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Synergy between NF-κB1/p50 and Notch2 during the Development of Marginal Zone B Lymphocytes

Stewart T. Moran, Annaiah Cariappa, Haoyuan Liu, Beth Muir, Dennis Sgroi, Cristian Boboila, and Shiv Pillai

NF-κB1 and Notch2 are both required for the development of marginal zone (MZ) B cells. Analysis of B lymphocyte development in mice that are doubly heterozygous at the Notch2 and NF-κB1 loci revealed synergy between Notch2 and NF-κB1 during MZ B cell development. Two known transcriptional targets of the Notch pathway, Hes-5 and Deltex-1, were found to be preferentially expressed in MZ B cells and regulated by NF-κB1. These studies provide in vivo evidence for a genetic interaction between the Notch and NF-κB pathways. *The Journal of Immunology*, 2007, 179: 195–200.

The Notch and NF-κB protein families represent two major evolutionarily conserved pathways that affect cell fate and cell survival in all known metazoans, but genetic interactions between these pathways have not been hitherto described. Notch proteins are cell surface molecules that influence binary cell fate decisions in all multicellular organisms. The activation of a Notch receptor results in the proteolytic liberation of its intracellular domain, which is then translocated to the nucleus. The cleaved intracellular domain of Notch forms ternary complexes with recombination signal binding protein-Jκ (RBP-Jκ) and mastermind-like proteins, resulting in the conversion of RBP-Jκ from a transcriptional repressor to an activator of transcription that ultimately mediates the induction of a set of target genes (reviewed in Ref. 1).

The NF-κB pathway, like the Notch pathway, also influences cell fate decisions in metazoans. It is also relevant in terms of the induction of gene expression in a number of other biological contexts, including inflammation and cancer. There are five distinct NF-κB proteins in vertebrates and these exist as homodimers or heterodimers that are retained in the cytosol by IκB proteins before activation. Following the ubiquitination and proteasomal degradation of IκB, NF-κB dimers are released and enter the nucleus. IκB is marked for ubiquitination following its phosphorylation by the IκB kinase (IKK) complex, which is comprised of two catalytic subunits, IKKα/IKK-1 and IKKβ/IKK-2, and a regulatory subunit, IKKγ/NEMO (NF-κB essential modulator). Two of the five members of the NF-κB family, NF-κB1/p50 and NF-κB2/p52, possess DNA-binding Rel-homology domains, but lack a transactivation domain. The other three members, p65/RelA, RelB, and c-Rel, all possess DNA binding as well as transactivation domains (2). As a result, NF-κB activates the transcription of target genes as a heterodimer, generally with p65 or c-Rel, and less frequently with RelB. NF-κB p50 homodimers lack the ability to activate transcription and may recruit histone deacetylases resulting in transcriptional repression (3).

Both Notch and NF-κB are key players in lymphocyte development. Although Notch1 is a crucial mediator of the T vs B lymphoid cell fate decision (4, 5), Notch2 is required at the mature follicular (FO) vs marginal zone (MZ) B lymphoid cell fate transition. MZ B cells are lost but FO B cells are preserved in mice in which Notch2 or RBP-Jκ are conditionally deleted in the B lineage (6, 7). In addition, the deletion of the gene that encodes Delta-like-1, a ligand for Notch proteins, or the overexpression of a dominant-negative mutant of mastermind-like-1, also results in the absence of MZ B cells (8, 9), while the loss of MINT (Msx2 interacting nuclear target protein), a negative regulator of the Notch pathway, contributes to an increase in MZ B cells (10). Signaling via Notch2 is therefore required for the development of MZ B cells. The role of Notch2 during peripheral B cell development may not be restricted to the generation or maintenance of MZ B cells. Although conditional Notch2–/− mice exhibited no defect in peritoneal B-1 B cells (6), examination of Notch2+/− B cells in another study implicated Notch2 in peritoneal B-1 B cell development (11).

Mice that lack NF-κB1 have a marked reduction of MZ B cells (12). A less prominent reduction in MZ B cell numbers was noted in Rag-2−/− mice that were reconstituted with p65−/− hemopoietic stem cells, or in mice lacking c-Rel (12). These data suggest that while NF-κB1/p65 and NF-κB1/c-Rel both contribute to MZ B cell development, the requirement for NF-κB1 as a component of these heterodimers is stringent. Alternatively, it remains formally possible that NF-κB1 homodimers are required to repress crucial targets to permit MZ B cell differentiation. Regardless of whether NF-κB1 functions as a transcription activator or a repressor during MZ B cell development, it is also possible that it is required for MZ B cell generation because it functions as a negative regulator of the BCR/Bruton’s tyrosine kinase (Btk) pathway. The loss of negative regulators of BCR signaling, such as Aiolos and CD22, can contribute to a block in MZ B cell development (13, 14).
Many other genes contribute to the generation or maintenance of MZ B cells, some at the level of cell survival and others in maintaining MZ B cells in their niche (14). Signaling via the B cell-activating factor of the TNF family (BAFF) receptor (BAFF-R) may drive MZ development as suggested by the expansion of MZ B cells in BAFF-transgenic mice (15); BAFF may contribute to the activation of the canonical NF-κB pathway and thus contribute not just to MZ B cell development but possibly to maintenance as well (16). Other proteins that are believed to influence MZ B cell survival include CD19 (17) and PI3Kp110 (18, 19). The LFA-1 pathways interact.

FIGURE 1. The loss of MZ B cells in NF-κB1/p50 mutant mice is not a result of enhanced Btk-derived signals. MZ B cells (IgM<sup>high</sup>IgD<sup>low</sup>CD21<sup>high</sup>) were analyzed from NF-κB1/p50<sup>−/−</sup>, NF-κB1/p50<sup>−/+</sup>, and NF-κB1/p50<sup>−/−</sup>/xid mice. Numbers indicate the percentage of cells that are of a MZ B cell phenotype in each gate. The table below shows the absolute numbers of MZ B cells in each mouse strain. Three mice were analyzed in each group. Values of p for differences between groups were determined by the Mann-Whitney U test.

TagMan RT-PCR analysis

Splenocytes from C57BL/6 and p50 mutant mice were stained as described above with anti-IgM, IgD, and CD21 Abs; IgM<sup>high</sup>IgD<sup>low</sup>/CD21<sup>high</sup> MZ B cells, IgM<sup>low</sup>IgD<sup>high</sup>/CD21<sup>low</sup> FO type I (FO-I), and IgM<sup>low</sup>IgD<sup>high</sup>/CD21<sup>low</sup> FO type II (FO-II) were sorted. Total RNA was isolated from each sorted fraction by the Absolutely RNA Microprep kit (Stratagene) and converted into cDNA. TaqMan quantitative RT-PCR and primer design was performed as previously described (32). Primers used were: Deltx1 forward TGTTCCGGCTATACACCCGACA, reverse CCACCCGCACCTTTCA AG; TaqMan probe CTTAGGAGCGCAACAGGCCTAAT. Hex5 forward CTGATGCCGCTACAG, reverse CATGACACCCCATACAA; TaqMan probe TCTCCAGGTGACCTTAAAGGATT.

Materials and Methods

Mice

p50-null mice were obtained from The Jackson Laboratory and have been described previously (12). Notch2<sup>−/−</sup> mice were provided by Dr. Y Hamada (Institute for Basic Biology, Okazaki Japan). C57BL/6 mice and xid/(CBA/N) mice were purchased from The Jackson Laboratory. Xid/p50-null double mutants were generated in a manner similar to that described for Aiolos<sup>−/−</sup> mice (13). p50<sup>−/−</sup> mice were mated with Notch2<sup>−/−</sup> mice and Notch2 function synergistically during MZ B cell development as revealed by the analysis of splenic B cell development in mice haploinsufficient for both these genes, but no evidence was found for a genetic interaction between Notch2 and NF-κB1 during B-1 B cell development. These studies further our understanding of an important cell fate decision in lymphocytes and provide the first in vivo evidence that the Notch and NF-κB pathways interact.

Flow cytometric analysis and FACS

Single-cell suspensions from the spleen were obtained using standard methods. Peritoneal B cells were harvested by injecting 10 ml of PBS containing 0.2% BSA into the peritoneal cavity followed by aspiration with an 18-gauge needle. Flow cytometry and flow sorting were performed as previously described (29). The following murine mAb conjugates were used: r-PE (R-PE)-1B4B1 (anti-IgM, rat IgG), and biotinylated-11-26 (anti-IgD, rat IgG) purchased from BD Pharmingen. Biotinylated Abs were revealed using streptavidin-allophycocyanin (BD Pharmingen). Flow cytometric analysis was performed on a dual-laser FCS500 (Beckman Coulter) and sorting was performed on a MoFlo sorter (DakoCytemation). The purity of sorted samples always exceeded 96%. Gates in the spleen were set according to Hardy et al. (30) and Cariappa et al. (13, 31). Processed sample data were analyzed using FloJo version 8.2 software (Tree Star).

NF-κB1/p50 AND Notch2 SYNERGISM IN MZ B CELL DEVELOPMENT
Results

The defect in MZ B cell development in NF-κB1-null mice is not linked to the activation of Btk

Aiolos-null mice present with an enhancement in BCR signal strength and a significant defect in MZ B cell development. In double mutant mice that lack Aiolos and also carry the xid mutation (an inactivating point substitution within the pleckstrin homology domain of Btk), the MZ B cell population is restored, presumably because Btk-dependent BCR signal strength is no longer enhanced (13). Like Aiolos-deficient mice, NF-κB1 mutant mice present with decreased numbers of MZ B cells (12). We used a similar genetic approach to the one used with Aiolos-null mice to determine whether the decreased numbers of MZ B cells in NF-κB1-null mice may be attributed to enhanced signaling via Btk.

We crossed the NF-κB1-null mutation onto the xid (CBA/N) background and, in contrast to the “reappearance” of the IgM<sup>high</sup>IgD<sup>low</sup>CD21<sup>high</sup> MZ B cell fraction observed in xid/Aiolos<sup>−/−</sup> mice (13), xid/NF-κB1<sup>−/−</sup> mice present with a virtually complete absence of MZ B cells (Fig. 1). Although there appears to be an increase in the newly formed (NF/T) B cell population in the double mutant mice (right side panel of Fig. 1), this is an apparent increase that reflects the paucity of MZ B cells. We conclude that the loss of MZ B cells in NF-κB1 mutant mice cannot be attributed to increased Btk-derived signals.

NF-κB1 is required for B-1 B cell development

NF-κB1 is activated downstream of the BAFF-R in developing and mature B cells (16). Although Notch2 may be required for

Table I. Absolute numbers of splenic B cells in wild-type and mutant mice<sup>a</sup>

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Phenotype&lt;sup&gt;b&lt;/sup&gt;</th>
<th>C57BL/6</th>
<th>p50&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>N2&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>p50&lt;sup&gt;−/−&lt;/sup&gt; × N2&lt;sup&gt;−/−&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>NF/T1</td>
<td>IgM&lt;sup&gt;high&lt;/sup&gt;IgD&lt;sup&gt;low&lt;/sup&gt;/CD21&lt;sup&gt;low&lt;/sup&gt;</td>
<td>3.0 (0.058)</td>
<td>3.3 (0.212)</td>
<td>2.4 (0.351)</td>
<td>3.7 (0.173)</td>
</tr>
<tr>
<td>FO-II</td>
<td>IgM&lt;sup&gt;high&lt;/sup&gt;IgD&lt;sup&gt;high&lt;/sup&gt;/CD21&lt;sup&gt;low&lt;/sup&gt;</td>
<td>3.7 (0.472)</td>
<td>7.2 (0.007)</td>
<td>4.8 (0.723)</td>
<td>5.8 (1.168)</td>
</tr>
<tr>
<td>FO-I</td>
<td>IgM&lt;sup&gt;low&lt;/sup&gt;IgD&lt;sup&gt;high&lt;/sup&gt;/CD21&lt;sup&gt;int&lt;/sup&gt;</td>
<td>14.8 (1.266)</td>
<td>14.3 (0.707)</td>
<td>22.9 (6.140)</td>
<td>12.6 (0.916)</td>
</tr>
<tr>
<td>MZP</td>
<td>IgM&lt;sup&gt;high&lt;/sup&gt;IgD&lt;sup&gt;high&lt;/sup&gt;/CD21&lt;sup&gt;high&lt;/sup&gt;</td>
<td>1.8 (0.208)</td>
<td>3.0 (0.212)</td>
<td>1.4 (0.100)</td>
<td>0.7 (0.208)</td>
</tr>
<tr>
<td>MZ</td>
<td>IgM&lt;sup&gt;high&lt;/sup&gt;IgD&lt;sup&gt;low&lt;/sup&gt;/CD21&lt;sup&gt;high&lt;/sup&gt;</td>
<td>4.0 (0.404)</td>
<td>5.5 (0.141)</td>
<td>1.0 (0.251)</td>
<td>0.3 (0.058)</td>
</tr>
</tbody>
</table>

<sup>a</sup> n = 3 mice in each group.
<sup>b</sup> See Refs. 13, 30, and 31 for gating strategy.
<sup>c</sup> Value of p < 0.05 when compared to N2<sup>−/−</sup> or p50<sup>−/−</sup>, or C57BL/6.
B-1 B cell development (11), BAFF knockout mice exhibit normal B-1 B cell development (33). It was of some interest to therefore examine whether NF-κB1-null mice have a defect in B-1 B cell development. As can be seen from Fig. 2, peritoneal IgM<sup>hi</sup>CD5<sup>+</sup> B-1a B cell development is markedly compromised in the absence of NF-κB1. A less striking reduction in peritoneal IgM<sup>hi</sup>Mac-1<sup>+</sup> B-1b B cells was also noted in these mice.

**NF-κB1 and Notch2 synergize during the development of MZ B cells**

NF-κB1-null mice, Notch2 conditionally null mice, as well as Notch2<sup>+/−</sup> mice all present with a significant reduction in MZ B cells (6, 11, 12). We therefore entertained the possibility that Notch2 and NF-κB1 might cooperate to influence MZ B cell development. To test this hypothesis, we analyzed FO and MZ B cell development in NF-κB1<sup>−/−</sup>, Notch2<sup>−/−</sup>, and NF-κB1<sup>−/−</sup>/Notch2<sup>−/−</sup> doubly heterozygous mice.

We have previously suggested that IgM<sup>hi</sup>IgD<sup>hi</sup>CD21<sup>int</sup> B cells may represent the last common precursor of MZ and IgD<sup>hi</sup>IgM<sup>lo</sup> mature FO B cells (14, 34, 35). The majority of these cells are long-lived posttransitional B cells (A. Cariappa, H. Liu, C. Boboila, S. T. Moran, and S. Pillai, submitted for publication). These cells are present in mutants, such as the Aiolos-null and Notch2<sup>−/−</sup>/− mice, that lack MZ B cells, but are also preserved in xid mice and other mutants in the Btk pathway in which IgD<sup>hi</sup>IgM<sup>lo</sup> mature FO B cells fail to develop or are lost. We currently refer to IgM<sup>hi</sup>IgD<sup>hi</sup>CD21<sup>int</sup> B cells as FO-II cells. We distinguish them from IgD<sup>hi</sup>IgM<sup>lo</sup> mature FO B cells, which we refer to as FO-I B cells. Consistent with previous reports, NF-κB1<sup>−/−</sup> mice have no obvious defect in splenic B cell development and Notch2<sup>−/−</sup> mice have a reduction in a presumed MZ precursor (MZP; IgM<sup>hi</sup>IgD<sup>hi</sup>CD21<sup>hi</sup>) population and in MZ (IgM<sup>hi</sup>IgD<sup>lo</sup>CD21<sup>hi</sup>) B cells, while other B cell populations (NF/T1, IgM<sup>hi</sup>IgD<sup>lo</sup>CD21<sup>lo</sup>; FO-II, IgM<sup>hi</sup>IgD<sup>hi</sup>CD21<sup>int</sup>; and IgM<sup>lo</sup>IgD<sup>hi</sup>CD21<sup>int</sup> FO-I B cells) are not reduced (6, 11, 12) (Fig. 3). Strikingly, in NF-κB1<sup>−/−</sup>/Notch2<sup>−/−</sup> doubly heterozygous mice, there is an almost complete absence of MZP and MZ B cells (Fig. 3; see Table I for absolute numbers). These data establish that the NF-κB1 and Notch2 genes interact genetically and function synergistically to promote MZ B cell development.

**FIGURE 4.** NF-κB1 and Notch2 do not cooperate in the generation of peritoneal B1 B cells. Peritoneal B cells from NF-κB1/p50<sup>−/−</sup>, Notch2<sup>−/−</sup>, NF-κB1/p50<sup>−/−</sup>/Notch2<sup>−/−</sup>, and NF-κB1/p50<sup>−/−</sup>/Notch2<sup>−/−</sup> mice were analyzed. Three mice were analyzed in each group.

**FIGURE 5.** Expression of Hes5 and Deltex1, is regulated by NF-κB1. A, The IgM<sup>hi</sup>IgD<sup>hi</sup>CD21<sup>int</sup> FO-II fraction is preserved in p50<sup>−/−</sup> mice when compared with wild-type mice (n = 3 in each group). B, Expression of Hes5 and Deltex1 in wild-type and NF-κB1 mutant FO-II B cells as well as in wild-type MZ and FO-I B cells was analyzed by a real-time PCR approach described in Materials and Methods. Error bars denote 1 SD from the mean; n.s., no signal.
However, in contrast, examination of peritoneal B-1 B cell development in NF-κB1+/−/Notch2+/− and NF-κB1+/−/Notch2+/− mice revealed no evidence of synergism between Notch2 and NF-κB1 during B-1B cell development (Fig. 4).

Hes5 and Deltex1 are regulated by NF-κB1

There are a number of potential ways in which the Notch2 and NF-κB1 transcriptional regulators could potentially synergize during MZ B cell development. One possible mechanism is the cooperative transcriptional regulation of target genes. The Deltex1 (Dtx1) gene is known to be positively regulated by Notch2 and to be expressed at higher levels in MZ B cells than in other B cell populations (6). The hairy/enhancer of split homolog 5 (Hes5) is also expressed most strongly in MZ B cells (6). We tested the hypothesis that these known Notch targets might represent NF-κB1-regulated genes in B cells. We performed quantitative real-time RT-PCR on RNA obtained from wild-type and NF-κB1 mutant FO-II cells (the cell type that represents the presumed last common precursor for both MZ B cells and FO-I B cells) as well as on RNA extracted from wild-type MZ and FO-I B cells. Fig. 5A shows that the IgM+IgD+CD21+ FO-II B cell population is preserved in NF-κB1−/− mice even though the MZP B cell population is reduced. As shown in Fig. 5B, Hes5 and Dtx1 are more highly expressed in wild-type FO-II cells than in NF-κB1-null FO-II cells, suggesting that NF-κB1 is a positive regulator of these genes. In addition, quantitative real-time RT-PCR analysis confirmed that both Hes5 and Dtx1 are more highly expressed in wild-type MZ B cells relative to FO-I B cells. These data suggest that in differentiating B cells, some genes, such as Deltex1, may be dually regulated by both Notch2 and NF-κB1.

Discussion

We have observed a strong genetic interaction between two hemizygous loci. Such collaboration between two distinct heterozygous null alleles has very rarely been observed in studies of mouse development. Although Notch2+/− mice do have a clearly discernible reduction in MZ B cells, Notch2−/−/NF-κB1+/− mice present with a far more striking phenotype. Our genetic analyses clearly reveal synergism between NF-κB1 and Notch2 during MZ B cell development. There are many potential ways in which Notch2 and NF-κB1 may function synergistically. Given that both these proteins are transcriptional regulators, Notch2 could potentially affect the expression of NF-κB1; alternatively, NF-κB1 could potentially regulate the expression of Notch2 or of components of the Notch pathway. Yet another possibility is that target genes could be synergistically regulated by Notch2 and NF-κB1. Our studies suggest that Notch2 and NF-κB1 might cooperate at the level of target gene expression in splenic B cells and this may explain their synergism during the development of MZ B cells. Cooperative interactions between the Notch and NF-κB pathways have not been described in any other developmental context in metazoans.

As has been reported earlier (6, 11), we observed an increase in FO-I B cell numbers in Notch2−/− mice, but this increase was no longer observed in Notch2−/−/p50−/− double heterozygotes (Table I). We do not precisely understand why normal FO-I B cell numbers are observed in the double heterozygotes; these data are consistent with the view that Notch2 drives MZ B cell development at the expense of FO-I B cell maturation, but that the increase in FO-I B cells in the absence of Notch2 may depend in part on some aspect of NF-κB signaling. The synergism between Notch2 and NF-κB1 during MZ B cell development does not extend to the regulation of FO B cell numbers or to the development of B-1 B cells.

We have shown that both Deltex1 and Hes5 are expressed at relatively high levels in MZ B cells and that both these genes are expressed in the B lineage in an NF-κB1-dependent manner. The dependence of Deltex1 expression on Notch2 is clear but the evidence for Notch2-dependent regulation of Hes5 is weaker (6). Our data are consistent with the possibility that a set of genes may be coordinately regulated by Notch2 and NF-κB to orchestrate a program that potentially commits developing B cells to a MZ B cell fate. Clearly a large set of genes, and not merely Dtx1 (and possibly Hes5), may be dual Notch and NF-κB targets. It will be necessary to perform chromatin immunoprecipitation assays at specific stages of B cell development to examine the in vivo occupancy by transcription factors of regulatory sites in putative target genes to fully reveal the transcriptional targets of Notch2 and NF-κB that contribute to MZ B cell development.

What signaling receptor is responsible for the induction of NF-κB1-containing heterodimers during MZ B cell development? The actual signaling pathway that contributes to the activation of NF-κB during MZ B cell development remains unclear. Although basal or low-level BCR signaling may be the source of NF-κB activation during MZ B cell development, the participation of NF-κB1 does not appear to depend on the activation of the BCR/Btk/ phospholipase Cy pathway, because this latter pathway is not required for MZ B cell development. A likely source of NF-κB activation in the context of MZ B cell development is the BAFF-R. BAFF-null mice present with a severe reduction of both MZ and FO B cell populations (33) and this defect is phenocopied by conditional IKKγ-null mice defective in the canonical NF-κB-signaling pathway (36). Constitutively active IκKB can rescue the BAFF-R defect, indicating that canonical NF-κB signaling during B cell development is largely mediated by the BAFF-R (16). BAFF signaling may mediate the survival of peripheral B cells by posttranslationally down-regulating the expression of Bim (37). However, it might separately contribute to the transcriptional induction of target genes in collaboration with Notch2 to mediate MZ B cell development or survival.

It is interesting that there appears to be an absolute requirement for NF-κB1, an NF-κB protein that lacks an activation domain, during MZ B cell development. We assume that the activation in vivo of a subset of genes required for MZ B cell development depends on NF-κB1-containing heterodimers, and that c-Rel homodimers for instance, cannot functionally replace NF-κB1 containing heterodimers in this context, although they may suffice for FO B cell survival. These stringent in vivo requirements are consistent with the growing appreciation that the composition and posttranslational modifications of NF-κB dimers critically influence target gene activation (2, 38). We have previously demonstrated that the absence of c-Rel or of p65/RelA only partly reduces MZ B cell numbers (12), suggesting that both NF-κB1/c-Rel and NF-κB1/p65 heterodimers may contribute to MZ B cell development. The requirement for NF-κB1 is particularly intriguing when one considers that BAFF signaling via the alternative pathway is likely to contribute to the processing of p100 to yield NF-κB2 in all B cells in lymphoid follicles (39), suggesting indirectly that NF-κB2 cannot substitute for NF-κB1 during MZ B cell development. Nevertheless, there appears to be an independent B cell-intrinsic as well as a B cell-extrinsic requirement for RelB during MZ B cell development (40), suggesting a requirement for both the canonical and alternative NF-κB pathways during MZ B cell development and/or survival.

Our studies suggest that Notch2 and NF-κB1 function in a different way during B-1 B cell development, distinct from their synergistic roles in MZ B cells. The role of Notch2 in B1 B cell
development remains unclear—a defect was not seen in conditional Notch2−/− mice but was observed in a separate study examining Notch2+/− heterozygotes. How exactly NF-κB1 functions in B-1 B cell development is also unclear, and given that BAFF-R is dispensable during the development of this lineage, the cell surface receptor activating NF-κB1 during B-1 B cell development is presumably not this TNF family receptor. Given the stringent requirement for relatively strong BCR signaling and for the Btk pathway during B-1 B cell development (in contrast to the limited role, if any, for Btk during MZ B cell development), it may well be that the BCR/Btk pathway induces the activity of NF-κB1-containing heterodimers during B-1 B cell development.

In conclusion, our results indicate that Notch2 and NF-κB1 function synergistically in vivo during the development of MZ B cells. The identification of the specific Notch and NF-κB1 targets that are required during MZ B cell development will be essential to obtain a detailed molecular elucidation of the programs that drive the development of cells of this B lymphoid lineage.

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Disclosures

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