APRIL-Deficient Mice Have Normal Immune System Development

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APRIL (a proliferation-inducing ligand) is a member of the tumor necrosis factor (TNF) superfamily. APRIL mRNA shows high levels of expression in tumors of different origin and a low level of expression in normal cells. APRIL shares two TNF receptor family members, TACI and BCMA, with another TNF homolog, BlyS/BAFF. BlyS is involved in regulation of B-cell activation and survival and also binds to a third receptor, BR3/BAFF-R, which is not shared with APRIL. Recombinant APRIL and BlyS induce accumulation of B cells in mice, while BlyS deficiency results in severe B-cell dysfunction. To investigate the physiological role of APRIL, we generated mice that are deficient in its encoding gene. APRIL−/− mice were viable and fertile and lacked any gross abnormality. Detailed histological analysis did not reveal any defects in major tissues and organs, including the primary and secondary immune organs. T- and B-cell development and in vitro function were normal as well, as were T-cell-dependent and -independent in vivo humoral responses to antigenic challenge. These data indicate that APRIL is dispensable in the mouse for proper development. Thus, BlyS may be capable of fulfilling APRIL’s main functions.

Various aspects of the development and activity of the mammalian immune system are regulated by proteins that belong to the tumor necrosis factor (TNF) ligand family (reviewed in references 1, 11, 15, 36, and 43). Most members of the TNF ligand family are type II transmembrane proteins with a receptor-binding motif located at their C terminus. Except LTA, which is expressed only as a soluble molecule, TNF family members are expressed as cell surface proteins acting in a juxtacrine and autocrine manner. Proteolytic processing of some of the ligands generates their corresponding soluble forms. The majority of proteins of the TNF receptor family are composed of type I transmembrane molecules. Many of these receptors also exist in soluble forms generated by proteolytic cleavage of the cell surface protein or transcribed by alternative splicing mechanisms from the genes encoding the full-length receptors. The ligand-binding motif of the TNF receptor family consists of tandem cysteine-rich domains of about 40 amino acids in length. Each cysteine-rich domain contains several cysteines (typically six) and certain other residues in conserved positions.

APRIL (a proliferation-inducing ligand, also known as TRDL-1, TALL-2 [12, 35], and TNFSF13A) is a member of the TNF family that has been shown to be capable of inducing the proliferation of certain tumor cell lines in vitro and in vivo (9). Together with a related member of the TNF family, BlyS (B-lymphocyte stimulator, also known as BAFF, TALL-1, zTNF4, THANK, and TNFSF13B) (22, 23, 32, 35), APRIL shares two common receptors, TACI and BCMA (21, 29, 40, 45). However, unlike APRIL, BlyS also binds to BR3 (BlyS receptor 3 or BAFF-R), the least-conserved member of the TNF receptor family (39, 48). Both APRIL and BlyS are expressed by macrophages, monocytes, dendritic cells, and T cells (25, 32, 35, 37). Both ligands exist in cell surface as well as soluble forms.

Like most other TNF family members, soluble BlyS is created by cleavage of a transmembrane cell surface protein (18, 22, 32). In contrast, soluble APRIL is produced in the Golgi apparatus within the cell by a furin convertase (16). Furthermore, the transmembrane form of APRIL (named TWE-PRIL) is an unusual fusion product of two alternatively spliced RNAs, composed of exons encoding intracellular and transmembrane domains from the neighboring family member TWEAK [also called Apo3L or TNFSF12 (4, 20)] and exons from APRIL encoding the extracellular part of the molecule (28). BCMA, TACI, and BR3 are type III transmembrane proteins, lacking N-terminal signal sequences. BCMA and TACI contain intracellular TRAF binding motifs (reviewed in reference 17). The signaling mechanisms of these receptors are not fully characterized; however, they activate the NF-κB and mitogen-activated protein kinase pathways (reviewed in reference 17). All three receptors are expressed on B cells, while TACI and BR3 are also detected on the surface of some T cells (14, 39, 41, 46, 48).

While several reports document direct involvement of BlyS, TACI, and BR3 in regulating the development and function of B cells in vivo (reviewed in reference 17), the role of APRIL in immune regulation is not well defined. Alteration in the expression of BlyS or BR3 in the mouse (by gene knockout or naturally occurring mutation, respectively) leads to diminished numbers of mature B cells due to a block at the T1 stage of development (7, 31, 40, 49). In contrast, knockout of TACI results in accumulation of B cells, particularly pronounced in older mice with homogeneous genetic background (34). Elevated levels of BlyS in transgenic mice upregulate B-cell activity, leading to the development of a lupus-like autoimmune disorder (8, 13, 18). Humans with severe B-cell disorders or

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immunodefiency virus infection have elevated serum levels of BLyS (3, 6, 38, 52).

The role of APRIL in the immune system was previously investigated by analysis of APRIL transgenic mice (37). These mice display augmented T-cell-independent B-cell responses and increased survival of T cells. To assess whether APRIL is important for embryonic development and postnatal immune function, we deleted the mouse APRIL gene by homologous recombination.

MATERIALS AND METHODS

Cloning of the mouse APRIL gene and adjacent region. A 129/SvJ mouse genomic bacterial artificial chromosome (BAC) library screening with a mouse TWEAK cDNA was done by Genome Systems Inc. (St. Louis, Mo.) (H. Maecker, unpublished data). Analysis of the sequence of TWEAK-positive BAC clone 19145 revealed the organization of the TWEAK and APRIL genomic locus, as shown in Fig. 1.

Generation of APRIL-deficient mice. An APRIL targeting vector was constructed based on the TNLOX1-3 vector (5) by replacing 2.5 kb of the APRIL gene, encompassing the first and all five downstream exons, with a PGK-neo cassette. The construct contained two DNA stretches derived from the mouse genome: a 3.1-kb fragment encompassing the sixth and the seventh exons of TWEAK and part of exon one of APRIL, placed 5' of the neo cassette, and a 4.1-kb fragment encompassing the first and second SMT3IP1 exons placed 3' of the PGK-neo' cassette.

R1 embryonic stem cells (24) were transfected with the linearized vector by electroporation, and G418-resistant clones were screened for the presence of the expected recombination event by Southern blot analysis with 5'- and 3'-specific

FIG. 1. Targeting of the mouse APRIL gene. (A) Structure of the mouse APRIL genomic locus. Boxes correspond to the genomic regions containing the TWEAK (white bars), APRIL (black bars), and SMT3IP1 (grey bars) genes. The orientations of the three genes are marked by arrows. (B) Schematic representation of the targeting construct designed to replace part of the first exon and all downstream exons of the APRIL gene with a neo cassette. (C) Structure of the mutated region in the APRIL gene. The positions of the 5' and 3' external probes used for Southern blot analysis of ES cell are indicated by bars. The positions of the primer sets used for genotype analysis of mouse tail DNA are indicated by black (external) and grey (internal) arrowheads. (D) Southern blot analysis of recombination of the APRIL gene. Analysis of BsmI (DI) and SpeI (DII) digested DNA derived from several ES cell clones. DNA was digested and fractionated on a 0.7% agarose gel, blotted onto a nylon membrane, and hybridized with 5' (DI) and 3' (DII) probes. (E) Genotyping of APRIL−/− mice by PCR. Tail-derived genomic DNA was subjected to PCR amplification with nested external and internal sets of primers to visualize wild-type and deletion mutant APRIL genes as 3.2-kb and 3.0-kb fragments, respectively. (F) Expression of APRIL in total splenocytes derived from APRIL+/+ and APRIL−/− mice was determined by fluorescence-activated cell sorting with anti-mouse APRIL monoclonal antibody (black) or an isotype-matched irrelevant antibody control (grey line and filled area). (G) Quantitative real-time PCR analysis of APRIL (black bars), TWEAK (white bars), and SMT3IP (grey bars) mRNA expression in spleens of APRIL+/+ (WT), APRIL+−/− (HET), and APRIL−−/− (KO) mice. All values were normalized to an RPL19 RNA internal control. Standard deviations were calculated from triplicate reactions.
DNA probes (as shown in Fig. 1). Two independent APRIL-/- cell lines were microinjected into C57BL/6 blastocysts. Germ line transmission in mice generated by crossing chimeric males with C57BL/6 females was detected by coat color and confirmed by two-step genomic PCR (Fig. 1) with the following external (E) and internal (I) primer sets: E forward, GCCCCTAGGCGTCTACACCCTATCC; E reverse, TGGGCCTAGAAGATCTATCACACCGAACTAC; I forward, GGAGCCTAGGCGTCTACACCCTATCC; I reverse, AAGATTTCTCAGCACAGCAAGACG. 

Ex vivo KLH-specific T-cell responses. Six-week-old APRIL-/- and APRIL+/- mice were immunized via the hind footpads with 100 μg of trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) in complete Freund's adjuvant that contained Mycobacterium tuberculosis strain H37Ra (1 mg/ml, Difco Laboratories, Detroit, Mich.). After 5 days, the pooled lymph nodes were removed and cell suspensions were prepared as described above. T cells (5 × 10^6/well) were stimulated with increasing amounts of KLH. For interleukin-2 measurement, culture supernatants were collected after 4 h and analyzed by ELISA (BD). Interleukin-2 and gamma interferon levels in the linear range of detection were used to determine relative immunoglobulin levels in APRIL-/- and APRIL+/- mice.

Histologic analysis of APRIL-/- mice. Sections from spleens (A) and mesenteric lymph nodes (B) from APRIL-/- mice (left panel) and APRIL+/- mice (right panel) were stained with hematoxylin and eosin. Stained sections were photographed under a light microscope at 40×. 

FIG. 2. APRIL-/- mice have normal lymphoid organ structure. Histologic analysis of APRIL-/-mice. Sections from spleens (A) and mesenteric lymph nodes (B) from APRIL-/- mice (left panel) and APRIL+/- mice (right panel) were stained with hematoxylin and eosin. Stained sections were photographed under a light microscope at 40×.
values indicate the percentage of cells within the lymphocyte population. For spleen and lymph node, values are $10^6$ cells. The data are representative of at least four analyses.

We engineered a gene-targeting vector that replaced part of the APRIL gene. Sections were analyzed for structure of germinal centers. Peanut agglutinin (Vector Research, Burlingame, Calif.)-stained frozen sections were analyzed for structure of germinal centers.

RESULTS AND DISCUSSION

Generation of APRIL knockout mice. We engineered a gene-targeting vector that replaced part of the first and all of the five downstream exons of APRIL with a neomycin resistance cassette (Fig. 1A and B). This construct inactivates both the secreted and the recently discovered transmembrane form of APRIL (TWE-PRIL) (28) (Fig. 1C). The vector was introduced into RI embryonic stem (ES) cells (24) by electroporation. Screening of 400 neomycin-resistant ES cell clones for homologous recombination by Southern blot analysis revealed 6 positive clones with targeted mutation of the APRIL gene (Fig. 1D).

Germ line-competent chimeric mice were generated by injection of two independent targeted ES cell lines into C57BL/6 blastocysts. These founders were used to generate two lines of APRIL$^{−/−}$ mice, which were born at the expected Mendelian ratio and with indistinguishable phenotypes, as described below. Further comparison of the phenotypes of two independent lines of APRIL$^{−/−}$ mice APRIL gene deficiency was verified by genomic PCR (Fig. 1E). In addition, fluorescence-activated cell sorting analysis with specific anti-mouse APRIL antibodies revealed lack of APRIL protein expression in total splenocytes isolated from APRIL-deficient mice but not from aged-matched wild-type littersmates (Fig. 1F). To ensure that our APRIL targeting construct did not alter the TWEAK or SMT3IP1 genes, we analyzed the mRNA expression of all three genes in selected tissues by real-time PCR. This revealed altered expression of APRIL but not of TWEAK or SMT3IP1 (Fig. 1G).

Our observation that APRIL-deficient mice are viable appears to contradict a recent comment by Mackay et al., who noted in a review article that they have observed embryonic lethality of APRIL knockout mice (17). The striking discrepancy between these two knockout studies is difficult to reconcile without further details. Knockout of the same gene in different strain backgrounds on rare occasions may lead to distinctive phenotypes. For example, mice with knockout of the manganese superoxide dismutase gene generated on the C57BL/6j background die in utero due to dilated cardiomyopathy, while the same mutation introduced on the DBA/2J background leads to the development of a distinct severe phenotype and survival over several weeks after birth (10).

![FIG. 3. APRIL$^{−/−}$ mice have normal T-cell development. Thymic T-cell populations in APRIL$^{+/+}$ and APRIL$^{−/−}$ mice were determined by fluorescence-activated cell sorting. Numbers indicate the percentages of cells within the lymphocyte forward and side scatter gates. The data are representative of at least four analyses.](image)

| TABLE 2. B-cell subpopulations in APRIL$^{+/+}$ and APRIL$^{−/−}$ mice$^a$ |
|---|---|---|---|---|---|
| Location | Cell type | APRIL$^{+/+}$ | APRIL$^{−/−}$ |
| | | 1 | 2 | 3 | 1 | 2 | 3 |
| Bone marrow (%) | Pro-B cells (B220+ CD43+ IgM$^+$) | 5.8 | 11.6 | 4.7 | 9.5 | 7.1 | 11.9 |
| | Pre-B cells (B220+ CD43+ IgM$^+$) | 29.8 | 36.0 | 33.0 | 28.1 | 28.9 | 18.3 |
| | Immature B cells (B220+ IgM$^+$ IgD$^+$) | 18.6 | 28.4 | 36.4 | 28.7 | 27.5 | 24.8 |
| | Recirculating mature cells (B220+ IgD$^+$ IgM$^+$) | 5.9 | 8.1 | 5.9 | 9.1 | 5.0 | 7.1 |
| Spleen ($10^6$) | Total B cells (B220$^+$) | 42.0 | 48.0 | 42.0 | 40.0 | 48.0 | 54.0 |
| | Mature B cells (B220$^+$ IgD$^+$ IgM$^+$) | 11.4 | 17.0 | 16.0 | 15.6 | 11.5 | 13.0 |
| | Marginal zone B cells (B220$^+$ CD21$^+$ CD23$^+$) | 17.6 | 20.0 | 18.3 | 16.7 | 20.8 | 23.2 |
| | Type I B cells (IgM$^+$ IgD$^+$ CD21$^+$) | 3.3 | 2.4 | 1.9 | 2.3 | 2.0 | 1.6 |
| | Type II B/follicular cells (IgM$^+$ IgD$^+$ CD21$^+$) | 38.3 | 45.5 | 40.1 | 37.4 | 45.7 | 52.1 |
| Mesenteric lymph node ($10^6$) | Total B220$^+$ | 0.25 | 0.37 | 0.14 | 0.18 | 0.16 | 0.46 |

$^a$ Distribution of various B-cell populations in the bone marrow, spleen, and lymph nodes of APRIL$^{+/+}$ and APRIL$^{−/−}$ mice was analyzed by FACS. For bone marrow, values indicate the percentage of cells within the lymphocyte population. For spleen and lymph node, values are $10^6$ cells. The data are representative of at least four analyses done with three animals (animals 1, 2, and 3) per group.
Embryonic lethality may also be due to the very sensitive nature of the embryonic stem cells, which are able to acquire unrecognized mutations during in vitro manipulations. Alternatively, modification of the gene of interest may unexpectedly affect the expression of neighboring genes.

Of note, no other TNF family gene knockout reported to date caused embryonic lethality. Moreover, we have generated mice lacking both TWEAK and APRIL together by direct genetic manipulations in ES cells (rather than through crossing of single-gene knockout animals); these mice were also viable. Hence, it is unlikely that inadvertent deletion of TWEAK together with APRIL would cause lethality. On the other hand, SMT3IP1 encodes an isopeptidase involved in modification of SMT3b, a member of the sentrin family of ubiquitin-like proteins (26). It remains formally possible that unintended change in the structure or control of SMT3IP1 downstream of the APRIL locus may cause embryonic lethality. Regardless, our data suggest that APRIL is not critical for embryonic development.

Gross phenotypic and histologic analysis of APRIL $^{-/-}$ mice. To avoid potential phenotypic bias due to inbred genetics, we backcrossed the knockout animals 5 times into the C57/BL/6 strain background. APRIL $^{-/-}$ mice appeared phenotypically normal and fertile. APRIL signals by binding and activating two receptors: BCMA, expressed only on B lymphocytes (14, 19), and TACI, expressed both on T and B cells (41, 46).
BCMA binds with high affinity to murine APRIL but not BLyS (33). Mice without BCMA lack any detectable immune abnormality (31, 47). In contrast, knockout of mouse TACI, a receptor that prefers binding to mouse BLyS over APRIL (33), leads to B-cell expansion and splenomegaly (42, 50). Surprisingly, administration of recombinant APRIL to mice results in a phenotype similar to that of TACI knockout (51). This probably results from exposure to high, nonphysiological APRIL doses that may trigger otherwise silent BCMA activity or perhaps prevent BLyS-TACI association, thereby directing BLyS more toward BR3.

Detailed necropsy and histologic analysis of various organs from APRIL-null animals compared to wild-type littermates, including primary and secondary lymphoid organs such as spleen and mesenteric lymph nodes (Fig. 2), did not reveal any detectable abnormalities. We also did not observe any notable differences between aged APRIL-deficient and wild-type animals. Thus, APRIL does not have a critical contribution to the development and structural organization of primary and secondary lymphoid organs. Other tissues examined and considered histologically normal included: brain, liver, kidneys, intestines, pancreas, bones, and gonads.

Normal lymphocyte development in APRIL-deficient mice. In vivo administration of recombinant APRIL protein to mice as well as costimulation of T and B cells with APRIL in vitro results in significant activation of lymphocytes (51). In addition, transgenic expression of APRIL under control of the T-cell-specific promoter lck in mice promotes T-cell survival in vitro and in vivo (37). Analysis of T cells in APRIL transgenic mice revealed an increase in CD62L+ CD4+ and CD8+ cells. Furthermore, the percentage of both CD4+ and CD8+ T cells was decreased in the peripheral lymph nodes of APRIL transgenic mice, most likely due to reduced homing capacity of T cells caused by decreased CD62L expression (37). Thus, APRIL may contribute to proper development and maintenance of the immune system.

To assess if this contribution is essential, we first examined the effect of APRIL gene inactivation on T- and B-cell develop-
development. Fluorescence-activated cell sorting analysis of cells derived from the thymus, spleen, lymph nodes and peripheral blood with monoclonal antibodies to cell-specific markers did not reveal any gross differences in the number of T cells, neutrophils, natural killer (NK) cells or monocytes between APRIL-null and wild-type mice (Table 1, Fig. 3, and data not shown). Expression of the T-cell activation markers CD62L, CD44, CD25, and CD69 was similar on the surface of APRIL knockout and wild-type T cells (data not shown).

Flow cytometric analysis of B-cell types, including pro- (B220⁺, CD43⁻, IgM⁻), pre- (B220⁺, CD43⁻, IgM⁻), immature (B220⁺, IgM⁺, IgD⁻), and recirculating mature (B220⁺, IgM⁻, IgD⁻) B cells from bone marrow also did not reveal differences in cell number (Table 2). In contrast to the uniform increase of cellular components in the B-cell compartment in secondary lymphoid organs observed in TACI-null mice, the number of mature B cells in APRIL⁻/⁻ mice was indistinguishable from that of the wild-type age-matched controls (Table 2). Together, these data suggest that elimination of the APRIL gene does not impair development, maturation and distribution of T and B lymphocytes.

T and B cells derived from APRIL-deficient mice have normal function in vitro. Costimulation of T and B cells in vitro by recombinant APRIL significantly increases their proliferation rates (51). T cells derived from T-cell-specific APRIL transgenic mice also show elevated proliferation capacity (37). In addition, knockout of the TACI gene in mice leads to hyperresponsiveness of B cells to mitogenic and costimulatory signals (42, 50), suggesting negative regulation of B cells by TACI. Total splenic T cells from APRIL-null mice and wild-type age-matched controls were cultured in the presence of concanavalin A or anti-CD3 antibodies alone or together with anti-CD28 antibodies. The proliferative responses of APRIL-deficient cells were similar to those of the wild-type cells (Fig. 4A and data not shown). Interleukin-2 production also appeared unaltered in APRIL-null T cells (not shown).

To examine B-cell function in vitro, we stimulated purified splenic B cells with lipopolysaccharide or anti-CD40 antibody combined with recombinant interleukin-4. We did not observe any significant difference in proliferation rates between APRIL⁻/⁻ and APRIL⁺/+ B cells (Fig. 5B). Analysis of immunoglobulin production revealed similar levels of secretion of
IgG1, IgG2a, IgE, and IgM by B cells (Fig. 4C and data not shown). Thus, APRIL signaling is dispensable for T-cell proliferation and/or survival in vitro. APRIL also does not appear crucial to the regulation of B-cell function in vitro.

**APRIL-deficient mice have normal function of antigen-specific T cells in vivo.** Injection of recombinant TACI-Fc or BCMA-Fc proteins in antigen-challenged mice inhibits antigen-specific T-cell responses in vivo, suggesting a role for BlyS and/or APRIL in modulating T-cell function (44, 51). To assess the specific importance of APRIL in T-cell activation in vivo, we immunized APRIL-null and APRIL-wild-type mice via footpads with keyhole limpet hemocyanin protein in complete Freund’s adjuvant. Five days later, draining lymph nodes were removed (all of which appeared to be of similar size in both groups of mice). KLH restimulation in vitro of draining lymph node T cells from APRIL−/− and wild-type animals induced similar rates of proliferation (Fig. 5A). The cells also secreted equivalent amounts of gamma interferon, interleukin-2, and interleukin-4 (Fig. 5B to D). These data suggest that APRIL is not uniquely required for activation and priming of antigen-specific T cells in vivo.

**APRIL-deficient mice have normal humoral responses.** Transgenic APRIL expression results in increased serum levels of IgM but not IgG (37). Conversely, TACI gene knockout leads to decreased levels of IgM and not IgG (50). However, in another study, TACI deficiency marginally increased levels of IgM and some IgGs (42). Comparison of total IgG levels in the serum of naïve APRIL-null and wild-type mice did not reveal any significant difference (Fig. 6A). Challenge of APRIL transgenic mice with specific antigens resulted in a significant increase in thymus-independent type 2 T-cell-independent antibody responses, manifested by elevated production of IgM and IgGs, while only IgM levels show increases in the case of thymus-dependent B-cell triggering (37). Consistent with activation of TACI-mediated signaling by transgenic APRIL expression, TACI knockout mice show severe diminution of B-cell type 2 T-cell-independent responses (42, 50). In one of these reports, however, considerable elevation of immunoglobulin titers due to thymus-dependent B-cell responses was noted only for IgGs (50). We compared thymus-dependent responses in APRIL−/− and wild-type mice by challenging them with nitrophenyl-conjugated chicken gamma globulin. Titers of both high-affinity (Fig. 6B) and total (Fig. 6C) antibodies of the IgM, IgG1, IgG2a, IgG2b, IgG3, IgA, and IgE classes were similar in both groups of mice. The type 2 T-cell-independent B-cell responses in APRIL−/− and wild-type mice examined by injecting TNP-conjugated KLH were also similar (Fig. 6D).

The above T-cell-independent antigens are soluble and stimulate B cells directly, which might undermine a need for myeloid-derived costimulation through BlyS or APRIL. To rule out this possibility, we immunized mice with a particulate T-cell-independent antigen (phosphorylcholine-expressing *Streptococcus pneumoniae*). This bacterium is captured by myeloid dendritic cells (and macrophages), which subsequently secrete BlyS or APRIL and promote antigen-specific B-cell expansion and survival (2). Both the high and low antigen doses gave similar numbers of phosphorylcholine-specific plasmablasts 3 days after immunization of APRIL−/− and wild-type mice, suggesting that BlyS alone is capable of supporting this T-cell-independent B-cell response (Fig. 7). These findings suggest that APRIL is not essential for the generation of B-cell antibody responses in vivo.

**Concluding remarks.** Abolition of APRIL gene expression did not cause any significant developmental defects or immune deficiency. The finding that APRIL is not an essential gene is perhaps not surprising. First, only knockout of the common receptor, TACI, but not the murine APRIL-favored receptor, BCMA, leads to immune abnormality (31, 42, 47, 50). Second,
changes in the structure of immune organs and the development and functional status of immune cells induced by transgenic expression of APRIL, BLyS, and soluble versions of their common receptors also did not identify any unique APRIL-associated activity that could not be ascribed to BLyS signaling. Indeed, in contrast to the marked accumulation of B cells, enlargement of lymphoid organs, and development of lupus-like disease seen in BLyS transgenic animals (8, 13, 18), APRIL transgenic mice with systemic APRIL expression show only significant elevation of type 2 T-cell-independent B-cell responses (37), an effect observed also in BLyS transgenic animals. Moreover, transgenic expression of soluble human TACI, which binds to both mouse ligands, but not expression of mouse BCMA, which apparently interacts only with murine APRIL, mimics BLyS deficiency to a great extent (7, 31). Interestingly, transgenic mice overexpressing murine BCMA do not display an abnormal phenotype (31, 47).

While APRIL does not appear to play an obligatory role in development or immune function, its elevated expression in tumors and ability to promote cell proliferation remains intriguing (9). Constitutive expression of APRIL in mouse NIH 3T3 fibroblasts enhances their proliferation in vitro and tumorigenicity in vivo (9, 29). Since 3T3 cells lack expression of TACI and BCMA, this raises the possibility that an APRIL-specific yet unidentified receptor is expressed by these cells (29). In addition, it has been suggested that APRIL provides prosurvival signals in a glioblastoma cell line by inhibiting proapoptotic caspase activity and promoting expression of the inhibitor of apoptosis protein XIAP (30). In 3T3 fibroblasts and HT29 colon adenocarcinoma cells, APRIL induces expression of the prosurvival molecules Bel-2 and Bel-xl (17). Future studies will make use of APRIL knockout mice to investigate whether APRIL plays a specific role in causing cancer.

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