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Review

BAFF, APRIL and human B cell disorders

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Abstract

B cells require signals from multiple sources for their development from precursor cells, and differentiation into effector cells. BAFF has been identified as a critical regulator of B cell development and differentiation. Defects in the production of BAFF and/or expression of its receptors have been associated with a diverse array of human immunopathologies characterised by perturbed B cell function and behaviour, including autoimmunity, malignancy, and immunodeficiency. This review will discuss the role of BAFF in the pathogenesis of these human immune disorders. It will also highlight relevant differences between the function of BAFF in humans and mice and the impact of this on the therapeutic utility of BAFF antagonists in the treatment of different human diseases.

Keywords: Human B cells; BAFF; APRIL; Autoimmunity; Malignancy; Immunodeficiency

1. Overview

The generation of the mature B cell pool involves the stepwise development of hematopoietic stem cells into pro-B cells, which mature into pre-B cells and then immature B cells [1–3]. Immature B cells are then exported to the periphery as transitional B cells which undergo further selection and developmental events, represented as discrete subsets of transitional cells (type 1 [T1], type 2 [T2], type 3 [T3]) to yield mature B cells [4,5]. When mature B cells encounter T-cell dependent (TD) antigen (Ag), they differentiate into high affinity effector cells, namely memory B cells and immunoglobulin (Ig)-secreting cells (ISC) or plasma cells (PC) [3,6]. This process occurs within specialized structures called germinal centres (GC) in secondary lymphoid tissues [3,6] (Fig. 1). Thus, mature B cells are responsible for the generation of humoral immunity and long-lived serological memory. The co-ordinated differentiation of B cells at these different stages of development and maturation is influenced by multiple factors, such as stromal cells and cytokines provided by the bone marrow (BM) environment, exposure to Ag, and interactions between the B cells, Ag-specific T cells and dendritic cells (DC) in the periphery [1,3,6].

Differentiation of mature B cells into effector cells must be strictly regulated to ensure sufficient specific humoral immunity whilst simultaneously avoiding the production of autoantibodies. Receptor–ligand pairs of the tumour necrosis factor receptor (TNF-R)/TNF superfamily play critical roles in humoral immunity by regulating responses of activated B cells. Interactions between CD40 [7], CD27 [8], CD134 (OX40) [9] and TNF-R [10] on B cells and their respective ligands (CD40L, CD70, CD134L, TNF-α), usually on $CD4^+$ T cells, promote B cell proliferation, differentiation and Ig secretion, while ligation of CD30 [11] and CD95 [12] negatively regulate B cell behaviour. During the past 7 years, BAFF, another ligand of the TNF superfamily, has emerged as a potent regulator of multiple functions of human and murine B cells and, to a lesser extent, T cells. This review will discuss the potential role of BAFF, the related molecule APRIL, and their respective receptors, in the pathogenesis of a variety of human immune disorders.

2. BAFF: B cell activating factor belonging to the TNF family

BAFF (also known as BLyS, TALL-1, zTNF4, THANK; TNFSF13B) was independently identified in 1999 by several research groups based on its homology to the TNF superfamily [13–16]. BAFF exhibits greatest homology to another member of the TNF family, a proliferation-inducing ligand (APRIL) [17]. BAFF is produced predominantly by myeloid cells (monocytes,

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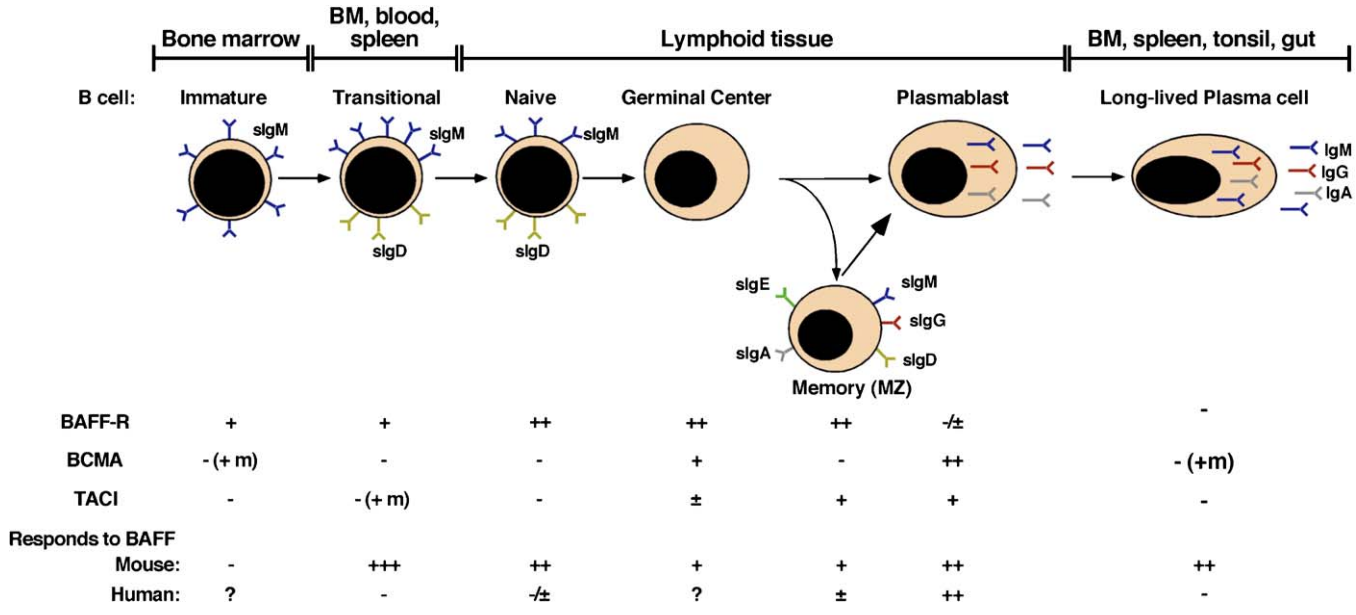

Fig. 1. Differential expression of BAFF receptors during human B cell development and differentiation. The expression of BAFF-R, TACI and BCMA at the different stages of B cell development (immature → transitional B cell) and differentiation (mature B cell → memory B cell/PC), as well as the ability of murine or human B cells to respond to BAFF, are indicated. The parentheses indicate differences in expression of BAFF receptors between murine and human B cells.

macrophages, DCs, astrocytes) [18–21] and neutrophils [22,23]. The amount of BAFF produced *in vitro* can be increased following stimulation of myeloid cells with CD40L, IL-10, IFN-α or IFN-γ [18–21], while G-CSF and other pro-inflammatory mediators increased production by neutrophils [22,24]. BAFF is also expressed by human B cells following either infection with the human herpes virus Epstein Barr virus (EBV) [25] or *in vitro* stimulation with anti-Ig and CD40L [26]. BAFF mRNA has been detected in resting and stimulated T cells [23,27], stromal cells [28], follicular DC (FDC) [29] and mast cells [23,30]; however expression of BAFF by these cells awaits confirmation at the protein level.

3. Functions of BAFF

3.1. B cell survival

Initial studies found that treating mice with BAFF increased the numbers of splenic B cells, particularly transitional B cells, and enhanced humoral immune responses to both T-cell independent (TI) and TD Ag [14,31,32]. Consistent with this was the ability of BAFF to augment proliferation and Ig secretion by B cells activated through the B cell receptor (BcR) [13,14] (Fig. 2). Although early studies proposed that BAFF improved B cell responses by acting as a co-stimulator of proliferation [13], it was subsequently revealed that BAFF achieved this effect by sustaining B cell survival [31–34].

BAFF is capable of binding to all peripheral murine B cells [31,35–38]. However, it does not influence the survival of all B cell subsets equally. BAFF has no effect on immature BM B cells (Fig. 1) or B1 cells in the peritoneal cavity [33,39]. Furthermore, the effect of BAFF on B cell survival and longevity was limited to late transitional (type 2–3) and mature B cells (Fig. 1) [31,36]. This led to the proposal that signals delivered by BAFF, coupled with engagement of the BcR, promotes the positive selection of transitional B cells and allows for their continued maturation [31,33]. This was supported by the findings that signalling through the BcR increased binding of BAFF to murine B cells [36], and that an intact BcR signal transduction pathway is required for acquisition of BAFF-responsiveness in developing B cells [40]. In contrast to murine B cells, the effects of BAFF on the survival of resting transitional and mature human B cells, at least *in vitro*, are less pronounced (Fig. 1) [41–44]. Despite this species difference, BAFF sustains the viability of plasmablasts generated from both human memory or GC B cells *in vitro* [29,41,45] and murine marginal zone (MZ) B cells *in vivo* [32,46]. Because CD40L and IL-10 are required for the generation of human plasmablasts *in vitro* [47], and also increase BAFF production by myeloid cells [18–20], activated $CD4^+$ T cells would thus be capable of providing an environment conducive to the initial generation, and subsequent survival, of effector cells (Fig. 3a). BAFF also supports the survival of murine, but neither human nor monkey, PC [38,41,48,49]. Overall, with the exception of some species-specific differences, BAFF can support the survival of murine and human B cells at different stages of development and differentiation (Fig. 1).

BAFF maintains B cell survival by modifying expression of pro- and anti-apoptotic molecules. Expression of the anti-apoptotic genes Al, bcl-2, bcl-xL [31,32] and Mcl-1 [38] was increased following *in vitro* exposure of murine B cells or PC to BAFF, while that of the pro-apoptotic molecules Bak [32], Blk [50] and Bim [51,52] was reduced. Thus, BAFF improves the survival of responsive B cells by altering the ratio between pro-survival and pro-apoptotic molecules. The inability of human transitional B cells to respond to BAFF [42–44] is reminiscent of murine Tl B cells [31,33]. Similarly, all human transitional and

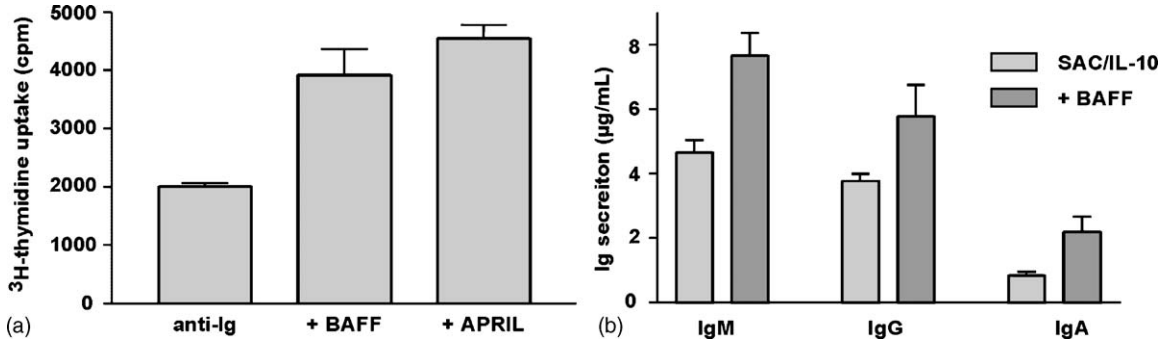

Fig. 2. BAFF enhances proliferation and Ig secretion by human B cells co-stimulated through the BcR. B cells were purified from normal peripheral blood and cultured with (a) F(ab)′$_2$ fragments of anti-human Ig alone (2.5 μg/mL; Jackson Research Labs), or in combination with BAFF (2 μg/mL) or APRIL (500 ng/mL; Peprotech), or (b) *Staphylococcus aereaus* Cowan (SAC; Calbiochem) and IL-10 in the absence or presence of BAFF: (a) cell proliferation was measured by determining the incorporation of ^{3}H-thymidine into newly synthesised DNA during the final 18 h of a 4 day culture and (b) Ig secretion was determined using heavy chain specific ELISAs after 12–14 days of culture. Each value represents the mean ± S.E.M. of (a) triplicate samples or (b) five to six replicates.

murine Tl B cells lack expression of bcl-2, bcl-xL and Al [43,53]. Thus, it is possible that the absence of pro-survival molecules from these transitional B cells compromises the ability of BAFF to increase their survival.

3.2. Class switch recombination

Following Ag stimulation, naïve IgM$^+$IgD$^+$ B cells can undergo class switch recombination (CSR) to express and produce IgG, IgA or IgE. This allows versatility in both function and distribution of the Ig, while retaining antigenic specificity [3]. CSR occurs in response to signals delivered through CD40, usually in combination with specific cytokines, or Toll-like receptors. BAFF and APRIL also induce CSR in human and murine B cells (Fig. 3b). Human B cells undergo the molecular events characteristic of CSR from Cμ to Cγ and Cα when stimulated with BAFF or APRIL alone, and this was greatly increased when the cultures were supplemented with IL-4 or IL-10 [19]. BAFF may further contribute to Ig isotype switching by inducing B cells to secrete IL-10 [54], a known switch factor for the production of IgG and IgA by human B cells [7]. Although CSR from Cμ to Cε was not observed when human B cells were exposed to BAFF or APRIL alone, switching to IgE did occur when combined with IL-4 [19]. Interestingly, BAFF or APRIL alone were not sufficient to induce the secretion of switched Ig isotypes by stimulated human B cells; rather, Ig secretion required additional signals, such as cross-linking of the BcR [19]. Similar findings have been reported for murine naïve B cells, inasmuch that they will undergo CSR to IgG and IgA in response to BAFF or APRIL alone, and to IgE in the presence of IL-4 [30,55]. Unlike human B cells [19], the secretion of switched Ig isotypes by BAFF or APRIL-stimulated murine B cells did not require BcR engagement [30,55].

DCs can enhance Ig isotype switching by human naïve B cells *in vitro* [56]. The finding that human B cells undergo an increased level of CSR when cultured with DCs that had been pre-activated with CD40L, IFNα or IFN-γ compared to those cultured with unstimulated DCs led to the suggestion that Ig isotype switching induced by activated DCs is BAFF-dependent. This was confirmed experimentally when it was discovered that CD40L and IFN's enhance BAFF production by myeloid cells [18–20], and that DC-mediated CSR could be inhibited when co-cultures of DCs and B cells were performed in the presence of reagents that neutralised the function of BAFF [19]. This may also explain the ability of IFN-α-stimulated DCs to induce murine B cells to undergo Ig isotype switching *in vivo* [57]. The ability of EBV to induce production of BAFF and APRIL by human B cells may explain how EBV can activate CSR, since EBV-mediated switching from IgM to IgG, IgA and IgE is dependent on endogenous BAFF production [25]. Thus, in addition to enhancing B cell survival, another function of BAFF (and APRIL) is to activate CSR.

Many of the findings established from *in vitro* investigations into the effects of BAFF on human and murine B cells have been substantiated by the phenotypes of mice rendered transgenic (Tg) or deficient for BAFF. BAFF Tg mice exhibit lymphoid hyperplasia, their spleens contain increased numbers of T2 and mature B cells, and serum levels of IgM, IgG and IgA are 20-, 2–20- and >1000-fold, respectively, higher than controls [58–60]. Notably, B cells in these mice express elevated levels of bcl-2 compared to controls [59]. On the other hand, BAFF-deficient mice have a severe reduction in peripheral B cells and are hypogammaglobulinemic [39,61]. Based on the functions of BAFF, it is likely that the increased levels of serum Ig in BAFF Tg mice, as well as those injected with BAFF [14,32,58–60], results from the combined effect of sustaining the viability of Ig-secreting plasmablasts [41,45,46] coupled with induction of CSR in naïve B cells [19,30,55].

3.3. T-cell activation

While most studies on BAFF have focussed on its B cell stimulatory function, an additional function as a co-stimulator of T cell activation has emerged. When human or murine T cells were stimulated with anti-CD3 mAb, proliferation and cytokine secretion were greatly increased in the presence of exogenous BAFF [23,27,62,63]. The level of the T cell response was comparable to that induced by anti-CD3 plus anti-CD28 mAb, demonstrating the potency of combined signals delivered by BAFF and Ag [23,62]. BAFF could enhance the *in vitro*

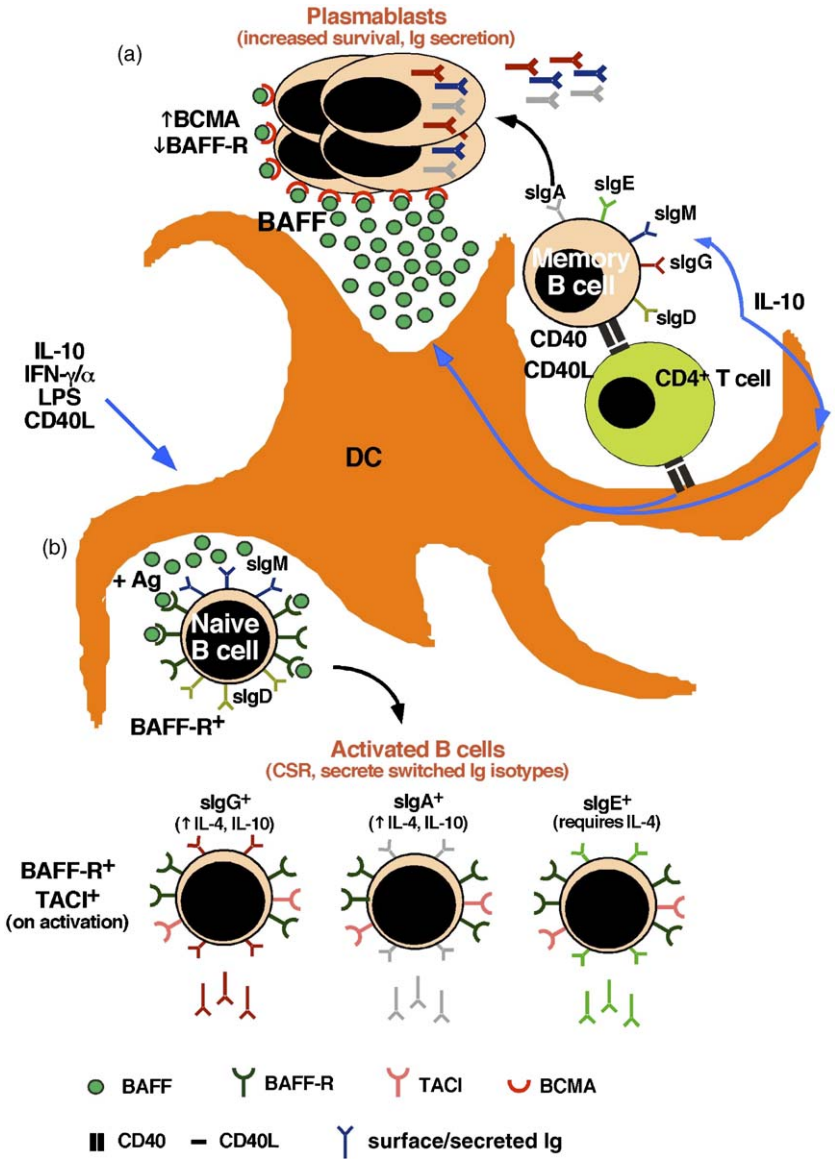

Fig. 3. Distinct effects of BAFF on activation of human B cells. Schematic representation of how interactions between T cells, DCs and (a) memory or (b) naïve B cells contribute to the generation of plasmablasts (a) or initiate the molecular events required for Ig CSR (b). The same T cell derived signals that drive B cell proliferation, differentiation and Ig isotype switching (i.e. CD40L and IL-10), as well as LPS and IFNs, stimulate DC to produce BAFF. Thus, DC-derived BAFF may act as (a) a survival factor for plasmablasts generated from memory B cells, and (b) a switch factor for the production of IgG, IgA or IgE (±appropriate cytokines) by naïve B cells.

response of $CD4^+$ and $CD8^+$ T cells, as well as naïve and effector T cells [62]. An effect of BAFF on T cells is consistent with the accumulation of activated T cells in BAFF Tg mice [59]. Thus, BAFF can augment the responses of not only B cells, but also T cells.

4. Identification and expression of BAFF receptors

BAFF binds three receptors, which all belong to the TNF-R superfamily—BAFF receptor (BAFF-R/BR3) [64,65], transmembrane activator of and calcium modulator and cyclophilin ligand (CAML) interactor (TACI), and B cell maturation antigen (BCMA) [60,61,66–68]; the latter two receptors also bind APRIL.

The expression of BAFF receptors has been characterised by two approaches—first, soluble BAFF was used as a probe, which does not discriminate between expression of BAFF-R, TACI and BCMA, and second, generating mAb specific for the different receptors. Recombinant BAFF binds all populations of murine B cells, including transitional, follicular, MZ and PC in spleen, LN and BM [31,35–38]. In contrast, BAFF does not bind to immature B cells in BM, yet it does react with transitional and recirculating mature B cells present in this tissue [31]. Consistent with these findings, BAFF-R was found to be absent from pro-B and pre-B cells in murine BM, but was detected at low levels on early transitional (T1) B cells and up-regulated on late transitional (T2/3), follicular and MZ B cells [69], thereby confirming previous findings obtained by PCR analysis of sort-purified pop-

ulations of murine splenic B cells [31]. TACI exhibited a similar expression profile as BAFF-R, as it was also low or absent from splenic TI B cells, induced at the follicular stage, and further increased on late transitional and MZ B cells [23,31]. In contrast to both BAFF-R and TACI, BCMA message was detected in Tl B cells in murine spleen, but then down-regulated at later stages of B cell maturation [31]. While neither BAFF-R nor TACI were detected by PCR analysis of PC in murine BM, these cells did contain mRNA for BCMA [38].

The expression of BAFF receptors on different subsets of human B cells has also been examined. Transitional, naïve, GC and memory B cells present in human spleen, tonsils, peripheral blood and BM are all capable of binding soluble BAFF. Transitional and naïve B cells bind BAFF exclusively through BAFF-R, because TACI and BCMA are absent from these cells. On the other hand, memory B cells can interact with BAFF through either BAFF-R or TACI [23,29,41,43,48,70–72]. In contrast, BCMA is not expressed by human transitional, naïve or memory B cells [29,41,42,48,70]. GC B cells in human tonsil predominantly express BAFF-R, albeit at a lower level (∼2-fold) than naïve and memory B cells present in the same tissue. GC B cells lack TACI expression, however a subset of them express BCMA [23,29]. Human plasmablasts acquire expression of BCMA and TACI yet down-regulate BAFF-R [29,41,45,73]. However, unlike the murine situation [38], human primary PC do not bind BAFF, lack surface expression of all known BAFF receptors and do not respond to BAFF with increased survival or Ig secretion *in vitro* [41,48]. This has also been noted for several human PC lines [13]. Thus, it would appear that normal human B cells first express BAFF receptors at the transitional stage of development and remain capable of receiving BAFF-dependent signals at least until they terminally differentiate into PC (see Fig. 1).

Although BAFF-R is expressed on the majority of B cells, its expression is not restricted to the B-lineage. A small subset of resting human and murine T cells have been found to bind soluble BAFF through BAFF-R, and the expression of BAFF-R increases following *in vitro* stimulation [23,63,68]. In contrast, neither resting nor stimulated T cells expressed detectable levels of TACI or BCMA [23,63].

5. BAFF-R, TACI and BCMA have distinct functions on B cells

The functions of the different BAFF receptors, as well as of BAFF and APRIL, have been investigated by examining mice harbouring mutations in the genes encoding these molecules. Analysis of these strains has revealed that each receptor has a distinct role following interaction with BAFF (summarised in Table 1).

5.1. BCMA

BCMA$^{-/-}$ mice exhibit normal B cell development and Ab responses following immunisation with TD or TI Ag, suggesting BCMA has a redundant role in B cell development and function [39,74,75]. Consistent with this, B cell development was unaffected in mice that were either deficient or transgenic for APRIL [55,76,77]. Furthermore, Ig production induced following culture of BCMA$^{-/-}$ B cells with BAFF or APRIL was normal, excluding a role for BCMA in this process [30]. Despite these findings, it is possible that BCMA is involved in specific aspects of B cell biology relating to their differentiation into effector cells, and thus the maintenance of humoral immune responses. For instance, BCMA$^{-/-}$ mice exhibit an impaired generation of BM PC *in vivo* [38]. Similarly, BAFF and APRIL support the survival and subsequent persistence of plasmablasts generated from murine splenic MZ B cells [46] or human memory B cells [29,41,45]. In these latter studies, the plasmablasts were BAFF-R^{-} BCMA^{+} (Fig. 3a), suggesting that BAFF may enhance humoral immunity by promoting plasmablast survival through a mechanism dependent on BCMA, rather than BAFF-R. More recently, it was shown that BCMA, but not TACI and BAFF-R, could enhance Ag presentation when over-expressed in a murine B cell line [78], providing further evidence for a role of BCMA in the effector function of mature B cells, rather than in their development (Table 1). It is also possible that BCMA has a distinct function in human B cell differentiation because human BCMA binds APRIL and BAFF with an affinity much higher than that for the interaction between murine BCMA and BAFF [75]. Although murine models show little disruption to B cell development and humoral immune responses in the absence of BCMA [39,74,75], this may not represent the same ligand–receptor interactions that occur in humans.

Table 1
Specific functions of BAFF-R, TACI and BCMA on human and murine B cells

BAFF receptor	Function	References
BAFF-R/BR3	Survival of murine transitional and murine and human mature B cells	[35,41,64,65]
	Sustains longevity of GC reaction	[112,113]
	CSR to produce IgG, IgA and IgE in response to BAFF	[30,55]
	Co-stimulates proliferation and cytokine secretion by human and murine T cells	[23,63]
TACI	Negative regulator of murine B cells	[79,80]
	Supports TI Ab responses	[35,89]
	CSR to produce to IgG, IgA and IgE in response to APRIL; to IgA in response to BAFF and APRIL	[30,55,83]
BCMA	Sustain survival of plasmablasts	[29,41,45,46]
	Enhance Ag presentation by mature B cells	[78]

5.2. TACI

In contrast to BCMA, TACI$^{-/-}$ mice exhibited a dramatic phenotype, inasmuch that they have increased peripheral B cells, and these cells exhibit greater proliferation and Ig production *in vitro* than B cells from control mice [79–81]. TACI$^{-/-}$ mice have altered levels of serum Ig, with reports of reduced IgM and IgA [80], as well as mildly increased IgM and IgG3 [79]. Furthermore, TACI$^{-/-}$ mice develop autoimmunity and

fatal lymphoproliferation [81]. Thus, TACI appears to be an inhibitory receptor of BAFF signalling. On the other hand, and despite the increased cellularity, TACI$^{-/-}$ mice have impaired responses following immunisation with Ficoll [79,80], suggesting an important role for TACI in regulating TI immune responses. This was further supported by the finding that TI responses were largely intact in BAFF-R$^{-/-}$ and BCMA$^{-/-}$ mice [35,74]. Thus, TACI appears to exert both positive and negative effects on the activation of murine B cells (Table 1). It is possible that TACI exerts some of its positive effect on humoral immunity by interacting with APRIL. First, responses to TI Ag [76] and serum levels of IgA [82] are elevated in APRIL Tg mice. Second, TACI$^{-/-}$ mice do not undergo CSR to produce IgG, IgA and IgE following *in vitro* stimulation with APRIL [30]. Third, APRIL-deficient mice are defective in CSR to IgA *in vivo* [55]. Despite this, TACI also contributes to CSR by interacting with BAFF. B cells from A/WySnJ mice underwent isotype switching to IgG and IgA in response to stimulation with BAFF, as well as APRIL [30]. Furthermore, TACI appears to be exclusively responsible for CSR to IgA, because TACI$^{-/-}$ mice failed to produce this isotype in response to either BAFF or APRIL [30]. Similar findings were noted for humans with loss-of-function mutations in TACI (see Section 6.3) whose B cells failed to secrete IgA in response to activation with either APRIL or BAFF [83]. Thus, TACI has multiple functions in regulating B cell differentiation (Table 1).

5.3. BAFF-R/BR3

Based on the observations that neutralising BAFF *in vivo* resulted in impaired B cell development [61,75], yet B cell development was intact in TACI$^{-/-}$ [79,80], BCMA$^{-/-}$ [39,74,75] and TACI$^{-/-}$ BCMA$^{-/-}$ [35] mice, it was anticipated that BAFF exerted its pro-survival effects on B cells through an additional receptor. Indeed, this led to the discovery of BAFF-R [64]. Coincidentally, a well-characterised immunodeficient mouse strain – A/WySnJ – was found to express a naturally mutated form of BAFF-R due to an insertion mutation in the intracellular domain [65,84]. A/WySnJ mice have severely reduced numbers of transitional and mature splenic B cells, but normal numbers of immature B cells in the BM, as well as peritoneal B1 cells [85–87]. This phenotype was due to an intrinsic defect in the survival of mature B cells [87,88]. These studies, together with those performed using mice that lack BAFF or BAFF-R due to gene targeting [35,89], established the unique role played by the BAFF/BAFF-R signalling pathway in regulating B cell survival and homeostasis. The impaired survival of developing B cells in mice deficient for BAFF or BAFF-R could be overcome by enforced expression of bcl-2 [89] or bcl-xL [84], supporting the proposal that BAFF maintains B cell survival by modulating expression of anti-apoptotic molecules (Table 1).

BAFF-R is also capable of mediating CSR in murine B cells. When stimulated with BAFF and IL-4, BCMA$^{-/-}$ or TACI$^{-/-}$ B cells produced comparable amounts of IgG1 and IgE as control B cells [30], implying that BAFF-R can initiate switching to these Ig isotypes independently of functional TACI or BCMA. Lastly, consistent with detection of BAFF-R, but not TACI, on T cells [23], BAFF-R is responsible for co-stimulating activated T cells [23,63] (Table 1).

6. Aberrant expression of BAFF, APRIL and BAFF receptors in human disease

The findings that (a) BAFF Tg mice develop symptoms typical of several human autoimmune diseases, such as systemic lupus erythematosus (SLE) and Sjogren's syndrome (SjS) [58–60,90], (b) the level of BAFF is elevated in the serum of autoimmune-prone mice [60], and (c) some BAFF Tg mice develop lymphomas [91] led to the suggestion that dysregulated expression and/or function of BAFF or its receptors may result in different human disease states. A wealth of information has been generated which indeed implicates BAFF (and APRIL) in the development, progression and pathogenesis in diseases as diverse as autoimmunity, B cell malignancies and immunodeficiencies.

6.1. Autoimmunity

BAFF, APRIL, and BAFF/APRIL heterotrimers have been found to be elevated in the serum and synovial tissue of some patients with systemic autoimmune diseases such as SLE, rheumatoid arthritis (RA) and SjS [90,92–99]. BAFF is also elevated in serum of patients with Wegener's granulomatosis, a chronic form of vasculitis [100], as well as in neurological lesions of patients with multiple sclerosis [21]. The BAFF and BAFF/APRIL trimer detected in autoimmune patients was biologically active [93,99] and, in general, the increased levels of serum BAFF were associated with increased levels of autoantibodies [92–94,96,98].

The cell-type responsible for the increased production of BAFF in some of these autoimmune conditions has been identified. BAFF is produced by T cells and, to a lesser extent, macrophages that infiltrate inflamed salivary glands in SjS [101], and by astrocytes [21] and monocytes [102] in MS. In RA patients, macrophages exclusively produced BAFF, while DC secreted APRIL, but neither of these cell types secreted the alternative cytokine [98]. Neutrophils, which can be detected in inflamed synovium, may also contribute to elevated levels of BAFF in RA [98]. Several other cytokines, such as soluble CD40L, IL-4, IL-6, IFN-α and IL-10, are elevated in autoimmune conditions [96,103–106]. Furthermore, there is a large increase in the level of expression of CD40L on T cells and B cells from SLE patients [107]. Interestingly, most of these cytokines can augment production of BAFF by macrophages, monocytes and DC *in vitro* [18–21]. Thus, although it has not been examined formally, it is likely that myeloid cells exposed to CD40L, IFN-α and IL-10 are responsible for the increased serum levels of BAFF in SLE.

In autoimmune diseases, the affected tissues are usually non-lymphoid, and are thus devoid of immune cells. However, inflamed synovium tissue and salivary glands from patients with RA and SjS contains large numbers of infiltrating lymphocytes [108]. In ∼30% of patients, the infiltrating cells form GC-like structures comprised of B cells, T cells, DC and FDC [108,109].

The formation of these ectopic GC-like structures is believed to result from the aberrant expression of soluble factors, namely TNF, lymphotoxin (LT), CXCL13, CCL19 and CCL21, that are required for normal lymphoid neogenesis, including the formation of GCs [109–111]. It has been recently revealed that the incidence of ectopic GC in inflamed tissues from patients with RA and SjS correlates not only with the levels of LT and CCL19, but also with the levels of serum BAFF and APRIL [95,96,98,105]. By using a xenogeneic model whereby NOD SCID mice are engrafted with synovial tissue from RA patients, it was demonstrated that the development of ectopic GC in these lesions could be abrogated by a soluble TACI-Fc decoy receptor [98]. The loss of GC's in TACI-Fc treated chimeric mice was accompanied by a large reduction in the levels of human IgG, IFN-γ, CCL19 and TNF-α, as well as the numbers of infiltrating B cells, T cells and FDC in the transplanted tissues grafts [98]. Interestingly, BAFF-deficient mice also exhibit a reduced FDC network [112,113]. These results therefore suggest that BAFF and/or APRIL plays a critical role in lymphoid neogenesis and the maintenance of ectopic GC's in autoimmune patients, eliciting effects on B cells, T cells and FDC. This is consistent with the findings that BAFF co-stimulates T cells [23,27,62,63], spleens from BAFF Tg mice contain increased numbers of effector T cells [59], and the accelerated dissolution of GC's in the spleens of mice with impaired BAFF/BAFF-R signalling compared to control mice [112,113].

6.2. B cell malignancies

The findings that BAFF and APRIL are elevated in several disease states that involve B cells, and that BAFF promotes B cell survival, led to the hypothesis that BAFF, APRIL and their receptors may be involved in the development and pathogenesis of some B cell malignancies. Evidence has now been presented suggesting that BAFF/APRIL may contribute to malignancies of mature B cells (non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (B-CLL)) and PC (multiple myeloma (MM), Waldenstrom's macroglobulinaemia (WM)). Malignant B cells from patients with B-CLL [71,114], NHL [26,72,115,116], MM [70,73], and WM [117] are all capable of binding soluble BAFF and, in some cases, APRIL. However, the receptor expression profile of these malignant B cells differed as NHL [116], B-CLL [71,114] and WM [117] B cells co-express BAFF-R and TACI, while MM cells preferentially expressed BCMA and TACI [70,73,118]. In contrast to NHL, B-lymphomas in the central nervous system exhibited a heterogeneous phenotype, with variable expression of BAFF-R, TACI and BCMA [21]. The ability of BAFF and APRIL to bind to malignant B cells was functionally significant because exogenous BAFF or APRIL promoted the proliferation and/or survival of B-CLL [71,114,119], NHL [72,115,116], MM [70,73] and WM [117] B cells *in vitro*.

The effects of BAFF/APRIL on malignant B cells, and the expression of BAFF-R and TACI on NHL and B-CLL B cells, resembled normal human B cells [23,29,41,43,48,70]; however, expression of BCMA by MM cells differs from normal primary human PC which lack expression of all known BAFF receptors [41,48]. Thus, the sustained expression of BCMA in MM represents divergence from the normal differentiation pathway and possibly provides an alternative survival signal for the malignant cells in this disease. Apart from this possibility, the ability of malignant mature B cells to respond to BAFF and APRIL *in vitro* does not explain the mechanism by which these cytokines may contribute to the disease process. However, a possible role of BAFF and APRIL in human B cell malignancies may lie in their aberrant expression and production in these diseases. First, BAFF and APRIL were found to be expressed by B-CLL [71,82,114], NHL [26,72] and MM cells [70,73,119] at levels greater than that observed for normal B cells. Second, levels of serum BAFF and APRIL were found to be elevated in patients with NHL [115,116], B-CLL [82,114] and WM [117] compared to healthy donors. Notably, those NHL patients with the highest levels of serum BAFF had reduced survival and poorer prognosis than those with lower levels [116]. Interestingly, APRIL Tg mice develop lymphoid tumours of B1 (i.e. CD5+) B cells [82], which corresponds to the malignant cell in B-CLL [120]. A complimentary mechanism whereby BAFF may contribute to the development of some lymphomas came from the observation that EBV infection of primary human B cells induces expression of both BAFF and APRIL [25]. The significance of this finding is that several B cell malignancies, such as Burkitt's lymphoma, develop following infection and immortalisation of normal human B cells by EBV [121]. Interestingly, patients with SjS are predisposed to B cell malignancies, particularly NHL [96]. Because patients with NHL have increased levels of serum BAFF [115,116], it is probable that increased levels of serum BAFF in SjS contributes not only to the development of autoimmune manifestations of this syndrome, but also to B cell malignancies frequently observed in these patients [96]. Third, the microenvironment of the malignancy may also exacerbate the survival and persistence of malignant B cells. For instance, BAFF was detected in the BM of myeloma patients at higher levels than that observed for samples of normal BM [70]; macrophages that infiltrate tissue-restricted lymphoma's secrete high levels of BAFF which promotes the viability of B-lymphoma cells [122]; and nurse-like cells, which can be isolated from B-CLL patients, contain abundant amounts of BAFF and APRIL and improve the survival of B-CLL B cells in a BAFF/APRIL-dependent manner [119].

BAFF protects normal B cells from apoptosis by modulating expression of members of the bcl-2 family of molecules [31,32,38,50–52]. This is also likely to be the mechanism by which BAFF and APRIL preserve the viability of malignant B cells. *In vitro* exposure of B cells from patients with NHL, B-CLL or MM to BAFF or APRIL increased expression of the pro-survival proteins bcl-2, mcl-1 and bcl-xL and decreased the pro-apoptotic regulator bax [72,73]. Similarly, survival and proliferation of, as well as expression of survival proteins by, malignant B cells were reduced when cultured in the presence of soluble BAFF antagonists [26,71,73]. Taken together, it is possible that aberrant and/or excessive production of BAFF or APRIL by malignant B cells themselves (i.e. autocrine) or by supporting cells present within the microenvironmental niche occupied by the malignant B cells (i.e. paracrine) may facilitate their growth and survival. Consequently, blocking interactions

between BAFF and APRIL and their receptors may be a feasible therapeutic approach for treating some B cell malignancies. Interestingly, B-CLL and MM cells express higher levels of pro-survival proteins than their normal B cell counterpart [120,123]. Based on the effect of BAFF and APRIL on expression of bcl-2, bcl-xL and mcl-1 by malignant B cells *in vitro*, it is possible that they may also be responsible for this *in vivo* feature of the malignant clone in B-CLL and MM. Lastly, EBV-induced transformation of B cells and the subsequent development of some B cell malignancies may result from synergistic effects between host and viral-derived mediators. The genome of EBV encodes survival genes, including a Bcl-2 homologue [121], and a viral homologue of human IL-10 (vIL-10; [124]). Thus, following infection, EBV may provide its host cell with several important survival factors.

6.3. Immunodeficiency

Studies of gene-targetted mice, the A/WySnJ strain, as well as *in vivo* neutralization of BAFF, have all demonstrated a critical role for BAFF and BAFF-R in B cell development and homeostasis [35,39,61,75,85,86,89]. For this reason, it was hypothesized that mutations in *baff-r* may manifest as a human immunodeficiency characterised by B cell deficiency. In a cohort of 48 patients with common variable immunodeficiency (CVID), heterozygous sequence variants were detected in *baff-r* mRNA in ∼20% of patients [125]. However, these variants were also detected at a similar frequency in the normal population, as well as healthy parents of the CVID patients suggesting that they were polymorphisms rather than disease-causing mutations [125]. Despite this, one individual with hypogammaglobulinemia and homozygous deletions in the transmembrane domain of BAFF-R was identified from a cohort of 50 CVID patients [126]. To date, limited information is available on the clinical presentation of this patient, however he does apparently have peripheral B cells [126]. In contrast, mutations in *TACI* have been convincingly found to be associated with familial as well as sporadic CVID and IgA deficiency (IgAD) ([83,127]; reviewed in [128]). Six different mutations (two nonsense, three missense, one frame-shift) have been identified in 8 families, as well as in a further 10 unrelated patients with no family history of CVID, yielding a current cohort of 24 patients [83,127,128]. These mutations were located throughout *TACI*, being positioned in the extracellular (C104R, S144X, frameshift), transmembrane (A181E) and intracellular (S194X, R202H) domains. Only the S144X mutation affected surface expression of TACI, while the C104R mutant had a reduced ability to bind soluble BAFF and APRIL [83,127]. B cell development was normal in the majority of these patients. Interestingly, most patients were heterozygous with respect to their *TACI* mutations, although two individuals were homozygous for S144X, and one patient was a compound heterozygote [83,127]. It is currently unclear how mutations in one TACI allele would result in an immunodeficiency. Because TACI is expressed as a trimer, it is possible that the stoichiometry of such a complex would be disrupted in the presence of a mutant TACI protein. Similarly, mutations in the intracellular domain of TACI may diminish its capacity to recruit mediators of downstream signaling pathways, thus compromising the quality of the signal delivered through the mutant receptor. In other words, a mutant TACI protein may act in a dominant negative manner.

A particular curiosity of individuals with TACI mutations was that the extent of disease was quite variable. For instance, unrelated individuals with the same heterozygous mutation developed either selective IgAD, CVID, or CVID with associated lymphoproliferation/malignancy and/or autoimmunity. Furthermore, there was one individual (A181E heterozygote) who was normal, and a TACI-null patient, due to S144X homozygosity, who had only mildly reduced levels of serum Ig [127,128]. This was also reflected when B cell development was examined—one S144X homozygote was B cell deficient, while their healthy sibling had normal numbers of peripheral B cells, including memory B cells [127]. Similarly, a CVID patient with compound heterozygous *TACI* mutations, and is therefore presumably TACI-null, had normal frequencies of peripheral B cells, including memory cells [83]. Thus, the penetrance of disease must be contingent upon not only mutations in *TACI*, but additional genetic or environmental modifiers. Despite this variability in disease presentation, identification and characterisation of these patients underscores the importance of BAFF and/or APRIL in the function of human B cells *in vivo*, with excessive signalling by BAFF causing B cell mediated immunopathology and insufficient signalling resulting in B cell immunodeficiency. Because TACI, but not BAFF-R, appears to be capable of initiating CSR to IgA in response to BAFF or APRIL [30], it would be anticipated that mutations in *TACI* would have a greater effect on serum IgA levels than would *BAFF-R* mutations. Thus, BAFF and/or APRIL may be therapeutic candidates for treating patients with humoral immunodeficiencies, particularly IgAD.

7. Differences in the BAFF/APRIL system in humans and mice

Excessive production of BAFF in both humans and mice results in B cell mediated disease—consequently, the therapeutic utility of BAFF inhibitors is enormous, a fact that has not been underestimated by pharmaceutical companies. However, it is important to appreciate important differences that may exist between human diseases and murine models. Several of these are outlined below.

7.1. TACI deficiency

Although there were some similarities between CVID patients with *TACI* mutations and TACI$^{-/-}$ mice, such as lymphoid hyperplasia, splenomegaly and reduced responses to TI Ag in some patients [79,80,83,127,128], there were also some striking differences. TACI$^{-/-}$ mice exhibit increased numbers of B cells, and these cells are hyperresponsive [79–81]. Despite this, basal serum Ig levels are generally similar to controls [79,80]. TACI$^{-/-}$ mice also develop lymphomas and SLE-like disease [81]. In contrast, humans with CVID associated with *TACI* mutations have normal numbers of peripheral B cells, yet reduced levels of serum Ig, especially IgG and IgA; some

patients are even agammaglobulinaemic [83,127]. Furthermore, none of these CVID patients exhibit clinical features of SLE [128]. Thus, although the data derived from analysis of TACI$^{-/-}$ mice suggested that TACI may act as a negative regulator of murine B cell function, it is possible that TACI predominantly delivers a positive signal to human B cells in response to BAFF and/or APRIL and thus plays an important role in B cell activation and differentiation [128]. These differences also highlight the need to re-evaluate the phenotype of mice heterozygous for TACI deficiency.

7.2. *Effect of BAFF on B cell development in humans and non-human primates*

There are several lines of evidence to suggest that B cells from humans and non-human primates are less dependent on BAFF for their development and survival. Unlike murine B cells [33,34], human B cells exhibit minimal responses to the pro-survival effects of BAFF *in vitro*, despite expressing BAFF-R [41–44]. Consistent with these results were the findings that when cynomolgus monkeys were treated with either a neutralising anti-BAFF mAb or soluble BAFF antagonist (BAFF-R-Fc), putative transitional B cells were unaffected while the frequency of mature peripheral B cells was reduced <2-fold compared to control animals [49,111,129]. Furthermore, B cell development appears to occur in the single CVID patient with mutations in BAFF-R [126]. In contrast, B cell numbers were reduced ∼10-fold in mice incapable of signaling through BAFF/BAFF-R [35,39,61,75,85,86,89]. Similarly, BAFF has very little effect on the survival and function of terminally differentiated PC present in spleens and BM of humans and cynomolgus monkeys [41,48,49], unlike its effect on murine PC [38]. The reduced responsiveness of developing human B cells to BAFF may explain why only ∼30% of RA patients exhibited some improvement in disease score following treatment with anti-BAFF mAb [111]. B cell depletion is accelerated in mice treated concomitantly with anti-CD20 mAb and BAFF-R-Fc [130]. Thus, it may be beneficial to combine therapies to improve the efficacy of BAFF antagonists for the treatment of RA.

7.3. *Cytokines in human autoimmune diseases*

Another feature to be aware of when using animal models of human disease is the species-specific effect of particular cytokines on B cell behaviour. An example of this is IL-10, which potently induces the growth and differentiation of human, but not murine, B cells [124]. A characteristic of human SLE, RA and SjS is the presence of high levels of IL-10 in patients' serum [104,106]. Because IL-10 induces BAFF production by myeloid cells [18,20], it is possible that elevated levels of serum BAFF in these diseases are secondary to the elevated levels of serum IL-10. Consistent with this, it was found that the disease activity in SLE was dramatically ameliorated following treatment with neutralizing anti-IL-10 mAb [131]. Thus, targeting BAFF together with IL-10 may have therapeutic benefit for the outcome of autoimmune diseases where there is a likely involvement of IL-10 and BAFF.

8. Conclusion

Since its discovery in 1999, a wealth of information regarding the role of BAFF in B cell development and differentiation has been reported. These findings have shed new light on some basic processes of immunology. More importantly, these studies have revealed potential mechanisms that underlie the pathogenesis of a diverse array of diseases characterised by perturbed B cell behaviour. With this knowledge, it should now be possible to improve treatment of antibody-mediated systemic autoimmune diseases and B cell malignancies in a manner similar to the way anti-CD20 mAb (Rituximab) revolutionised therapy of RA and NHL [111,132]. Although studies from murine models suggest excess BAFF levels alone are responsible for disease development, the greatest clinical effect of BAFF antagonists in humans may be observed when used together with therapies that target B cells (e.g. Rituximab) and/or cytokines (TNF-α, IL-10). Such regimes will hopefully provide improved outcomes for individuals diagnosed with debilitating B cell mediated dyscrasias.

Note added in proof

Since the submission of this review, another patient with CVID and a TACI mutation has been reported [133], while another study examining a BAFF neutralising mAb (Belimumab) also found that B-cell numbers in the lymphoid tissues of cynomolgus monkeys was reduced approximately two-fold when treated with this reagent for 26 weeks [134].

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