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B Cell-Activating Factor Belonging to the TNF Family (BAFF)-R Is the Principal BAFF Receptor Facilitating BAFF Costimulation of Circulating T and B Cells

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BAFF (B cell-activating factor belonging to the TNF family) is a cell survival and maturation factor for B cells, and overproduction of BAFF is associated with systemic autoimmune disease. BAFF binds to three receptors, BAFF-R, transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B cell maturation Ag (BCMA). Using specific mAbs, BAFF-R was found to be the predominant BAFF receptor expressed on peripheral B cells, in both humans and mice, and antagonist mAbs to BAFF-R blocked BAFF-mediated costimulation of anti-μ responses. The other BAFF receptors showed a much more restricted expression pattern, suggestive of specialized roles. BCMA was expressed by germinal center B cells, while TACI was expressed predominantly by splenic transitional type 2 and marginal zone B cells, as well as activated B cells, but was notably absent from germinal center B cells. BAFF was also an effective costimulator for T cells, and this costimulation occurs entirely through BAFF-R. BAFF-R, but not TACI or BCMA, was expressed on activated/memory subsets of T cells, and T cells from BAFF-R mutant A/WySnJ mice failed to respond to BAFF costimulation. Thus, BAFF-R is important not only for splenic B cell maturation, but is the major mediator of BAFF-dependent costimulatory responses in peripheral B and T cells. The Journal of Immunology, 2004, 173: 807–817.

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4 Abbreviations used in this paper: BAFF, B cell-activating factor from the TNF family (BAFF); TACI, BAFF-R (BR3), and B cell maturation Ag (BCMA) (13–17). Another TNF family member termed a proliferation-inducing ligand (APRIL) also binds to TACI and BCMA (14, 18). BAFF-R appears to be particularly important for the regulation of B cell survival and maturation in the spleen, because A/WySnJ mice expressing a defective BAFF-R have disrupted B cell maturation, similar to that seen in BAFF-deficient mice (17, 19). The BCMA receptor appears not to play a role in B cell maturation but is involved in plasma cell survival (20). In TACI-deficient mice, there are increased B cell numbers, marked splenomegaly, and mice develop autoimmune disorders (21). Antigenic challenge in these mice results in enhanced Ab production (21). Because an agonistic mAb to TACI inhibited B cell proliferation, signaling through TACI may in fact serve to down-regulate B cell activity (21).

BAFF and possibly APRIL also act on T cells. In vitro studies of human T cells showed that BAFF provided a complete costimulatory signal together with anti-TCR stimulation (22, 23). In mice, a TACI-Fc fusion protein blocked activation of T cells in vitro, and
inhibited T cell priming in vivo (24). Also, treatment with TACI-Fc substantially inhibited inflammation, as well as bone and cartilage destruction, in a mouse model of rheumatoid arthritis (24). T cells from transgenic mice that overexpressed human APRIL showed greatly enhanced survival in vitro and in vivo (25). In autoimmune mice, inappropriate costimulation of T and B cells by BAFF may be an important component of disease pathogenesis. The receptor for BAFF on T cells is largely unknown, although one study reported TACI expression on a subset of activated T cells using a polyclonal Ab (26). Expression of BCMA and BAFF-R is thought to be restricted to B cells (16, 17, 27, 28).

The critical functions of BAFF for B and T cell biology are facilitated by the regulated expression of BAFF receptors. Accordingly, we have investigated the capacity of BAFF to stimulate B and T cells, and have used specific antagonistic mAbs as well as strains of mice with mutant BAFF receptors to ascertain precise expression patterns and functional roles for the three receptors.

**Materials and Methods**

**Reagents and flow cytometry**

Soluble forms of human BAFF and BAFF receptors were supplied by Apotheon (Epalinges, Switzerland) and P. Schneider (Institute of Biochemistry, University of Bern, Switzerland). Denatured BAFF controls were prepared by incubation at 95°C for 2 h. Unconjugated and FITC-, PE-, CyChrome-, PerCP-, PE-Cy7-, allophycocyanin-Cy7- and allophycocyanin-conjugated mAbs to various cell surface markers were from BD Biosciences (San Diego, CA), with the exception of CCRT7-FITC (R&D Systems, Minneapolis, MN). Immunofluorescent staining was performed using standard procedures with appropriate secondary staining reagents (Jackson ImmunoResearch Laboratories, West Grove, PA), and cells were analyzed using BD FACS Calibur or LSRII flow cytometers (BD Biosciences). Six-color flow cytometric analysis to assess BAFF-R expression on naive and memory subsets of CD4+ and CD8+ cells used anti-human BAFF-R (h.BAFF-R) biotin (mAb 9-1-1) streptavidin-PE, anti-CCRT7-FITC, anti-CD3-PerCP, anti-CD8RO- allophycocyanin, anti-CD4-PE-Cy7, and anti-CD8-allophycocyanin-Cy7. A biotinylated mlgGl (BD Pharmingen, San Diego, CA) was used as an isotype control for the BAFF-R Ab.

**Animals, lymphocyte preparations, and T and B cell stimulations**

All human and mouse experiments were performed with approval of St. Vincent’s campus human or animal ethics committees. Human PBMCs were isolated from human blood by Ficoll gradient centrifugation. Human splenocytes or tonsillar cells were obtained from resected human spleen or tonsil, and were prepared by gentle teasing with forceps. Human CD3+ T cells were isolated from PBMC preparations by magnetic separation (MACS; Miltenyi Biotec, Sydney, Australia). CD3+ cells were then isolated in a magnetic field, to >98% purity. For human B cell stimulation, PBLs were incubated in 96-well plates (10^5 cells/well in 100 μl RPMI 1640 supplemented with 10% FBS) for 24 h with 75 ng/ml BAFF (Biogen Idec, Cambridge, MA), in the presence of 2 μg/ml goat anti-mouse μ-chain Ab (Jackson ImmunoResearch Laboratories), and with the indicated concentrations of previously described reagents, including polyclonal hlgG (Novartis Pharmaceuticals, East Hanover, NJ), h.BCMA-Fc, or anti-mouse BAFF-R (m.BAFF-R) mAbs (29). For Ag-specific T cell assays, splenic T cells from DO11.10 TCR transgenic mice were purified by magnetic separation, and 5 × 10^4 cells/well were cultured with 1 × 10^6 cells/well of mitomycin C-inactivated APC, together with OVA peptide in 96-well U-bottom plates. BAFF costimulation of T cells was performed by adding 4 μg/ml soluble BAFF or denatured BAFF to wells. For inhibition assays, cells were cultured with anti-m.BAFF-R mAbs (2 μg/ml), h.BCMA-Fc, or hlgG (2 μg/ml).

For anti-CD3 T cell proliferation assay, human T cells were activated using plate-bound anti-human CD3 mAb (mAb TR66), and mouse T cells were activated with anti-mouse-CD3 Ab 145-2C11 (BD Pharmingen). Anti-CD3 mAb and BAFF were coated onto plates overnight in PBS at 4°C either separately or in combination, followed by two PBS washes. A total of 2 × 10^6 PBMC (human) or CD3+ (human or mouse) cells/ml were added to tissue culture plates containing immobilized anti-CD3 ± plate-bound or soluble BAFF, and harvested after 72 h. For all mouse and human proliferation measurements, cultures were pulsed with [3H]thymidine (1 μCi/well) 18 h before harvesting and quantified using a beta-scintillation counter.

**Production and specificity of mAbs to human and mouse BAFF receptors**

An expression DNA construct containing the human TACI cDNA sequence was kindly provided by P. Schneider, and a human BAFF-R expression construct was developed using the p-Tracer vector, as per manufacturer’s protocol ( Invitrogen, Mt. Waverley, Australia). Cell surface receptor-expressing transfectants were made using the rat basophilic leukemia (RBL) mast cell line (human and mouse TACI, mouse BAFF-R), or mouse B cell lymphoma L1.2 cells (human BAFF-R). Anti-h-TACI mAb 1A11 (IgG1) was generated using immunizing Wistar rats with 2 × 10^6 irradiated TACI-transfected RBL cells, 6 times at 2-wk intervals. Cell fusion was performed as described (31). Similarly, anti-h-BAFF-R mAb 11C1 (mouse IgG1) was generated using the same procedures except that BAFF-R.L1.2 transfectants were used to immunize C57BL/6 mice. In addition, anti-h-BAFF-R (clone 9-1) and hamster anti-human BCMA (clone C4E2) mAbs were used. These were generated by immunizing with re- ceptor-Ig fusion proteins, as detailed previously (28). The rat anti-mouse TACI mAbs, 8F10 (IgG2a) and SL7 (IgG1), were generated by immunizing Wistar rats with soluble TACI extracellular domain protein, 6 times at 2-wk intervals. Anti-m.BAFF-R mAbs B2G1 and P188 were produced by immunizing hamsters with murine BAFF-Fc protein. Rat anti-m.BAFF-R mAbs 7H22-E16 and 3H4 (both IgG1) were produced by immunizing Wistar rats with soluble m.BAFF-R-Fc protein, 5 times at 2-wk intervals.

For immunohistochemical visualization of BAFF-R expression, Ag retrieval was performed by immersing 4-μm-thick paraffin sections of palatine tonsils in an EDTA-based retrieval solution (pH 9.0) and heating for 20 min at 95-99°C in a water bath. After cooling, the slides were incubated at 4°C, and sections were incubated sequentially for 30 min with anti-BAFF-R mAb (11C1) and Mouse EnVision+ HRP (DakoCytomation). Anti-BAFF-R binding was visualized using Liquid diaminobenzidine+ (DakoCytomation).

**GeneChip microarray analysis**

Affymetrix, Millenium Science, Victoria, Australia), and real-time LightCycler PCR (Roche Molecular Biochemicals, Sydney, Australia)

Total RNA was isolated from harvested cells using Qiagen RNeasy Total RNA Isolation kit (Valencia, CA). Total RNA (2 μg) was then used for cDNA synthesis, with 4.5 U AMV reverse transcriptase and MgCl2-containing buffer (Promega, Madison, WI). 20 nmol dNTPs (Promega), and 0.02 nmol oligo(dT)p15 primer (Roche Molecular Biochemicals) and incubated at 42°C for 90 min. cDNA was used for LightCycler PCR (Roche Molecular Biochemicals) with the LightCycler FastStart Master SYBR Green I kit (Roche Molecular Biochemicals), using 3 mM MgCl2, and 0.5 μM individual primers with the following specific protocol: 10 min 95°C activation; 45 cycles with FastStart Taq polymerase (Roche Molecular Biochemicals), and 40 cycles of 95°C for 15 s, 63°C for 5 s, and 72°C for 21 s. The primers were designed from the first 3′ untranslated region sequence using Primer3 (http://www-genome.wi.mit.edu/cgi-bin/ primer/ primer3 www.cgi). LightCycler analyses used the crossing point data for each gene during the logarithmic amplification program. The crossing point for each gene in each sample was normalized to the crossing point of GAPDH. Subsequent genes were then compared between two samples and expressed as a fold change.
For GeneChip assessment of transcript expression, cRNA was prepared using the methods as described (32). Hybridization to the Affymetrix U133A and B GeneChips and subsequent scanning and analysis was conducted exactly according to Affymetrix protocols. GeneChip analyses included human Th1 and Th2, purified eosinophils, cultured mast cells, IgE-activated mast cells, purified neutrophils, and splenic memory B cells. A full description of the preparation of these cells, and access to the full GeneChip results, is available at http://www.garvan.unsw.edu.au/public/microarrays.

Results

Production of mAbs to human and mouse BAFF receptors

mAbs were raised to human TACI, BAFF-R, and BCMA, and to mouse TACI and BAFF-R, to determine the functional significance of these three receptors for B and T cells, and to determine their precise expression pattern. Various immunization strategies

![Image of Figure 1](http://www.jimmunol.org/...)

**FIGURE 1.** Production and specificity of mAbs to human and mouse BAFF receptors. A. Reactivity of various mAbs against transfectants of human TACI and BAFF-R. The different shaded profiles represent different BAFF-R transfectants; lighter profiles are human BAFF-R transfectants (in mouse L1.2 cells, a B lymphoma line), and darker profiles are human TACI transfectants (in rat RBL cells). Negative control staining used mAbs of irrelevant specificity, and anti-h.TACI or anti-h.BAFF-R staining on irrelevant transfectants resembled this staining (data not shown). B, Mouse TACI and BAFF-R transfectants (both in rat RBL cells); lighter profiles are m.BAFF-R transfectants and darker profiles are m.TACI transfectants. In all instances, Ab staining of nontransfected cells resembled negative control staining. C, ELISA of anti-human BAFF-R, TACI, and BCMA mAbs against human TACI-Fc, BAFF-R-Fc, or BCMA-Fc fusion proteins. The mAbs used were anti-h.TACI mAb 1A1, anti-h.BCMA mAb C4E2.2, and anti-h.BAFF-R mAb 9-1. Values are shown as relative absorbance after subtraction of background values.
were used, including receptor fusion proteins and transfectants expressing high levels of these receptors. All of the ensuing mAb raised against either human or mouse BAFF-R or TACI stained their respective receptor transfectants (Fig. 1, A and B) or reacted specifically against receptor-Ig fusion proteins by ELISA (Fig. 1C), without cross-reacting with the other BAFF receptors. The specificity of a mAb raised against human BCMA-Fc, C4E2.2, has been reported previously (28). Anti-human BAFF-R mAb 9-1 blocked BAFF binding to BAFF-R expressing cells (data not shown). Similarly, anti-murine BAFF-R mAb B2G1 was blocking, while mAb P1B8 was nonblocking (see below).

**BAFF-R is the predominant BAFF receptor expressed on mouse T and B cells, whereas TACI marks maturing splenic B cell subsets**

mAb to mouse BAFF-R stained the vast majority of mouse splenic B cells (CD4-negative cells, Fig. 2A) and lymph node B cells (data not shown). A few resting T cells were clearly BAFF-R⁺ (Fig. 2A) and PCR analysis of mouse T cells and T cell lines revealed expression of BAFF-R and absence of TACI and BCMA (data not shown). In experiments using BAFF costimulation of anti-μ-mediated B cell proliferation, an antagonistic mAb to mouse BAFF-R blocked proliferation to the same level as did the positive control BCMA-Fc (Fig. 2B), indicating that in mice, BAFF-R is the principal costimulatory BAFF receptor for resting mature B cells.

TACI expression was detected on T cells using a polyclonal Ab (26), however, a kinetic analysis of TACI expression on T cells failed to detect TACI expression on 48-h-activated T cells (Fig. 2C), or on resting T cells or T cells at other activation time points (data not shown). However, TACI was expressed on a subset of peripheral B220⁺ B cells, and, as expected, mAb to TACI was unreactive with all B cell subsets from TACI⁻/- mice. The most notable expression of TACI was by maturing subsets of splenic B cells. Transitional type 1 (T1), transitional type 2 (T2), and marginal zone (MZ) B cells were distinguished using multicolor flow cytometry using IgM, CD21, and CD23 (see Ref. 33). In wild-type (WT) mice, T2 and MZ B cells expressed very high levels of TACI (>95% positive, Fig. 2D), whereas on other splenic B cell subsets such as follicular B cells and T1 B cells, TACI expression was low or absent, respectively. However, TACI expression by T2 or MZ B cells was not essential for B cell maturation in the spleen, because all B cell subsets developed in TACI⁻/- mice, although...
these mice displayed splenomegaly and peripheral B cell hyperplasia, as described for other strains of TACI−/− mice (21, 34, 35). When splenic B cells were stimulated with anti-μ for 24 h, TACI was strongly up-regulated (36) and the expression profile resembled that shown for MZ or T2 B cells.

**BAFF costimulation of T cells in mice is mediated by BAFF-R**

We next assessed BAFF responses by mouse T cells, using an OVA peptide-specific proliferation by DO11.10 TCR transgenic mouse T cells. Fig. 3A shows that an antagonistic mAb recognizing murine BAFF-R (mAb B2G1) inhibited T cell proliferation by ~20%, indicating that endogenous BAFF participates in T cell costimulation, for an optimal response (Fig. 3A), as previously shown by others (23). As a control, BCMA-Fc inhibited proliferation to the same extent. Furthermore, addition of exogenous soluble BAFF also increased T cell proliferation in the presence of APC (Fig. 3B). The effects of BAFF on B cells in mice occur, at least in part, through the up-regulation of the survival factor Bcl-2 (33, 37). Bound BAFF, but not denatured BAFF, also resulted in an up-regulation of Bcl-2 in purified mouse T cells suboptimally stimulated for 72 h with anti-CD3 (Fig. 3C).

To confirm that BAFF-R is facilitating BAFF-mediated effects on T cells, we studied BAFF costimulation of anti-CD3-costimulated T cell proliferation using two mutant strains of mice, TACI-deficient mice, and A/WySnJ mice with a defective BAFF-R (17, 19). Fig. 3D shows that the costimulatory effect of BAFF on purified T cells was abolished in A/WySnJ mice, in contrast to T cells from WT mice of the same genetic background expressing a functional BAFF-R, which responded to BAFF costimulation. T cells from A/WySnJ mice were costimulated by anti-CD28, similar to T cells from control A/J mice (Fig. 3D) indicating that T cells from A/WySnJ mice were competent and responded normally. Therefore, the defect in the ability to respond to BAFF costimulation was due to a lack of functional BAFF-R

![Fig. 3A](http://www.jimmunol.org/)

**FIGURE 3.** BAFF costimulates mouse T cells through BAFF-R. A. Inhibition of BAFF costimulation by h.BCMA-Fc and anti-m.BAFF-R blocking mAb B2G1. Purified DO11.10 T cells were stimulated with 0.05 μg/ml OVA peptide, together with 2 μg/ml anti-m.BAFF-R mAbs (P1B8, nonblocking; B2G1, blocking), or 2 μg/ml h.BCMA-Fc. B. Soluble BAFF (BAFF), but not denatured BAFF (DN BAFF), costimulates Ag-specific T cell proliferation at suboptimal concentrations of OVA peptide (0.01 μg/ml). BAFF was used at 4 μg/ml. C. Up-regulation of Bcl-2 in 72 h anti-CD3-stimulated, purified splenic T cells upon incubation with bound BAFF (B BAFF). Shaded and dotted profiles are similarly treated T cells, stimulated with denatured BAFF or no BAFF. D. T cell costimulation by BAFF is compromised in BAFF-R mutant mice (strain A/WySnJ). T cell costimulation by BAFF of purified T cells from the relevant A/J control background (■), and lack of costimulation of A/WySnJ T cells (■). T cells obtained from A/WySnJ mice responded well to anti-CD28 (2 μg/ml) costimulation. These T cells were purified from spleens by magnetic sorting, and cultures were activated with 1 μg/ml plate-bound anti-CD3, ± an optimal amount of plate-bound BAFF (4 μg/ml). E. T cells form TACI−/− mice, stimulated with anti-CD3 ± BAFF (■), compared with T cells from control littersmates (■) (mixed B6/129 background).
expression, rather than an intrinsic defect in T cell function. In addition, others have reported that APC function and T cell proliferation in A/WySnJ mice is normal (38). It is noteworthy that proliferation of T cells from A/J mice, without addition of exogenous BAFF, was consistently higher than for A/WySnJ mice, suggesting that activated T cells may be a source of BAFF (see microarray data below). In contrast, T cells from TACI-deficient mice were unaffected in their response to BAFF costimulation (Fig. 3E), indicating that BAFF does not costimulate T cells through this receptor. Similar to the previous study (22), we also found that anti-CD3-stimulated T cells responded to BAFF only when it was immobilized to plastic, although soluble BAFF was able to costimulate T cells in the Ag-specific assay in the presence of APCs (Fig. 3B).

**BAFF-R is the predominant BAFF receptor expressed on human blood B cells**

The studies described above established BAFF-R as the predominant BAFF receptor for mouse B and T cells. The expression pattern of BAFF-R, BCMA, and TACI was also assessed on subsets of blood and tonsil B cells in humans. On blood B cells, BAFF-R was expressed at a high level on all CD19⁺ B cells, whereas BCMA was absent (Fig. 4A). TACI was expressed, but only on a proportion of blood B cells, and at a much lower level than BAFF-R. We confirmed that BAFF-R was also the principal stimulatory receptor for human blood B cells, by inhibition of BAFF costimulation of anti-µ-treated blood B cells using a blocking mAb to BAFF-R. Fig. 4B shows that anti-BAFF-R mAb 9-1, which interferes with binding of BAFF to BAFF-R, inhibited BAFF costimulation of B cells. A proportion of these B cells did express low levels of TACI (Fig. 4A); however, TACI is known to repress B cell activation, and TACI is dispensable for BAFF stimulation of B cell responses to anti-µ (21).

**B cells with a germinal center (GC) phenotype show altered expression of BAFF receptors**

The staining of human tonsil cells with mAbs to BAFF-R, BCMA, and TACI revealed that BAFF-R was also the predominant receptor expressed on tonsil B cells. Nevertheless, BAFF-R did show a variation in staining intensity between different B cell subsets (Fig. 5A). B cells with a GC phenotype (CD38⁺, CD27⁺, CD39⁻, CD24⁻, and IgM⁻) expressed lower levels of BAFF-R, and this was clearly evident through immunohistochemical staining of B cell follicles in tonsil (Fig. 5B). In contrast to the blood, where no B cells expressed BCMA, a proportion of tonsil B cells did express low levels, and multicolor flow cytometry revealed that these BCMA⁺ B cells displayed a phenotype consistent with GC B cells (Fig. 5A). Strikingly, TACI and BCMA were expressed on different subsets of the CD19⁺ B cell population, with the TACI⁻ subset being CD38⁺, CD27⁺, CD39⁻, CD24⁻, and IgM⁻ (i.e., a non-GC phenotype). The distinct difference between the TACI⁻ and BCMA⁻ subsets was further illustrated by a direct two-color analysis (Fig. 5C), which showed that the vast majority of BCMA⁺ B cells were TACI⁻; this was particularly evident when B cell blasts were gated and analyzed (Fig. 5C).

**Expression of BAFF-R by human T cells**

BAFF has been reported to costimulate T cell responses in humans (22), as well as in mice (see above). However, mAb staining suggested that TACI and BCMA were absent from human blood T cells.
cells, and that BAFF-R was expressed at very low levels (Fig. 6A).
To further define the precise expression pattern of the receptors, particularly BAFF-R, a sensitive multicolor flow cytometric analysis was performed using a BD LSRII or FACScalibur. All human T cell populations examined, including blood T cells, in vitro anti-CD3-activated T cells (24 and 48 h, data not shown), and tonsillar T cells were found to be consistently negative for BCMA and TACI (Fig. 6A, and data not shown). In contrast, a fraction of T cells expressed BAFF-R, especially T cells activated for 72 h with anti-CD3 (Fig. 6A). This led us to examine expression of BAFF-R on naive, central memory, and effector memory CD4 and CD8 cells as defined using the markers CCR7 and CD45RO. Expression of BAFF-R was determined for the various populations and is represented by different shaded profiles, as indicated (Fig. 6B).

BAFF-R was expressed predominantly by central and effector memory T cells, and not naive T cells, in keeping with the up-regulation of BAFF-R on activated T cells.

RT-PCR showed that mRNA for BAFF-R, but not TACI or BCMA, was demonstrable in several T cell RNA samples, BJAB and RAJI cells were used as control (Fig. 6C). We also used the Affymetrix data-mining tool to assess the presence of BCMA and TACI transcripts in numerous T cell subsets (BAFF-R was not represented on the Affymetrix human U133 chips). TACI and BCMA were absent from all human T cell subsets assessed, including effector memory and central memory T cells, Th1 and Th2 cells generated in vitro, and specialized subsets such as T follicular-homing cells (Fig. 6D). The GeneChip results also showed that TACI and BCMA were undetectable in all nonlymphoid leukocyte types, such as mast cells, neutrophils, and eosinophils, and staining by flow cytometry with the specific mAbs confirmed these results (data not shown). BCMA and TACI were detectable in certain B cell populations, particularly memory cell subsets (Fig. 6D). Interestingly, all of the T cell subsets analyzed did show expression of BAFF. Transcripts of BAFF were also expressed strongly in mast cells, eosinophils, and particularly neutrophils, which is consistent with a recent report on production of BAFF by neutrophils (39). Transcripts of APRIL were also detected in mast cells, eosinophils, and neutrophils but at much lower levels than for BAFF (Fig. 6D); transcripts of APRIL were absent from all T cell subsets.
Costimulatory effects of BAFF on human T cell proliferation

Similar to the mouse results and previous study in humans (22), BAFF increased cell proliferation of suboptimally anti-CD3-treated human T cells, to a level similar to that observed with anti-CD28 stimulation (Fig. 7A). However, we observed that the ability of BAFF to costimulate human T cell responses was variable, and was highly dependent on T cell purity. We next used Ig fusion proteins of TACI, BCMA, and BAFF-R to block BAFF (and APRIL) activity in a suboptimal anti-CD3 T cell activation assay (Fig. 7B). All three fusion proteins inhibited T cell proliferation in response to anti-CD3, demonstrating the importance of endogenous BAFF for normal T cell stimulation. Neutralization of BAFF rather than APRIL appeared to be responsible for the observed effect, because BAFF-R-Fc (BAFF specific) reduced proliferation to the same extent as TACI-Fc or BCMA-Fc. Although it has been reported BCMA-Fc has a lower affinity for BAFF (29), we found all three fusion proteins showed a similar ability to block BAFF activity, presumably because these fusion proteins were used at high concentrations (30 μg/ml). We did observe a degree of person to person variation, as TACI-Fc failed to inhibit T cell proliferation in one individual of five (data not shown). In addition, unlike the mouse experiments described above, anti-h.BAFF-R mAb (2–30 μg/ml used) failed to inhibit T cell proliferation, suggesting that the way BAFF-R signals in B and T cells is different and possibly activated by a different interaction between BAFF and BAFF-R.

Discussion

The expression of BAFF receptors by subsets of B cells and T cells regulates survival or costimulation at critical stages of maturation or functional responses. Discerning the precise role of the various BAFF receptors is important to understanding how BAFF and APRIL influence immune responses, and how and why overproduction of BAFF causes autoimmune disease. This study establishes BAFF-R as the important BAFF costimulatory receptor on circulating T and B cells, and shows that BCMA and TACI display restricted expression patterns suggestive of specialized roles.

The role of BAFF-R in peripheral B cell survival and costimulation has been difficult to gauge using BAFF-R mutant mice, because splenic B cell maturation is halted at the T1 stage (17). As BAFF-R was the only known BAFF receptor detectable on most...
mature resting B cells, and anti-BAFF-R mAbs completely inhibited BAFF-mediated costimulation of B cells, we conclude that positive BAFF responses up to the stage of CD38\textsuperscript{+}/H11001 plasmablasts occur exclusively through this receptor. An assessment of BCMA and TACI deficient mice supports this conclusion. Early studies with BCMA-deficient mice found no unusual phenotype (30, 40, 41), although recent studies showed impaired survival of long-lived bone marrow plasma cells (20). Our expression analysis also showed that BCMA is most likely relevant for later stages of B cell maturation or survival, i.e., for CD38\textsuperscript{+}/H11001 plasmablasts (28) and GC B cells (this report). BCMA is also up-regulated on mouse plasma cells (20). Likewise, TACI was expressed only weakly on a small subset of peripheral B cells. TACI-deficient mice showed increased B cell numbers, and splenomegaly (Refs. 21, 34, 35, and this report). TACI has been proposed to play a role as a negative regulator for B cells, rather than as an essential survival-related receptor (21). However, TACI may play an important role in some aspects of B cell maturation or function within the spleen, because T2 and MZ B cells expressed high levels of TACI. MZ B cells are essential for T-independent immune responses (42), and TACI knockout mice have impaired T-independent but normal T-dependent responses (34). TACI was clearly dispensable for splenic B cell maturation, as shown by the production of mature B cells in TACI\textsuperscript{−/−} mice. Nevertheless, BAFF (or APRIL) signaling through TACI at the T2 or MZ stages may constrain B cell proliferation at critical stages of tolerance induction. The T1-T2 maturation step is likely an important immune tolerance checkpoint for maturing B cells (43). The MZ has been suggested as a refuge for autoreactive B cells (44, 45), and increased TACI expression facilitating repression of B cell proliferation might help contain autoreactive cells within this population.

Although BAFF and its receptors have been associated mostly with B cell responses, we showed that BAFF also had profound effects on T cell costimulation, occurring through BAFF-R. However, immobilized BAFF rather than soluble BAFF provided these costimulatory signals to T cells (this report and Ref. 22) suggesting that BAFF signaling through BAFF-R on T cells requires membrane expression by APC. It is also conceivable that APCs immobilize and present soluble BAFF. BAFF-R has been identified as a survival receptor for B cells and it may serve a similar role for T cells, such as effector and central memory subsets. Enhanced T cell survival would then augment the number of T cells capable of activation. The activity of BAFF as an important component of costimulation (or survival factor) for T cell proliferation is intriguing because of the very high levels of BAFF in inflammatory lesions and blood of certain patients with autoimmune diseases (7, 9, 10, 46), although whether BAFF in such patients affects T cell biology is uncertain. Interestingly, the cell types that are expanded in BAFF transgenic mice include mature B cells, splenic T2 and MZ B cells, and also effector memory T cells (4, 33). Our in vivo data

FIGURE 7. BAFF costimulates human T cells. A, Human T cells were purified from blood by magnetic separation and activated with anti-CD3 (1 μg/ml) for 72 h in the presence or absence of BAFF. BAFF was used either as plate-bound (B), soluble (S), or denatured (DN) at 5 μg/ml. B, Inhibition of BAFF costimulation by BAFF receptor fusion proteins. PBMCs were activated with anti-CD3 for 72 h, together with fusion proteins of h.BAFF-R-Fc, h.BCMA-Fc, TACI-Fc, or a hIgG control (30 μg/ml). C, Anti-h.BAFF-R failed to inhibit T cell proliferation. PBMCs were activated with anti-CD3 in the presence of anti-h.BAFF-R, h.TACI-Fc, and h.IgG (30 μg/ml) were used as controls. All cultures were pulsed with [3H]thymidine for 18 h.
has also shown that BAFF enhances the delayed-type hypersensitivity reaction, which is a classical T-dependent immune response (our unpublished data). Taken together, these results clearly indicate that BAFF is not only an important B cell factor; it is also a critical factor for T cell responses. The reason why only immobilized BAFF costimulated T cells is unresolved; it is possible that immobilization of BAFF on the plastic plate reproduces elements of membrane expression by APCs. A final feature of BAFF-R worthy of comment is that BAFF-R binds BAFF but not APRIL, and so the reported effects of APRIL on T cells (25) presumably occur through an unidentified receptor, or through indirect mechanisms.

BAFF receptors were regulated during B cell differentiation to GC cells. The most obvious expression of BCMA was by B cells in tonsil with a phenotype consistent with GC B cells, i.e., CD38+, CD27+, CD39+, and IgM+ (47, 48). BAFF is important for the GC reaction, because blocking BAFF in vivo attenuates the GC reaction (49), and those GCs that do form in BAFF-decient mice have impaired maturation and function (50). BAFF-R was also expressed on GC B cells, but at lower levels compared with mature B cells. Interestingly, TACI was largely absent from GC B cells, suggesting that its proposed role as a negative regulator of B cell activation (21, 35) does not extend to the GC reaction. Its loss from GC B cells would be consistent with the down-regulation by GC B cells of another inhibitory receptor, leukocyte-associated immunoglobulin-like receptor-1 (51) and the phosphatase Src homology 2-containing protein tyrosine phosphatases 1 (52). This would serve to limit the function of inhibitory receptors, and thus reduce the threshold for activation and proliferation in the course of B cell selection and differentiation within GCs. TACI mAb stained subsets of mature naïve B cells, memory B cells, and activated B cells, suggesting that TACI regulates the responses of each of these B cell subsets, but not GC B cells. The acquisition of BCMA by GC B cells (and plasmablasts) (28) presumably plays a role in regulating their survival, although the high expression of BAFF-R, albeit at lower levels compared with circulating B cells, implies a role for this receptor as well. The reason for BCMA up-regulation on GC cells is uncertain, but one consequence might be the acquired ability to signal in response to APRIL as well as BAFF.

The basis for the development of BAFF-associated autoimmune diseases is uncertain, but several possibilities exist. One is that during splenic maturation, BAFF corrupts B cell tolerance (33), possibly through excessive BAFF signaling through BAFF-R (17). However, BAFF receptors, particularly BAFF-R, are expressed abundantly at most stages of peripheral B cell differentiation. The GC reaction is another conceivable point where newly generated B cells with novel BCR specificities might escape tolerance induction, particularly in the presence of excess BAFF. However, in a recent study from our laboratory, TNF−/− mice that lack GC, when crossed to BAFF transgenic mice, still developed autoimmune disorders, indicating that corruption of tolerance in BAFF transgenic mice occurs independently of the GC reaction (36). BAFF might also costimulate autoreactive T cells. Blocking BAFF (and/or APRIL) severely inhibits inflammation in a mouse model of rheumatoid arthritis vivo (24); however, whether this effect is through T or B cells is uncertain. Indeed the relative contribution of T cells, B cells, and macrophages to disease pathogenesis in many autoimmune diseases is still unclear. In any event, our results clearly establish BAFF-R as the predominant BAFF receptor for circulating T and B cell responses, with BCMA and TACI showing much more restricted expression patterns and, by inference, having more specialized functional roles in B cell responses. Thus BAFF-R, as a widely expressed receptor of important functional relevance for T and B cell responses, represents an attractive target for intervention in autoimmune diseases.

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