The tumor necrosis factor family members BAFF and APRIL induce Ig isotype switching in human B cells. We analyzed the ability of BAFF and APRIL to induce isotype switching in murine B cells to IgG1, IgA, and IgE. APRIL and BAFF each engage two receptors, transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI) and B cell maturation antigen (BCMA), on B cells. In addition, BAFF engages a third receptor on B cells, BAFF-R. To determine the role of these receptors in isotype switching, we examined B cells from mice deficient in TACI, BCMA, and BAFF-R. The results obtained indicate that both TACI and BAFF-R are able to transduce signals that result in isotype switching.

Class switch recombination (CSR) in B cells requires two signals (1). The first is normally delivered by cytokines, which target specific C_H genes for transcription; the second is delivered in the case of T-dependent (TD) antigens by interaction of CD40 on B cells with its ligand CD40L on activated T cells. CSR is severely impaired in patients and mice deficient in CD40L or CD40 (2, 3), although low levels of IgG and variable levels of IgA are still detected in serum. Exposure to LPS derived from Gram-negative bacteria may account for some of this residual CSR in mice, but not in humans since LPS does not activate CSR in human B cells. EBV infection triggers CSR in human B cells independently of CD40L and CD40 (4) and may contribute to residual CSR in humans with CD40L and CD40 deficiency. B cell–activating factor of the TNF family (BAFF) and A proliferation–inducing ligand (APRIL) are two TNF family members that have been shown to activate CSR in human B cells (5) and hence may contribute to residual CSR in CD40L and CD40 deficiency. BAFF is expressed mainly by monocytes and dendritic cells. APRIL is expressed in a large variety of tissues that include monocytes/macrophages, dendritic cells, and activated T cells. APRIL and BAFF both bind to two receptors, B cell maturation antigen (BCMA) and transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI), which are members of the TNF receptor family. BCMA is exclusively expressed on B cells, whereas TACI is expressed on B cells and activated T cells. A third receptor, BAFF receptor (BAFF-R), that is unique for BAFF is expressed mainly on B cells but also on T cells (6). To identify the receptors that are involved in the induction of Ig class switching by BAFF and APRIL, we ascertained that these ligands activate CSR in mouse B cells and then examined their activity on B cells from TACI-, BCMA-, and BAFF-R–deficient mice.

RESULTS AND DISCUSSION

BAFF and APRIL activate IgG1, IgA, and IgE isotype switching in mouse B cells

We examined the capacity of BAFF and APRIL to induce IgG1, IgA, and IgE switching in mice. Splenic B cells from CD40−/− mice were negatively sorted and consisted of 96% sIgM+ sIgD−, 3–6% CD11b+, and undetectable CD3+ cells. APRIL and BAFF induced IgG1, IgA but no detectable IgE synthesis in these cells (Fig. 1 A). IL-4 enhanced the induction of IgG1 synthesis by BAFF and APRIL and synergized with these two ligands to induce IgE synthesis. As expected, B cells synthesized large amounts of IgG1 and IgE in response to LPS + IL-4, and TGFB synergized with LPS to induce IgA switching. Neutraliza-
tion of TGFβ had no effect on IgA secretion in response to BAFF and APRIL (unpublished data). Failure to block induction of IgA secretion by αTGFβ suggests that BAFF and APRIL induce germ line transcripts (GLTs) independently of TGFβ, or they induce TGFβ, but not all of it is accessible to neutralization by the antibody. IL-6 neutralization had no effect on IgG1 or IgA induction by BAFF or APRIL (unpublished data). IL-10 neutralization partially inhibited IgG1 secretion by BAFF (~40%) and APRIL (~60%) and IgA secretion by these ligands (~10% and ~30%, respectively). As another measure of CSR, we examined the induction of expression of surface IgG1. There were virtually no sIgG1+ cells in the negatively sorted B cells (Fig. 1 B). APRIL and BAFF alone and with IL-4 induced IgG1 surface expression in these B cells. Together these results suggest that APRIL and BAFF activate CSR in murine B cells.

CSR has been linked to cell division (7). APRIL- and BAFF-induced proliferation of negatively sorted B cells in a [3H]thymidine uptake assay and of splenic B220+ B cells in a 5- and 6-carboxyfluorescein diacetate succinimidy ester (CFSE) dye dilution assay (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20032000/DC1). Induction of CSR by APRIL and BAFF was not due to contamination with endotoxin because the preparations used contained <1 endotoxin U/μg protein; and polymyxin B, which inhibits LPS activation (8), failed to inhibit induction of IgG1 synthesis by APRIL and BAFF (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20032000/DC1).

Molecular events involved in CSR include expression of GLTs, expression of the gene for activation-induced deaminase (AID), followed by deletional switch recombination and expression of Iμ-Cα transcripts. APRIL and BAFF induced γ1GLT and αGLT, but no detectable εGLT, in negatively sorted B cells from CD40−/− mice (Fig. 1 C). APRIL, and to a lesser extent, BAFF induced AID gene expression. APRIL and BAFF synergized with IL-4 in inducing εGLT. Digestion circularization (DC)–PCR analysis revealed that APRIL and BAFF induced Sμ→Sγ1 and Sμ→Sα but not Sμ→Se deletional switch recombination (Fig. 1 D). IL-4 synergized with APRIL and BAFF to in-
were stimulated with (A) APRIL, (B) BAFF, and (C) controls. Results represent mean and SD of three experiments.

Figure 2. Role of TACI and BCMA in inducing IgG1, IgA, and IgE synthesis. Negatively sorted B cells from WT, TACI−/−, and BCMA−/− mice were stimulated with (A) APRIL, (B) BAFF, and (C) αCD40 and LPS as controls. Results represent mean and SD of three experiments.

Figure 2. Role of TACI and BCMA in inducing IgG1, IgA, and IgE synthesis. Negatively sorted B cells from WT, TACI−/−, and BCMA−/− mice were stimulated with (A) APRIL, (B) BAFF, and (C) αCD40 and LPS as controls. Results represent mean and SD of three experiments.

were unable to detect in these B cells induction of molecular events involved in CSR because these cells synthesized IgG1, IgA, and IgE in response to APRIL (Fig. 2 B). This was confirmed by the presence of GLTs, AID, and mature transcripts, unless IL-4 was added (Fig. 1 C). Since we used negatively sorted B cells from CD40−/− mice, these results indicate that APRIL and BAFF induce CSR in naive B cells.

Our findings extend previous results on human B cells positively sorted for IgD expression (5). Our observation that BAFF and APRIL activate CSR in B cells from CD40−/− mice definitively establishes that CSR, mediated by these ligands is independent of CD40L–CD40 interactions (Fig. 1). In the case of human B cells, BAFF/APRIL induction of secretion of the switched isotypes requires additional signals that include cross-liking of the B cell receptor and the cytokines, such as IL-10 and IL-15 (5). One possibility is that mouse B cells survive better in culture to the stage where they are able to secrete Igs.

**TACI mediates class switching by APRIL**

We next examined negatively sorted splenic B cells from mice that lack BCMA or TACI. B cells from WT mice were used as controls with results similar to those obtained with CD40−/− cells. BCMA−/− B cells synthesized IgG1, IgA, and IgE in response to APRIL and BAFF in amounts that were not significantly different from those secreted by WT B cells (Fig. 2, A and B). Intact CSR in BCMA−/− B cells was confirmed by examination of molecular events involved in CSR to IgG1, IgA, and IgE (Fig. 3).

TACI−/− B cells virtually failed to synthesize IgG1, IgA, and IgE in response to APRIL (Fig. 2 A). This was not due to an intrinsic defect in CSR because they synthesized IgG1 and IgE in response to LPS + IL-4 and αCD40 + IL-4 and IgA in response to LPS + TGFB (Fig. 2 C). Examination of molecular events confirmed the inability of APRIL to activate CSR in TACI−/− B cells (Fig. 3). In some experiments, CγGLT and AID were faintly detected in unstimulated B cells from TACI−/− mice. This may be related to the B cell activation observed in these mice in vivo (9). However, these faint CγGLT and AID transcripts were not up-regulated by APRIL. These results suggest that APRIL induction of CSR is mediated by TACI.

**Both TACI and BAFF-R mediate class switching by BAFF**

In contrast to their total inability to class switch in response to APRIL, TACI−/− B cells synthesized IgG1 and IgE in response to BAFF + IL-4 in amounts that were not significantly different from those secreted by normal B cells (Fig. 2 B). This was confirmed by the presence of γ1 and αGLTs, AID, and mature transcripts, unless IL-4 was added (Fig. 1 C). Since we used negatively sorted B cells from CD40−/− mice, these results indicate that APRIL and BAFF induce CSR in naive B cells.

We next examined negatively sorted splenic B cells from mice that lack BCMA or TACI. B cells from WT mice were used as controls with results similar to those obtained with CD40−/− cells. BCMA−/− B cells synthesized IgG1, IgA, and IgE in response to APRIL and BAFF in amounts that were not significantly different from those secreted by WT B cells (Fig. 2, A and B). Intact CSR in BCMA−/− B cells was confirmed by examination of molecular events involved in CSR to IgG1, IgA, and IgE (Fig. 3).

TACI−/− B cells virtually failed to synthesize IgG1, IgA, and IgE in response to APRIL (Fig. 2 A). This was not due to an intrinsic defect in CSR because they synthesized IgG1 and IgE in response to LPS + IL-4 and αCD40 + IL-4 and IgA in response to LPS + TGFB (Fig. 2 C). Examination of molecular events confirmed the inability of APRIL to activate CSR in TACI−/− B cells (Fig. 3). In some experiments, CγGLT and AID were faintly detected in unstimulated B cells from TACI−/− mice. This may be related to the B cell activation observed in these mice in vivo (9). However, these faint CγGLT and AID transcripts were not up-regulated by APRIL. These results suggest that APRIL induction of CSR is mediated by TACI.

Both TACI and BAFF-R mediate class switching by BAFF

In contrast to their total inability to class switch in response to APRIL, TACI−/− B cells synthesized IgG1 and IgE in response to BAFF + IL-4 in amounts that were not significantly different from those secreted by normal B cells (Fig. 2 B). This was confirmed by the presence of γ1 and αGLTs, AID, and mature transcripts, unless IL-4 was added (Fig. 1 C). Since we used negatively sorted B cells from CD40−/− mice, these results indicate that APRIL and BAFF induce CSR in naive B cells.

We next examined negatively sorted splenic B cells from mice that lack BCMA or TACI. B cells from WT mice were used as controls with results similar to those obtained with CD40−/− cells. BCMA−/− B cells synthesized IgG1, IgA, and IgE in response to APRIL and BAFF in amounts that were not significantly different from those secreted by WT B cells (Fig. 2, A and B). Intact CSR in BCMA−/− B cells was confirmed by examination of molecular events involved in CSR to IgG1, IgA, and IgE (Fig. 3).

TACI−/− B cells virtually failed to synthesize IgG1, IgA, and IgE in response to APRIL (Fig. 2 A). This was not due to an intrinsic defect in CSR because they synthesized IgG1 and IgE in response to LPS + IL-4 and αCD40 + IL-4 and IgA in response to LPS + TGFB (Fig. 2 C). Examination of molecular events confirmed the inability of APRIL to activate CSR in TACI−/− B cells (Fig. 3). In some experiments, CγGLT and AID were faintly detected in unstimulated B cells from TACI−/− mice. This may be related to the B cell activation observed in these mice in vivo (9). However, these faint CγGLT and AID transcripts were not up-regulated by APRIL. These results suggest that APRIL induction of CSR is mediated by TACI.

Both TACI and BAFF-R mediate class switching by BAFF

In contrast to their total inability to class switch in response to APRIL, TACI−/− B cells synthesized IgG1 and IgE in response to BAFF + IL-4 in amounts that were not significantly different from those secreted by normal B cells (Fig. 2 B). This was confirmed by the presence of γ1 and αGLTs, AID, and mature transcripts, unless IL-4 was added (Fig. 1 C). Since we used negatively sorted B cells from CD40−/− mice, these results indicate that APRIL and BAFF induce CSR in naive B cells.

We next examined negatively sorted splenic B cells from mice that lack BCMA or TACI. B cells from WT mice were used as controls with results similar to those obtained with CD40−/− cells. BCMA−/− B cells synthesized IgG1, IgA, and IgE in response to APRIL and BAFF in amounts that were not significantly different from those secreted by WT B cells (Fig. 2, A and B). Intact CSR in BCMA−/− B cells was confirmed by examination of molecular events involved in CSR to IgG1, IgA, and IgE (Fig. 3).

TACI−/− B cells virtually failed to synthesize IgG1, IgA, and IgE in response to APRIL (Fig. 2 A). This was not due to an intrinsic defect in CSR because they synthesized IgG1 and IgE in response to LPS + IL-4 and αCD40 + IL-4 and IgA in response to LPS + TGFB (Fig. 2 C). Examination of molecular events confirmed the inability of APRIL to activate CSR in TACI−/− B cells (Fig. 3). In some experiments, CγGLT and AID were faintly detected in unstimulated B cells from TACI−/− mice. This may be related to the B cell activation observed in these mice in vivo (9). However, these faint CγGLT and AID transcripts were not up-regulated by APRIL. These results suggest that APRIL induction of CSR is mediated by TACI.

Both TACI and BAFF-R mediate class switching by BAFF

In contrast to their total inability to class switch in response to APRIL, TACI−/− B cells synthesized IgG1 and IgE in response to BAFF + IL-4 in amounts that were not significantly different from those secreted by normal B cells (Fig. 2 B). This was confirmed by the presence of γ1 and αGLTs, AID, and mature transcripts, unless IL-4 was added (Fig. 1 C). Since we used negatively sorted B cells from CD40−/− mice, these results indicate that APRIL and BAFF induce CSR in naive B cells.
signals are indispensable for the activation of the Iκ promoter and induction of IgA switching.

**APRIL induction of CSR is independent of BAFF–BAFF-R interaction**

Given the observation that BAFF-R engagement can activate CSR, it was important to rule out the possibility that APRIL-mediated switching involved, in addition to TACI, engagement of BAFF-R by BAFF which may be endogenously made by B cells. Unstimulated B cells expressed small amounts of BAFF mRNA as assessed by RT-PCR (Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20032000/DC1). Stimulation with APRIL or APRIL + IL-4 caused no detectable increase in BAFF mRNA expression. More importantly, we examined the ability of APRIL to induce isotype switching in B cells from A/WySnJ mice, which carry a mutation in BAFF-R (10). These mice have very few peripheral B cells with a decreased proportion of mature CD23+ B cells. To examine CSR under culture conditions similar to those used for WT, BCMA−/−, and TACI−/− B cells (i.e., same cell number and density), we examined B cells from pooled splenocytes of 4 A/WySnJ mice. APRIL and BAFF induced IgG1 and IgA secretion in B cells from these mice (Fig. S3). These results suggest that APRIL-mediated CSR does not involve autocrine BAFF–BAFF-R interactions and that TACI engagement is sufficient to induce CSR. We cannot rule out the possibility that TACI synergizes with a putative APRIL-specific receptor to cause CSR.

Binding of TRAF2 and/or TRAF3 is essential for CD40-mediated CSR, whereas TRAF6 is important in plasma cell differentiation (11, 12). TACI, like CD40, recruits TRAF 2, 5, and 6 (6). This may explain its ability to activate CSR. BAFF-R binds TRAF3 but no other TRAF protein (6). TRAF3 may be important for CSR induced by the EBV protein LMP-1 (13). It is possible that CSR induced by BAFF in TACI−/− B cells involves a cooperative interaction between BAFF-R and BCMA, which recruits TRAF1, 2, and 3 proteins (6). The fact that BCMA fails to activate CSR may be due to the fact that the majority of BCMA has a low surface density and most of it is intracellular (6). Alternatively, non-TRAF signals may be important for CSR but may not be delivered by BCMA.

A clue to the physiological role of APRIL- and BAFF-mediated CSR is provided by results obtained on mice deficient in these ligands and their receptors. BAFF−/− and BAFF-R−/− mice are severely deficient in B cells (6) and not informative. BCMA−/− mice have normal serum Ig levels and normal antibody responses (14). This is consistent with our data that B cells from these mice switch normally in response to BAFF and APRIL. TACI−/− mice have low serum IgA and deficient antibody responses to immunization with type II T-independent antigens (15, 16). This is consistent with the failure of B cells from these mice to secrete IgA in response to BAFF and APRIL. We have shown that APRIL−/− mice have a selective IgA deficiency and decreased serum IgA antibody responses to mucosal immunization with TD antigen (17). This suggests that APRIL and BAFF play nonredundant roles in IgA switching in vivo. Since serum IgA levels are normal in CD40−/− mice (11, 18), APRIL–BAFF–TACI interactions play an important role in physiologic IgA switching and could be manipulated therapeutically to enhance antibody responses to oral vaccines.

**MATERIALS AND METHODS**

**Mice.** CD40−/−, BCMA−/−, and TACI−/− mice were described previously (14, 16, 19). A/WySnJ mice that carry a mutation in BAFF-R were purchased from Jackson Laboratories. All mice were kept in a specific pathogen-free animal facility.
In vitro isotype switching. Spleen cells from CD40−/−, BCMA−/−, and TACI−/− mice were labeled with a cocktail of biotin-conjugated mAbs to CD43, CD11b, Thy1.2, CD138, IgG1, IgG2a, IgG2b, IgG3, IgA, and IgE and negatively sorted with Streptavidin magnetic beads (Dynal). B cells were cultured at 10^6/ml in RPMI containing 10% FCS, 1-glutamine, and 50 μM 2-ME (complete medium). For Ig synthesis, B cells were cultured in complete medium alone or in the presence of 1 μg/ml sAPRIL (R&D Systems), 1 μg/ml tBAFF (Alexis), IL-4 (50 μg/ml; R&D Systems), TGFB (R&D Systems), 10 μg/ml LPS (Sigma-Aldrich), or 1 μg/ml αCD40 (BD Biosciences). Neutralizing antibodies to IL-6, IL-10, and TGFβ (R&D Systems) were used as suggested by the manufacturer. After 6 d, supernatants were assayed for IgA, IgE, and IgG1 by ELISA (11), and genomic DNA was prepared for DC-PCR.

IgG1 surface expression. B cells stimulated for 6 d as above were stained with αB220-FITC and trlG1 biotin–conjugated mAbs followed by staining with PE-conjugated Streptavidin and FACS analysis.

RT-PCR for GLT, AID, and post switch mRNAs. RNA was extracted from 4-d-cultured B cells using TRIzol (Invitrogen) and was reverse transcribed by Supercrypt II RT (Invitrogen). PCR primers used for γ1, e, and αGLT, (μ-εC), μe, (μ-εC), μα-Cα, AID, and B-2-microglobulin were as described previously (11, 20). All PCR reactions were performed on three dilutions of cDNA (1:1, 1:3, and 1:9) for semiquantitative evaluation. Amplified products were separated on agarose gel and stained with ethidium bromide.

DC-PCR. Genomic DNA isolated from cultured B cells on day 6 was digested with EcoRI, circularized, and used as template for PCR using primers as reported previously for Sq-Sy1, Sq-Sx, Sq-Sr, and the nicotinic acetylcholine receptor β unit (11, 21). All PCR reactions were performed on three dilutions of circularized DNA (1:1, 1:3, and 1:9) for semiquantitative evaluation.

Online supplemental material. Figs. S1–S3 show additional analysis of BAFF- or APRIL-stimulated B cells. Supplemental Materials and methods describe CFSE staining, [3H]thymidine incorporation assay, polymyxin B treatment, RT-PCR for BAFF mRNA, and isolation of IgM+ and IgD+ B cells. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20032000/DC1.

This work was supported by National Institutes of Health grants AI31136 and AI31541, the March of Dimes, and the Wallace Fund.

The authors have no conflicting financial interests.

Submitted: 19 November 2003
Accepted: 19 November 2004

REFERENCES