BAFF: a local and systemic target in autoimmune diseases

I. Moisini and A. Davidson
Center for Autoimmune and Musculoskeletal Diseases, Feinstein Institute for Medical Research, Manhasset, NY, USA

Summary
BAFF (a B lymphocyte activating factor of the tumour necrosis factor family) is a vital homeostatic cytokine for B cells that helps regulate both innate and adaptive immune responses. Increased serum levels of BAFF are found in a number of different autoimmune diseases, and BAFF is found in inflammatory sites in which there is lymphoid neogenesis. BAFF antagonism has been used in several autoimmune disease models, resulting in B cell depletion, decreased activation of T cells and dendritic cells (DC) and a reduction in the overall inflammatory burden. BAFF, through its interaction with BAFF-R, is required for survival of late transitional, marginal zone and mature naïve B cells, all of which are depleted by BAFF blockade. Through their interactions with TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) and BCMA (B cell maturation protein), BAFF and its homologue APRIL (a proliferation-inducing ligand), support the survival of at least some subsets of plasma cells; blockade of both cytokines results in a decrease in serum levels of immunoglobulin (Ig)G. In contrast, neither BAFF nor APRIL is required for the survival or reactivation of memory B cells or B1 cells. BAFF also helps DC maturation and interleukin (IL)-6 release and is required for proper formation of a follicular dendritic cell (FDC) network within germinal centres, although not for B cell affinity maturation. The clinical efficacy of BAFF blockade in animal models of autoimmunity may be caused both by the decline in the number of inflammatory cells and by the inhibition of DC maturation within target organs. Blockade of BAFF and its homologue APRIL are being explored for human use; several Phases I and II clinical trials of BAFF inhibitors for autoimmunity have been completed and Phase III trials are in progress.

Keywords: BAFF, APRIL, autoantibodies, rheumatoid arthritis, SLE, multiple sclerosis, Sjögren’s syndrome

Introduction
B cells are considered culprits in autoimmune diseases both because they produce pathogenic autoantibodies and because they have multiple effector functions, including antigen uptake and transport [1], antigen presentation to T cells [2,3], production of cytokines [4] and chemokines [5] and migration to sites of inflammation [6–8]. One way to modulate B cell function is to antagonize the B cell survival molecule BAFF (α-B lymphocyte activating factor of the tumour necrosis factor family). Therapeutic antagonism of BAFF and its homologue APRIL (a proliferation-inducing ligand) has been based on the discoveries that BAFF provides an important homeostatic signal for B cell survival and selection [9–12] and that soluble BAFF and APRIL are expressed at high levels in the serum and in the target organs of individuals with established autoimmune diseases [13–19]. In this review we will examine the physiology of BAFF and its receptors, its role in autoimmunity and its potential as a therapeutic target.

BAFF and its receptors
BAFF is a member of the tumour necrosis factor (TNF) family and is expressed on the surface of monocytes, dendritic cells (DC), neutrophils, stromal cells, activated T cells,
malignant B cells and epithelial cells (reviewed in [20]). BAFF binds to three different receptors, BAFF-R, TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) and BCMA (B cell maturation protein), that are expressed differentially at various times during B cell ontogeny [21]. Most of the expressed BAFF is cleaved from the cell surface and circulates as a soluble active homotrimer [22] that binds to BAFF-R. A small proportion of circulating BAFF self-associates into dimers of 2ng trimers that bind to and activate TACI [23]. The homologous molecule APRIL binds to TACI and BCMA but not to BAFF-R. TACI is activated by multimerized or membrane-bound BAFF and multimerized APRIL, but not by homotrimers. BCMA is a high-affinity receptor for APRIL; in humans BCMA binds BAFF with low affinity, whereas mouse BCMA is APRIL-specific (reviewed in [24]). APRIL also interacts with sulphated proteoglycans that aggregate APRIL on the cell surface [25]. TACI can bind to syndecans through a site separate from the BAFF binding site. The role of this interaction is not yet clear [26] (Fig. 1).

BAFF, TACI and BCMA expression does not become evident until the transitional stage, with BAFF-R being the predominant receptor on naive and memory B cells, TACI the predominant receptor on short-lived plasma cells and BCMA the predominant receptor on long-lived plasma cells [27,28]. Each receptor activates its own set of signalling pathways. BAFF-R is the only BAFF receptor to activate the alternative nuclear factor kappa B (NF-kB) pathway; BAFF enhances long-term B cell survival primarily through this pathway by up-regulating anti-apoptotic proteins (reviewed in [21,29]). BAFF-R ligation also results in signals through Pim2 that increase metabolic fitness by inducing a metabolic bias toward glycolysis [29,30]. In this manner B cells are prompted to respond to BCR activation. TACI and BCMA signal through the classic NF-kB pathway and through the Mek (mitogen-activated protein extracellular signal-related kinase) pathway to up-regulate anti-apoptotic and down-regulate pro-apoptotic pathways, and through JNK/p38 (c-Jun N-terminal kinase) to drive class-switching (reviewed in [21]).

Functions of BAFF and APRIL

BAFF and APRIL mediate several important B cell functions. The interaction of BAFF with BAFF-R is essential for survival of B2 cells past the transitional type 1 (T1) stage with a minor contribution from TACI and none from APRIL or BCMA [31–33]. BAFF acts as a rheostat for B cell selection such that increased competition for BAFF results in more stringent deletion of autoreactive B cells. Conversely, decreased competition for BAFF in the context of B cell lymphopenia or increased levels of circulating BAFF result in relaxation of B cell selection and release of more autoreactive naïve B cells. BCR signalling strength controls the amount of BAFF signal because it generates p100, a substrate for the non-classical NF-kB signalling pathway used by BAFF-R. The presence of p100 is the green light for signalling through BAFF-R leading to B cell survival. Lack of p100 causes B cell death because of insufficient BAFF-R signalling. BCR signals generate little p100 in early T1 cells because their immature rafts contain insufficient cholesterol; T1 cells are therefore selected negatively. In contrast, the amount of p100 generated by BCR signalling is sufficient for BAFF-instructed survival of late transitional and follicular B cells and absence of BAFF leads to distinctly decreased survival of these cells [34]. BAFF-R expression is up-regulated by B cell receptor (BCR) ligation on mature B cells [35] and it is the predominant receptor expressed on resting memory B cells [36]. However, it is clear that survival and reactivation of B cell memory is BAFF-independent. Plasma cells express TACI and/or BCMA and their survival can be supported by either BAFF or APRIL that are secreted by multiple cell types within the lymph node or bone marrow microenvironment [37]. In contrast, B1 cells do not require BAFF or APRIL for survival [31].

BAFF plays an important role in humoral immunity. T cell-independent type II responses require the interaction of BAFF 60-mer or membrane BAFF with TACI [23,38,39]. This interaction is also vital for T cell-dependent immunoglobulin (Ig)M responses [27], whereas IgG responses are much less BAFF-dependent. BAFF-deficient mice fail to develop a proper FDC (follicular dendritic cell) network leading to smaller and unstable germinal centres in which class-switching and somatic hypermutation still occur, but with diminished IgG and secondary responses [40] (reviewed in [41]). Class-switching to IgA appears to be dependent upon the interaction of APRIL, multimerized by proteoglycans, with TACI [42,43]; a deficiency of IgA is the major phenotypic abnormality of APRIL-deficient mice.
suggesting that it functions in the maintenance of mucosal immunity [44]. An ongoing puzzle is the regulatory role of TACi, with the development of autoimmunity and lymphomas in TACi-deficient mice. Several alternate mechanisms have been suggested for this effect [45].

BAFF is also an essential component of the innate immune response and is induced in myeloid DC by type I interferons (IFNs) [46]. BAFF up-regulates Toll-like receptor (TLR) expression, promotes B cell survival and, together with IL-6, promotes Ig class-switching and plasma cell differentiation [47,48]. Activation of intracellular TLRs in B cells by immune complexes containing nucleic acids up-regulates expression of BAFF receptors, particularly TACi [47,49], and increases BCR-mediated signalling. In contrast, activation of B cells via TLR-4 up-regulates BAFF-R preferentially and renders the cells sensitive to Fas-mediated apoptosis [50]. The triad of TLRs, type I IFNs and BAFF creates an amplification loop that propagates production of IgG autoantibodies to nucleic acids in the absence of T cell help [49] (Fig. 2).

The role of BAFF receptors on cell types other than B cells is just beginning to be appreciated. Although BAFF-R is expressed on T cells [51], its role in T cell co-stimulation is still controversial and T cell numbers are normal in BAFF-deficient mice. DC also express BAFF receptors (mainly TACi) and, when transfected with an siRNA that silences BAFF, remain in an immature state and fail to produce the IL-6 required for the differentiation of T helper type 17 (Th17) cells [52]. Human myeloid DC stimulated with BAFF up-regulate co-stimulatory molecules, lose their phagocytic ability and produce inflammatory cytokines and chemokines including IL-1, IL-6, monocyte chemoattractant protein (CCL2) and CCL5, inducing Th1 responses in vitro [53].

These studies suggest that BAFF acts on DC to help them recruit immune cells to inflammatory sites and to enhance the proinflammatory activity of T cells. Whether or not this is an autocrine function of DC in inflamed tissues is currently not known.

Induction of BAFF by Type I IFNs and TNF blockade

It is recognized increasingly that the innate immune system can initiate and perpetuate autoimmunity. A small number of patients undergoing therapy with type I IFNs for cancer or viral infections developed systemic lupus erythematosus (SLE) [54]. Similarly, IFN-α accelerates SLE in some murine models [55] and this is associated with increased serum levels of BAFF. TNF-α blockade also induces increased levels of BAFF via up-regulation of type I IFNs and has been associated with the development of anti-nuclear antibodies (ANAs) in up to 50% of patients and clinical SLE in a much smaller proportion [56]. Interestingly, IFN-β, used for the treatment of multiple sclerosis, also induces BAFF (but not APRIL) in a manner that correlates highly with the induction of IFN-inducible genes [57]. IFN-β therapy is associated with the induction of autoantibodies [58], but has been associated only rarely with clinical SLE in humans [59]. Drug-induced SLE associated with increased BAFF is usually reversible after the offending drug is discontinued. B cell depletion in patients treated with anti-CD20 (Rituximab) also results in high levels of BAFF in an attempt to maintain B cell homeostasis [60]. Similarly, high-dose cyclophosphamide induces increased BAFF levels (unpublished data). A potential consequence of high BAFF levels could be the emergence of autoreactive B cells and enhanced adaptive immune responses through stimulation of DC maturation. The implications of this with respect to relapse or progression of autoimmune disease are yet to be explored.

BAFF and APRIL are therapeutic targets in autoimmunity

Several strategies have been developed to block BAFF and APRIL in vivo. Selective inhibition of BAFF can be achieved with a soluble BAFF-R-Ig fusion protein or with an antibody to BAFF; soluble TACi-Ig or BCMA-Ig fusion proteins can block both BAFF and APRIL (reviewed [61]). Because the phenotype of APRIL transgenic (Tg) and APRIL knock-out
mice is much less dramatic than that of the BAFF Tg and knock-out mice there has been much less interest in developing APRIL-specific drugs, although APRIL may be involved preferentially in humoral mucosal immunity. Clinical trials of a selective antibody to BAFF (Belimumab) and with the BAFF/APRIL inhibitor TACI-Ig (Atacicept) are currently in progress [62–64]. There are still unresolved issues regarding their mechanism of action as well as the therapeutic benefits of BAFF and APRIL blockade versus blockade of BAFF alone. BAFF may be a therapeutic target in several different diseases.

Rheumatoid arthritis (RA). Increased serum BAFF levels are found in RA patients [65] and are associated with anti-collagen type II antibodies in collagen arthritis (CIA), an animal model of RA [52,66]. BAFF protein is also expressed highly in DCs in the early stages of disease in the CIA model. Silencing of BAFF specifically in the synovium of mice pre-immunized with collagen does not alter systemic humoral immune responses to collagen, but attenuates the production of IL-6 by DCs and abrogates local inflammation by decreasing local Th17 and plasma cell accumulation [52]. High levels of both BAFF and APRIL, along with their receptors, are found in the rheumatoid synovium [15] with APRIL being produced by synovial DCs and BAFF by tissue macrophages [18] and synovial fibroblasts. Both cytokines are also produced by synovial B cells [67,68]. Using human synovium–severe combined immunodeficiency (SCID) synovial grafts, Seyler et al. demonstrated that BAFF/APRIL blockade destroys the FDC network within ectopic germinal centres which then decrease in size. TACI-Ig seemed to have no effect on Ig production in the synovial samples lacking germinal centres, suggesting that synovial plasma cells are resistant to BAFF/APRIL blockade. Interestingly, this treatment resulted in increased IFN-γ production from T cells suggesting a switch from Th17 to Th1 responses in the joint [18].

Multiple sclerosis (MS). With the emerging view that B cells are equal offenders with T cells in the pathogenesis of MS, the role of BAFF has also been investigated. B cells infiltrate the plaques and clonally expanded populations produce antibodies that are responsible for intrathecal oligoclonal bands [69]. In mouse models, B cell depletion leads to collapse of CD4 and CD8 T cell numbers and disappearance of ectopic lymphoid structures from the meninges. Of interest, B cell depletion with Rituximab depletes B cells from the cerebrospinal fluid (CSF) but does not affect plasma cells; nevertheless, treatment has a long-lasting clinical benefit [70]. BAFF is expressed by astrocytes that are associated closely with BAFF-R-expressing cells [13] and within ectopic lymphoid follicles in the meninges [14], suggesting that BAFF is also a potential target in multiple sclerosis. In a study, BAFF/APRIL blockade in EAE resulted in B cell depletion, a subsequent decrease in T cells and activated DC and a concomitant decrease of brain and spinal cord infiltration. However, the effects of this treatment were strain-dependent and greater clinical efficacy was achieved with preventive therapy than with treatment of established disease [71]. A Phase II clinical trial of TACI-Ig in MS is currently in process.

Sjögren’s syndrome. BAFF Tg mice develop a Sjögren’s syndrome (SS)-like disease with enlarged salivary glands, leucocyte infiltrates and destruction of acinar cells [16]. High levels of BAFF were detected in the serum and epithelial cells of SS patients which add to the local BAFF produced by lymphocytes infiltrating salivary glands [72–74]. This may be a consequence of TLR stimulation and type I IFN release in the glands [73,75]. SS patients have higher numbers of Bcl-2 positive peripheral B cells compared to healthy controls and a lower incidence of apoptosis [76]. Similar to the collagen-induced arthritis (CIA) model, BAFF levels correlate with autoantibody levels [77]. Thus BAFF may be responsible for increased B cell survival and exaggerated Ig production in SS.

SLE. The pathogenic role of BAFF in SLE was revealed early in BAFF Tg mice that develop a lupus-like illness with the production of anti-DNA antibodies and the development of glomerulonephritis [22,78]. This was followed by the observation that BAFF blockade delayed SLE onset in SLE models [22] and the subsequent discovery that patients with SLE have high serum levels of BAFF and APRIL, that in some studies correlated with disease activity [79–81]. Because BAFF antagonists can inhibit the development of disease in lupus-prone mice, drugs that inhibit BAFF and/or APRIL have been developed for clinical use in SLE patients.

Interestingly, studies of BAFF Tg mice have shown that SLE in these mice is completely dependent on the presence of myeloid differentiation primary response gene 88 (MyD88), the key adaptor molecule for TLR signalling, and SLE develops even if CD4+ T cells are completely absent [49]. Thus, via the amplification loop shown in Fig. 2, extreme excess BAFF can drive the development of autoimmunity through activation of the innate immune system alone. Similarly, BAFF may help perpetuate disease in a manner that is independent of cognate T cell help. Recent studies have shown that activation of human memory B cells can be driven by the combination of BAFF, cytosine-guanine dinucleotide (CpG) ligation and cytokines. Thus, generalized inflammation and high levels of BAFF may drive continued production of plasma cells producing pathogenic autoantibodies [82,83].

Mechanisms of action of BAFF and APRIL blockade in murine SLE

We have performed extensive studies of the mechanism of action of BAFF blockade in three different models of murine
BAFF inhibition in autoimmunity

The effect of BAFF blockade on target organ injury

It is clear from studies of multiple autoimmune diseases that BAFF is expressed in inflamed target organs and can be produced by both parenchymal cells and infiltrating leukocytes. BAFF is expressed by renal mononuclear phagocytes in the lupus kidney, some of these cells being normal residents in this organ [19]. Within target organs, BAFF may play a role in DC maturation [52,53], as well as in support of ectopic germinal centers and lymphoid infiltrates. It is therefore likely that some of the therapeutic effects of BAFF blockade are mediated directly through inhibition of inflammation within the target organs themselves. This may be a result of local B cell depletion or, as described above, via a direct effect on DC that express BAFF receptors [52].

Relevance to human disease and clinical trials of BAFF blockade in humans

Our murine studies in sum show that blockade of BAFF alone is sufficient to achieve disease prevention or remission in all our three models of SLE with no extra benefit of blocking APRIL. However, there is considerable strain variation with respect to efficacy, especially disease remission, with some strains requiring a second synergistic agent. Despite B cell depletion of up to 50%, germinal centre reactions still form after BAFF blockade. There is considerable heterogeneity in BAFF- or APRIL-dependence of plasma cells in different models; this may depend upon the presence or absence of other cytokines able to support plasma cells and the nature of the microenvironment in which these cells reside. Although BAFF blockade does not deplete autoantibodies in three of the four SLE models that have been studied, it does decrease the inflammatory response to immune complex deposition.

Human studies of selective and non-selective BAFF blockade are just beginning

Rheumatoid arthritis. Targeting of BAFF alone in patients with RA results in prompt depletion of transitional and naïve B cells, with sparing of memory B cells and plasma cells and only modest reductions in serum immunoglobulin levels. These results are consistent with the murine studies. A 24-week Phase II study of Belimumab, an anti-BAFF antibody given to patients with rheumatoid arthritis, has been completed and showed a twofold increase in the percentage of patients who achieved an ACR20 response with Belimumab over placebo [93]. A Phase I study of non-selective BAFF/APRIL blockade using TACI-Ig (Atacicept) for RA has also been completed. The degree of B cell depletion was similar to that observed with Belimumab but serum IgM
levels decreased by >50%, consistent with the dependence of short-lived plasma cells on both BAFF and APRIL. In contrast, serum IgG levels were decreased only modestly, consistent with the murine studies. Atacicept decreased rheumatoid factor levels by 40% and anti-citrullinated peptide (CCP) levels by 25% but did not however, decrease circulating levels of IgG anti-tetanus or anti-diphtheria antibodies when these were compared at the beginning and end of the study [64,94]. These data suggest that in humans, only a proportion of short-lived plasma cells is dependent on BAFF/APRIL for survival and that long-lived plasma cells are maintained in the absence of BAFF/APRIL.

SLE. An initial Phase II clinical trial of Belimumab in SLE has been completed. Levels of B cell depletion and effects on serum Ig levels were similar to those observed in patients with RA. Autoantibody titres decreased by approximately 30% over a long time-period and in a small subset of patients they disappeared completely, consistent with the heterogeneity of responses observed in the mouse models [63][Jacobi, submitted]. Although the primary end-points of the trial were not met, there appeared to be some improvement in treated patients at 56 and 76 weeks when a post-hoc analysis using a composite disease outcome measure was performed [63,84]. If B cell depletion has anti-inflammatory effects in humans, as it does in mice, a decrease in clinical activity of SLE might be expected over a long time-period, given the slow kinetics of B cell depletion in humans. For this reason, the Phase III studies currently in progress for SLE are of relatively long duration (up to 76 weeks). Similar effects on serum Ig levels were observed in a phase I study of Atacicept in SLE patients [62]. However, hypogammaglobulinaemia has been observed in some SLE patients treated with both Atacicept and the T cell inhibitor mycophenolic acid, indicating that plasma cell survival may be compromised when T cell-derived cytokines are also depleted. Depletion of marginal zone B cells and short-lived IgM-secreting plasma cells by TACI-Ig [84] may confer an increased risk of bacterial infections over inhibition of BAFF alone. Further studies of Atacicept in SLE are ongoing.

Despite considerable immunological differences between rodents and humans, the results of the human studies are consistent with those of the mouse studies. In both mice and humans BAFF blockade depletes naïve and transitional B cells preferentially but has little effect on memory B cells and long-lived plasma cells. Selective BAFF blockade has less effect on serum Ig levels than does blockade of both BAFF and APRIL, and both agents decrease serum levels of IgM preferentially. Clear therapeutic effects of BAFF blockade are evident in patients with RA, but a therapeutic effect of BAFF blockade in SLE has not yet been established. This may reflect the resistance of established disease to immunological intervention as we have observed in some of the murine models. Much useful information has been obtained from the animal studies, and further studies of drug combinations may eventually allow us to maximize therapeutic benefits of BAFF/APRIL blockade in humans with autoimmune diseases.

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Disclosure

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