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

Encarnacio Montecino-Rodriguez and Kenneth Dorshkind

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 slgM⁺CD11b⁺CD5⁺ B-1a and slgM⁺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 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
FIGURE 1. Most sIgM+ B cells in neonatal SPL are immature transitional cells. A, Representative FACS analyses showing T1 and T2/T3 transitional, FO, MZ, and B-1 cells in SPLs of 6-wk- and 5-d-old SW mice. B, Age-related changes in the relative frequency of B cell populations within sIgM+ cells in SPLs. T1 and FO cells were defined according to their levels of sIgM and CD21 expression as illustrated in Supplemental Fig. 1. C, Representative FACS analyses of B-2, B-1a, and B-1b cells in the PerC of 6-wk- and 5-d-old SW mice. D, Age-related changes in the relative frequency of B cell populations within sIgM+ cells in the PerC. n indicates the number of animals processed at the indicated time points. Average frequency and SD values for older animals are provided in Supplemental Table I.

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 cell production observed in various NF-κB-deficient strains of mice to be placed into a coherent cellular context.

Materials and Methods

Mice

Swiss Webster (SW, Igh-6a), CB17.SCID (Igh-6b, SCID), and RAG-2/SIL [B6.SJL(129S6)-PtprcαtBocCrtTac-Rag2<sup>2m</sup>, CD45.1] mice were obtained from Taconic Farms. BAFFR<sup>−/−</sup> [B6(Cg)-Tnfrsf13ctm1Mass/J, CD45.2] mice and their B6 (C57BL/6J) controls, NF-kB<sup>−/−</sup> mice and NF-κB<sup>B1</sup>−/− mice from Taconic Farms. BAFFR<sup>−/−</sup> mice were transplanted into RAG-2/SJL 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<sup>−/−</sup>) cells by immunostaining.

Immunostaining and flow cytometry

Spleen cell suspensions were prepared by crushing tissue between frosted slides in Ca<sup>2+</sup>, Mg<sup>2+</sup>-free PBS. Peritoneal B cell suspensions were prepared by lavage with Ca<sup>2+</sup>, Mg<sup>2+</sup>-free PBS. When necessary, RBCs were lysed by guest on April 14, 2017 http://www.jimmunol.org/ Downloaded from

Table I. sIgM+ frequencies and numbers in SPLs of SW, BAFFR<sup>−/−</sup>, NF-κB<sup>B1</sup>−/−, 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 x 10&lt;sup&gt;6&lt;/sup&gt; ± SD</th>
</tr>
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<tr>
<td>SW</td>
<td>Neonates</td>
<td>3 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.5 ± 0.9</td>
<td>0.9</td>
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<tr>
<td></td>
<td></td>
<td>8 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.1 ± 10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.0 ± 18.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.9 ± 3.1</td>
<td>32.7 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.4 ± 4.7</td>
<td>26.1 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>5 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.7 ± 1.9</td>
<td>40.4 ± 8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.9 ± 7.9</td>
<td></td>
</tr>
<tr>
<td>BAFFR&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Neonates</td>
<td>5 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7 ± 1.1</td>
<td>1.1</td>
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<tr>
<td></td>
<td></td>
<td>7 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.4 ± 5.2</td>
<td>5.2</td>
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<td></td>
<td></td>
<td>10 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.9 ± 7.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>11 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6 ± 2.9</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td>NF-κB&lt;sup&gt;B1&lt;/sup&gt;−/−</td>
<td>Neonates</td>
<td>6 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.8 ± 1.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6 ± 1.6</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.4 ± 5.1</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>8 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.1 ± 8.1</td>
<td>23.8 ± 7.2</td>
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<tr>
<td></td>
<td></td>
<td>10 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.0 ± 5.8</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.9 ± 0.9</td>
<td>7.2 ± 2.0</td>
</tr>
<tr>
<td>B6</td>
<td>Adults</td>
<td>10 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.3 ± 1.9</td>
<td>28.1 ± 2.9</td>
</tr>
<tr>
<td>WT</td>
<td>Adults</td>
<td>8 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.0 ± 3.4</td>
<td>39.6 ± 15.2</td>
</tr>
<tr>
<td>CBA</td>
<td>Adults</td>
<td>12 wk&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.2 ± 7.6</td>
<td>20.4 ± 3.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of animals.

<sup>b</sup>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, Igμ-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 ClqR; 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_{11} 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⁺CD21loCD93⁺ FO and sIgM⁺CD23⁺CD21hiCD93⁺ MZ B cells remained low in that tissue for the first 2 wk of life (Fig. 1A,1B). In contrast, sIgM⁺CD23⁺CD21hiCD93⁺ B-1 cells were observed in neonatal SPLs (Fig. 1A,1B), and sIgM⁺sIgD⁺CD11b⁺ CD5⁺ B-1a and sIgM⁺sIgD⁺CD11b⁺CD5⁻ 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⁺CD21loCD93⁺ T1 and sIgM⁺CD23⁺CD21hiCD93⁺ 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...
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_{H}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 sIgM⁺CD93⁺CD23⁻ T1 and sIgM⁺CD93⁺CD23⁺ 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 sIgM⁺sIgDa/lo B-2 cells, and the few B-1 cells in particular, is reduced (Fig. 4k). However, defects in classical NF-κB signaling, but there is a pronounced deficiency in B-2 cells (Fig. 2B). In contrast, transitional cells isolated from adult SPLs preferentially generated sIgM⁺sIgDa/lo 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 B-2 cells (Fig. 3). B-1 transitional cells develop in BAFFR⁻/⁻, NF-κB1⁻/⁻, and xid mice

Simultaneous inactivation of the classical and alternative NF-κB 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-κB signaling, but there is a pronounced deficiency in B-2 cells (Fig. 4A). However, defects in classical NF-κB 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-κB1⁻/⁻ mice (34), whereas B-2 cells are detected readily (Fig. 4B, 4C).

We next examined how these defects in alternative or classical NF-κB signaling affect B-1 transitional cells. Adult BAFFR⁻/⁻ mice had a profound deficiency in the frequency of sIgM⁺ FO and MZ B-2 cells in their SPLs (Table I), and the few sIgM⁺ cells present were mostly transitional B cells (Fig. 5A). In contrast, sIgM⁻ 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-κB1-deficient mice showed no major alterations in the frequencies of sIgM⁺, 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-κB1⁻/⁻ neonates up to 12 d of age showed that they had normal frequencies of sIgM⁺ 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-κB1 signaling.

B-1 transitional cells from BAFFR⁻/⁻ and NF-κB1⁻/⁻ 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-κB1⁻/⁻ 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-κB1 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\(^+\)sIgM\(^+\)hisIgD\(^-\) 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-\(\kappa\)B1\(^{-/-}\) 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-\(\kappa\)B1 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-\(\kappa\)B 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
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<sup>−/−</sup> 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<sup>−</sup> 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<sup>−/−</sup> mice (37) have normal frequencies and numbers of B-1 cells. Thus, although activation of the BAFF/BAFFR/NF-κB 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<sup>−/−</sup> result in reduced frequencies of B-1 cells, and B-1a B cells in particular, but not their complete absence.

FIGURE 6. B-1 transitional cells from BAFFR<sup>−/−</sup> and NF-κB1<sup>−/−</sup> mice mature into B-1 cells. A, Representative FACS analyses of the PerC cells from RAG-2/SJL mice transplanted with T1 and T2/T3 SPL cells from 5-d- and 8-wk-old BAFFR<sup>−/−</sup> donors. B, Relative frequencies of B-1 and B-2 cells within donor CD45.2<sup>+</sIgM<sup>+</sup> cells in the PerC of recipients of BAFFR<sup>−/−</sup> transitional cells from 5-d- (neonates) and 8-wk-old (adults) donors. C, Representative FACS analyses of the PerC cells from RAG-2/SJL mice transplanted with T1 and T2/T3 SPL cells from 7-d-old NF-κB1<sup>−/−</sup> donors. D, B-1 and B-2 potential of 7-d-old NF-κB1<sup>−/−</sup> T1 and T2/T3 SPL cells. The upper panels show the frequencies of B-1 and B-2 cells reconstituted, and the lower panels show the frequencies of B-1a and B-1b cells within the B-1 populations in the upper panels. The data in all of the panels represent relative frequencies within donor CD45.2<sup>+</sIgM<sup>+</sup> cells. n indicates the number of recipients processed in each group.
to reconcile a series of puzzling observations made in various NF-kB–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.

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