primary mechanism of the deficient MZB cell generation in Sh2d3c<sup>-/-</sup> mice.

Earlier work indicated that in Jurkat cells Sh2d3c was phosphorylated upon TCR engagement, and that overexpression of Sh2d3c increased JNK activation and boosted IL-2 production (6). Moreover, Cas-L (41) and Pyk2 (33, 42, 43), which associate with Sh2d3c, were also implicated in T cell activation and IL-2 production. Although there was a modest decrease in the number of splenic T cells in Sh2d3c<sup>-/-</sup> mice, associated with a general reduction in the cellularity of this organ, T cell subpopulations in the thymus and blood of Sh2d3c<sup>-/-</sup> mice did not show significant changes and splenic T cells responded normally to TCR-mediated activation in vitro. Together, our data suggest that Sh2d3c does not play a critical role in T cell development or function. It is possible that the role of Sh2d3c in T lineage cells is redundant and alternative pathways are in place to compensate for its absence, or that the signaling mechanism has been affected in cell lines, such as Jurkat.

In conclusion, our results reveal a nonredundant role for Sh2d3c in MZB cell development and function. We propose that Sh2d3c is required for proper localization of cells, particularly MZB cells in vivo, and support the concept that Sh2d3c is a component of a signaling pathway that also includes Pyk2 and Cas-L. Although our data show a clear and relatively selective role for Sh2d3c in MZB cell formation, the exact role of Sh2d3c in downstream signaling pathways of integrins or chemokine receptors is yet to be determined. The decrease in polysaccharide binding on MZB cells in Sh2d3c-deficient mice suggests a reduced function for complement receptors on these cells, but the underlying causes are not yet clear. Finally, in light of the apparent absence of a nonimmunological phenotype in Sh2d3c null mice, the significance of alternative splicing of Sh2d3c RNA and the relatively ubiquitous expression of one of the described Sh2d3c isoforms remains to be defined.

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References


