

## **Maturation of Marginal Zone and Follicular B Cells Requires B Cell Activating Factor of the Tumor Necrosis Factor Family and Is Independent of B Cell Maturation Antigen**

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### **Abstract**

B cells undergo a complex series of maturation and selection steps in the bone marrow and spleen during differentiation into mature immune effector cells. The tumor necrosis factor (TNF) family member B cell activating factor of the TNF family (BAFF) (BLyS/TALL-1) plays an important role in B cell homeostasis. BAFF and its close homologue a proliferation-inducing ligand (APRIL) have both been shown to interact with at least two receptors, B cell maturation antigen (BCMA) and transmembrane activator and cyclophilin ligand interactor (TACI), however their relative contribution in transducing BAFF signals *in vivo* remains unclear. To functionally inactivate both BAFF and APRIL, mice transgenic for a soluble form of TACI were generated. They display a developmental block of B cell maturation in the periphery, leading to a severe depletion of marginal zone and follicular B2 B cells, but not of peritoneal B1 B cells. In contrast, mice transgenic for a soluble form of BCMA, which binds APRIL, have no detectable B cell phenotype. This demonstrates a crucial role for BAFF in B cell maturation and strongly suggests that it signals via a BCMA-independent pathway and in an APRIL-dispensable way.

Key words: TNF • transgene • receptor • ligand • lymphocyte

### **Introduction**

In adult mice, B cell precursors in the bone marrow rearrange their Ig genes to yield immature B cells, which subsequently undergo negative selection steps before differentiation into mature B cells through at least two successive transitional stages (T1 and T2). Newly formed B cells leaving the bone marrow have a short half-life and remain largely confined to blood circulation and defined areas of the spleen, as they lack adhesion molecules necessary for extravasation in peripheral lymphoid organs such as lymph nodes. Only a fraction of these newly formed B cells eventually enters the pool of long-lived, recirculating follicular B cells populating lymphoid organs, which are referred to as B2 B cells. Upon antigen encounter and T cell help, follicular B cells undergo expansion, affinity maturation, isotype

switching, and differentiation into memory cells or antibody-secreting plasma cells (1–3). In contrast, B1 B cells represent a self-renewing population of the peritoneal and pleural cavities, which mainly produce natural antibodies against bacterial antigens in a T cell-independent manner. These cells are believed to give rise to IgA-secreting plasmocytes in the intestine and to play an important role in the innate immune response (1, 4, 5). Moreover, distinct extrafollicular naive and memory B2 B cell populations reside in the marginal zone of the spleen at the boundary between red and white pulp and participate in early T cell-independent antibody responses against blood-borne particulate antigens (6).

The signals promoting survival and differentiation of newly formed B cells are not well understood. A B cell activating factor of the TNF family (BAFF) (BLyS, TALL-1, zTNF4), is a ligand that is clearly involved in the regulation of B cell homeostasis, as BAFF transgenic mice display severe B cell hyperplasia, hyperglobulinemia, and autoimmune manifestations (7–9). BAFF has been demonstrated

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to interact with two receptors, B cell maturation antigen (BCMA) and transmembrane activator and cyclophilin ligand interactor (TACI), both of which also bind to a second ligand, a proliferation-inducing ligand (APRIL; references 7, 10, and 11). To gain insight into the role of individual proteins within this complex system, targeted inactivation of selected receptors or ligands is a valuable approach. BCMA-deficient mice have been recently reported to be normal (12), whereas TACI-deficient mice display moderate B cell hyperplasia with a partial defect in T cell-independent humoral responses, but are otherwise largely normal (13, 14). In this study, we have generated BAFF- and APRIL-deficient mice by overexpressing soluble forms of their receptors. We describe a severe B lymphopenia in mice lacking functional BAFF, which results from a blockade of B cell maturation in the periphery.

## Materials and Methods

**Transgenic Mice.** A transgenic vector containing human  $\alpha 1$  antitrypsin promoter (nucleotides -6,602 to -5,328 as HindIII/XbaI fragment) and the 3' end of rabbit  $\beta$ -globin gene (nucleotides 421-1,592 as XbaI/XhoI fragment, comprising the end of exon 2, intron 2, exon 3, and the poly A addition signal) was provided by K. Araki and M. Araki, Kumamoto University, Kumamoto, Japan (15). A cassette encoding the signal peptide of human Ig G heavy chain, the extracellular domain of human TACI (amino acids 2-118) or murine BCMA (amino acids 1-46) and the Fc domain of human Ig G1 was inserted in the transgenic vector using the EcoRI site of  $\beta$ -globin exon 3. Transgenic mice were generated by microinjection of the HindIII/XhoI excised construct into fertilized (C57BL/6  $\times$  DBA/2) F2 oocytes, and screened by PCR using oligonucleotides 5' CAGACCCCA-CATAAAGAGCCTAC3' and 5' CCGATGGAAAAATGG-AGC3'. Expression of the transgene was determined by Western blot analysis of 0.5  $\mu$ l of serum using horseradish peroxidase-coupled goat anti-human IgG.

**ELISA Assays.** Receptor-ligand ELISA. ELISA plates were coated overnight with mouse anti-human IgG (5  $\mu$ g/ml in 50 mM carbonate buffer, pH 9.6; Jackson ImmunoResearch Laboratories). After blocking, the following additions were performed, all for 1 h at 37°C and separated by washing steps: (i) saturating concentrations of receptor:Fc (100- and 25-fold diluted serum for transgenic muBCMA:Fc and hTACI:Fc, respectively, corresponding to 250 ng/ml of recombinant receptors); (ii) indicated concentrations of Flag-tagged murine BAFF, APRIL, or TNF; (iii) biotinylated anti-Flag M2 antibody (250 ng/ml; Sigma-Aldrich); and (iv) horseradish peroxidase-coupled streptavidin (1/4,000). Cloning and expression of Flag-tagged ligands has been described previously (16).

**Antibody Quantification.** Quantification of Ig isotypes in mouse sera was performed by ELISA. Briefly, plates were coated with goat anti-mouse IgG plus IgM (2  $\mu$ g/ml; Caltag) and serial dilutions of sera or of purified mouse IgG1, 2a, 2b, 3, M, and A standards were added. Captured antibodies were revealed with biotinylated anti-IgG1, 2a, 2b, 3, M, and A (1/2,000; Caltag), respectively, followed by horseradish peroxidase-coupled streptavidin (1/4,000). Concentrations were inferred from the IC<sub>50</sub> values.

**FACS<sup>®</sup> Analysis.** The following antibodies were purchased from BD PharMingen: anti-CD3 $\epsilon$ -FITC (17A2); anti-CD4-Cy-Chrome (RM4-5); anti-CD5-PE (53-7.3); anti-CD16/CD32 (2.4G2, Fc Block<sup>™</sup>); anti-CD21 (7G6); anti-CD23-FITC

(B3B4); anti-CD43-FITC (S7); anti-B220-PE; anti-B220-Cy-Chrome (RA3-6B2); anti-IgMa plus b-PE (R6-60.2); anti-BP1-PE (6C3); anti-TCR- $\alpha\beta$ -FITC (H57-597); and anti-TCR- $\gamma\delta$ -PE (GL3). Anti-IgD-PE (11-26) was from Southern Biotechnology Associates and anti-CD62L-PE (MEL-14) was from Caltag. Anti-CD8-Cy5 (53-6.7), anti-CD24-APC (M1/69), and biotinylated anti-CD21 (7G6) were purified from hybridoma culture supernatant and conjugated in our laboratory according to standard procedures. Cells were treated with anti-CD16/CD32 (2.4G2, as culture supernatant) before staining, and analyzed using a four color FACSCalibur<sup>™</sup> and CELLQuest<sup>™</sup> program (Becton Dickinson).

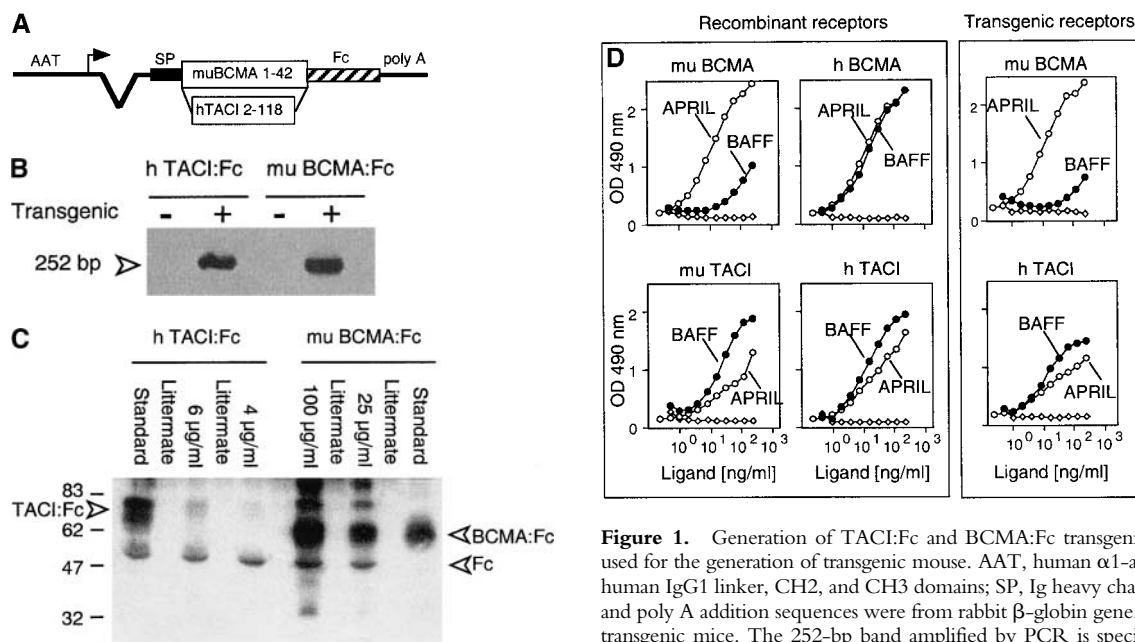
**Immunohistochemistry.** Antibodies for immunohistochemistry included anti-CD3 $\epsilon$ -biotin (145-2C11), anti-CD11b (M1/70), anti-CD11c-biotin (HL3), anti-CD35 (8C12), anti-B220 (RA3-6B2) (all from BD PharMingen), and anti-B220-biotin (RA3-6B2; Caltag). Acetone-fixed 7- $\mu$ m frozen sections were stained with horseradish peroxidase and alkaline phosphatase substrates according to standard procedures (17).

**NH<sub>2</sub>-Terminal Sequence Determination.** TACI:Fc (amino acids 2-148) and TACI:Fc (amino acids 2-118) were expressed in 293 cells and purified with protein A-Sepharose. Purified proteins (10  $\mu$ g) were reduced, blotted onto polyvinylidene difluoride membranes, and stained with Ponceau S. Bands of interest were submitted to automated Edman degradation using an ABI 120A gas phase sequencer coupled to an ABI 120A analyzer equipped with a PTH C18 2.1  $\times$  250 mm column. Data was analyzed using ABI 610 software.

## Results and Discussion

To investigate the role of BAFF and/or APRIL in vivo, we generated mice transgenic for soluble, secreted forms of either BCMA or TACI under the control of a liver promoter, with the expectation that both circulating transgenic proteins would act as BAFF and APRIL inhibitors and generate a similar phenotype. To this purpose, we used the entire extracellular domain of murine BCMA (amino acids 1-46) and the NH<sub>2</sub>-terminal portion of human TACI (amino acids 2-118), fused to the Fc portion of human IgG1 (Fig. 1 A and B). Although the extracellular domain of TACI comprises 144 amino acids, initial transfection experiments revealed a major proteolytic cleavage site at Arg<sup>122</sup> (sequence RRQR-SG), prompting us to use a shorter version. Despite this precaution, the transgenic TACI:Fc protein recovered at concentrations of 4 and 6  $\mu$ g/ml in sera of two transgenic lines was extensively processed (Fig. 1 C), probably at Lys<sup>108</sup> (CENK-LR) and Arg<sup>110</sup> (NKLR-SP), as inferred from Edman sequencing of the processed recombinant protein. In contrast, BCMA:Fc, which was expressed at higher concentrations in two lines of transgenic mice (25 and 100  $\mu$ g/ml, respectively), remained largely intact (Fig. 1 C).

Both transgenic soluble receptors in mouse sera were capable of binding recombinant APRIL in an ELISA assay, demonstrating that they were produced in an active form. While transgenic TACI:Fc also bound murine BAFF, binding of soluble BCMA to murine BAFF was marginal (Fig. 1 D). We can rule out that the weak binding observed between BCMA and BAFF is due to a missfolding artifact, because both proteins interacted well with another partner



**Figure 1.** Generation of TACI:Fc and BCMA:Fc transgenic mice. (A) Constructs used for the generation of transgenic mouse. AAT, human  $\alpha$ -antitrypsin promoter; Fc, human IgG1 linker, CH2, and CH3 domains; SP, Ig heavy chain signal peptide. Intron and poly A addition sequences were from rabbit  $\beta$ -globin gene. (B) Genomic screen of transgenic mice. The 252-bp band amplified by PCR is specific for human  $\alpha$ 1-antitrypsin promoter. (C) Western blot analysis of transgene expression. Serum (0.5  $\mu$ l) of two independent lines of each TACI:Fc and BCMA:Fc transgenic mice and of nontransgenic controls were analyzed under nonreducing conditions and revealed with horseradish peroxidase-coupled goat anti-human IgG antibody. Recombinant proteins (6 ng of BCMA:Fc and 12 ng of TACI:Fc) in normal serum were loaded as standard. For transgenic mice, the serum concentration of the transgenic protein is indicated. Molecular weight standard are in kDa. (D) Receptor-ligand interaction ELISA. Binding of recombinant murine BAFF (black circles), murine APRIL (white circles), or control murine TNF- $\alpha$  (white diamonds) to immobilized recombinant or transgenic BCMA:Fc and TACI:Fc was monitored by ELISA.

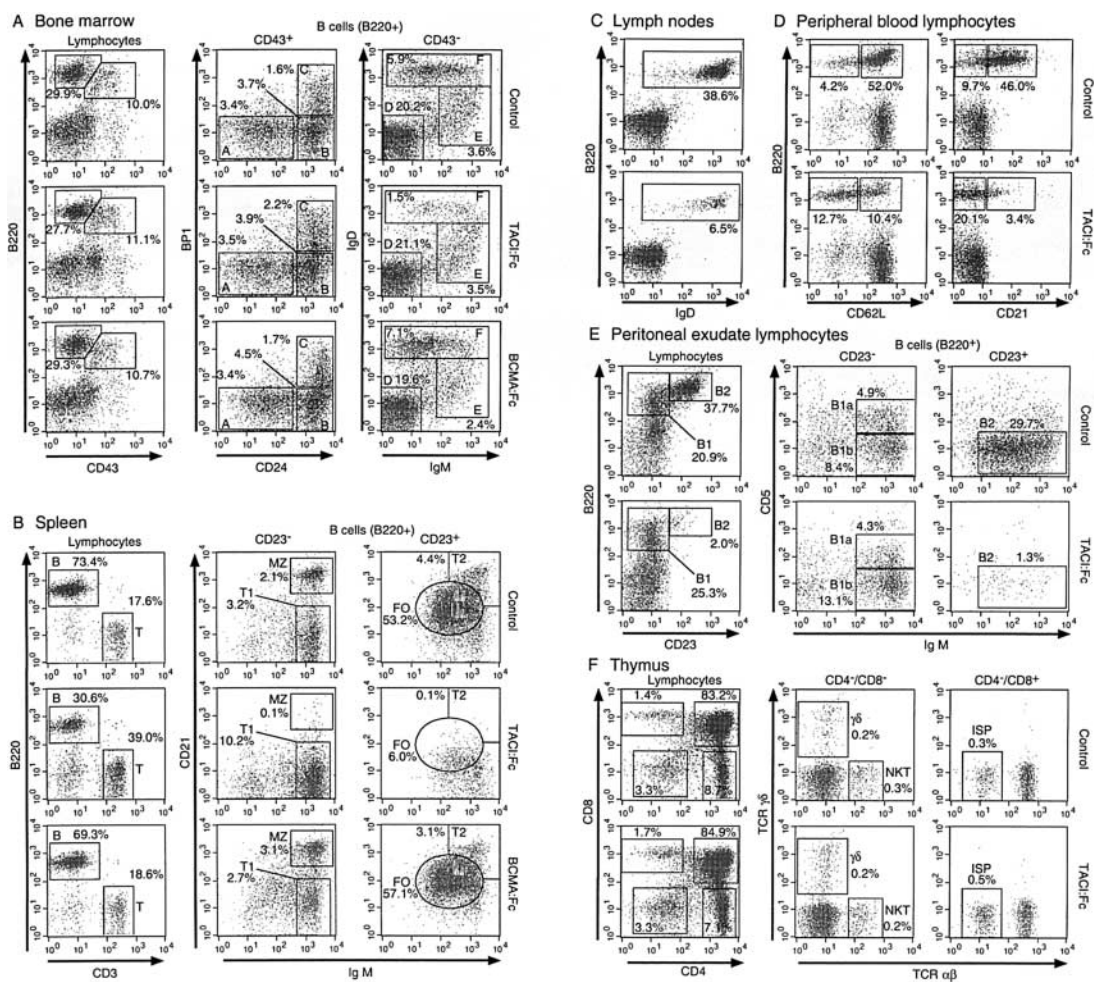
two independent lines of each TACI:Fc and BCMA:Fc transgenic mice and of nontransgenic controls were analyzed under nonreducing conditions and revealed with horseradish peroxidase-coupled goat anti-human IgG antibody. Recombinant proteins (6 ng of BCMA:Fc and 12 ng of TACI:Fc) in normal serum were loaded as standard. For transgenic mice, the serum concentration of the transgenic protein is indicated. Molecular weight standard are in kDa. (D) Receptor-ligand interaction ELISA. Binding of recombinant murine BAFF (black circles), murine APRIL (white circles), or control murine TNF- $\alpha$  (white diamonds) to immobilized recombinant or transgenic BCMA:Fc and TACI:Fc was monitored by ELISA.

(TACI for BAFF and APRIL for BCMA). The lack of a BCMA:Fc/BAFF interaction stands in contrast with previous experiments in which the function of murine BAFF was investigated using human BCMA:Fc (16). This apparent discrepancy can be explained by the fact that murine BCMA:Fc has a much lower affinity than human BCMA:Fc for murine BAFF (Fig. 1 D).

Based on these binding studies, it was anticipated that inactivation of BAFF may be considerably higher in TACI:Fc than in BCMA:Fc transgenic mice. Indeed, while BCMA:Fc transgenic mice displayed a normal B cell profile (Fig. 2 and data not shown), the analysis of TACI:Fc transgenic mice revealed a pronounced B cell lymphopenia phenotype, which is summarized in Table I and Fig. 2. Pre-pro-B, pro-B, pre-B, and transitional B cells were essentially present in normal numbers and relative proportions in the bone marrow, and only the population of mature recirculating B cells was strongly reduced in TACI:Fc mice, suggesting that the B cell defect occurred in the periphery (Fig. 2 A). The most immature B cell population of the spleen, transitional T1 B cells, was normal or even slightly increased in TACI:Fc mice (Fig. 2 B). This was in sharp contrast with the next maturation stage of transitional T2 B cells, and with the downstream populations of marginal zone and mature follicular B cells, which were either virtually absent or strongly reduced (Fig. 2 B). As a result, the lymphocyte number in the spleen was decreased by 60%, essentially due to the scarcity of the B cell compartment. Interestingly, the absolute number of splenic T cells was also reduced, although to a lesser extent. A reduction of mature B cells was also observed in lymph nodes (Fig. 2 C)

and in peripheral blood lymphocytes (Fig. 2 D). In the latter, most remaining B cells were CD21-negative and lacked the adhesion molecule L-selectin (CD62L), which is consistent with a transitional B cell phenotype. In peritoneal exudate lymphocytes of TACI:Fc mice, there was a marked decrease of B2 B cells, but both subpopulations of B1 B cells were still present (Fig. 2 E). As expected, populations of thymic lymphocytes were normal in TACI:Fc mice (Fig. 2 F). Immunohistochemical analysis of spleen, lymph node, and Peyer's patches sections confirmed the atrophy of the B cell compartment, but revealed that B and T cell segregation was not altered (Fig. 3). Follicular dendritic cells (CD35<sup>+</sup>), dendritic cells (CD11c<sup>+</sup>), macrophages (CD11b<sup>+</sup>), and germinal centers (PNA<sup>+</sup>, B220<sup>-</sup>) were distributed normally, except that cells and structures associated with B cells were decreased accordingly (Fig. 3 and data not shown). Consistent with the severe deficit of mature B cells in TACI:Fc transgenic mice, serum levels of most Ig isotypes were reduced compared with controls (Fig. 4). This was particularly significant for the IgG1 and IgM isotypes.

A crucial role of BAFF in B cell homeostasis has been firmly established by the generation of transgenic mice overexpressing BAFF, which all suffer from B cell hyperplasia (7–9). We have now generated a functional knockout of BAFF by transgenic expression of a soluble form of the receptor TACI. These mice have a severe defect in mature B cells, pointing to a crucial role for BAFF in B cell development. The phenotype of TACI:Fc mice is consistent with *in vitro* data implying that BAFF is a survival factor for transitional T2 B cells (18), and with the phenotype of BAFF transgenic mice in which transitional T2 and marginal zone B cells are



**Figure 2.** FACS<sup>®</sup> analysis of lymphoid organs of TACI:Fc and BCMA:Fc transgenic mice. The indicated percentages refer to gated lymphocytes. (A) Bone marrow. Lymphocytes expressing the B cell lineage marker B220 were separated according to CD43 expression and further analyzed with CD24 and BP-1 (for CD43<sup>+</sup> cells) and IgM and IgD (for CD43<sup>-</sup> cells). Populations A (pre-pro-B), B (pro-B), C (pro-B/PreB), D (pre-B), E (transitional B), and F (recirculating mature B) are labeled according to Hardy's nomenclature (reference 29). (B) Spleen. Analysis of splenic B (B220<sup>+</sup>) and T (CD3<sup>+</sup>) cell populations, and four color FACS<sup>®</sup> analysis of splenic B cell populations based on B220, CD23, CD21, and surface IgM expression (references 2 and 18). FO, follicular B cells; MZ, marginal zone B cells; T1, transitional T1 B cells; T2, transitional T2 B cells. (C) Inguinal lymph nodes. Analysis of B cell populations based on B220 and IgD. (D) Peripheral blood lymphocytes. Analysis of B cell populations based on the expression of B220, CD62L (L-selectin), and CD21. (E) Peritoneal exudate lymphocytes. Peritoneal B lymphocytes are classified into B1 and B2 cells based on the expression level of CD23, and further analyzed for CD5 and IgM expression. B1a, CD5<sup>+</sup> B1 B cells; B1b, CD5<sup>-</sup> B1 B cells. (F) Thymus. Thymocytes are analyzed based on the expression of CD4, CD8, TCR- $\alpha\beta$ , and TCR- $\gamma\delta$ . ISP, immature CD8 single positive precursor T cells; NKT, NK T cells.

the most enlarged populations (9, 18). In contrast, peritoneal B1 B cells were only marginally affected in TACI:Fc mice, suggesting that BAFF plays no major role in the generation of this particular B cell lineage. However, it is noteworthy that some BAFF transgenic mice have an enlarged B1 B cell compartment, whereas other have not (7, 8).

The mechanisms driving maturation of B2 B cells in the periphery are poorly understood. Signals through the B cell receptor on immature B cells trigger cell death, yet mutations affecting the B cell receptor or its signaling pathway prevent B cell maturation (1, 19). Our work offers a possible explanation for this paradox: signaling through the B cell receptor may be lethal for immature B cells unless the right level of antiapoptotic signal is delivered at the same time, possibly by BAFF. Because BAFF and the B cell receptor are both critical for B cell maturation, B cell differentiation in

the periphery might result from a finely tuned balance between life and death signals. Recently, *in vitro* studies demonstrated the specific survival effect of BAFF on transitional T2 B cells, but not on transitional T1 B cells from which they derive (18). Since BAFF has been shown to stimulate NF- $\kappa$ B activation in resting and activated B cells and to modulate the level of Bcl-2 family members, BAFF could provide an NF- $\kappa$ B-dependent, antiapoptotic signal to T2 B cells (20). This is consistent with the high expression of Bcl-2 monitored in B cells from BAFF transgenic mice (9), which develop an autoimmune disease very similar to that seen in mice with transgenic expression of Bcl-2 in the B cell compartment or in mice deficient in the Bcl-2 antagonist, proapoptotic protein Bim (21, 22). Moreover, immunodeficient (recombination activating gene [Rag]-1<sup>-/-</sup>) mice reconstituted with hematopoietic precursor cells from

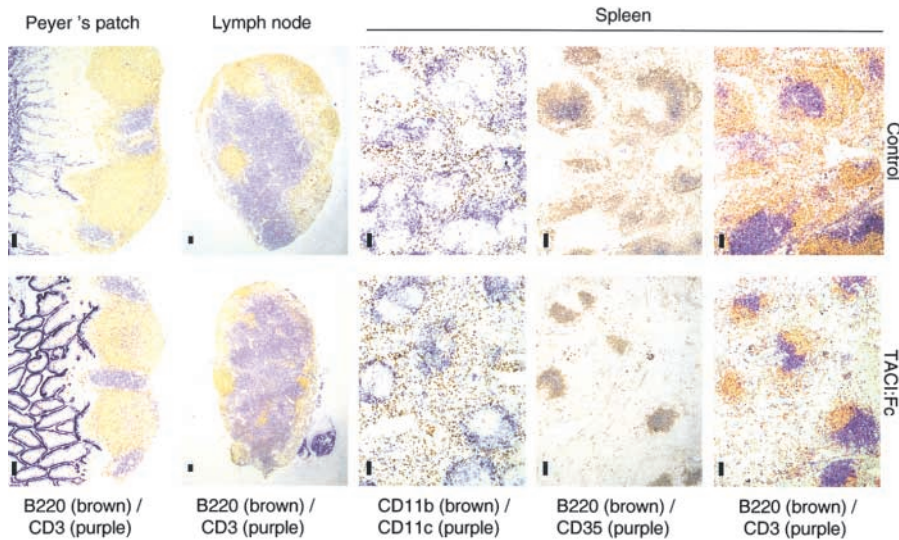
**Table I.** Lymphocytes Counts in TACI:Fc Transgenic Mice and Control Littermates

	Cell number ( $\times 10^6$ )					
	Controls	TACI:Fc	<i>P</i> value	Controls	TACI:Fc	<i>P</i> value
<b>Spleen</b>						
All cells	63.5 $\pm$ 7.1	25.0 $\pm$ 2.9	**	100	100	
T cells	12.3 $\pm$ 1.2	9.3 $\pm$ 1.4		19.5 $\pm$ 2.1	36.9 $\pm$ 1.8	**
CD4 <sup>+</sup>	9.1 $\pm$ 0.8	6.2 $\pm$ 0.6	*	14.4 $\pm$ 1.3	25.0 $\pm$ 1.1	**
CD8 <sup>+</sup>	3.3 $\pm$ 0.3	2.9 $\pm$ 0.9		5.3 $\pm$ 0.7	11.5 $\pm$ 2.1	*
B cells	44.8 $\pm$ 5.0	9.3 $\pm$ 3.7	**	70.5 $\pm$ 2.2	37.8 $\pm$ 5.1	**
T1	1.9 $\pm$ 0.3	3.3 $\pm$ 0.4		3.0 $\pm$ 0.3	13.5 $\pm$ 2.6	
T2	2.6 $\pm$ 0.1	0.045 $\pm$ 0.022	***	4.2 $\pm$ 0.3	0.19 $\pm$ 0.09	***
MZ	1.4 $\pm$ 0.3	0.050 $\pm$ 0.014	**	2.3 $\pm$ 0.6	0.21 $\pm$ 0.08	**
FO	36.1 $\pm$ 5.6	2.0 $\pm$ 0.3	**	56.6 $\pm$ 2.6	8.3 $\pm$ 1.7	**
<b>Thymus</b>						
All cells	122.3 $\pm$ 12.0	133.7 $\pm$ 14.4		100	100	
CD4 <sup>+</sup> /CD8 <sup>+</sup>	107.2 $\pm$ 10.5	113.5 $\pm$ 12.9		87.6 $\pm$ 0.3	85.0 $\pm$ 3.4	
CD4 <sup>+</sup>	0.84 $\pm$ 0.05	1.17 $\pm$ 0.33		6.9 $\pm$ 0.2	8.7 $\pm$ 2.2	
CD8 <sup>+</sup>	0.17 $\pm$ 0.04	0.24 $\pm$ 0.08		1.4 $\pm$ 0.4	1.8 $\pm$ 0.6	
Immat. CD8 <sup>+</sup>	0.049 $\pm$ 0.023	0.47 $\pm$ 0.005		0.41 $\pm$ 0.19	0.36 $\pm$ 0.08	
$\gamma\delta$	0.016 $\pm$ 0.003	0.021 $\pm$ 0.004		0.13 $\pm$ 0.01	0.16 $\pm$ 0.02	
<b>Bone marrow</b>						
All cells	44.8 $\pm$ 3.1	32.1 $\pm$ 3.0	*	100	100	
Pro-B/pre-B	4.6 $\pm$ 0.4	4.2 $\pm$ 1.4		10.2 $\pm$ 0.3	13.1 $\pm$ 3.3	*
Pre-B/Imm. B	13.6 $\pm$ 0.5	8.8 $\pm$ 0.8	*	30.6 $\pm$ 1.5	27.5 $\pm$ 1.8	
Recirculating B	3.0 $\pm$ 0.2	0.53 $\pm$ 0.09	***	6.6 $\pm$ 0.5	1.6 $\pm$ 0.1	***
<b>Lymph node</b>						
CD4 <sup>+</sup>	ND	ND		36.1 $\pm$ 2.4	48.6 $\pm$ 1.0	*
CD8 <sup>+</sup>	ND	ND		26.4 $\pm$ 2.3	33.5 $\pm$ 1.8	*
B cells	ND	ND		35.5 $\pm$ 2.0	5.6 $\pm$ 2.2	**
<b>PBLs</b>						
All cells	8.2 $\pm$ 1.1	5.5 $\pm$ 0.8	**	100	100	
	2.5 $\pm$ 0.3	2.9 $\pm$ 0.5		30.5 $\pm$ 1.8	51.7 $\pm$ 3.8	**
	1.5 $\pm$ 0.2	1.8 $\pm$ 0.3	*	18.6 $\pm$ 1.3	31.8 $\pm$ 2.2	**
	0.98 $\pm$ 0.15	1.1 $\pm$ 0.2		12.0 $\pm$ 0.6	19.2 $\pm$ 1.6	**
	4.8 $\pm$ 0.7	1.4 $\pm$ 0.1	**	58.1 $\pm$ 2.9	26.5 $\pm$ 5.6	**
	0.28 $\pm$ 0.08	0.47 $\pm$ 0.07	**	3.3 $\pm$ 0.7	8.7 $\pm$ 1.2	**
	3.4 $\pm$ 0.6	0.35 $\pm$ 0.04	**	41.0 $\pm$ 2.6	6.5 $\pm$ 0.5	***
<b>PEL's</b>						
B2/B1 ratio				Ratio		
				0.48 $\pm$ 0.16	0.056 $\pm$ 0.039	**

Cell numbers are in million per organ. For bone marrow, numbers are for one tibia and one femur. For PBLs, numbers are for 1 ml of blood. Three mice (9-wk-old females) were analyzed in each group.

*P* values: \*0.2  $\geq P \geq$  0.05; \*\*0.05  $\geq P \geq$  0.01; \*\*\**P* < 0.01.

Population definition: Spleen: T1 (B220<sup>+</sup>, CD23<sup>-</sup>, CD21<sup>-</sup>, IgM<sup>+</sup>); T2 (B220<sup>+</sup>, CD23<sup>+</sup>, CD21<sup>++</sup>, IgM<sup>++</sup>); MZ (B220<sup>+</sup>, CD23<sup>-</sup>, CD21<sup>++</sup>, IgM<sup>++</sup>); FO (B220<sup>+</sup>, CD23<sup>+</sup>, CD21<sup>+</sup>, IgM<sup>+</sup>). Thymus: Immature CD4<sup>+</sup> (CD4<sup>+</sup>, TCR- $\alpha\beta$ <sup>-</sup>);  $\gamma\delta$  (CD4<sup>-</sup>, CD8<sup>-</sup>, TCR- $\gamma\delta$ <sup>+</sup>). Bone marrow: pro-B/pre-B (B220<sup>+</sup>, CD43<sup>+</sup>); pre-B/immature B (B220<sup>+</sup>, CD43<sup>-</sup>, IgD<sup>-</sup> or <sup>low</sup>); recirculating B (B220<sup>+</sup>, CD43<sup>-</sup>, IgD<sup>+</sup>). PBLs: immature B (B220<sup>+</sup>, CD23<sup>-</sup>); mature B (B220<sup>+</sup>, CD23<sup>+</sup>). PELs: B2 (B220<sup>+</sup>, CD23<sup>+</sup>, CD5<sup>-</sup>); B1 (220<sup>+</sup>, IgM<sup>+</sup>, CD23<sup>-</sup>, CD5<sup>-</sup> or <sup>+</sup>).



**Figure 3.** Immunohistochemistry of frozen spleen, lymph node, and Peyer's patches sections. Spleen. Serial sections were double stained with B cells (anti-B220, brown), T cells (anti-CD3 $\epsilon$ , purple), follicular dendritic cells (anti-CD35/Cr1, purple), macrophages (CD11b, brown) and dendritic cells (CD11c, purple) markers, as indicated. Inguinal lymph nodes and Peyer's patches were doubly stained for B and T cells. Peyer's patches were present in normal number in TACI:Fc mice, but had a smaller size. Bars = 100  $\mu$ m.

c-Rel<sup>-/-</sup> RelA<sup>-/-</sup> double deficient mice display a block in B cell maturation remarkably similar, if not identical, to that observed in the TACI:Fc transgenic mice. This maturation arrest correlates with defects in the upregulation of Bcl-2 and A1 antiapoptotic proteins and is rescued to a large extent by enforced expression of Bcl-2 (23). Therefore, a likely scenario in the TACI:Fc transgenic mouse involves sequestration of BAFF by the transgenic decoy receptor, lack of BAFF-mediated NF- $\kappa$ B signals resulting in deficient expression of antiapoptotic proteins such as Bcl-2. As a result, newly formed T2 B cells express levels of antiapoptotic proteins that are below the threshold required for survival and differentiation into mature and/or marginal zone B cells.

In view of the fact that BAFF and APRIL share BCMA and TACI as receptors, an essential role of APRIL in B cell biology has been proposed (10, 11). However, comparison of TACI:Fc and BCMA:Fc mice suggest that APRIL is not essential for B cell development because BCMA:Fc mice

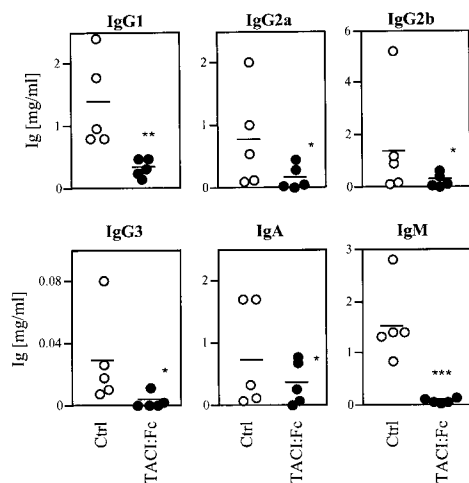
have no B cell phenotype. If, for some unknown reasons, endogenous APRIL is not inhibited by the two soluble receptors, the phenotype of TACI:Fc mice would still imply that APRIL cannot substitute for BAFF.

Inactivation of the receptor through which BAFF signals in transitional B cells is expected to result in a severe B cell deficiency. BCMA is unlikely to mediate these effects, first because of its low affinity for BAFF (Fig. 1), and second because the B cell compartment of BCMA-deficient mice is not affected (12). Thus, TACI, which binds well to BAFF, is likely to be responsible for the BAFF-mediated signals. Yet, the phenotype of TACI-deficient mice (13, 14) drastically differs from that of the functional BAFF knockout. In fact, rather than having less B cells, these mice display a moderate B cell hyperplasia. The recent cloning of a novel BAFF-specific receptor, which in contrast to TACI and BCMA does not interact with APRIL, provides an additional candidate for mediating BAFF effects on B cell maturation (24). Indeed, disruption of the BAFF-R locus in A/WySnJ mice is associated with a B cell phenotype closely related to that of the TACI:Fc transgenic mice or to the recently described BAFF<sup>-/-</sup> mice, strongly suggesting that BAFF mediates its B cell maturation signal through BAFF-R (24–27).

The fact that TACI-deficient mice have an increased number of B cells raises the hypothesis that TACI may fulfill an inhibitory or regulatory role. In this context, it is of interest that the two sites involved in proteolytic processing of TACI:Fc in the transgenic mice are conserved between human and mouse sequences. Therefore, it is not excluded that shedding of membrane-bound TACI, similar to TNF receptors (28), may have a physiological regulatory function.

We thank C. Agosti, G. Badic, and E. Sauerli for technical support, C. Ambrose and J.S. Thompson for mouse BCMA cDNA, K. Araki and M. Araki for the transgenic vector, and H. Acha-Orbea and J.-P. Kraehenbühl for reagents and stimulating discussion.

This work was supported by grants from the Swiss National Science Foundation (to P. Schneider and J. Tschopp).



**Figure 4.** Reduced Ig levels in TACI:Fc mice. Ig levels were determined in normal serum of TACI:Fc transgenic mice and matched control littermates. *P* values: \*0.2 > *P*  $\geq$  0.05; \*\*0.05 > *P*  $\geq$  0.01; \*\*\**P* < 0.01.

Submitted: 30 July 2001  
Revised: 24 September 2001  
Accepted: 4 October 2001

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