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Genetic Analyses of NFKB1 and OCA-B Function: Defects in B Cells, Serum IgM Level, and Antibody Responses in Nfkb1−/− Oca-b−/− Mice

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Defined patterns of gene expression during cell differentiation are likely to be ensured by multiple factors playing redundant roles. By generating mice deficient in both NFKB1 and OCA-B, we show here that the two transcription factors are required for B-1 cell differentiation and serum IgM production. In addition, relative to Nfkb1−/− or Oca-b−/− mice, the Nfkb1−/−Oca-b−/− mice show a decrease in conventional B cell frequencies in the spleen and augmented reductions in T-independent and T-dependent Ab responses. These results suggest that NFKB1 and OCA-B play compensatory roles in multiple aspects of B cell differentiation. The Journal of Immunology, 2000, 165: 6825–6832.

In the past few years, targeted gene disruption studies in mice have implicated many genes in B cell differentiation. In addition to the signaling and receptor proteins, a host of transcription factors that ultimately regulate expression of such proteins have been found to be essential for normal B cell differentiation (1). Thus, disruptions of genes encoding transcription factors PU.1, Ikaros, early B cell factor, E2A, and B cell lineage-specific activator protein (BSAP)3 (Pax5) all block early B cell differentiation in the bone marrow. In contrast, genes encoding transcription factors such as Spi-B, Oct-2, NF-κB, Pip-1, and OCA-B are required for Ag-dependent B cell differentiation in the periphery.

OCA-B (also called Bob1 and OBFl-1) is a transcription coactivator that functions in conjunction with Oct family transcription factors Oct-1 and Oct-2 to generate full activation of Ig promoters in vitro and in transient transfection assays (2–6). Surprisingly, mice lacking OCA-B can undergo normal Ag-independent B cell differentiation and produce normal levels of serum IgM, indicating that OCA-B function is not required for early B cell development and serum IgM production (7–9). However, OCA-B is essential for the Ag-dependent phase of B cell differentiation, as Oca-b−/− mice show severe deficiencies in serum IgG, IgA, and IgE levels, reduced Ab responses to T-independent (TI) and T-dependent (TD) Ags, and an inability to form germinal centers (7–9). Because Oca-b−/− B cells can undergo normal isotype switching when cultured in the presence of LPS and IL-4, but show reduced levels of transcripts from corresponding loci (7), the reduced levels of secondary isotypes appear to result from defects in transcription from switched loci.

Consistent with indications that transcription of heavy chain genes is driven by an intronic enhancer in early B cell differentiation and by the 3′ enhancer in late B cell differentiation, it was recently shown that the IgH 3′ enhancers are less active in Oca-b−/− B cells than in normal cells under certain conditions (10). This may reflect direct function of OCA-B through octamer sequence elements present in the 3′ IgH enhancer (11) or an enhancer-specific synergy with OCA-B-activated promoters. Because B cell activation by CD40 and IL-4 induces OCA-B expression (12, 13), OCA-B also may regulate factors required for B cell activation that in turn induce 3′ IgH enhancer function. Oca-b−/− splenic B cells are deficient in B cell receptor (BCR) signaling, because mutant B cells show decreased proliferation following surface IgM cross-linking (7). Furthermore, Oca-b−/− B cells, unlike normal B cells, do not exhibit repression of the 3′ IgH enhancer following surface IgG cross-linking (10). Because a number of studies suggest that BCR signaling is required for normal differentiation and maintenance of B cells in the bone marrow and periphery (14, 15), inefficient BCR signaling by Oca-b−/− B cells is likely to affect the differentiation and survival capabilities of these cells.

Studies of mice lacking members of the NF-κB/Rel transcription factor family have shown that, like OCA-B, these proteins function in late B cell differentiation (16, 17). Thus, B cells in Nfkb1 (p50/p105) null mutant mice undergo normal Ag-independent differentiation, but show a severely reduced ability to proliferate in response to LPS stimulation and a moderately reduced ability to proliferate upon surface IgM cross-linking (18). Like OCA-B-deficient mice, mice lacking NFKB1 have reduced levels of serum IgG, IgA, and IgE but normal levels of IgM. These mutant mice do not respond well to TD Ags and are more susceptible to certain bacterial infections. However, in contrast to Oca-b−/− B cells, Nfkb1−/− B cells are defective in both transcription at IgG3 and IgE germline loci and isotype switching to IgG3, IgE, and IgA (19), but not in germinal center formation. Double-null mutations in Nfkb1 (p50/p105) and Nfkb2 (p52/p100) result in a B cell differentiation block earlier than that seen with single-null mutations, as the double-mutant mice contain severely reduced levels of B cells in the spleen (20). Adoptive transfer studies showed that this
defect is B cell autonomous and that double-null B cells are functionally deficient and, like Oca-b−/− mice, unable to form germinal centers. Therefore, NFKB1 and NFKB2 play compensatory roles in generating normal numbers of splenic B cells and in germinal center formation.

It has been proposed that cell lineage originates from stochastic low-level expression of lineage-affiliated genes in the progenitor cells (21–23). Defined patterns of gene expression within a lineage may be ensured by redundancies and feedback mechanisms involving multiple proteins. The criteria for functional redundancy are that mutations in single genes individually produce no phenotype, whereas mutation of all paralogs produces a strong phenotype (21). Therefore, a protein may be involved in pathways that are not disrupted by a mutation in that protein if its normal function is fully compensated by other factors playing redundant roles. Consequently, analyses of single mutations produce only a partial list of protein functions, and more extensive insight into a protein function can be gained by creating multiple mutations in genes that are suspected of playing redundant roles. Such functional redundancy has been observed not only among proteins within the same family (i.e., NFKB1 and NFKB2) but also between the two unrelated transcription factors early B cell factor and E2A (24). Considering the fact that both NFKB1 and OCA-B are required for late B cell differentiation, we wished to determine whether these two transcription factors participate in redundant pathways during late B cell differentiation. To this end, we generated mice lacking both NFKB1 and OCA-B by crossing the Nfkb1−/− mice with Oca-b−/− mice. Interestingly, the double-mutant mice showed severe deficiencies in B-1 cell development and serum IgM. Moreover, conventional B (B-2) cell frequencies in the spleen also were reduced in Nfkb1−/−Oca-b−/− mice. These results suggest that NFKB1 and OCA-B are involved in functionally redundant pathways that ensure proper B cell differentiation.

Materials and Methods

Generation of the Nfkb1−/− Oca-b−/− mice

Oca-b−/− mice (7) were mated with the Nfkb1−/− mice (18 mice) (stock number 002849; B6, 129-Nfkb1tm1Bal. The Jackson Laboratory, Bar Harbor, ME) to generate mice heterozygous for Oca-b and Nfkb1 mutations. Intercrossing the heterozygotes generated homozygous double-knockout mice. All mice including the wild type were maintained in a C57BL and 129SV hybrid background in specific pathogen-free conditions. The following primers were used to identify the genotypes by PCR: primer set A, sense, TGCGAAATCCGAGAACCAC; antisense, CGTGGTCG CGGCTCCTCGCCGTC; set B, sense, CGTGGACATTGATTGATGGT GA GGGTGA, antisense, CCATACGGGCGTGTCCTGA; set C, sense, CTCCCTACCCGGTTAGATTCG; antisense, GTGGTGGTCG CGGCTCCTCGCCGTC; set D, sense, GGCTGTCG CGGCGTCTCCGCGG; antisense, CTGCGCTGAGTCGCTG.

B cell purification and RT-PCR

B cells were purified from spleen by immunomagnetic cell sorting as follows. A single-cell suspension from spleen was washed twice with PBS/0.3% BSA, fixed with 1% formaldehyde (methanol-free EM grade; Polysciences, Warrington, PA), and analyzed on a FACScan using CellQuest software (Becton Dickinson, Mountain View, CA). Lymphocytes were gated according to the forward- and side-scatter patterns to exclude large granular cells and debris. For each experiment, isotype controls were used as the negative staining controls. Quadrants were drawn according to the negative controls and confirmed by subsequent controls. Numbers represent the percentage of cells in a region or quadrant. Data are illustrated as dot plots or 20% probability contour plots. In each case, a representative data set out of five separate experiments is shown. The following antisera recognizing mouse cell surface markers were used: from PharMingen, anti-B220, clone RA3-6B2; anti-IgM, clone II/41; anti-CD4, clone H129.19; anti-CD8, clone 53-6.7; anti-IgD, clone M1/70; anti-IgA, clone J1-26; anti-CD5, clone 53-73; anti-CD43, clone S7; anti-CD23, clone B3B4; from Southern Biotechnology Associates (Birmingham, AL), goat anti-IgM R-PE conjugate; and from Caltag (San Francisco, CA), anti-CD11b Tricolor conjugate, clone M170.15.

Immunization and determination of serum Ig levels

Total Ig levels (IgM and IgG) were determined by sandwich ELISA using a mouse isotyping kit (Southern Biotechnology Associates). For IgA measurements, anti-mouse IgA (Southern Biotechnology Associates) was used as a capture Ab, and biotinylated anti-mouse IgA (Sigma) followed by avidin-HRP conjugate was used as detecting Ab. For all experiments, IgA was serially diluted sera and standard Igs were added to the plate. Ig concentrations were determined using a standard curve generated from standard Igs. For TI Ab responses, mice were i.p. injected with 50 μg DNP-conjugated Ficoll (Biosearch Technologies, Novato, CA). Blood was collected before and 10 days after the immunization. TD responses were analyzed by injecting mice with 100 μg DNP-keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, CA) with CFA. After 3 wk, mice were injected again with 10 μg DNP-KLH with IFA. Blood was collected before and 10 days after the first and second injections. Ab to DNP was measured by ELISA using plates coated with either DNP-OVA (OVA; Biosearch Technologies) or DNP-gammaglobulin (Calbiochem). Eight 1:5 serially diluted sera were used to determine the titer. The DNP-specific titer was defined as fold serum dilution that resulted in background absorbance.

Mice lacking both NFKB1 (p50/p105) and OCA-B were made by crossing Oca-b−/− mice with Nfkb1−/− mice (Fig. 1). As expected, the Nfkb1−/−Oca-b−/− mice expressed neither NFKB1 nor OCA-B mRNA, as determined by RT-PCR assays (Fig. 1B). Recently, it was reported that the promoter of the B cell-specific chemokine receptor gene Blr-1 (Cxcr-5) contains NF-κB and ocameron sites and that this promoter is synergistically activated by the NFKB1/RelA heterodimer and the Oct/OCA-B complex (25). The BLR-1 mRNA level was decreased 2- to 3-fold in the Oca-b−/− splenic B cells, indicating that OCA-B is indeed necessary for full activation of this promoter (Fig. 1B, data not shown). In contrast, the Nfkb1−/− splenic B cells expressed normal levels of BLR-1 mRNA, suggesting that NFKB1 is not required for activation of the Blr-1 promoter. In the Nfkb1−/−Oca-b−/− splenic B cells, the
In lymphoid organs by flow cytometry. Similar numbers of nucleated bone marrow cells were obtained from wild-type and mutant mice (24 ± 9 × 10⁶ for wild type, 19 ± 8 × 10⁶ for Nfkb1⁺⁄⁻, 25 ± 18 × 10⁶ for Oca-b⁺⁄⁻, and 26 ± 8 × 10⁶ for Nfkb1⁺⁄⁻Oca-b⁻⁄⁻). Previous studies indicated that B cell differentiation in the bone marrow proceeds normally in the absence of NFKB1 or OCA-B (7–9, 18). To find out whether NFKB1 and OCA-B play redundant roles in early B cell differentiation, we analyzed B cell differentiation in the bone marrow of Nfkb1⁺⁄⁻Oca-b⁻⁄⁻ mice. The double-mutant mice contained slightly reduced proportions of B220⁺ IgM⁺ immature B cells (Fig. 2A). However, analyses of B220 and CD43 expression did not show any obvious differences (data not shown), indicating that early B cell differentiation is largely normal in the absence of both NFKB1 and OCA-B. Previous studies of Oca-b⁻⁄⁻ mice indicated that there is a reduction of the recirculating B cell population in bone marrow (8–9, 27). This deficiency in recirculating B cells was most pronounced in young (3- to 4-wk-old) mice, while in older (20-wk) mice the recirculating B cell population was largely restored. In the present study, when mice were analyzed between 5 and 9 wk of age, the recirculating B cell population (defined as B220⁺CD43⁺ IgD⁺ cells) was significantly reduced in both Nfkb1⁺⁄⁻ and Oca-b⁻⁄⁻ mice, as well as in Nfkb1⁺⁄⁻Oca-b⁻⁄⁻ mice (Fig. 2A).

The immature B cells that have completed differentiation in the bone marrow exit to the blood stream and homed to the spleen. In the spleen, only a fraction of immature B cells from bone marrow are selectively included in the long-lived recirculating B cell pool. The total number of nucleated splenocytes was reduced 2- to 3-fold in the mutant mice (24 ± 11 × 10⁶ for wild-type, 13 ± 6 × 10⁶ for Nfkb1⁺⁄⁻, 9 ± 5 × 10⁶ for Oca-b⁺⁄⁻, and 12 ± 4 × 10⁶ for Nfkb1⁺⁄⁻Oca-b⁻⁄⁻). However, frequencies of the B220⁺ IgM⁺ and IgM⁺IgD⁺ B cell populations were quite normal in the Nfkb1⁺⁄⁻ and Oca-b⁻⁄⁻ spleens (Fig. 2B). Interestingly, the Nfkb1⁺⁄⁻Oca-b⁻⁄⁻ mice showed 2- to 3-fold reductions in frequencies of both the B220⁺ IgM⁺ and IgM⁺IgD⁺ B cell populations (Fig. 2B). Therefore, NFKB1 and OCA-B play redundant roles in the generation and/or maintenance of normal splenic B cell frequencies.

B splenocytes can be further subdivided according to IgM and IgD expression patterns (28). Transitional B cells recently arrived from the bone marrow are IgM⁺IgD low (T1) and subsequently IgM⁺IgD high (T2), whereas mature recirculating B cells are IgM⁺IgD high. An increase in the transitional B cell population with a concomitant decrease in the mature B cell population would indicate that there is a developmental block preventing B cell progression from the transitional to the mature B cell stage. For example, B cell differentiation is blocked at the T2 to mature B cell transition in B220 null and X-linked immunodeficiency (XID) mice (28). However, in the present case the Nfkb1⁺⁄⁻Oca-b⁻⁄⁻ mice exhibited defects in both transitional and mature B cell populations (Fig. 2B). This suggests that the developmental block is at or before the arrival of the transitional B cells in the spleen of Nfkb1⁺⁄⁻Oca-b⁻⁄⁻ mice. Alternatively, there might be a defect in survival rate of the mutant B cells. T cell differentiation to CD4 or CD8 single-positive stages occurs normally in the double-knockout thymus (data not shown). However, there is up to a 2-fold increase in the frequency of CD8⁺ cells in the Nfkb1⁺⁄⁻Oca-b⁻⁄⁻ spleen (Fig. 2B). This altered CD4⁺/CD8⁺ frequency in the Nfkb1⁺⁄⁻Oca-b⁻⁄⁻ spleen might indicate a dependency of CD4⁺ T cell maintenance on B cell function. However, we cannot exclude the possibility that NFKB1 and OCA-B might directly function in redundant T cell pathways that maintain normal CD4⁺/CD8⁺ frequencies.
B-1 cell development is defective in the absence of NFKB1 and OCA-B

In addition to the well-studied conventional B cells (B-2 cells), there is a separate population of B cells (B-1 cells) that is found mainly in the adult peritoneal and pleural cavities (29–32). B-1 cells make a major contribution to serum IgM, IgG3, and IgA levels and respond well to TI Ags such as bacterial coat Ags and autoantigens. Recent studies showed that natural IgM produced by B-1 cells is crucial for complement-mediated inflammatory diseases (33). B-1 cells are subdivided into B-1a and B-1b populations on the basis of cell surface markers and progenitor sources (34). All B-1 cells in the peritoneum are CD11b(MAC1)⁺, IgM⁻, and CD23⁻. B-1a cells express CD5 on the cell surface, whereas B-1b cells do not. Adoptive transfer studies show that the precursors for B-1a cells are restricted to fetal liver and omentum, while progenitors for B-1b cells are found in adult bone marrow as well as in fetal liver and omentum (34). An alternative view proposes that B-1 cells are generated from activated B-2 cells rather than from a separate lineage (35, 36). In addition, recent studies using the hen egg lysozyme transgenic mouse model system have shown that anergic B cells can assume a B-1a cell phenotype by up-regulating the expression of CD5, which inhibits the BCR signal induced by the autoantigen (37). Regardless of the origin of the B-1 cells, several recent studies showed that B-1 cell differentiation and survival are critically dependent on positive selection through BCR signaling (38–40).

We studied B-1 cell development in the various mutant mice by analyzing cell surface expression of CD5 and CD11b in the peritoneum (Fig. 3). Staining for the total B-1 cells (CD11b⁺IgM⁻) indicated that Oca-b⁻/⁻ mice contained an increased frequency of B-1 cells in the peritoneum (Fig. 3A). In contrast, there was a 2- to 3-fold reduction in the frequency of CD11b⁺IgM⁻ B-1 cells in mice lacking NFKB1 (Fig. 3A). In fact, the absolute numbers of CD11b⁺IgM⁺ cells were 2- to 3-fold elevated in the Oca-b⁻/⁻ mice and 2- to 3-fold reduced in the Nfkbi⁻/⁻ mice (1.2 ± 0.6 × 10⁶ for wild-type, 0.5 ± 0.3 × 10⁶ for Nfkbi⁻/⁻, 3 ± 2 × 10⁵ for Oca-b⁻/⁻, and 0.05 ± 0.04 × 10⁵ for Nfkbi⁻/⁻Oca-b⁻/⁻). Three-color flow cytometry showed that this reduction in the total B-1 cell population in Nfkbi⁻/⁻ mice is specifically due to a severe reduction in the CD5⁺IgM⁺ B-1a cell population, whereas the CD5⁻IgM⁺ cell population appears to be unaffected by the Nfkbi mutation (Fig. 3B, upper panel). The CD11b expression patterns of the IgM⁺CD5⁻ gated fraction showed that Nfkbi⁻/⁻ mice contained a normal proportion of B-1b (CD11b⁺CD5⁻) IgM⁻ cells (Fig. 3B, lower panel). Thus, our analyses indicate that NFKB1 is selectively required for B-1a cell differentiation. In Oca-b⁻/⁻ mice, there was an increase in the B-1a (CD5⁺IgM⁻) population (Fig. 3B, upper panel) and a slight decrease in the CD11b⁻CD5⁻IgM⁺ population (Fig. 3B, lower panel). Interestingly, there was a drastic loss of the IgM⁺CD11b⁻CD5⁻ population in the Oca-b⁻/⁻ mice (boxed, Fig. 3B, lower panel), indicating that a subset of B-1b cells are reduced in the absence of OCA-B. Importantly, Nfkbi⁻/⁻Oca-b⁻/⁻ mice had severe deficiencies in both the B-1a and the B-1b cell populations in the peritoneum (Fig. 3, A and B). In fact, the reduction in B-1 cells in Nfkbi⁻/⁻Oca-b⁻/⁻ mice was comparable to that seen in the XID mice (Fig. 3, A and B). Taken together, these results suggest that NFKB1 and OCA-B are required for B-1 cell differentiation and...
that each factor selectively affects either B-1a (NFKB1) or B-1b (OCA-B) cell differentiation.

Mice lacking OCA-B and NFKB1 produce severely reduced levels of Igs, including IgM, and deficient TI and TD Ab responses

We measured the total serum Ig levels to see whether the reduced number of Nfkb1−/−Oca-b−/− B cells in the periphery function normally (Fig. 4). As expected from the single-knockout phenotypes, the secondary heavy chain isotypes were all reduced, albeit to variable degrees, in the Nfkb1−/−Oca-b−/− mice. Thus, whereas IgG1 and IgG2b levels were slightly increased in Nfkb1−/−Oca-b−/− mice compared with Oca-b−/− mice, IgG2b, IgG3, and IgA levels were decreased even further. It is unclear whether these slight differences are functionally significant. Surprisingly, however, the IgM levels in the Nfkb1−/−Oca-b−/− mice were greatly reduced, to <10% of the wild-type level. Because single-knockout mice produce normal levels of IgM, this is another phenotype that reflects redundant functions of OCA-B and NFKB1. Considering that B-1 cells produce a major portion of serum IgM, the reductions in IgM levels might be due to the virtual absence of B-1 cells in the Nfkb1−/−Oca-b−/− mice.

To further test whether the Nfkb1−/−Oca-b−/− B cells are functional in vivo, we measured Ab responses to TI (DNP-Ficoll) and TD (DNP-KLH) Ags. As shown in Fig. 5A, the Oca-b−/− mice showed diminished IgM (18-fold) and IgG3 (90-fold) responses to TI Ag. This is in agreement with previous reports (8). In the present study, mice lacking NFKB1 also showed decreased TI responses (4-fold for IgM and 8-fold for IgG3), although the degree of reduction was less than in Oca-b−/− mice (Fig. 5A). Moreover, the Nfkb1−/−Oca-b−/− mice showed synergistic reductions in TI responses, as no DNP-specific antisera were detected above preimmune levels. Similarly, and consistent with previous reports, TD responses were greatly reduced in both the Nfkb1−/− (6-fold for IgM, 60-fold for IgG1, and 400-fold for IgA responses) and the Oca-b−/− (4-fold for IgM, 500-fold for IgG1, and 600-fold for IgA responses) mice (Fig. 5B). Again, Nfkb1−/−Oca-b−/− mice showed even greater defects and produced very little DNP-specific antisera. These results suggest that NFKB1 and OCA-B play functionally redundant roles in TI and TD Ab responses.

FIGURE 3. B-1 cell differentiation is defective in Nfkb1−/−Oca-b−/− mice. A. Flow cytometry of CD11b-PE and IgM-FITC double-stained peritoneal cells. As a comparison, peritoneal cells from XID mice (CBA/J-2-129P7-2/J; The Jackson Laboratory) exhibiting a severe reduction in B-1 cells are shown. B. Peritoneal cell staining with anti-CD5-PE, anti-IgM-FITC, and anti-CD11b-Tricolor. Lower panels show profiles of cells gated on the CD5+ IgM+ population (boxed). Frequencies of cells within the IgM+CD11b+ fraction indicated by the rectangular regions in the lower panel are: 32% (wild type), 41% (Nfkb1−/−), 11% (Oca-b−/−), 2% (Nfkb1−/−Oca-b−/−), and 7% (XID).

FIGURE 4. Nfkb1−/−Oca-b−/− mice contain severely reduced levels of serum IgM, IgG, and IgA. Isotype-specific ELISA showing the serum Ig levels. Wild type, ○; Nfkb1−/−, △; Oca-b−/−, □; Nfkb1−/−Oca-b−/−, ●. Bars denote the average values. *, Two mice had IgG2a levels below the detection limit.
Previous studies showed that $Oca-b^{-/-}$ mice lacked germinal centers in both spleen and lymph nodes, whereas $Nfkb1^{-/-}$ mice could form germinal centers in response to TD Ags. The $Nfkb1^{-/-} Oca-b^{-/-}$ mice did not form any germinal centers when immunized with the TD Ag DNP-KLH (data not shown). Therefore, OCA-B is still required for germinal center formation in an NFKB1-deficient background.

**Discussion**

This study describes analyses of B cell differentiation and immune functions in mice lacking both OCA-B and NFKB1. Both of these transcription factors have been shown to be crucial for late B cell maturation and function and both are induced upon B cell activation. Therefore, these two transcription factors are likely to function in redundant or interacting feedback loops that ensure proper B cell responses during the Ag-driven maturation process. As detailed below and summarized in Table I, the analyses of the double-null mutation have revealed functional redundancies between NFKB1 and OCA-B and novel roles for these two key transcription factors in B cell differentiation.

**B-1 cell differentiation and serum IgM levels**

Our present study shows that $Nfkb1^{-/-}$ mice have a severely reduced B-1a subpopulation (CD5$^+$CD11b$^+$IgM$^+$) but normal levels of B-1b cells (CD5$^+$CD11b$^+$IgM$^+$) (Fig. 3, A and B). In contrast, $Oca-b^{-/-}$ mice contain elevated numbers of B-1a cells and reduced numbers of IgM$^{high}$ B-1b cells (Fig. 3, A and B). Interestingly, the double-knockout mice contain drastically reduced levels of both B-1a and B-1b cells (Fig. 3). This suggests that NFKB1 and OCA-B are required for B-1 cell differentiation. This also suggests that although B-1a and B-1b cells are considered to be closely related, each are under a distinct transcriptional control. Currently, only limited information is available on the transcriptional regulation of B-1 cell differentiation. Analyses of mice lacking either Oct-2 or Pax-5 (BSAP) have shown that these transcription factors are required for B-1 cell differentiation (41, 42). However, in these studies on Oct-2$^{-/-}$ and Pax-5$^{-/-}$ mice, only CD5$^+$ B-1a cells were analyzed, and no information is available on the requirement of these factors in B-1b cell differentiation. Therefore, ours is the first report to show that two distinct transcription factors regulate B-1a or B-1b cell differentiation. There is
Table I. Genetic Analysis of B Cell Pathways Mediated by NFKB1 and OCA-B

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<th>Pathways</th>
<th>Disruption in Nfkbl&lt;sup&gt;−/−&lt;/sup&gt; Mice</th>
<th>Disruption in Oca-b&lt;sup&gt;−/−&lt;/sup&gt; Mice</th>
<th>Disruption in Nfkbl&lt;sup&gt;−/−&lt;/sup&gt; Oca-b&lt;sup&gt;−/−&lt;/sup&gt;</th>
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<tr>
<td>Immature B cells in bone marrow</td>
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<td>B-2 cells in spleen</td>
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<tr>
<td>Recirculating B cells in bone marrow</td>
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<td>B-1 Cells</td>
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<td>+ (B-1b)</td>
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<sup>−</sup>, Disrupted in the mutant mice; <sup>+</sup>, not disrupted in the mutant mice; ?, not analyzed.

Growing evidence that positive selection through the BCR plays a crucial role in B-1 cell differentiation (38–40). Therefore, the B-1 cell deficiencies observed in the mutant mice might be due in part to defective BCR signaling in the mutant B cells (7, 10, 18).

It has been proposed that B-1 cells make a major contribution to serum IgM levels. For example, depletion of B-1 cells by anti-IL-10 treatment results in a 90% reduction in serum IgM levels, whereas IgM responses to TD Ag by conventional B cells are normal (43). Therefore, it is likely that reductions in serum IgM levels in the Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> mice are, in large part, a consequence of the severe reduction in the B-1 populations. The inability of conventional Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> B cells to generate specific IgM Abs against TI and TD Ags (Fig. 5) may contribute further to the deficiencies in the total serum IgM levels.

Conventional (B-2) B cell differentiation

In the spleen, B220<sup>+</sup>IgM<sup>+</sup> B cell frequencies are relatively normal in the single-knockout mice (Fig. 2B). In contrast, the double mutation causes a 2- to 3-fold reduction in the B220<sup>+</sup>IgM<sup>+</sup> B cell frequency. These results suggest that OCA-B and NFKB1 play redundant roles in the generation and/or maintenance of normal frequency of conventional B cells in the periphery. While analyzing the cell surface expression of IgM and IgD in the spleen, we noticed that the mean intensity of both IgM and IgD was consistently reduced in Oca-b<sup>−/−</sup> and Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> mice. In fact, this reduction is quite obvious in the contour plot of the double staining (Fig. 2B). We do not know the precise reason for this reduction, but it is possible that the peripheral B cells in the Oca-b<sup>−/−</sup> and the Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> mice are abnormal. Thus, in addition to being reduced in number, the peripheral B cells in Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> mice may be functionally distinct from the B cells in wild-type mice. Detailed cellular analyses are under way to study the activation processes in mutant B cells.

Defects in switched isotype levels, Ab responses, and germinal center formation

Single-null mutations in either Nfkbl or Oca-b lead to decreased serum IgG and IgA levels. In the Oca-b<sup>−/−</sup> mice these defects may reflect essential OCA-B function both in germinal center formation (7–9) and in optimal 3' enhancer-dependent expression of switched heavy chain loci (10). In contrast, the decreased serum isotype levels in Nfkbl<sup>−/−</sup> mice may reflect the fact that unlike OCA-B, NFKB1 is required for germline immunoglobulin transcription and isotype switching, but not for germinal center formation (18, 19). It is currently unknown whether NFKB1 plays a role in transcription of the switched isotype loci through the 3' IgH enhancer. However, the presence of functional NF-κB sites along with octamer sites in the 3' IgH enhancer (11) suggests that transcription of switched isotype loci might be coordinately regulated by NFKB1 and OCA-B.

Ab responses to either TI (DNP-Ficoll) or TD (DNP-KLH) Ags are significantly reduced in the single-knockout mice. In the Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> mice, the responses are further reduced, with very little anti-DNP antisera detectable (Fig. 5). Functional redundancy between NFKB1 and OCA-B is obvious in both TI and TD responses, as the single-knockout mice produce significantly reduced (4- to 600-fold) yet measurable amounts of DNP-specific antisera, whereas the Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> mice produce very little anti-DNP antisera above the preimmune level. These results suggest that OCA-B and NFKB1 play functionally redundant roles in the production of TI and TD Ab responses. The roles played by these factors in isotype switching, germinal center formation, and 3' IgH enhancer-dependent transcription may be part of this process. However, the degree of the defect produced by the double-null mutation is more the simple sum of defects caused by the single mutations. Therefore, it is likely that NFKB1 and OCA-B play additional roles in generating specific Ab responses.

Collaboration between NFKB1 and OCA-B

Currently, the only known target gene that is coordinately regulated by NFKB1 and OCA-B is the B cell chemokine receptor Blr-1 (25). Our analyses of BLR-1 mRNA levels in the single- and double-mutant B cells suggest that NFKB1 function on this promoter is compensated by other NF-kB family proteins (Fig. 1B). Considering the defects in the Nfkbl<sup>−/−</sup>Oca-b<sup>−/−</sup> mice, it is possible that there are other target promoters/enhancers that are directly and synergistically regulated by NFKB1 and OCA-B. It is also possible that NFKB1 and OCA-B activate separate target genes that regulate the same downstream genes within a B cell differentiation pathway. A large-scale gene expression profiling of the mutant mice should yield interesting information on potential target genes and pathways coordinately regulated by NFKB1 and OCA-B.

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References


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