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B Cell-Activating Factor Belonging to the TNF Family Acts through Separate Receptors to Support B Cell Survival and T Cell-Independent Antibody Formation

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The TNF-related ligand, B cell-activating factor belonging to the TNF family (BAFF), is necessary for normal B cell development and survival, and specifically binds the receptors transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), B cell maturation Ag (BCMA), and BAFF-R. Similarities between mice completely lacking BAFF and A/WySnJ strain mice that express a naturally occurring mutant form of BAFF-R suggest that BAFF acts primarily through BAFF-R. However, the nearly full-length BAFF-R protein expressed by A/WySnJ mice makes unambiguous interpretation of receptor function in these animals impossible. Using homologous recombination we created mice completely lacking BAFF-R and compared them directly to A/WySnJ mice and to mice lacking BAFF. BAFF-R-null mice exhibit loss of mature B cells similar to that observed in BAFF^{-/-} and A/WySnJ mice. Also, mice lacking both TACI and BCMA simultaneously exhibit no B cell loss, thus confirming that BAFF-R is the primary receptor for transmitting the BAFF-dependent B cell survival signal. However, while BAFF-R-null mice cannot carry out T cell-dependent Ab formation, they differ from BAFF-deficient mice in generating normal levels of Ab to at least some T cell-independent Ags. These studies clearly demonstrate that BAFF regulates Ab responses *in vivo* through receptors in addition to BAFF-R. *The Journal of Immunology*, 2004, 173: 2331–2341.

Several lines of evidence suggest that B cell-activating factor belonging to the TNF family (BAFF³; also called BLyS, TALL-1, THANK, and zTNF4), promotes the generation and survival of mature B cells (1–5). *In vitro*, exogenous BAFF increases the life span of splenic B cells and stimulates transcription of *bcl-2* family members (6–8). *In vivo*, overproduction of soluble BAFF in transgenic mice increases the number of B cells in the spleen, lymph nodes, and peripheral blood (5, 9, 10). Consistent with these observations, treatment of mice with rBAFF protein expands B cell regions in the spleen and increases the concentration of serum IgM and IgA (2).

BAFF binding is not detectable on early immature B cells, but increases during maturation and may be mediated by three receptors (8). BAFF-R (also known as BR3), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), and B cell maturation Ag (BCMA) are all BAFF-interacting proteins variably expressed by B cells, although both TACI and BAFF-R have been reported on some T cell populations as well (5, 11–15). BAFF is the only known ligand for BAFF-R, whereas both

TACI and BCMA also bind the BAFF-related protein, a proliferation-inducing ligand (APRIL, also called TRDL-1) (5, 16–18).

The central importance of BAFF-R for BAFF function was first suggested by the analysis of A/WySnJ mice that express a mutated form of the receptor. A/WySnJ animals exhibit a significant loss of mature B cells and reduced Ab responses to T cell-dependent (TD) Ags, suggesting that signaling through BAFF-R is a positive regulator of survival (19–21). However, this phenotype is readily distinguishable from that observed in BAFF-null mice, which also have few mature B cells and exhibit attenuated Ab responses to both TD and T cell-independent type II (TI-II) Ags (9, 15, 22). The complex nature of the A/WySnJ mutation makes it impossible to clearly define through the study of these animals which functions of BAFF are mediated by BAFF-R. A transposon insertion resulted in the replacement of the C-terminal 8 aa of BAFF-R with 21 unrelated residues (13). Thus, the largely intact receptor in A/WySnJ mice may retain at least some signaling functions or act as a partially dominant-negative component in heteromeric signaling complexes.

The roles of the other known BAFF receptors in regard to BAFF function are still only partially understood. Surface expression of BCMA is absent or weak in most cells, and BCMA-null mice exhibit normal numbers of B cells as well as normal primary Ab responses to both TD and TI-II Ags (11, 23, 24). However, BCMA is more highly expressed on human plasmablasts and murine long-lived plasma cells, and has recently been shown to enhance the survival of these cells (25, 26). The third known BAFF receptor, TACI, has been suggested to function as a negative regulator of B cell homeostasis. *In vitro*, purified B cells from TACI-null mice exhibit enhanced proliferation, and Ab secretion as compared with wild-type (WT) controls (27). *In vivo*, TACI-deficient B lymphocytes are significantly expanded in number and produce enhanced Ab responses to TD Ags (27, 28). Intriguingly, however, mice lacking TACI are compromised in their ability to respond to TI-II

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³ Abbreviations used in this paper: BAFF, B cell-activating factor belonging to the TNF family; TACI, transmembrane activator and calcium-modulator and cyclophilin ligand interactor; BCMA, B cell maturation Ag; T1, transitional type 1; T2, transitional type 2; TD, T cell-dependent; TI-II, T cell-independent type II; MZB, marginal zone B; GC, germinal center; hBAFF, human BAFF; CGG, chicken γ globulin; KLH, keyhole limpet hemocyanin; AP, alkaline phosphatase; TNP, 2,4,6-trinitrophenyl; APRIL, a proliferation-inducing ligand; ES, embryonic stem; KO, knockout; WT, wild type.

Ags (28). However, as both TACI and BCMA also bind the BAFF-related protein APRIL (18, 29–31), it is possible that TACI- and BCMA-dependent functions may reflect APRIL signaling.

In this report, we show that mice completely deficient in BAFF-R exhibit the same reduction in mature B cell number as observed in mice lacking BAFF. The failure of BAFF-R^{-/-} mice to respond to exogenous BAFF treatment, and the lack of B cell loss in TACI;BCMA double mutant mice strongly supports the model that BAFF-R is the sole receptor mediating the B cell survival signal from BAFF. However, in contrast to BAFF-null mice, BAFF-R-null animals are capable of normal titer TI-II Ag responses. These results indicate that BAFF functions through at least one receptor other than BAFF-R to support or regulate the generation of Abs to TI-II Ags.

Materials and Methods

Mice

To create a BAFF-R-targeting construct, genomic 129/SvJ DNA isolated from a Lambda Library (Stratagene 946313; Stratagene, La Jolla, CA) was subcloned into a high-copy number plasmid and targeted by homologous recombination in bacteria (32) to insert a human CD2 reporter gene lacking cytoplasmic residues at the initiating ATG. The final construct was deleted in 530 nt of genomic DNA encoding the first 95 residues of BAFF-R; the deletion ends in the second exon and removes the putative ligand-binding and transmembrane domains. This construct was used to target the BAFF-R locus of E14.2 embryonic stem (ES) cells (33), and correctly targeted cells were injected into C57BL/6 blastocysts to generate chimeric mice. Chimeras were crossed to C57BL/6 mice to generate founder mice. BAFF-R- and BAFF-null mice (9) analyzed were of a C57BL/6-129/Sv mixed background. A/J and A/WySnJ mice were maintained as homozygous strains via in-house breeding from founders purchased from The Jackson Laboratory (Bar Harbor, ME). All studies were performed following guidelines of the Biogen Idec Institutional Animal Care and Use Committee on 8- to 12-wk-old mice housed under specific pathogen-free conditions.

RT-PCR

Total splenic RNA purified using TRI reagent (Molecular Research Center, Cincinnati, OH) was treated with RQ1 RNase-free DNase (Promega, Madison, WI) and cDNA synthesized using random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Promega). PCR amplification (45 cycles, with steps of 30 s each at 94°C and 63°C, and of 1 min at 72°C) was used to detect cDNAs of indicated sizes using specific primers: CD4 (590 bp; 5'-CAGCCCTGGATCTCCTTCTCC-3' and 5'-TGGCTCTTCTGCATCCGGTGG-3'); BAFF-R (521 bp; 5'-GACATG GGCGCCAGGAGACTCCGGGTCCGA-3' and 5'-TGGGCCAGCTG TCTTGGT GGTCACCAGCTC-3'); BAFF (397 bp; 5'-ATGG ATGAGTCTGCAAAGACCCTGCCACCA-3' and 5'-TCCTCTGGT CCCTGGAAAGCGCG-3').

FACS analyses

Single-cell suspensions were prepared from spleens ground between frosted slides into HBSS with 3% serum, strained through a 70- μ m nylon screen (Fisher Scientific, Hampton, NH) and counted with a Beckman Z2 Coulter Counter (Beckman Coulter, Fullerton, CA). After erythrocytes were lysed (6), cells were washed, and resuspended with CD16/32 Fc-Block (BD Pharmingen, San Diego, CA) in FACS buffer (3% serum, 10 mM HEPES, 0.02% sodium azide in PBS, pH 7.6). A total of 2×10^7 cells/ml were stained in FACS buffer with the following anti-mouse Abs: anti-CD21-FITC, anti-CD8a-FITC, anti-CD62L-FITC, anti-CD4-PE, anti-IgD-PE, anti-B220-PerCP, anti-CD44-allophycocyanin, anti-IgM-allophycocyanin, anti-TCR β -allophycocyanin, anti-CD23-biotin, anti-TCR β -biotin, anti-TNP-biotin, anti-TACI-biotin (clone 8P2 and 5L7) and anti-BAFF-R (clone B2D8 and PIB8). Biotinylated Abs were detected with PE-, PerCP-, or allophycocyanin-conjugated streptavidin. Anti-BAFF-R (clone B2D8) was detected with PE-conjugated anti-hamster Ab. All Abs and streptavidin conjugates were purchased from BD Pharmingen, except anti-IgD-PE (Southern Biotechnology Associates, Birmingham, AL), anti-BAFF-R, and anti-TACI. Anti-BAFF-R was generated from a murine BAFF-R human IgG fusion protein immunization of Armenian hamsters and subsequent fusion with the P3X63Ag.653 murine myeloma line (34) following standard procedures (35). Anti-TACI was generated from a murine TACI immunization of rats using similar methods. BAFF-binding to lymphocytes was assayed by incubation with biotinylated recombinant human BAFF (hBAFF), followed by detection with PE-conjugated

streptavidin. After fixation with Cytotfix (BD Pharmingen), at least 50,000 lymphocytes per sample were analyzed on the BD FACSCalibur (BD Biosciences, San Jose, CA). Data analysis was performed using FlowJo software (Tree Star, Ashland, OR).

Immunohistochemistry

B220/CD3 double immunohistochemical staining procedures were performed on formalin-fixed paraffin-embedded spleen sections. After heat induced epitope retrieval using 1 mM EDTA, pH 8.2, unconjugated rat mAbs from Serotec (Oxford, U.K.) were used to recognize CD3 (rat anti-human CD3, clone CD3-12) and CD45R/B220 (rat anti-mouse CD45R, clone RA3-6B2). Primary Abs were detected via the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) using biotinylated anti-rat immunoglobulins followed by avidin-biotin-HRP complexes; immunostaining procedures were performed sequentially using diaminobenzidine substrate (Vector Laboratories) to detect T cells and Vector VIP (Vector Laboratories) to detect B cells. B220/MOMA-1 double immunohistochemical staining procedures were performed on acetone-fixed cryosections of spleen. Unconjugated rat anti-mouse mAbs from Serotec were used to recognize metallophilic macrophages (clone MOMA-1), and CD45R/B220 (clone RA3-6B2). Primary Abs were detected as above, using diaminobenzidine for metallophilic macrophages, and Vector VIP for B cells. Tissue sections stained for B220/MOMA-1 were counterstained with methyl green. For germinal center (GC) analysis, SRBC immunizations, immunofluorescent staining, and GC quantification were as previously described (36, 37).

Immunization and ELISA

Mice were immunized with 100 μ g of keyhole limpet hemocyanin (KLH; Sigma-Aldrich, St. Louis, MO) or chicken γ globulin (CGG; Jackson ImmunoResearch Laboratories) precipitated in alum (Pierce, Rockford, IL), and boosted without adjuvant on day 35. Serum was separated from blood collected from anesthetized mice via retro-orbital bleeding and assayed for Ag-specific Abs by ELISA. Serum Abs captured on KLH- or CGG-coated plates were detected with alkaline phosphatase (AP)-conjugated anti-mouse IgM or pooled AP-conjugated anti-mouse IgG1, IgG2a, IgG2b, and IgG3 subclass Abs (Jackson ImmunoResearch Laboratories) and subsequent incubation with *p*-nitrophenyl phosphate (Sigma-Aldrich). Relative serum Ab titers were determined from OD₄₀₅ values by comparison to values obtained from dilution of the appropriate IgM or IgG standard captured on goat anti-mouse Ig-coated plates (Southern Biotechnology Associates). Serum dilutions used to calculate relative Ab titer were those that gave OD₄₀₅ values within the linear range of the standard curve. Immunization and ELISA for TI-II analyses were performed as above, except mice were immunized once with either 50 μ g of 2,4,6-trinitrophenol (TNP)-Ficoll (Biosearch Technologies, Novato, CA) or 5 μ g of Pneumovax (Merck, West Point, PA) and serum Abs were captured on either TNP-BSA- (Biosearch Technologies) or Pneumovax-coated plates.

To measure total Ig levels, serum Abs collected from unimmunized mice were captured on plates coated with goat anti-mouse Ig (Southern Biotechnology Associates). IgM Abs were detected with AP-conjugated goat anti-mouse IgM, and IgG subclass Abs were detected with individual AP-conjugated anti-mouse IgG-subclass Abs (Southern Biotechnology Associates). Absolute Ab concentrations were determined from OD₄₀₅ values by comparison to dilutions of the appropriate IgM or IgG subclass standard (Southern Biotechnology Associates) captured on plates coated with goat anti-mouse Ig. Serum dilutions used to calculate Ab concentrations were chosen as described above.

Statistics

All statistical analyses were performed using Student's *t* test.

Results

Mice completely lacking BAFF-R or BAFF have similar B lymphocyte numbers

To create mice completely deficient in BAFF-R, we prepared a gene-targeting vector that removed most of the first two exons of coding sequence (Fig. 1A). Two mouse ES cell lines heterozygously mutated at the BAFF-R locus were identified by Southern blotting (Fig. 1B) and injected into C57BL/6 blastocysts. Chimeric male mice derived from both clones transmitted the deleted BAFF-R allele to their progeny. Intercrosses of heterozygous mice derived from both clones yielded homozygotes in the expected Mendelian proportions (our unpublished observations). More extensive characterization of one line revealed a lack of detectable

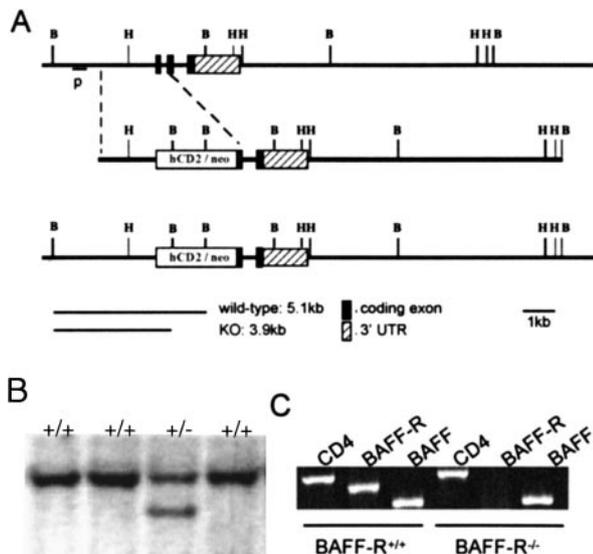


FIGURE 1. Gene inactivation of murine BAFF-R. *A*, WT BAFF-R locus (*top*) with gene-targeting construct and resulting targeted locus (*bottom*). Dashed lines indicate replacement of endogenous sequence with a human CD2 reporter gene and neomycin resistance cassette (hCD2/neo). Restriction enzyme sites for *Bgl*II (B) and *Hind*III (H) are shown, although additional sites are also present. Size of *Bgl*II restriction digest products for WT and targeted knockout (KO) locus DNA hybridizing with indicated probe (p) is shown. *B*, Southern blot analysis of BAFF-R targeted ES cell clones. *Bgl*II-digested genomic DNA from BAFF-R^{+/+} (+/+) and BAFF-R^{+/-} (+/-) ES cell clones is shown hybridized with DNA probe (A). *C*, RT-PCR detection of indicated genes from splenic RNA from BAFF-R^{+/+} and BAFF-R^{-/-} mice. CD4 expression is assayed as a positive control.

BAFF-R mRNA as assayed by RT-PCR (Fig. 1C); this line (BAFF-R5) was used for all subsequent analysis.

Analyses of BAFF-R^{-/-} mice revealed that B lymphocyte populations were altered in a manner largely consistent with previous characterizations of BAFF^{-/-} mice (9, 22): severe reduction of mature B cell populations in lymph nodes, peripheral blood, and the bone marrow, but no loss of marrow pre- and pro-B cells (Fig. 2A, and our unpublished observations) (9, 22). Immunohistochemical staining of spleen sections from BAFF-R^{-/-} and BAFF^{-/-}

mice showed that both exhibit similar preservation of CD3⁺ T cells and severe loss of B220⁺ B cells (Fig. 3). Staining for B220 and the metallophilic macrophage marker MOMA-1 also indicates that BAFF-R^{-/-} mice exhibit a dramatic loss of marginal zone B (MZB) cells consistent with that observed in BAFF^{-/-} mice (Fig. 3, and Ref. 9). FACS analyses of splenic lymphocytes from BAFF-R^{-/-} mice were performed in parallel with BAFF^{-/-} mice and the naturally occurring BAFF-R mutant A/WySnJ mice to directly compare the effects of all three mutations. The numbers of total B220⁺ cells in the three mutant strains were reduced ~5-fold compared with the corresponding WT controls, with particularly severe losses in the IgD^{high}IgM^{low} mature population (Fig. 2B, Table I, and Refs. 9 and 20). Similar levels of B cell loss in each mutant strain were also observed for transitional type 2 (T2, IgD^{high}IgM^{high}) and MZB/transitional type 1 (T1) (IgD^{int}IgM^{high}) populations (Fig. 2B, Table I, and Ref. 15). Although our analysis directly comparing all three mutants suggested a loss of T1 B cells in BAFF-R mutant mice not exhibited by BAFF^{-/-} mice, repeated FACS stainings revealed no significant difference between each mutant strain and the respective WT control mice (Fig. 2B, Table I, and our unpublished observations). Consistent with the observed similar phenotypes of BAFF- and BAFF-R-deficient mice, we find that B cells from BAFF-R^{-/-} mice exhibit little or no BAFF binding (Fig. 2C). Finally, although a reduction in at least some T cell populations has been reported in BAFF^{-/-} mice (22), we do not observe a significant loss of any T cell populations for either BAFF- or BAFF-R-mutant mice (Table I).

Although these results suggest that the A/WySnJ mutation is equivalent to a BAFF-R-null mutation in regard to loss of specific B cell populations, they do not rule out the possibility that the truncated receptor expressed in the A/WySnJ mice retains some signaling activity. Therefore, we compared B cell CD21 and CD23 surface expression in A/WySnJ mice with that in BAFF^{-/-} and BAFF-R^{-/-} mice, as expression of both genes has been shown to be BAFF-dependent (15). FACS analysis of IgD^{high}IgM^{low} mature B cells revealed that expression of CD21 was reduced similarly in all three mutant strains (our unpublished observations). However, whereas mature B cells from BAFF^{-/-} mice exhibit markedly lower CD23 expression than cells from the respective WT control animals, the same cell population in A/WySnJ mice has essentially

Table I. Splenic B and T cell numbers^a in BAFF and BAFF-R mutant mice^b

	BAFF		BAFF-R		A/WySnJ	
	Wild type	Mutant	Wild type	Mutant	Wild type	Mutant
Splenocytes	131.9/10.9	73.9/6.7	151.0/10.7	87.7/24.6	127.1/18.0	69.4/10.8
B cells (B220 ⁺)	76.3/11.5	17.7/2.9	88.7/10.1	17.7/10.6	72.5/10.2	12.0/2.9
Mature ^c	45.7/6.2	3.8/0.6	52.1/6.5	5.0/2.9	45.1/6.4	5.7/1.4
Marginal zone/T1 ^c	6.0/1.8	3.6/0.7	8.2/0.9	2.4/1.8	4.7/0.8	1.3/0.4
T1 ^c	1.8/0.7	2.0/0.5	2.9/1.0	1.0/0.7	1.5/0.3	0.6/0.2
T2 ^c	7.0/1.4	1.9/0.4	8.0/1.0	2.7/2.0	4.6/0.7	1.2/0.4
T cells (TCRβ ⁺)	28.1/3.2	27.4/1.4	30.8/3.7	34.6/5.0	21.0/1.8	25.7/2.3
CD4 ⁺	14.1/1.2	13.0/0.7	15.8/1.9	17.1/2.5	10.7/1.2	13.3/1.2
CD8 ⁺	13.1/2.2	13.4/0.9	14.0/2.3	16.4/2.7	9.9/0.9	11.8/1.6
Naïve ^d	28.3/2.9	30.1/1.9	28.5/6.1	38.0/5.6	17.3/1.9	24.3/2.9
Effector ^e	10.8/1.5	8.9/0.8	9.3/2.1	9.3/2.6	3.1/0.5	5.6/0.8
Memory ^f	4.6/0.5	4.6/0.2	7.3/1.1	7.6/3.0	4.6/0.6	7.6/2.0

^a Cell numbers are × 10⁻⁶.

^b Values shown indicate the mean and SD for five mice analyzed in each group.

^c Gated as shown in Fig. 2B.

^d CD62L^{high}CD44^{low}

^e CD62L^{high}CD44^{high}

^f CD62L^{low}CD44^{high}

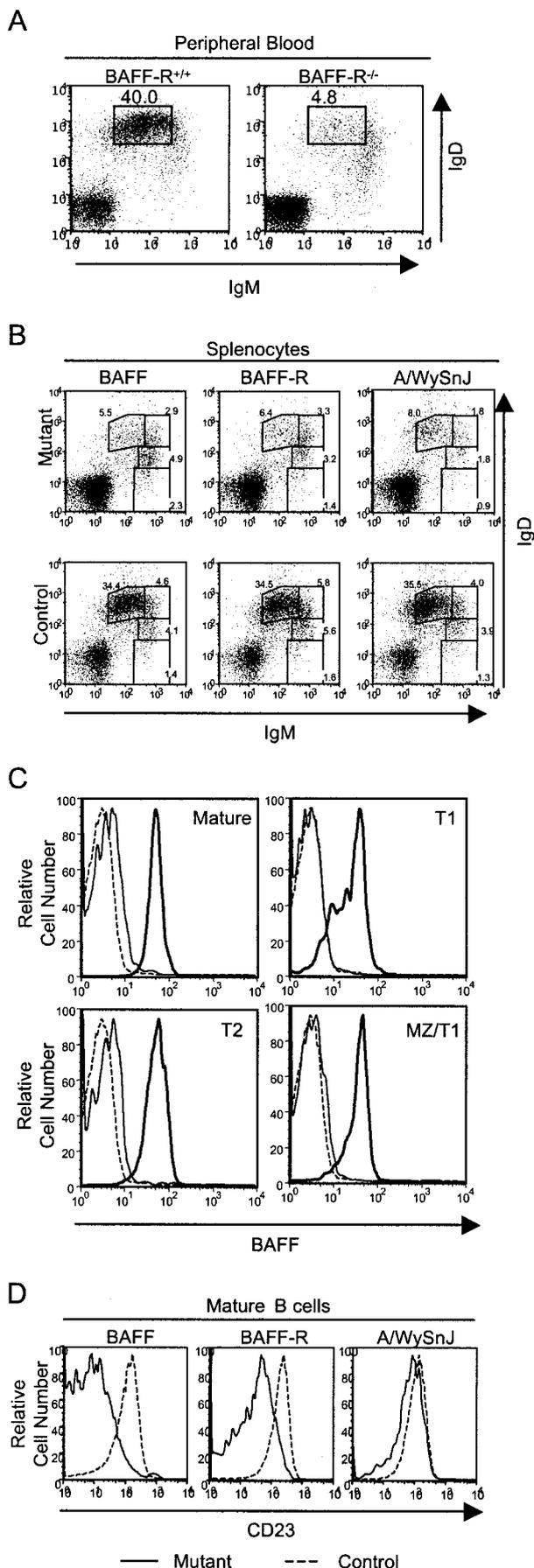


FIGURE 2. FACS staining of splenocytes and peripheral blood from BAFF and BAFF-R mutant mice. Plots (A and B) and histograms (C and D) are representative of four to five mice analyzed for each strain.

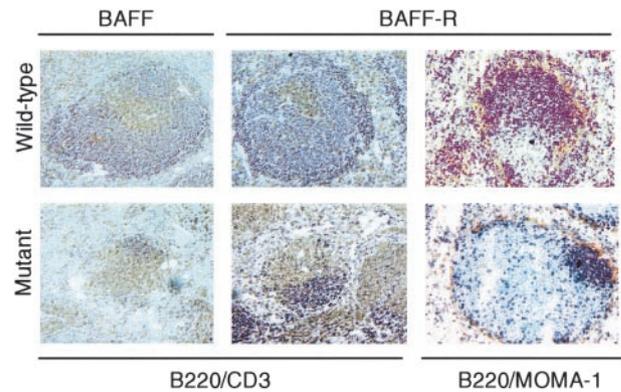


FIGURE 3. Immunohistochemical staining of splenic B cell follicles in BAFF and BAFF-R mutant mice. B220 (purple) and CD3 (brown) or MOMA-1 (brown) staining of spleen sections from indicated mutant (BAFF^{-/-}, BAFF-R^{-/-}) and respective WT control mice (magnification, $\times 400$). Images are representative of four mice analyzed for each strain.

the same expression level as cells from the parental A/J strain, (Fig. 2D, and Ref. 15). Interestingly, BAFF-R-deficient cells showed intermediate levels of CD23 (Fig. 2D).

BAFF-R is the primary receptor for BAFF-dependent B cell survival

The similar degree of B cell loss observed in BAFF^{-/-} and BAFF-R^{-/-} mice suggest BAFF-R is the primary receptor responsible for regulating the BAFF-dependent positive B cell survival signal. Consistent with this idea, we find that mice simultaneously lacking both of the other known BAFF receptors exhibit no B cell loss. In fact, TACI^{-/-};BCMA^{-/-} mice exhibit the same expansion of mature B cell populations as TACI^{-/-} mice (Table II) (27, 28). This result demonstrates that the lack of B cell loss in TACI^{-/-} mice and BCMA^{-/-} mice is not the result of redundancy between these two receptors, and that BAFF-R itself is sufficient to transmit the BAFF-dependent B cell survival signal in naive mice.

To confirm that TACI and BCMA do not mediate a BAFF-dependent survival signal, we assayed B cell populations in BAFF-R^{-/-} mice treated with recombinant hBAFF protein. Previously published reports demonstrated that BAFF-treatment of WT mice results in expansion of the B cell population (2, 38). If BAFF were capable of promoting survival through either TACI or BCMA, we would expect to see B cell expansion in hBAFF-treated BAFF-R^{-/-} mice. However, whereas a 72-h hBAFF treatment in BAFF^{-/-} mice expanded the total splenic B220⁺ population by 3-fold, no increase was observed in treated BAFF-R^{-/-} mice (our

D) are representative of four to five mice analyzed for each strain. A, Staining for indicated Ags on peripheral blood from BAFF-R^{-/-} and BAFF-R^{+/+} mice. Numbers within panels indicate percentage of scatter-gated lymphocytes that are recirculating mature B cells. B, Staining for indicated Ags on total splenocytes from mutant mice (*upper panels*) and parental strain controls (*lower panels*). Numbers within panels indicate percentage of scatter-gated lymphocytes that are mature (IgD^{high}IgM^{low}), T1 (IgD^{high}IgM^{high}), T2 (IgD^{high}IgM^{high}), and marginal zone/T1 (IgD^{int}IgM^{high}) B cells. C, FACS analysis of recombinant hBAFF binding to splenic B cells from BAFF-R^{-/-} (thin solid line) and BAFF-R^{+/+} (heavy solid line) mice. Indicated B cell subsets were gated as in B. Dashed line indicates FACS signal from a secondary Ab-staining control. D, CD23 surface expression on IgD^{high}IgM^{low} mature splenic B cells from mutant and parental control mice shown within each panel.

Table II. Splenic B cell numbers^a in TACI^{-/-}; BCMA^{-/-} mice^b

	Wild type	TACI ^{-/-}	TACI ^{-/-} ; BCMA ^{-/-}
Splenocytes	175.5/21.0	282.0/37.5	327.6/91.4
T cells (TCRβ ⁺)	35.0/5.2	33.6/5.2	40.7/12.2
B cells (B220 ⁺)	95.0/16.0	194.8/28.8	230.9/63.4
Mature B cells			
IgD ^{high} IgM ^{low}	66.7/11.2	139.3/21.6	162.8/40.7
Marginal	12.4/3.6	19.5/3.8	30.7/10.0
Zone ^c			

^a Cell numbers are ×10⁻⁶.

^b Values shown indicate the mean and SD for five mice analyzed in each group.

^c CD21^{high}CD23^{int}.

unpublished observations). Furthermore, hBAFF-treatment increased the total number of IgD^{high}IgM^{low} mature B cells by 5-fold in BAFF^{-/-} mice, but had no effect on this population in BAFF-R^{-/-} mice (Fig. 4). These observations demonstrate that TACI and BCMA do not function to support the B cell survival signal from either endogenous or exogenous BAFF, and thus support the model that BAFF-R is solely responsible for transmitting the BAFF-dependent survival signal in naive mice.

B cell loss in BAFF-R^{-/-} mice is a dominant phenotype

Mice lacking TACI exhibit increased numbers of peripheral B cells (27, 28). To test whether repression of TACI signaling could compensate for the loss of BAFF-R, we created double mutant mice lacking both TACI and BAFF-R. These animals are born at expected Mendelian frequencies and survive to adulthood (our unpublished observations). FACS analysis of splenocytes strongly suggests that the loss of B cells in BAFF-R^{-/-} mice is dominant to the increase in B cell number observed in TACI^{-/-} mice; the numbers of total and mature splenic B cells in the double mutant animals are essentially identical with that observed in BAFF-R^{-/-} mice (Table III). Thus, loss of TACI does not result in a survival signal capable of compensating for BAFF-R function.

The dominance of the BAFF-R^{-/-} phenotype over the TACI^{-/-} phenotype (in regard to numbers of splenic B cells) suggests that the positive survival signal from BAFF-R is stronger than the negative signal from TACI. Consistent with this possibility, we find that multiple Abs against BAFF-R yield a stronger FACS signal on splenic B cells than do Abs against TACI (Fig. 5, and our unpublished data). BAFF-R is expressed at high levels on both mature and transitional B cells, with the highest expression being on T2 and mature B cells (Fig. 5, and Ref. 15). Although some marginal zone and/or T1 cells do not express TACI, at least some cells in each of the transitional and mature B cell populations do express TACI, albeit at much lower levels than seen for BAFF-R.

Ab responses in BAFF-R^{-/-} mice significantly differ from those in BAFF^{-/-} mice

To assess B cell Ab production in BAFF-R^{-/-} mice, we measured both baseline serum Ig levels in naive mice, as well as Ab responses to specific Ags. These analyses were performed in parallel with BAFF^{-/-} and A/WySnJ mice to directly compare the effects of each mutation on Ab formation. Consistent with our previous report (9), Ig levels of IgM and all IgG subclasses were significantly reduced in naive BAFF^{-/-} mice as compared with WT control animals (Fig. 6A). Although the average serum levels of all Abs analyzed were also reduced in BAFF-R^{-/-} mice, these levels differed significantly from WT levels only for IgM and IgG1. Interestingly, IgM levels in BAFF-R^{-/-} mice were significantly higher than in BAFF^{-/-} mice (Fig. 6A, $p < 0.01$). In this analysis,

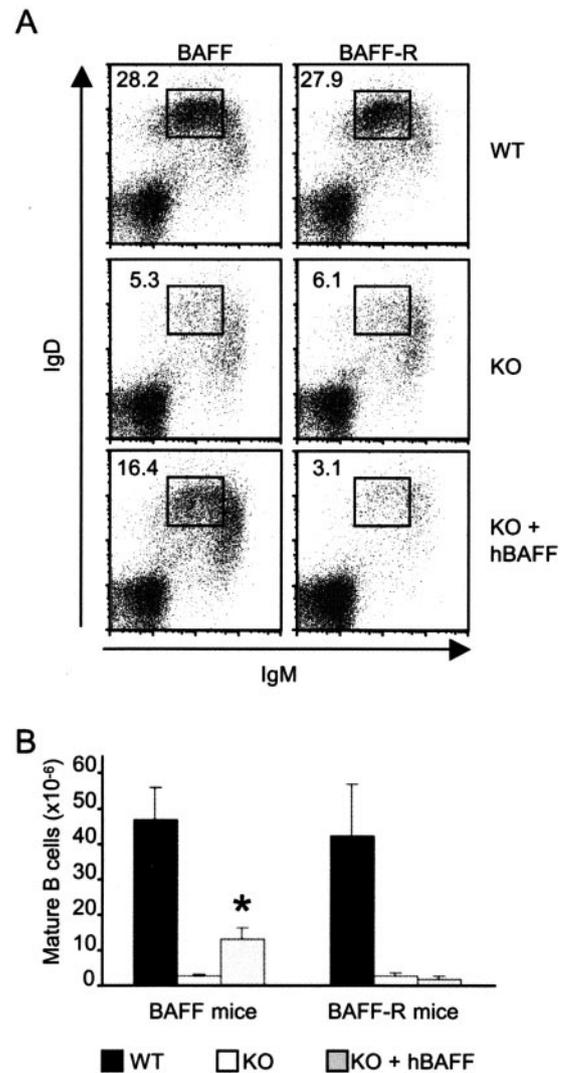


FIGURE 4. rBAFF treatment of BAFF^{-/-} and BAFF-R^{-/-} mice. *A*, IgD and IgM FACS staining of scatter-gated splenic lymphocytes from WT mice, PBS-treated gene-targeted mice (KO), and gene-targeted mice treated with recombinant hBAFF for 72 h (KO + hBAFF). Numbers within panels indicate percentage of scatter-gated lymphocytes that are mature B cells (IgD^{high}IgM^{low}). Plots shown are representative of five mice analyzed for each group. *B*, Absolute numbers of IgM^{low}IgD^{high} mature splenic B cells from indicated mice. Data bars indicate the mean value for five mice per group, and error bars indicate + 1 SD. *, $p < 0.001$, as compared with PBS-treated KO mice of the same genotype.

and all immunization studies described below, the ratio of Ab levels in A/WySnJ mice to A/J mice was equivalent to that between BAFF-R^{-/-} and BAFF-R^{+/+} mice.

Further differences between BAFF- and BAFF-R-null mice were revealed by analysis of Ag-specific Ab responses in mice immunized with TI-II Ags. We initially immunized mice with TNP-Ficoll, because A/WySnJ mice were previously shown to mount a normal titer IgM response to this Ag (21), and then measured TNP-reactive Ab levels up to 14 days after immunization. BAFF-R^{-/-} and A/WySnJ mice responded with Ag-specific IgM and IgG levels that were statistically indistinguishable from those in WT control mice (Fig. 6B, and our unpublished data). In contrast, BAFF^{-/-} mice exhibited severely attenuated production of anti-TNP Ab, particularly for IgM Abs (Fig. 6B, and Ref. 9).

Analysis of mice immunized with the Pneumovax vaccine confirmed the finding that TI-II Ab production is BAFF-dependent,

Table III. Splenic B cell numbers^a in BAFF-R^{-/-}; TACI^{-/-} mice^b

	Wild type	BAFF-R ^{-/-}	BAFF-R ^{-/-} ; TACI ^{-/-}
Splenocytes	112.7/13.8	63.9/9.3	70.6/20.6
B cells (B220 ⁺)	54.0/7.3	9.5/2.2	11.4/2.1
Mature B cells			
IgD ^{high} IgM ^{low}	39.9/4.0	3.5/1.5	5.0/1.5
IgD ^{high} CD24 ^{int}	36.1/5.7	2.0/0.4	3.4/1.5

^a Cell numbers are $\times 10^{-6}$.

^b Values shown indicate the mean and SD for five mice analyzed in each group.

but independent of BAFF-R. By comparison to WT control mice, animals lacking BAFF produced 7- to 10-fold less Ab specific for the Pneumovax Ags. In contrast, BAFF-R^{-/-} and A/WySnJ animals generated equally high levels of Ag-specific IgM as WT control mice (Fig. 6C, and our unpublished data). Thus, for immunizations with two different TI-II Ags, BAFF-R-mutant mice exhibited responses nearly identical with WT mice, whereas mice lacking BAFF exhibited severely reduced responses that differed significantly from those in WT animals.

Analysis of mice immunized with TD Ags revealed different Ag-specific IgM responses between BAFF and BAFF-R mutant animals. At days 7 and 14 following KLH immunization, BAFF-R^{-/-} mice exhibit levels of anti-KLH IgM that are statistically indistinguishable from that in WT control mice, whereas Ag-specific IgM levels in BAFF^{-/-} mice were reduced on average ~10-fold (Fig. 7A). Ag-specific IgM responses to SRBC immunizations were also less attenuated in BAFF-R^{-/-} mice than in BAFF^{-/-} mice ($p < 0.01$), although no difference was observed between these mice in response to CGG immunization (Fig. 7A). In contrast to IgM responses to KLH and SRBC, both primary and secondary IgG responses to KLH and CGG were similarly reduced in BAFF^{-/-} and BAFF-R^{-/-} mice as compared with respective WT controls (Fig. 7, B and C). Immunizations with multiple Ags thus clearly reveal differences between ligand- and receptor-deficient mice in their abilities to respond to TI-II Ags, and in IgM responses to at least two TD Ags.

Recently published work has demonstrated a requirement for BAFF in sustaining GCs formed in response to immunizations with SRBC and (4-hydroxy-3-nitrophenyl)acetyl (37, 39). To investigate the role of BAFF-R in mediating this signal, we assayed the kinetics of GC survival in BAFF-R^{-/-} and WT mice. Histological examination of spleens reveals that BAFF-R^{-/-} and WT mice exhibit a similar density of GCs 6 days after SRBC immunization. However, whereas control mice maintain, or increase, the number of GCs for least another 7 days, the number of GCs in BAFF-R^{-/-} mice decreases thereafter (Fig. 8). These data indicate that signaling through BAFF-R is required for the maintenance, but not the formation, of GCs in response to Ag.

MZB cells from BAFF-R^{-/-} mice fail to capture TNP-Ficoll

It has been proposed that the immune response to TI-II Ags uses both MZB cells and peritoneal B1 B cells (40–44). To investigate the function of MZB cells in the response of BAFF-R^{-/-} mice to TI-II Ags, we determined whether cells from the small MZB population in these mice could capture i.v.-injected TNP-Ficoll. Spleens were harvested 30 min after immunization, and cells stained with anti-TNP were analyzed by FACS to detect B cell populations with surface-bound Ag. Anti-TNP binding to cells in the IgM^{high}IgD^{int}-gated MZB/T1 population is shown in Fig. 9 (left panels). This analysis reveals that MZB cells from both BAFF^{-/-} and BAFF-R^{-/-} immunized mice exhibit the same lack of anti-TNP-staining as do unimmunized mice. Interestingly, al-

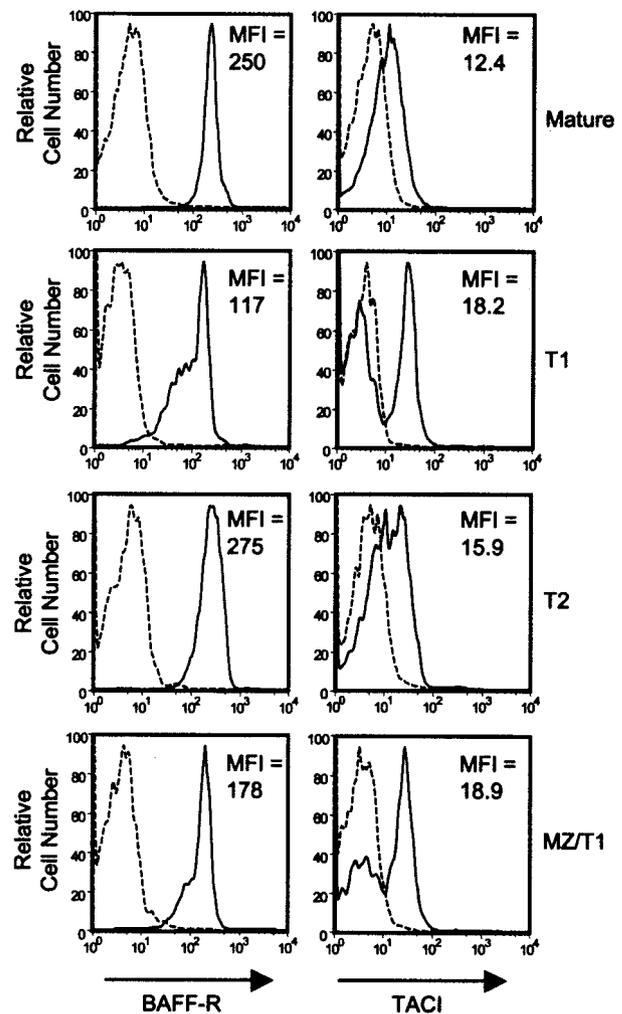


FIGURE 5. Expression of BAFF-R and TACI on splenic B cells. FACS analysis of anti-BAFF-R Ab and anti-TACI Ab staining of splenic B cell subsets gated with IgM/IgD as in Fig. 2B. The dashed line indicates staining with the appropriate isotype control Ab. Numbers within panels indicate mean fluorescence intensity (MFI) of anti-BAFF-R or anti-TACI staining for four mice.

though TACI^{-/-} mice fail to mount a normal immune response to TI-II Ags (28), MZB cells from TACI^{-/-} mice capture Ag nearly as efficiently as those from WT mice (Fig. 9, lower left panel). Consistent with published data that CD21 is required for complement-coated Ag capture by MZB cells (45), the efficiency of Ag capture for all three mutant strains correlates with the level of MZB CD21 expression (Fig. 9, right panel). These results indicate that the TI-II defect in TACI^{-/-} mice does not result from poor Ag capture by MZB cells, and furthermore that efficient Ag capture by MZB cells is not required for the normal anti-TNP IgM titer observed in immunized BAFF-R^{-/-} mice.

Preservation of peritoneal B1 cells in BAFF-R^{-/-} mice

The possibility that differences between peritoneal B cells in BAFF^{-/-} and BAFF-R^{-/-} mice contributes to their different TI-II Ag responses led us to compare these populations from the respective mutant mice. FACS analysis revealed that in BAFF-R^{-/-} mice, as well as BAFF^{-/-} mice (9, 22), B2 populations are dramatically reduced, but that B1 populations appear well preserved (Fig. 10A). Furthermore, both strains of mutant mice exhibit a

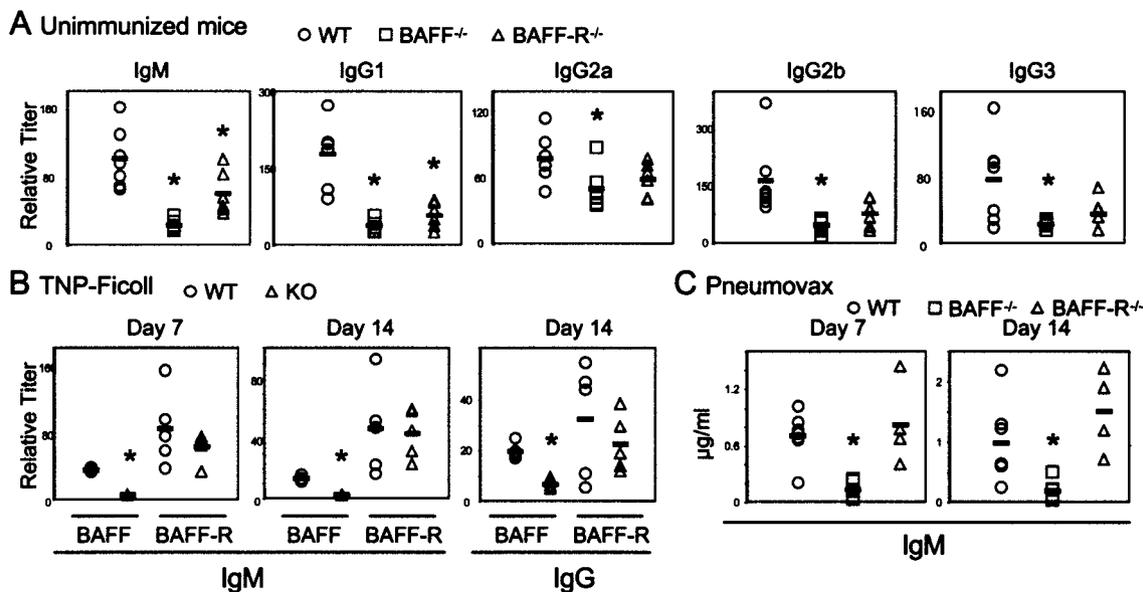


FIGURE 6. Ab production in immunized and naive $\text{BAFF}^{-/-}$ and $\text{BAFF-R}^{-/-}$ mice. **A**, ELISA analysis of total serum IgM and IgG subclasses in naive $\text{BAFF}^{-/-}$ and $\text{BAFF-R}^{-/-}$ mice. Data points indicate relative Ab titer for individual mice, and horizontal bars represent mean values for each group. Naive $\text{BAFF-R}^{+/+}$ mice (WT) serve as controls for both groups of mutants. The data are representative of three separate experiments. *, $p < 0.05$, as compared with WT control mice. **B**, Ag-specific Ab production in TNP-Ficoll-immunized BAFF and BAFF-R mutant mice. ELISA analysis of sera from mice that are WT or gene-targeted (KO) at the indicated gene locus (BAFF or BAFF-R) at indicated days following immunization. Data points indicate relative Ab titer of IgM (left and center panels) or IgG (right panel) for individual mice, and horizontal bars represent mean values for each group. Differences in Ab responses between the two WT groups may reflect the different number of C57BL/6 backcrosses between the BAFF and BAFF-R mice. The data are representative of two separate experiments. *, $p < 0.001$, as compared with respective WT control mice. **C**, Ag-specific IgM Ab production in Pneumovax-immunized BAFF and BAFF-R mutant mice. ELISA analysis of sera from $\text{BAFF}^{-/-}$, $\text{BAFF-R}^{-/-}$, and $\text{BAFF-R}^{+/+}$ (WT) mice at indicated days following immunization. Data points indicate serum Ab concentration for individual mice, and horizontal bars represent mean values for each group. The data are representative of two separate experiments. *, $p < 0.01$, as compared with WT control mice.

similar ratio of B1a to B1b cells (Fig. 10A). Thus, the discrepancy in TI-II responses between $\text{BAFF}^{-/-}$ and $\text{BAFF-R}^{-/-}$ mice does not appear to result from gross differences in the peritoneal B cell populations. However, it is possible that a more subtle defect in B1 cell function contributes to the reduced TI-II immune response observed in $\text{BAFF}^{-/-}$ and $\text{TACI}^{-/-}$ mice. As a first step in investigating the potential role of BAFF-R and TACI in peritoneal B1 cell function, we examined the cell surface expression of both receptors. Anti-TACI and anti-BAFF-R staining of peritoneal B1 cells revealed that both receptors are expressed on these cells (Fig. 10B). However, whereas BAFF-R is expressed at similar levels on both B1 and B2 cells, TACI expression is notably higher on B1 cells, suggesting that TACI may have a B1 cell-specific function.

Discussion

In this paper, we have characterized mice completely lacking the BAFF receptor BAFF-R. Comparison of these mice to animals lacking BAFF indicates that both exhibit a similar loss of peripheral B cell subsets, but that there are significant differences in Ab responses. These results provide important insights into which receptors are used to mediate various BAFF-dependent signals to B cells. Additionally, comparison of A/WySnJ mice to $\text{BAFF-R}^{-/-}$ mice suggests that the partial mutation of BAFF-R in A/WySnJ mice is sufficient to prevent the BAFF-signaling required for efficient B cell survival, but that it is not absolutely equivalent to a complete null mutation.

The similarly low number of mature B cells in BAFF and BAFF-R mutant mice strongly supports the model that BAFF-R is the sole receptor mediating the BAFF-dependent survival signal. Consistent with the idea that neither TACI nor BCMA are respon-

sible for mediating this signal, both $\text{BAFF-R}^{-/-}$ and A/WySnJ mice fail to respond to treatment with exogenous BAFF protein (38). Importantly, we also show that mice simultaneously lacking both TACI and BCMA exhibit no loss of mature B cells. This result demonstrates that the lack of B cell loss in the TACI and BCMA single mutant animals (24, 27, 28) does not merely reflect redundancy between these receptors in regard to B cell survival. However, consistent with the findings of others, we find that TACI is indeed expressed on the cell surface of at least some subsets of transitional and mature B cells (46). TACI expression is low compared with that of BAFF-R, consistent with our observation that B cells lacking BAFF-R fail to exhibit robust binding of BAFF.

Nonetheless, these findings do not rule out the possibility that receptors other than BAFF-R mediate some BAFF-dependent functions. Most obviously, both BAFF and TACI are required for TI-II Ab responses (9, 28) that we show are not dependent on BAFF-R. Additionally, BCMA has been implicated in the BAFF-dependent survival of mouse plasma cells and human plasmablasts (25, 26). Differences in GC maintenance between immunized $\text{BAFF}^{-/-}$ and $\text{BAFF-R}^{-/-}$ mice provide additional evidence that some BAFF-dependent functions are not totally dependent on BAFF-R. Both mutant strains generate normal splenic GC density in response to immunization, and both exhibit more rapid loss of GCs than in WT animals (37). However, comparison of data previously published with that in this report reveals the lack of BAFF has a more deleterious effect on GC maintenance than does lack of BAFF-R (37). Finally, the receptor responsible for BAFF-dependent class-switching activity has not yet been identified, leaving open the possibility that a receptor other than BAFF-R may mediate this activity (47).

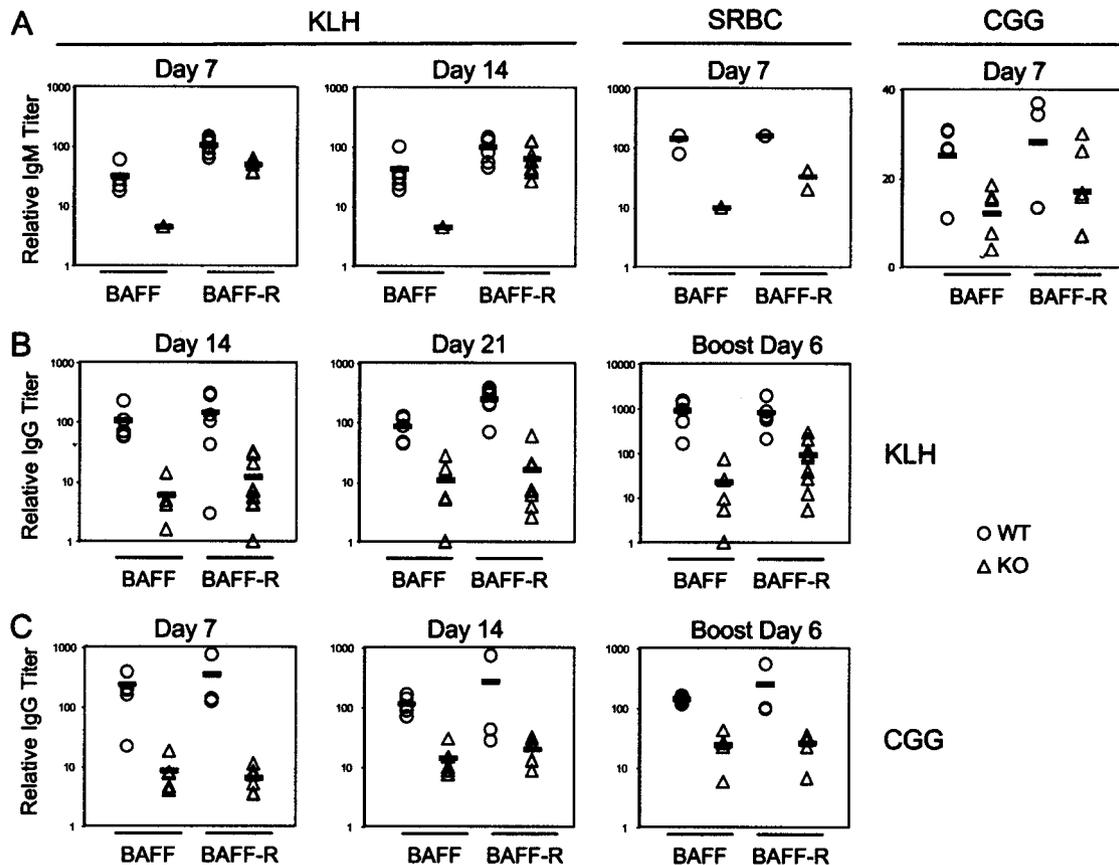


FIGURE 7. Ag-specific Ab production in KLH-immunized BAFF and BAFF-R mutant mice. ELISA analysis of sera from mutant mice (BAFF^{-/-} and BAFF-R^{-/-}; △) and respective WT control mice (BAFF^{+/+} and BAFF-R^{+/+}; ○) immunized with indicated Ag (KLH, CGG, or SRBC) at indicated days following primary or secondary (Boost) immunization. Data points indicate relative Ab titer of IgM (A) or IgG (B and C) for individual mice, and horizontal bars represent mean values for each group. Ag-specific IgM levels for KLH-immunized BAFF^{-/-} mice are shown as the lowest value in the linear range of our assay, as values for these mice were too low to be accurately quantified by our assay. $p \leq 0.02$, between WT and mutant mice for all analyses except for IgM production in KLH-immunized BAFF-R^{-/-} mice.

The opposing functions of BAFF-R and TACI in regard to B cell survival led us to investigate the epistasis of mutations in these genes. We found that mice simultaneously lacking both genes exhibit the same reduction of mature B cells as observed in BAFF-R^{-/-} mice. One possible explanation of this result is that TACI suppresses B cell survival only after a certain developmental stage and that this stage is never achieved in the absence of BAFF-R signaling. Alternatively, BAFF-R^{-/-} mice may have too few B cells to activate pathways that negatively regulate B cell homeostasis, and thus the presence or absence of a negative regulator such as TACI would have no effect on B cell numbers.

We also compared BAFF-R^{-/-} mice to A/WySnJ animals. Because the BAFF-R mutation in A/WySnJ mice leaves over 95% of the protein intact and includes novel carboxyl-terminal residues (13), it has been unclear whether this mutation is equivalent to a null mutation of BAFF-R. Although comparisons between BAFF-R^{-/-} and A/WySnJ mice must be undertaken with caution due to differences in strain background, we observe that both mutations result in a similar loss of mature B cells (91 and 87%, respectively). Although this suggests that the BAFF-R mutation in A/WySnJ mice is sufficient to completely prevent transmission of the BAFF-dependent survival signal, analysis of B cell surface CD23 expression indicates a phenotypic difference between BAFF-R^{-/-} and A/WySnJ mice. The minimal consequence of the A/WySnJ mutation on CD23 expression is intriguing in light of the observation that BAFF is required for CD23 expression (15). Al-

though we cannot rule out the possibility that strain differences between the A/WySnJ and gene-targeted mice contribute to the differential CD23 expression, these data suggest either that the BAFF-dependent regulation of CD23 expression is not dependent on BAFF-R, or that the A/WySnJ mutation does not completely nullify BAFF-R signaling. The intermediate level of B cell CD23 expression in BAFF-R^{-/-} mice (i.e., between that of BAFF^{-/-} and A/WySnJ animals) supports both possibilities, suggesting

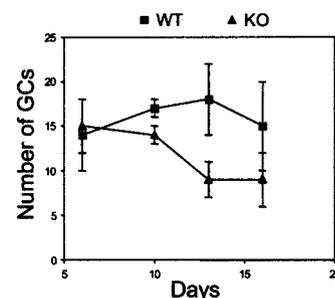


FIGURE 8. Kinetics of GC maintenance in BAFF-R^{-/-} mice. Mean number of GCs per $\times 10$ microscope field of a spleen from a WT or BAFF-R^{-/-} (KO) mouse at indicated days following immunization with sheep RBC. Mean values are determined from analysis of three mice of each strain at each time point and error bars represent ± 1 SD.

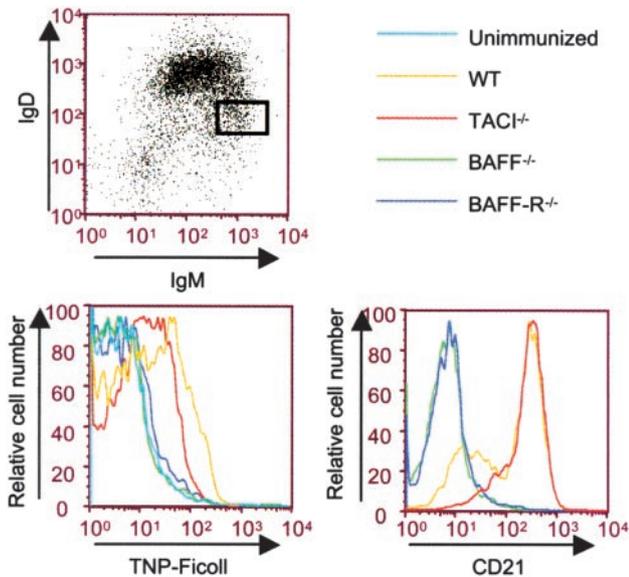


FIGURE 9. Analysis of T-independent Ag capture by MZB cells in BAFF-pathway mutant mice. TNP-Ficoll captured on IgD^{int}IgM^{high} marginal zone/T1 B cells (box, upper panel) in unimmunized WT, immunized WT, and immunized indicated mutant mice was detected by FACS-staining with anti-TNP Ab (lower left panel). CD21 surface expression on the MZB cell populations in the same mice is shown in the lower right panel. Data shown are representative of two mice analyzed per group.

BAFF may regulate CD23 expression through both BAFF-R and at least one other receptor.

Although all three mutant mice analyzed in this study share a similar phenotype in regard to mature B cell survival, previous analyses have suggested differential Ag-specific Ab responses between BAFF^{-/-} and A/WySnJ mice. Whereas Ag-specific IgM and IgG production is very reduced for both TD and TI-II Ags in BAFF^{-/-} mice (9, 22), the IgM responses to both the TD Ag KLH and the TI-II Ag TNP-Ficoll appear normal in A/WySnJ mice (21). Furthermore, BAFF^{-/-} mice exhibit a more rapid loss of GCs than do A/WySnJ mice (37). However, it has been impossible to determine whether these differences reflected a difference between BAFF and BAFF-R function, or merely the partial nature of the BAFF-R mutation in A/WySnJ animals. Our parallel analyses of null mutation mice allow direct comparison of the effects of complete loss of BAFF vs BAFF-R. Although both strains were on a mixed 129/Sv-C57BL/6 background, which contributes to non-BAFF or BAFF-R locus variation between the two mutants and their respective WT controls, our data indicate that BAFF-R is indeed dispensable for at least some TI-II Ag responses in mice. We also demonstrate that at least part of the BAFF-dependence for both IgM responses to TD-Ags and the maintenance of GCs do not require BAFF-R. These results provide definitive evidence for BAFF-dependent functions that are clearly not dependent on BAFF-R. The fact that BAFF^{-/-} and BAFF-R^{-/-} mice have similar numbers of B cells indicates that the TI-II defect in BAFF^{-/-} mice is not merely an indirect effect resulting from the relative absence of mature B cell populations. The BAFF requirement for TI-II responses may reflect the existence of a BAFF-dependent B cell subpopulation involved in the response, or possibly a BAFF signal directly required for Ab production. The most obvious receptor candidate for mediating these putative signals is TACI, as TACI^{-/-} mice exhibit a TI-II immune response defect similar to that observed in BAFF^{-/-} mice (28).

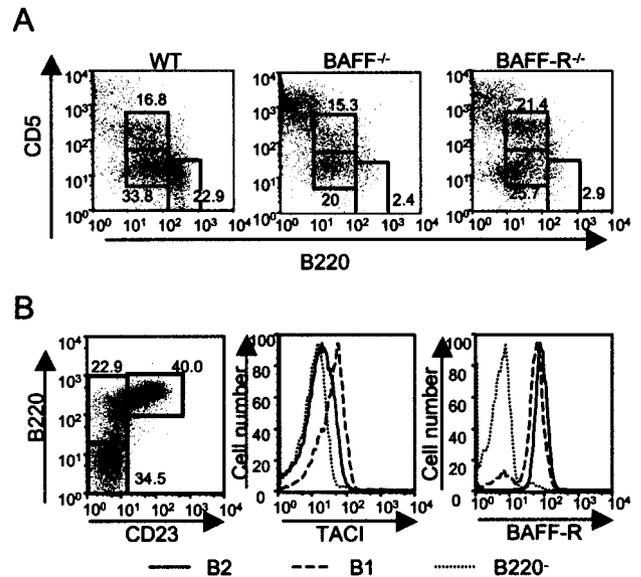


FIGURE 10. Peritoneal B cells in BAFF^{-/-} and BAFF-R^{-/-} mice and cell surface expression of BAFF receptors. *A*, FACS staining for indicated cell surface Ags on peritoneal lymphocytes harvested from WT and indicated mutant mice. Numbers within each panel indicated percentage of scatter-gated lymphocytes that are B2 cells (right box), B1a cells (upper left box), or B1b cells (lower left box). *B*, Peritoneal lymphocyte cell surface expression of TACI and BAFF-R. FACS-staining for TACI (center panel) and BAFF-R (right panel) expression on WT lymphocyte populations as defined by B220 and CD23 expression (left panel). Numbers within the left panel indicate percentage of scatter-gated lymphocytes that are B2 cells (right box), B1 cells (upper left box), and B220⁻ cells (lower left box). Cell numbers of TACI- and BAFF-R-stained populations (center and right panels) are normalized within each panel. FACS staining was performed on two separate occasions, each time with two to five mice per group.

How BAFF-R^{-/-} animals generate high titers of TI-II Ag Abs with such low numbers of mature B cells is not clear. One possibility is that a subpopulation of B cells responsible for this response is maintained at sufficient numbers despite the severe reduction in the global population of mature B cells. TI-II immune responses are believed to be mediated at least in part by MZB cells (41, 42). This model is supported by the observation of a TI-II defect in Pyk-2^{-/-} mice, which appear to be normal for all B lymphocyte populations except for a dramatic loss of MZB cells (43). However, our results indicate that loss of most MZB cells in itself does not prevent high titer IgG and IgM responses to at least some TI-II Ags. As a first order investigation into potential differences between MZB cells in BAFF-R- and BAFF-null mice, we assayed the Ag-binding capacity of these cells in each strain. Our finding that MZB cells from both mutant strains exhibit poor Ag binding suggests that differences in Ag capture by this population cannot explain the observed immune response differences. However, we cannot rule out the possibility that capture by marginal zone macrophages may be sufficient for efficient presentation to MZB cells, and that the different TI-II responses between BAFF^{-/-} and BAFF-R^{-/-} mice may be caused, at least in part, by differences in this capture or presentation (48, 49). Nevertheless, our results indicate that efficient Ag capture directly by MZB cells is not required for a robust response.

Evidence also exists for B1 cell function in the TI-II response (40, 42, 44), however, the role of these cells is more controversial, especially for the response against haptenated TI-II Ags such as

TNP-Ficoll (50, 51). Although previous reports have shown no gross loss of peritoneal B1 cells in BAFF- and TACI-null mice (9, 22, 28), these cells may have defects that contribute to the poor TI-II response. The possibility that the function of these cells is BAFF-R-independent simply because they normally do not express the gene was nullified by our finding that it is indeed expressed on peritoneal B cells. However, we do observe TACI expression on peritoneal B1 cells, consistent with the idea that BAFF-dependent TACI signaling in these cells mediates TI-II responses in normal mice. Significant further effort will clearly be required to determine the BAFF-dependent cells and pathways responsible for TI-II immune responses.

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References

- Schneider, P., F. MacKay, V. Steiner, K. Hofmann, J. L. Bodmer, N. Holler, C. Ambrose, P. Lawton, S. Bixler, H. Acha-Orbea, et al. 1999. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J. Exp. Med.* 189:1747.
- Moore, P. A., O. Belvedere, A. Orr, K. Pieri, D. W. LaFleur, P. Feng, D. Soppet, M. Charters, R. Gentz, D. Parmelee, et al. 1999. BLYS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* 285:260.
- Mukhopadhyay, A., J. Ni, Y. Zhai, G. L. Yu, and B. B. Aggarwal. 1999. Identification and characterization of a novel cytokine, THANK, a TNF homologue that activates apoptosis, nuclear factor- κ B, and c-Jun NH2-terminal kinase. *J. Biol. Chem.* 274:15978.
- Shu, H. B., W. H. Hu, and H. Johnson. 1999. TALL-1 is a novel member of the TNF family that is down-regulated by mitogens. *J. Leukocyte Biol.* 65:680.
- Gross, J. A., J. Johnston, S. Mudri, R. Enselman, S. R. Dillon, K. Madden, W. Xu, J. Parrish-Novak, D. Foster, C. Lofton-Day, et al. 2000. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* 404:995.
- Batten, M., J. Groom, T. G. Cachero, F. Qian, P. Schneider, J. Tschopp, J. L. Browning, and F. Mackay. 2000. BAFF mediates survival of peripheral immature B lymphocytes. *J. Exp. Med.* 192:1453.
- Rolink, A. G., J. Tschopp, P. Schneider, and F. Melchers. 2002. BAFF is a survival and maturation factor for mouse B cells. *Eur. J. Immunol.* 32:2004.
- Hsu, B. L., S. M. Harless, R. C. Lindsley, D. M. Hilbert, and M. P. Cancro. 2002. Cutting edge: BLYS enables survival of transitional and mature B cells through distinct mediators. *J. Immunol.* 168:5993.
- Schiemann, B., J. L. Gommerman, K. Vora, T. G. Cachero, S. Shulga-Morskaya, M. Dobles, E. Frew, and M. L. Scott. 2001. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 293:2111.
- Khare, S. D., I. Sarosi, X. Z. Xia, S. McCabe, K. Miner, I. Solovyev, N. Hawkins, M. Kelley, D. Chang, G. Van, et al. 2000. Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. *Proc. Natl. Acad. Sci. USA* 97:3370.
- Thompson, J. S., P. Schneider, S. L. Kalled, L. Wang, E. A. Lefevre, T. G. Cachero, F. MacKay, S. A. Bixler, M. Zafari, Z. Y. Liu, et al. 2000. BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population. *J. Exp. Med.* 192:129.
- Kayagaki, N., M. Yan, D. Seshasayee, H. Wang, W. Lee, D. M. French, I. S. Grewal, A. G. Cochran, N. C. Gordon, J. Yin, et al. BAFF/BLYS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF- κ B2. *Immunity* 17:515.
- Yan, M., Brady, J. R., Chan, B., Lee, W. P., Hsu, B., Harless, S., Cancro, M., Grewal, I. S., and Dixit, V. M. 2001. Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. *Curr. Biol.* 11:1547.
- von Bulow, G. U., and R. J. Bram. 1997. NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. *Science* 278:138.
- Gorelik, L., A. H. Cutler, G. Thill, S. D. Miklasz, D. E. Shea, C. Ambrose, S. A. Bixler, L. Su, M. L. Scott, and S. L. Kalled. 2004. Cutting edge: BAFF regulates CD21/35 and CD23 expression independent of its B cell survival function. *J. Immunol.* 172:762.
- Kelly, K., E. Manos, G. Jensen, L. Nadauld, and D. A. Jones. 2000. APRIL/TRDL-1, a tumor necrosis factor-like ligand, stimulates cell death. *Cancer Res.* 60:1021.
- Thompson, J. S., S. A. Bixler, F. Qian, K. Vora, M. L. Scott, T. G. Cachero, C. Hession, P. Schneider, I. D. Sizing, C. Mullen, et al. 2001. BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF. *Science* 293:2108.
- Yu, G., T. Boone, J. Delaney, N. Hawkins, M. Kelley, M. Ramakrishnan, S. McCabe, W. R. Qiu, M. Kornuc, X. Z. Xia, et al. 2000. APRIL and TALL-1 and receptors BCMA and TACI: system for regulating humoral immunity. *Nat. Immunol.* 1:252.
- Lentz, V. M., M. P. Cancro, F. E. Nashold, and C. E. Hayes. 1996. Bcmd governs recruitment of new B cells into the stable peripheral B cell pool in the A/WySnJ mouse. *J. Immunol.* 157:598.
- Miller, D. J., and C. E. Hayes. 1991. Phenotypic and genetic characterization of a unique B lymphocyte deficiency in strain A/WySnJ mice. *Eur. J. Immunol.* 21:1123.
- Miller, D. J., K. D. Hanson, J. A. Carman, and C. E. Hayes. 1992. A single autosomal gene defect severely limits IgG but not IgM responses in B lymphocyte-deficient A/WySnJ mice. *Eur. J. Immunol.* 22:373.
- Gross, J. A., S. R. Dillon, S. Mudri, J. Johnston, A. Littau, R. Roque, M. Rixon, O. Schou, K. P. Foley, H. Haugen, et al. 2001. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLYS. *Immunity* 15:289.
- Gras, M. P., Y. Laabi, G. Linares-Cruz, M. O. Blondel, J. P. Rigaut, J. C. Brouet, G. Leca, R. Haguenaer-Tsapis, and A. Tsapis. 1995. BcMAP: an integral membrane protein in the Golgi apparatus of human mature B lymphocytes. *Int. Immunol.* 7:1093.
- Xu, S., and K. P. Lam. 2001. B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensable for humoral immune responses. *Mol. Cell. Biol.* 21:4067.
- O'Connor, B. P., V. S. Raman, L. D. Erickson, W. J. Cook, L. K. Weaver, C. Ahonen, L. L. Lin, G. T. Mantchev, R. J. Bram, and R. J. Noelle. 2004. BCMA is essential for the survival of long-lived bone marrow plasma cells. *J. Exp. Med.* 199:91.
- Avery, D. T., S. L. Kalled, J. I. Ellyard, C. Ambrose, S. A. Bixler, M. Thien, R. Brink, F. Mackay, P. D. Hodgkin, and S. G. Tangye. 2003. BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. *J. Clin. Invest.* 112:286.
- Yan, M., H. Wang, B. Chan, M. Roose-Girma, S. Erickson, T. Baker, D. Tumas, I. S. Grewal, and V. M. Dixit. 2001. Activation and accumulation of B cells in TACI-deficient mice. *Nat. Immunol.* 2:638.
- von Bulow, G. U., J. M. van Deursen, and R. J. Bram. 2001. Regulation of the T-independent humoral response by TACI. *Immunity* 14:573.
- Rennert, P., P. Schneider, T. G. Cachero, J. Thompson, L. Trabach, S. Hertig, N. Holler, F. Qian, C. Mullen, K. Strauch, J. L. Browning, C. Ambrose, and J. Tschopp. 2000. A soluble form of B cell maturation antigen, a receptor for the tumor necrosis factor family member APRIL, inhibits tumor cell growth. *J. Exp. Med.* 192:1677.
- Wu, Y., D. Bressette, J. A. Carrell, T. Kaufman, P. Feng, K. Taylor, Y. Gan, Y. H. Cho, A. D. Garcia, E. Gollatz, et al. 2000. Tumor necrosis factor (TNF) receptor superfamily member TACI is a high affinity receptor for TNF family members APRIL and BLYS. *J. Biol. Chem.* 275:35478.
- Marsters, S. A., M. Yan, R. M. Pitti, P. E. Haas, V. M. Dixit, and A. Ashkenazi. 2000. Interaction of the TNF homologues BLYS and APRIL with the TNF receptor homologues BCMA and TACI. *Curr. Biol.* 10:785.
- Zhang, Y., J. P. Muylers, G. Testa, and A. F. Stewart. 2000. DNA cloning by homologous recombination in *Escherichia coli*. *Nat. Biotechnol.* 18:1314.
- Capecchi, M. R. 1989. Altering the genome by homologous recombination. *Science* 244:1288.
- Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky. 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J. Immunol.* 123:1548.
- Kennett, R. H., T. J. McKearn, and K. B. Bechtol. 1982. *Monoclonal Antibodies: A New Dimension in Biological Analyses*. Plenum Press, New York.
- Vora, K. A., K. M. Tumas-Brundage, V. M. Lentz, A. Cranston, R. Fishel, and T. Manser. 1999. Severe attenuation of the B cell immune response in Msh2-deficient mice. *J. Exp. Med.* 189:471.
- Rahman, Z. S., S. P. Rao, S. L. Kalled, and T. Manser. 2003. Normal induction but attenuated progression of germinal center responses in BAFF and BAFF-R signaling-deficient mice. *J. Exp. Med.* 198:1157.
- Harless, S. M., V. M. Lentz, A. P. Sah, B. L. Hsu, K. Clise-Dwyer, D. M. Hilbert, C. E. Hayes, and M. P. Cancro. 2001. Competition for BLYS-mediated signaling through Bcmd/BR3 regulates peripheral B lymphocyte numbers. *Curr. Biol.* 11:1986.
- Vora, K. A., L. C. Wang, S. P. Rao, Z. Y. Liu, G. R. Majeau, A. H. Cutler, P. S. Hochman, M. L. Scott, and S. L. Kalled. 2003. Germinal centers formed in the absence of B cell-activating factor belonging to the TNF family exhibit impaired maturation and function. *J. Immunol.* 171:547.
- Scher, I. 1982. The CBA/N mouse strain: an experimental model illustrating the influence of the X-chromosome on immunity. *Adv. Immunol.* 33:1.
- Balazs, M., F. Martin, T. Zhou, and J. Kearney. 2002. Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity* 17:341.

42. Martin, F., A. M. Oliver, and J. F. Kearney. 2001. Marginal zone and B1 B cells unite in the early response against T-independent blood-borne particulate antigens. *Immunity* 14:617.
43. Guinamard, R., M. Okigaki, J. Schlessinger, and J. V. Ravetch. 2000. Absence of marginal zone B cells in *Myk-2*-deficient mice defines their role in the humoral response. *Nat. Immunol.* 1:31.
44. Prior, L., S. Pierson, R. T. Woodland, and J. Riggs. 1994. Rapid restoration of B-cell function in XID mice by intravenous transfer of peritoneal cavity B cells. *Immunology* 83:180.
45. Haas, K. M., M. Hasegawa, D. A. Steeber, J. C. Poe, M. D. Zabel, C. B. Bock, D. R. Karp, D. E. Briles, J. H. Weis, and T. F. Tedder. 2002. Complement receptors CD21/35 link innate and protective immunity during *Streptococcus pneumoniae* infection by regulating IgG3 antibody responses. *Immunity* 17:713.
46. Ng, L. G., A. Sutherland, R. Newton, F. Qian, T. G. Cachero, M. Scott, J. S. Thompson, J. Wheway, T. Chtanova, J. Groom, et al. B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating B and T cells. *J. Immunol.* 173:807.
47. Litinskiy, M. B., B. Nardelli, D. M. Hilbert, B. He, A. Schaffer, P. Casali, and A. Cerutti. 2002. DCs induce CD40-independent immunoglobulin class switching through BlyS and APRIL. *Nat. Immunol.* 3:822.
48. Humphrey, J. H., and D. Grennan. 1981. Different macrophage populations distinguished by means of fluorescent polysaccharides: recognition and properties of marginal-zone macrophages. *Eur. J. Immunol.* 11:221.
49. Aichele, P., J. Zinke, L. Grode, R. A. Schwendener, S. H. Kaufmann, and P. Seiler. 2003. Macrophages of the splenic marginal zone are essential for trapping of blood-borne particulate antigen but dispensable for induction of specific T cell responses. *J. Immunol.* 171:1148.
50. Berland, R., and H. H. Wortis. 2002. Origins and functions of B-1 cells with notes on the role of CD5. *Annu. Rev. Immunol.* 20:253.
51. Mond, J. J., A. Lees, and C. M. Snapper. 1995. T cell-independent antigens type 2. *Annu. Rev. Immunol.* 13:655.