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Formation of B-1 B Cells from Neonatal B-1 Transitional Cells Exhibits NF-κB Redundancy

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The stages of development leading up to the formation of mature B-1 cells have not been identified. As a result, there is no basis for understanding why various genetic defects, and those in the classical or alternative NF-κB pathways in particular, differentially affect the B-1 and B-2 cell lineages. In this article, we demonstrate that B-1 B cells are generated from transitional cell intermediates that emerge in a distinct neonatal wave of development that is sustained for ∼2 wk after birth and then declines as B-2 transitional cells predominate. We further show that, in contrast to the dependence of B-2 transitional cells on the alternative pathway, the survival of neonatal B-1 transitional cells and their maturation into B-1 B cells occurs as long as either alternative or classical NF-κB signaling is intact. On the basis of these results, we have generated a model of B-1 development that allows the defects in B-1 and B-2 cell production observed in various NF-κB–deficient strains of mice to be placed into a coherent cellular context. The Journal of Immunology, 2011, 187: 5712–5719.

After production in the bone marrow, immature surface IgM+ (sIgM+) B lymphocytes migrate to the spleen (SPL) where they transit through distinct transitional cell stages, termed T1, T2, and T3, before maturing into mature follicular (FO) or marginal zone (MZ) B-2 B cells (1–4). MZ B cells are localized to the margins of splenic follicles and respond to blood-borne pathogens (5), whereas FO B cells are the predominant B cell population in adult SPL and lymph nodes and participate in adaptive immunity (1, 6, 7). The events involved in transitional cell survival and maturation are increasingly well defined and depend upon interactions between Ag and BCR and binding of BAFF to BAFFR (TNFRSF13C). Engagement of BAFFR in turn results in activation of the alternative NF-κB pathway (8–11).

The analysis of mice with deficient expression of genes known to affect transitional cell survival and maturation has revealed puzzling differences between the formation of mature B-2 cells and a second population of B lymphocytes referred to as B-1 cells. B-1 cells are involved in innate immune responses and preferentially localize to serous cavities wherein sIgM+CD11b+CD5+ B-1a and sIgM+CD11b+CD5− B-1b subsets can be distinguished (12, 13). In particular, defects in the expression of either BAFF (14), BAFFR (15), or NF-κB2 (16), the key transcriptional regulatory protein in the alternative NF-κB pathway, result in a significant deficiency in transitional cell numbers, a block at the T1 to T2 stage of development, and a severe depletion of mature B-2 cells (9, 17). However, the number of mature B-1 cells is not affected (10, 14, 16). In contrast to these observations, mice with defective expression of various intermediates in the classical NF-κB pathway, such as Btk, Bcl-10 (18, 19), MALT-1 (20, 21), Card11/CARMA1 (22, 23), or NF-κB1 (24), have a reduced number of B-1 cells, and B-1a cells in particular, whereas the size of their B-2 cell compartment is not affected significantly.

These observations indicate that the stages of development leading up to the formation of mature B-1 and B-2 cells must be regulated differentially. However, although the signals necessary for the formation of mature B-2 cells and the stages of transitional cell development at which they act have been defined, a comparable understanding of the events resulting in the generation of mature B-1 cells has not been attained. It has been proposed that B-1 cells also develop from transitional cell intermediates (25), but such populations have not been identified. As a result, there is no basis for understanding why deletion of specific genes in one or the other NF-κB pathways differentially affects the formation of mature B-1 and B-2 cells.

The layered immune system hypothesis (26) proposes that B-1 and B-2 cells belong to separate lineages that derive from distinct progenitors that emerge at different times during development. Our demonstration that B-1 cells are generated from lineage-negative CD45R−lowCD19+ B-1 restricted progenitors that arise in the embryo before B-2 committed progenitors provided support for this view. B-1 progenitor numbers decline by young adulthood as B-2 production is established (27–29). The emergence of B-1 progenitors before B-2 progenitors suggested that intermediate stages of development between B-1 progenitors and mature B-1 cells would be most abundant in the newborn. In order to test this possibility, we undertook a detailed examination of the kinetics of B cell emergence in neonates.

In this study, we show that B-1 cells are generated from transitional B-1 cell intermediates that emerge in a distinct wave of development in the neonate. This B-1 transitional cell wave is sustained for ∼2 wk after birth and then wanes as B-2 transitional cell production becomes dominant. We further demonstrate that, in contrast to the dependence of B-2 transitional cells on alternative NF-κB signaling, the survival of B-1 transitional cells and their maturation into B-1 B cells occur as long as either the...
alternative or the classical NF-κB pathway is intact. In view of these observations, we propose a model in which B-1 and B-2 cells are produced in staggered waves from lineage-specific transitional cells that are regulated differentially. This new developmental scheme allows the defects in B-1 and B-2 cells to be placed into a coherent cellular context.

Materials and Methods

**Mice**

Swiss Webster (SW, Igκ-6a), CB17.SCID (Igκ-6b, SCID), and RAG-2/SJL [B6.SJL(129S6)-Ptprcα/BoCrTac-Rag2tm1Mass/J, CD45.2] mice were obtained from Taconic Farms. BAFFR−/− [B6(Cg)-Tnfrsf13ctm1Mass/J, CD45.2] mice and their B6 (C57BL/6J) controls, NF-κB1−/− (B6, 129P2-NfkB1tm1Bal/J) mice and their wild-type controls [B6(Cg)-Tnfrsf13ctm1Mass/J, CD45.2] mice and their B6 (C57BL/6J) controls, NF-κB1−/− (B6, 129P2-NfkB1tm1Bal/J) mice and their wild-type controls [B6129F1/J Aw-J/(2)], CD45.2+IgM+ (BAFFR−/−/CD45.2+) B cells in their peritoneal cavities were used for the presence of mature donor sIgM+ (SW) or CD45.2+IgM+ (BAFFR−/−, NF-κB1−/−) B cells in their peritoneal cavities by immunostaining.

**Immunostaining and flow cytometry**

Spleen cell suspensions were prepared by crushing tissue between frosted slides in Ca2+,Mg2+-free PBS. Peritoneal B cell suspensions were prepared by lavage with Ca2+,Mg2+-free PBS. Peritoneal cavity (PerC) cells from neonates up to 21 d of age were pooled, but their SPLs were processed individually.

**Cell transplantsations**

A total of 0.1–1×106 T1 or T2/T3 transitional cells, purified by flow cytometry from the SPLs of SW, BAFFR−/−, and NF-κB1−/− mice at the ages indicated in the figures, were transplanted i.p. into immunodeficient recipients. SW cells were transplanted into SCID mice, whereas BAFFR−/− and NF-κB1−/− mice were transplanted into Rag2−/− mice. Only cells enriched >95% were used. Recipient mice were sacrificed 4–6 d after injection and tested for the presence of mature donor sIgM+ (SW) or CD45.2+IgM+ (BAFFR−/−, NF-κB1−/−) B cells in their peritoneal cavities by immunostaining.

**Table I.** sIgM+ frequencies and numbers in SPLs of SW, BAFFR−/−, NF-κB1−/−, and xid mice and their respective controls, SW, B6, WT, and CBA, at the indicated ages

<table>
<thead>
<tr>
<th>Strains</th>
<th>Age</th>
<th>n°</th>
<th>sIgM+ Cell Frequency ± SD (%)</th>
<th>sIgM+ Cells × 106 ± SD</th>
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</thead>
<tbody>
<tr>
<td>SW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonates</td>
<td>3 d°</td>
<td>12</td>
<td>19.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>8 d°</td>
<td>7</td>
<td>33.1</td>
<td>10.0</td>
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<td>12 d°</td>
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<td>33.0</td>
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</tr>
<tr>
<td></td>
<td>17 d 4</td>
<td>53.9±1.3</td>
<td>32.7±3.0</td>
<td></td>
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<td></td>
<td>21 d 8</td>
<td>35.4±4.7</td>
<td>26.1±4.4</td>
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<tr>
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<td>BAFFR−/−</td>
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<tr>
<td></td>
<td>Adults</td>
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<td>xid</td>
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<tr>
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<tr>
<td>Adults</td>
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<td>CBA</td>
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<td>2</td>
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</table>

*aNumber of animals.

*Neonate SPLs were pooled for immunofluorescence analyses; values provided for older animals indicate average ± SD.
with Tris-ammonium chloride (pH 7.2) before immunostaining. Cells were incubated with anti-CD16/CD32 (FcγRII-III, clone 2.4G2; eBioscience) to reduce nonspecific staining. Transitional T1 and T2/T3, FO, MZ, B-1, B-1a, and B-1b cells were resolved by flow cytometry after incubation with the appropriate combination of the following Abs: polyclonal rat anti-mouse IgD and goat anti-mouse IgM (Southern Biotechnology Associates), anti-mouse IgD (clone AMS-9.1; Biolegend), IgM (clone DS-1, IgM-6a; BD Pharmingen), CD5 (Ly-1, clone 53-7.3), CD11b (clone M1/70), CD21/CD35 (clone CR2/CR1), CD23 (clone FcεRII), CD45.2 (clone 104), and CD93 (AA4.1, clone C1qRq; all from eBioscience). Sample analyses were performed on a LSRII (BD Biosciences) located in the Broad Stem Cell Research Center (University of California at Los Angeles). Cell purifications were performed on a FACSAria (BD Biosciences) located in the Flow Cytometry Core at the Jonsson Comprehensive Cancer Center (University of California at Los Angeles). Values indicated on the FACS plots indicate the population frequencies within the gated cells.

cDNA preparation and PCR reactions

Total mRNA was extracted from flash-frozen cell pellets of purified neonatal or adult transitional T1 and T2/T3 cells with the RNeasy Plus Micro Kit (QIAGEN), and cDNA was prepared with the RT2 First Strand Kit (QIAGEN) according to the manufacturer’s instructions. IgH V H11 gene rearrangements were assessed by nested PCR as described (27).

Statistical analyses

Data are presented as mean ± SD. Differences in values between groups were tested using a two-tailed unpaired Student t test.

Results

Transitional B cells predominate in neonatal SPLs

Our strategy for detecting cellular intermediates between B-1 progenitors and mature B-1 cells is based on the hypothesis that B-1 development would dominate the interval immediately after birth. We therefore determined the kinetics with which mature B-1 and B-2 cells emerged in the SPLs of 3- to 21-d-old SW neonates by flow cytometry, as described in Supplemental Fig. 1, to determine if this is the case.

The results showed that sIgM +CD23+CD21intCD93 2 FO and sIgM+CD23 2 CD21hiCD93 2 MZ B cells remained low in that tissue for the first 2 wk of life (Fig. 1A,1B). In contrast, sIgM +CD23 2 CD21hiCD93 2 B-1 cells were observed in neonatal SPLs (Fig. 1A,1B), and sIgMhisIgDloCD11b+CD5+ B-1a and sIgMhi sIgDloCD11b+CD5 2 B-1b cells were detected readily in neonatal PerC during the first week after birth (Fig. 1C,1D). These results support the view that the majority of mature B cells present immediately after birth are B-1 cells.

However, we also observed that the total number of sIgM + cells in the SPLs of 3-d-old neonates exceeded the number of mature B cells (Table I). Further analysis revealed that the majority of neonatal sIgM + cells were CD23 2 CD21intCD93 + T1 and sIgM +CD23+CD21intCD93+ T2/T3 transitional cells (Fig. 1A,1B). This high frequency of transitional cells in neonatal SPLs contrasts with observations in adult SPLs where the transitional cell pool constitutes 5–10% of total B cells (Fig. 1B).

Neonatal transitional cells primarily generate B-1 cells

The high frequency of transitional cells in neonatal SPLs, combined with the fact that most mature B cells detected in that organ

![FIGURE 2.](#) Neuronal T1 and T2/T3 cells preferentially mature into B-1 cells. A, Ig V H11 usage in T1, T2/T3, and FO cells isolated from 5-d- and 8-week-old SW mice. B and C, Representative FACS analyses of PerC cells from SCID mice transplanted with T1 and T2/T3 SPL cells isolated from 4-d- (B) and 8-week-old (C) donors. Plots show populations gated on donor sIgM +sIgD + cells.

![FIGURE 3.](#) Age-related changes in the B-1 potential of T1 and T2/T3 neonatal SPL cells. A, Relative frequencies of B-1 and B-2 cells within donor sIgM + cells in the PerC of SCID mice transplanted with T1 and T2/T3 transitional cells isolated from the SPLs of 5- and 12- and 6-week-old SW mice. B, Relative frequencies of B-1a and B-1b cells within the donor B-1 cells shown in A. Note that neonatal transitional cells generate B-1a cells more efficiently than adult transitional cells. Differences in reconstitution between adult and neonatal populations were tested by two-tailed unpaired Student t test (a = 0.05). The p values are indicated in parentheses. n indicates the number of recipients processed in each group.
and the PerC are B-1 cells, raised the possibility that the first transitional cells to arise in the newborn might be B-1 specified. This hypothesis was substantiated further by the fact that transitional cells isolated from 5-d-old neonates used Ig genes of the \( V_{\mu}11 \) family (Fig. 2A), which is a characteristic of B-1 cells (30).

To test this hypothesis, we examined the potential of purified neonatal transitional cells to differentiate into B-1 or B-2 cells after i.p. injection into SCID mice. In initial experiments, we compared slgM\(^{-}\)CD93\(^{-}\)T1 and slgM\(^{-}\)CD93\(^{-}\)T2/T3 B cells isolated from the SPLs of 4-d- and 8-wk-old SW mice. Examination of the recipients 4–6 d later showed that neonatal T1 and T2/T3 B cells preferentially generated donor slgM\(^{+}\)slgD\(^{+}\) cells that included both CD11b\(^{+}\)CD5\(^{-}\)B-1a and CD11b\(^{+}\)CD5\(^{-}\)B-1b cells (Fig. 2B). In contrast, transitional cells isolated from adult SPLs preferentially generated slgM\(^{+}\)slgD\(^{+}\) B-2 cells, and the few B-1 cells produced were mostly CD5\(^{-}\)CD11b\(^{-}\) B-1b cells (Fig. 2C). Similar results were obtained when transitional cells were injected i.v., except that donor cell yields were reduced (data not shown).

Having established that neonatal transitional cells preferentially generate B-1 cells, we tested how this potential evolved with age. Transitional cells from SW mice up to 12 d of age retained robust B-1 potential, although the capacity of transitional cells isolated from older neonates to generate B-1a cells had declined (Fig. 3A, 3B). Furthermore, as B-1 potential waned, B-2 potential increased, and transitional cells from 6-wk-old SW mice generated primarily B-2 cells (Fig. 3A). Consistent with these data, the number of B-1 cells generated per transitional cell transplanted was higher for neonatal cells than that for adult cells, whereas the reverse was true for B-2 cell generation (E.M.R. and K.D., unpublished observations).

These results indicate that B-1 cells mature through transitional cell intermediates and that, in contrast to adult cells, the majority of transitional cells in neonatal SPLs are committed to the B-1 lineage. In addition, the fact that B-1 potential declines with age as B-2 transitional cells arise demonstrates that B-1 and B-2 cells are generated in two sequential, overlapping waves.

**B-1 transitional cells develop in BAFFR\(^{-/-}\), NF-\(\kappa\)B1\(^{-/-}\), and xid mice**

Simultaneous inactivation of the classical and alternative NF-\(\kappa\)B pathways results in a severe block in B cell development at the immature B cell stage, and as a result neither transitional nor mature B cells develop (31, 32). However, selective inactivation of one or the other pathway has distinct effects on the mature B-1 and B-2 compartments. For example, B-1 B cells are present in the PerC of adult BAFFR\(^{-/-}\) mice, which are deficient in alternative NF-\(\kappa\)B signaling, but there is a pronounced deficiency in B-2 cells (Fig. 4A). However, defects in classical NF-\(\kappa\)B signaling result in a reduced number of B-1 cells in the PerC, although it is important to emphasize that B-1 cells are still present. For example, the number of mature B-1 cells, and B-1a cells in particular, is reduced in Btk-deficient xid (33) and NF-\(\kappa\)B1\(^{-/-}\) mice (34), whereas B-2 cells are detected readily (Fig. 4B, 4C).

We next examined how these defects in alternative or classical NF-\(\kappa\)B signaling affect B-1 transitional cells. Adult BAFFR\(^{-/-}\) mice had a profound deficiency in the frequency of slgM\(^{-}\)FO and MZ B-2 cells in their SPLs (Table I), and the few slgM\(^{-}\) cells present were mostly transitional B cells (Fig. 5A). In contrast, slgM\(^{-}\) cells were present in the SPLs of BAFFR\(^{-/-}\) neonates at levels only slightly lower than those in wild-type mice during the first 10 d after birth, and these were mostly T1 and T2/T3 cells (Fig. 5B, Table I).

Comparable analyses of adult Btk and NF-\(\kappa\)B1-deficient mice showed no major alterations in the frequencies of slgM\(^{-}\), T1 and T2/T3 transitional, and FO cells in their SPLs (Fig. 5C, 5E, Table I). In addition, analyses of Btk-deficient xid and NF-\(\kappa\)B1\(^{-/-}\) neonates up to 12 d of age showed that they had normal frequencies of slgM\(^{+}\) cells in their SPLs (Table I) and that these mostly consisted of T1 and T2/T3 transitional cells (Fig. 5D, 5F).

Together, these observations indicate that the emergence of the B-1 transitional wave in the neonate is not affected by deficiencies in either BAFFR, Btk, or NF-\(\kappa\)B1 signaling.

**B-1 transitional cells from BAFFR\(^{-/-}\) and NF-\(\kappa\)B1\(^{-/-}\) mice mature normally**

We next determined the B-1 developmental potential of T1 and T2/T3 transitional cells isolated from the SPLs of BAFFR\(^{-/-}\) mice and NF-\(\kappa\)B1\(^{-/-}\) neonates by transplanting them into RAG-2/SJL recipients. Doing so was particularly important with the latter strain, because the reduced number of B-1 cells observed in those mice might have reflected a requirement for NF-\(\kappa\)B1 in transitional cell maturation into B-1 cells. Recipient mice were examined 4–6 d after transplantation.
T1 and T2/T3 B cells isolated from the SPLs of 5-d-old BAFFR−/− neonates as well as from 8-wk-old adults preferentially generated donor CD45.2+sIgMhiIgDlo cells that included both CD11b+CD5+ B-1a and CD11b+CD5− B-1b cells but not B-2 cells (Fig. 6A–6B). In addition, T1 and T2/T3 transitional cells isolated from 7-d-old NF-κB1−/− mice also reconstituted B-1 cells after transplantation in vivo (Fig. 6C–6D), and notably, these cells showed no deficiency in their ability to generate B-1a B cells (Fig. 6D).

These results, together with the observation in the previous section, indicate that the emergence, survival, and maturation of B-1 transitional cells into B-1a and B-1b cells are not affected by a deficiency in BAFFR, Btk, or NF-κB1 expression (Fig. 7).

Discussion
In this study, we demonstrate that mature B-1 cells are produced through sIgM+CD93+CD23− transitional cell intermediates that predominate in neonatal SPLs during the first 2 wk after birth. B-1 transitional cell production occurs in a single wave that tapers in young adults as B-2 transitional cell production emerges and ultimately predominates. On the basis of these results, we have formulated a new, layered model of peripheral B-1 and B-2 maturation shown in Fig. 7. We further demonstrate how this scheme allows the reconciliation of our data and a puzzling series of observations showing that defects in the classical and alternative NF-κB pathways have differential effects on the formation of mature B-1 and B-2 B cells.

This model integrates the neonatal B-1 transitional cells described in this article with the recent identification of B-1 specified common lymphoid progenitors (CLPs) (35) and B-1 specified progenitors (27). In this model, B-1 CLPs generate B-1 progenitors that in turn differentiate into immature B-1 cells. The latter cells then migrate to the SPL where they mature into B-1a and B-1b cells through B-1 transitional cell intermediates. This B-1 pathway is most robust at the earliest stages of immune system development, because all of these populations are present at their highest levels in the fetus and neonate and decline in number in the adult. The existence of this transient, neonatal wave of B-1 development distinct from the adult B-2 transitional cell wave is illustrated particularly well in BAFFR−/− mice. The neonatal B-1

FIGURE 5. Neonatal B-1 transitional cells emerge in BAFFR−/−, xid, and NF-κB1−/− mice. A–F, Age-related changes in the relative frequencies of B cell populations within sIgM+ cells in SPLs of adult (A) and neonatal (B) BAFFR−/− mice, adult (C) and neonatal (D) xid mice, and adult (E) and neonatal (F) NF-κB1−/− mice. n indicates the number of animals processed in each group. Average frequency and SD values for older animals are provided in Supplemental Table II.
wave appeared in normal numbers and kinetics in this strain, but the subsequent B-2 transitional cell wave did not emerge, and the few transitional cells present in adults were B-1 specified.

The application of this model to the analysis of various NF-κB–deficient strains of mice, along with a retrospective review of the literature, allowed us to identify the stages of B-1 development that are dependent on or independent of various NF-κB signals.

First, when all NF-κB signaling is abrogated, as in NF-κB1−/−NF-κB2−/− mice, the maturation of immature B-1 cells into B-1 transitional cells is blocked completely (Fig. 7, step 1). This conclusion is based on the fact that B cell development in these double knockout mice does not proceed beyond the immature sIgM+sIgD− stage of development and few if any CD23−expressing transitional B cells are detected in the SPLs of 2-wk-old double knockout mice (32), an age at which B-1 transitional cell numbers are expected to be at their peak. This study also shows that abrogation of all NF-κB signaling blocks the emergence of the adult B-2 transitional and mature B-2 cells (32), indicating that regulation of the immature B cell to transitional B cell progression is regulated similarly in the B-1 and B-2 lineages (Fig. 7, step 1).

However, selective deficiencies in one or the other NF-κB pathway have distinct effects on the formation of mature B-1 and B-2 cells. For example, although our data confirm a deficiency of mature B-2 cells but normal numbers of B-1 cells in mice with defects in the alternative pathway (16), why this is the case has not been clear. The results demonstrating that BAFFR expression is not required for the emergence, survival, or maturation of B-1 transitional cells provide a framework for understanding why mice with genetic defects in the expression of BAFFR and downstream mediators such as NF-κB–inducing kinase (36) or NF-κB2 (16) do not have defects in the number of mature B-1 cells. We also have observed in preliminary studies that adult p100−/− mice (37) have normal frequencies and numbers of B-1 cells. Thus, although activation of the BAFF/BAFFR/NF-κB2 cascade is critical for B-2 transitional cell survival and maturation (Fig. 7, step 2), this is not the case for B-1 transitional cells.

The conventional view is that classical NF-κB signaling is required for the formation of mature B-1 cells. In fact, it often is concluded that disruption of this pathway results in a complete block in B-1 development (11). However, it is important to note that the analysis of mature B-1 numbers in many studies of mice with defects in the expression of classical NF-κB components is often incomplete and has focused solely on the B-1a compartment (19, 22, 24). As we show, a different picture emerges when a comprehensive analysis of PerC B-1a and B-1b populations is conducted. In this case, it is clear that deficiencies in classical pathway intermediates such as Btk and NF-κB1−/− result in reduced frequencies of B-1 cells, and B-1a B cells in particular, but not their complete absence.
cannot be excluded. For example, committed B-2 transitional block cross talk between the two pathways (17, 39), are effects at

cation of B-1 transitional cells has provided the first opportunity

BCR signaling after antigenic exposure in SPL. However, the

strains. Furthermore, B-1 transitional cells harvested from NF-

showed that the neonatal B-1 transitional cell wave arose in both

mature B-1 cell pool after its formation (Fig. 7, step 3). In this

development. This view provides an explanation for why defective

B-1 transitional cells into B-1a or B-1b cells. Instead, classical signaling is

However, this does not compromise the emergence or maturation of B-1 transitional cells into B-1a or B-1b cells. Instead, classical signaling is

required for the maintenance of the mature B-1 pool and for B-1a cells in particular after their formation (step 3).

The analysis of neonatal Btk-deficient xid and NF-κB1−/− mice showed that the neonatal B-1 transitional cell wave arose in both strains. Furthermore, B-1 transitional cells harvested from NF-

κB1−/− mice matured normally into both B-1a and B-1b cells. In view of these results, it seems most likely that intact classical NF-

κB signaling is required for the homeostatic maintenance of the mature B-1 cell pool after its formation (Fig. 7, step 3). In this regard, B-1 cells are distinguished by their potential to self-renew (38). Therefore, we propose that, in the absence of NF-κB1 or Btk, this potential is compromised. A similar mechanism may explain why mice with defects in other classical pathway intermediates, such as BCL-10 (18, 19), MALT-1 (20, 21), and CARD11/ CARMA1 (22, 23), have reduced numbers of mature B-1 cells.

Taken together, the analyses of BAFFR−/−, xid, and NF-κB1−/− mice indicate that the emergence, survival, and maturation of B-1 transitional cells can occur as long as one of the NFκB pathways is intact, suggesting that classical and alternative NFκB signaling have redundant roles at the B-1 transitional cell stage of development. This view provides an explanation for why defective expression of Btk or NF-κB1 has no obvious effect on B-1 transitional cells. Only when this redundancy is eliminated, such as in NF-κB1−/− × NF-κB2−/− mice or in strains with defects that block crosstalk between the two pathways (17, 39), are effects at the B-1 transitional cell stage manifest.

In summary, we propose that the majority of B-1 cells are generated through committed B-1 transitional cell intermediates. However, the possibility that other routes into the B-1 pool exist cannot be excluded. For example, committed B-2 transitional cells could be selected into the B-1 population by the strength of BCR signaling after antigenic exposure in SPL. However, the efficiency with which such an event takes place in a physiological setting remains an open question. That issue aside, our identification of B-1 transitional cells has provided the first opportunity to reconcile a series of puzzling observations made in various NFκB-deficient strains of mice. Our data also provide additional support for the layered immune system hypothesis and demonstrate the implications of viewing B cell development from that perspective.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


