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Targeting BAFF in autoimmunity

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Abstract

BAFF and APRIL are TNF-like cytokines that support survival and differentiation of B cells. The early appreciation that overexpression of BAFF leads to B cell expansion and a lupus-like syndrome in mice, and the demonstration that BAFF inhibition delays lupus onset in spontaneous mouse models of SLE and other autoimmune diseases has rapidly led to the development of strategies for inhibiting both BAFF and APRIL. The commercialization of this new class of drugs has proceeded in parallel with the continuing elucidation of the biology of the cytokines and their receptors. Recent studies have uncovered a role for BAFF in enhancing both innate and adaptive immune responses and in amplifying aberrant pathways that arise during inflammation. Two phase III studies of an anti-BAFF antibody have yielded positive, although modest, results in SLE and alternate inhibitors are being tested in a variety of autoimmune diseases in which BAFF may play a pathogenic role.

Introduction

BAFF and APRIL are expressed by many cell types including monocytes, DCs, neutrophils, stromal cells, activated T cells, B cells and B cell tumors, and epithelial cells. BAFF binds to three receptors, BAFF-R, TACI and BCMA that are expressed on B cells at different developmental stages whereas APRIL binds to TACI and BCMA and has a proteoglycan binding site that facilitates its aggregation on cell surfaces (Figure 1). Increased serum levels of BAFF and APRIL are found in several autoimmune diseases, and both cytokines can be elaborated in inflammatory sites. Comprehensive descriptions of BAFF and APRIL and their receptors including the consequences of their overexpression or deletion have recently been published [1,2].

Expression of the BAFF/APRIL receptors first becomes functional at the transitional B cell stage with BAFF-R being the predominant receptor on naïve and memory B cells, TACI the predominant receptor on marginal zone B cells and short-lived plasma cells and BCMA the predominant receptor on long-lived plasma cells. Each receptor activates its own set of signaling pathways with BAFF-R being the only BAFF receptor to activate the alternative NF- κ B pathway (reviewed in [1–5]).

Selective antagonists of BAFF include a fully human anti-BAFF antibody that binds only soluble BAFF (belimumab - Human Genome Sciences) and other antibodies that block both soluble and membrane bound BAFF (K. Kikly, abstract 693, presented at American College

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of Rheumatology Meeting, Philadelphia, November 2009). A BAFF-R-Ig fusion protein is also under development, as is a depleting antibody to BAFF-R [6]. TACI-Ig is a non-selective antagonist of both BAFF and APRIL (atacept – EMD, Serono - Figure 1).

Variant forms of BAFF and APRIL

BAFF and APRIL are Type II transmembrane proteins that are cleaved by furin proteases to yield soluble homotrimers. APRIL is also expressed on the cell membrane as a fusion protein consisting of the extracellular domain of APRIL and the transmembrane and cytoplasmic domain of TWEAK (TWE-PRIL). BAFF is extensively cleaved but it is also expressed on the cell membrane either as full length BAFF or as an alternatively spliced form missing 57bp (Δ BAFF) that is not cleaved and acts as an inhibitor [7]. The physiologic role of membrane BAFF is important to understand because some BAFF inhibitors target the membrane form whereas others do not. Recent reports suggest that reverse signaling through membrane BAFF may occur [8,9]; the physiologic significance of this observation remains to be determined.

A small proportion of soluble BAFF multimerizes into a 20 trimer structure. While BAFF-R is activated by BAFF trimers, signaling through TACI requires multimerized ligands [10] such as membrane BAFF, circulating BAFF 60-mer, or multimerized APRIL. APRIL is multimerized by binding to proteoglycans but a possible role for TWE-PRIL in APRIL-TACI interactions has not been excluded. Of note, TACI-Ig blocks the binding of BAFF to BAFF-R indicating that it inhibits the function of the trimeric form of BAFF; it is possible that binding of TACI to monomeric BAFF may occur although this is not sufficient to initiate signaling through TACI. BAFF and APRIL can heterotrimerize, but the level of these heterotrimers is low [11] and their physiologic significance is not known. Other splice variants of the cytokines and their receptors continue to be identified [12].

Further analysis of the functional activities of BAFF and APRIL and their variant forms as well as clarification of their roles in supporting each of the known B cell subsets during periods of inflammation will undoubtedly be forthcoming as the various inhibitors are tested in human diseases.

BAFF supports naïve B cell survival and influences B cell selection

The interaction of BAFF with BAFF-R cooperates with BCR signaling at the late transitional B cell stage in determining whether or not a B cell will compete efficiently for survival and absence of either BAFF or BAFF-R results in substantial loss of mature B cells (reviewed [2]). BCR signaling upregulates expression of BAFF-R and also generates p100, an essential substrate for the non-classical NF- κ B signaling pathway used by BAFF-R [13]. This is dependent on the presence of Btk [14,15]. It is important to note however that signaling through the alternative NF κ B pathway is not sufficient to maintain B cell survival if BCR-mediated activation of the PI3K/Akt pathway is inhibited [16]. Partially tolerized autoreactive B cells compete less well for BAFF because they have immature rafts and may have downregulated expression of the BCR; they therefore require a stronger BCR signal to generate sufficient p100. Deregulation of the TRAF3-NIK axis that is downstream of p100 releases the brake conferred by competition for BAFF; this may play an important role in lymphomagenesis [17]. The mechanisms of BAFF-R/BCR cross talk have recently been reviewed [18].

BAFF-R deficient mice have normal serum IgA levels despite profound B cell depletion, and their mesenteric lymph nodes contain normal numbers of IgA expressing germinal center B cells [19]; mucosal IgA responses preferentially depend on the interaction of APRIL with TACI [20]. Increased transitional B cells, decreased mature B cells and normal

IgA levels have similarly been reported in two humans with complete BAFF-R deficiency [21]. Both BCR stimulation and CD40 ligation upregulate the expression of BAFF-R in follicular B cells and this compartment remains dependent on BAFF for its continued maintenance [22]. BAFF-R signals also regulate the expression of complement receptors on the B cell surface through a 7 amino acid intracytoplasmic site different from that required to support B cell survival [23].

While TACI does not play a major role in selection of the naïve B cell repertoire in adults, functional TACI mutations predispose to the development of common variable immunodeficiency. The most common TACI mutation A181E, in the transmembrane region, when expressed in mice, leads to deficiency of IgA and IgG1 and abnormal responses to T-independent antigens [24]. Another mutation, C104R, in the extracellular domain, is associated with hypogammaglobulinemia and a defect in memory B cell production [25,26]. TACI also facilitates class switching through a pathway involving the unique interaction of a conserved intracytoplasmic domain of TACI with the intermediary region domain of MyD88 [27]. TACI also has a negative regulatory role with respect to B cell activation; several mechanisms for this have been proposed [1]. The recent discovery that BAFF can propagate the expansion of a MZ-like subset of regulatory B cells that produce IL-10 [28] suggests another mechanism by which deficiency of TACI, that is highly expressed on the MZ subset, could lead to altered B cell homeostasis. The roles of TACI in maintaining B cell homeostasis on the one hand and in participating in immune responses on the other, could account for the seemingly contradictory observations that TACI deficiency is associated with B cell hyperactivity and autoimmunity (reviewed [1]) whereas inhibition of the TACI ligands is therapeutic in autoimmunity.

BAFF serum levels rise immediately following any period of B cell lymphopenia, subsiding again once B cell numbers return to normal. Since there are now several drugs available that deplete B cells it is important to determine whether an increase in availability of BAFF generates an autoreactive naïve B cell repertoire during B cell reconstitution. Studies in transgenic systems have shown that excess BAFF does not rescue very high affinity autoreactive B cells that are usually deleted in the bone marrow but alters the fate of autoreactive B cells with lower affinities that escape deletion in the bone marrow or that might otherwise have undergone receptor editing [29,30]. Kawabata et al used a mouse expressing an autoreactive class switched IgG2a transgene with specificity for dsDNA to demonstrate that B cell depletion results in selection of autoreactive B cells into the naïve repertoire during B cell reconstitution. Because these cells produce non-mutated but class switched antibodies that are capable of tissue penetration these authors were able to demonstrate that the germline encoded autoantibodies produced are of sufficient affinity to deposit in the kidneys [31].

The role of BAFF and APRIL during B cell activation

Myeloid dendritic cells, neutrophils and basophils release BAFF when stimulated by a variety of innate stimuli including cytokines (Type I IFN and TNF α) and Fc receptor ligation [32–36]. During antigen activation BAFF upregulates TLR expression, promotes B cell survival and, in collaboration with cytokines, costimulatory signals or TLR signals promotes Ig class switching [37,38]. Activation of intracellular TLRs in B cells upregulates expression of BAFF receptors, particularly TACI [39–41], and increases both BCR signaling and BAFF/APRIL sensitivity (Figure 2A). These findings help explain the dependence of T independent (type 2) responses on TACI. Since autoreactive B cells are more likely to internalize immune complexes containing autoantigens, particularly those containing nucleic acids, excess BAFF and APRIL may preferentially drive class switching of naïve autoreactive B cells. It is therefore conceivable that high levels of BAFF induced by viral

infections or following B cell depletion will induce pathogenic autoimmunity in susceptible individuals by enhancing both selection and class switching of naïve autoreactive B cells. Studies of BAFF transgenic mice that express 50-100 fold increased serum BAFF levels, have indeed shown that the SLE-like autoimmunity that ensues over time is a consequence of expansion and class switching of autoreactive marginal zone and/or B1 B cells and is independent of CD4 T cells but dependent on signaling through MyD88 [40]. In contrast, our own studies have shown that the 3 fold increase in serum BAFF level induced by administration of a small dose of Type I IFN is not sufficient to induce the production of pathogenic class switched autoantibodies in T cell depleted NZB/W mice (Z Liu and A Davidson, manuscript in press).

The germinal center environment contains low levels of BAFF and APRIL and germinal center B cells express only low levels of the receptors [42]. Germinal centers that support somatic mutation and class switching are initiated even if BAFF and APRIL are absent. Nevertheless both BAFF and BAFF-R are required to maintain optimal size and longevity of the germinal center response and the follicular dendritic cell network within the germinal center fails to fully mature when BAFF is absent, resulting in a decrease in magnitude of the antibody response (reviewed [43]). Whether increased serum levels of BAFF alter germinal center longevity or selection remains to be determined. As germinal B cells become either memory cells or plasma cells their differentiation program is associated with continued expression of BAFF-R and TACI (in the memory population) or with loss of BAFF-R and upregulation of BCMA (in plasma cells) [42]. Crosslinking of BCR and FcRIIB during humoral immune responses attenuates expression of BAFF-R thus blunting the effect of BAFF on cell survival [44].

BAFF and APRIL are not necessary for survival or reactivation of class switched memory B cells in vivo [45–47] but unswitched IgM memory B cells are still partially BAFF dependent [46]. Similarly, in SLE patients exposed to belimumab for up to 3 years IgM+ memory B cells decreased by a median of 76% but there was no effect on class switched memory B cells [48]. Nevertheless, two studies in humans have shown that BAFF collaborates with inflammatory cytokines IL-21 or IL-17 in driving the differentiation of memory B cells to plasma cells, thus providing a role for BAFF receptor expression on these cells [49,50]. In humans treated with either belimumab or atacicept an increase in the absolute number of circulating memory cells is observed during the first 8 weeks of treatment ([51,52], W Stohl, abstract 1160 presented at ACR meeting, San Diego, 2005). Whether this is due to mobilization of cells or to proliferation of cells has not yet been investigated.

In normal adult mice long-lived plasma cells in both the spleen and bone marrow are dependent on either BAFF or APRIL, consistent with their preferential expression of BCMA [47]. Survival of the earliest plasma cells in the bone marrow appears to be more dependent on APRIL than on BAFF and the poor survival of plasma cells in neonates may be due to the attenuated expression of TACI on B cells [53] and of APRIL by neonatal bone marrow stromal cells [54]. Similarly, short-lived IgM producing plasma cells that predominantly express TACI [55], can be supported by either BAFF or APRIL [47] and are therefore depleted by TACI-Ig but not by selective BAFF inhibition [56,57]. However in several SLE prone strains TACI-Ig has no effect on long-lived bone marrow plasma cells showing that other survival factors can maintain plasma cell survival under inflammatory conditions [56,58]. Importantly, elimination of plasma cells is not required in order to achieve a therapeutic effect with BAFF blockade in mice [56,57].

In human SLE, belimumab, has little effect on plasma cells and only a very modest effect on circulating IgG levels, as expected [48,59]. Even atacicept has a more profound effect on serum levels of IgM and IgA than on IgG with only a 20% decrease in total serum IgG

levels and little effect on antibody titers to recall antigens such as tetanus toxoid [51,52,60,61]. These observations in sum are consistent with the idea that fully differentiated bone marrow plasma cells in most humans are no longer entirely dependent on BAFF and APRIL. The reason why IgM-producing memory and plasma cells are more sensitive to BAFF/APRIL inhibition than are IgG-producing cells may involve signaling through the BCR as there are considerable differences in gene expression and an exaggerated calcium flux in IgG compared with IgM-bearing cells [62]. This may be due to differences in the rate of BCR clustering on the cell surface [63].

BAFF-R is expressed on some T cells; its possible role in T cell function has recently been reviewed [64]. Although T cell numbers are normal in BAFF deficient mice, T cells from SLE patients produce more IFN γ in response to BAFF than do T cells from normal individuals and monocytes from SLE patients produce more BAFF in response to IFN γ stimulation than do normal monocytes [65]. Mice with deficiency of lyn kinase, express a hyperactivated myeloid cell phenotype associated with elevated serum levels of BAFF. Adoptive transfer of BAFF-R deficient and wt T cells into these mice resulted in reduced activation and IFN γ production only in the BAFF-R deficient T cells [66].

BAFF also supports the survival of monocytes and enhances their differentiation into macrophages [67,68]. Human myeloid DCs stimulated with BAFF *in vitro* up-regulate co-stimulatory molecules, lose their phagocytic ability and produce inflammatory cytokines and chemokines including IL-1, IL-6, CCL2 and CCL5, inducing Th1 responses [67]. In a mouse model of arthritis, synovial dendritic cells transduced 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 [69].

These studies in sum, suggest an amplification loop in which BAFF acts on DCs to help them activate and recruit immune cells and directly enhances the proinflammatory activity of T cells; this in turn may elicit further production of BAFF (Figure 2A).

Murine studies of BAFF/APRIL inhibitors

BAFF or BAFF/APRIL inhibition have been used successfully in murine models of several autoimmune diseases including SLE, collagen induced arthritis, type I diabetes and multiple sclerosis. The studies in murine SLE have been instructive because several strains have been tested at both early and late stages of disease and both selective and non-selective inhibitors have been used [56,58]. In most SLE strains BAFF-R-Ig and TACI-Ig are equally effective at delaying disease onset but reversal of established disease is more difficult to achieve and depends on the amount of concomitant systemic inflammation [57]. Neither inhibitor prevents autoantibody formation or the deposition of autoantibodies in the kidneys however the rapid B cell depletion that accompanies BAFF inhibition results in shrinkage of secondary lymphoid organs and therefore a diminution in the total number of activated T cells and dendritic cells. This is associated with a decrease in production of the circulating inflammatory mediators that activate endothelial cells and enhance local inflammation. In the NZM2410 strain both BAFF-R-Ig and TACI-Ig are equally effective at reversing established nephritis, in part by decreasing the activation state of both circulating and renal mononuclear phagocytes. This appears to be an indirect effect of BAFF inhibition as it is sustained long after the treatment course is complete [57]. Similar results have been observed in the Lyn deficient mouse [66] although as mentioned above there is evidence in this strain that BAFF inhibition directly inhibits T cell activation, an effect that has not been observed in the other SLE strains. In contrast, in MRL/lpr mice, autoantibody producing plasma cells that are mostly generated in extrafollicular foci are highly dependent on BAFF and APRIL and serum IgG autoantibody levels plummet within 1–2 weeks of receiving

TACI-Ig; this is associated with a marked decrease in renal immune complex deposition and improved survival. T cell activation and interstitial nephritis are not affected by TACI-Ig in this strain [70]. These studies highlight the heterogeneity of the responses to BAFF and BAFF/APRIL inhibition in multiple murine models of SLE and suggest that there may be subsets of humans that respond better to BAFF inhibition than others.

Human studies of BAFF/APRIL inhibitors

There are few published reports of clinical trials of either selective BAFF or non selective BAFF/APRIL inhibitors in human autoimmune diseases; most of these studies have been reported in abstract form only. Nevertheless some trends are emerging that can be reviewed here. The inhibitors have been used in three diseases, rheumatoid arthritis, SLE and multiple sclerosis.

Both belimumab and atacicept have been used for the treatment of rheumatoid arthritis [51,52]. In Phase II studies belimumab had a modest effect on disease activity whereas no effect was observed in Phase II studies with TACI-Ig. Thus these drugs have not moved forward to Phase III studies. In a phase II study, moderate, but not high or low doses, of a different anti-BAFF antibody (LY2127399; Eli Lilly) that blocks both soluble and membrane BAFF had beneficial effects in RA similar to that of TNF blockers (M Genovese, abstract 1923 presented at American College of Rheumatology Meeting, Philadelphia, 2009). This result raises interesting questions about the potential benefits of inhibiting the various BAFF forms that will need to be formally addressed if this drug continues to show efficacy in larger trials.

BAFF overexpression has been detected in the brains of mice and patients with multiple sclerosis [71] and TACI-Ig had a beneficial effect in a mouse model of MS [72]. However a phase II study of atacicept for MS had to be terminated because of disease worsening (www.clinicaltrials.gov). These findings stand in stark contrast to the beneficial effects of global B cell depletion in multiple sclerosis using anti-CD20 antibodies. IFN β is standard treatment for MS and increases serum BAFF levels in MS patients [73]. Whether the negative effect of atacicept was due to a decrease in Type I IFN, or alterations in other cytokines such as IFN γ or IL-10 has not been determined. A clinical trial of LY2127399 began in 2009 and is ongoing.

The most exciting findings have been the positive results of two large Phase III studies of belimumab in SLE. Although a previous large Phase II study of this drug in SLE failed to meet its primary endpoints [59], the study prompted the development of a composite SLE responder index that was used as a primary endpoint in the subsequent Phase III studies [74]. In both Phase III studies, belimumab given together with standard of care therapy outperformed standard of care alone over a period of 52 weeks. This was associated with steroid sparing effects and a reduction in the number of severe flares (R.F. van Vollenhoven, abstract OP0068 presented at EULAR Congress, Rome 2010 and S. Navarra, abstract SAT0204 presented at EULAR Congress, Rome, 2010. By 76 weeks however, the difference between the two groups was no longer statistically significant although there was still some advantage to the belimumab group when other post-hoc analyses were performed (R. Furie, abstract presented at 9th International SLE Congress, Vancouver, 2010). Human Genome Sciences announced submission of a Biologics License Application for belimumab to the FDA in June 2010 that should be reviewed by the end of 2010. Mechanistic studies in humans have shown that, as predicted by the mouse physiology, and based on its selective inhibition of BAFF, belimumab depletes naïve and transitional B cells within the first 6 months of treatment and depletes IgM+ memory B cells and IgM producing plasma cells with delayed kinetics but has no effect on class switched memory B cells even after 2 years

of treatment [48,59]. The effect of drug on T cell activation pathways and on monocytes remains to be determined. An important difference between the mouse and human/primate studies is that the kinetics of B cell depletion takes much longer in humans and is associated with delayed shrinkage of lymphoid organs [75]. This is consistent with the apparently delayed onset of action of belimumab.

Conclusions

It has been a little over a decade since the discovery of BAFF, the homologous molecule APRIL and the three BAFF/APRIL receptors followed by the rapid development of several therapeutic BAFF inhibitors, one of which, belimumab, has recently demonstrated efficacy in two large Phase III clinical trials for human SLE. While this has certainly been reason to celebrate, enthusiasm has been tempered by the modest improvement in disease activity measures conferred by belimumab over time. Many questions remain about the mechanisms of action of BAFF/APRIL inhibitors, including their effects on each B cell subset during periods of disease quiescence and flare, their effects on cell types other than B cells, and whether drugs with improved efficacy can be designed based on the function of the various forms of the cytokines and their receptors. Clinical questions that need to be answered include whether it will be possible to identify subsets of patients that respond better than others, what is the optimal length of time to treat patients, which other drugs might be synergistic with BAFF/APRIL inhibition, and which autoimmune diseases will be responsive to or exacerbated by this new class of drugs. Most importantly, because SLE is a disease in which damage accumulates over time, the long term advantage of a class of drugs that even modestly decreases the frequency of major flares and spares exposure to other toxic drugs needs to be determined using appropriately designed clinical trials.

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Figure 1.

The BAFF/APRIL family and their receptors: BAFF and APRIL are cleaved by furin proteases to yield soluble homotrimers. BAFF and APRIL can also heterotrimerize. APRIL is expressed on the cell membrane when it is fused to the transmembrane and cytoplasmic portion of TWEAK (TWE-PRIL). BAFF is expressed on the cell membrane either as full length BAFF or as an alternatively spliced form missing 57bp (Δ BAFF) that is not cleaved. Other splice variants of various family members have been identified. Soluble BAFF can multimerize into a 20 trimer structure that is the preferential ligand for TACI. APRIL is multimerized by binding to proteoglycans. TACI can also bind to proteoglycans such as syndecan. Drugs that target the cytokines include belimumab that blocks soluble BAFF and atacicept that blocks both BAFF and APRIL.

Abbreviations: Abbreviations: APRIL, A proliferation inducing ligand; BAFF, B cell activating factor belonging to the TNF family; TACI, Transmembrane activator and calcium modulator ligand interactor; BCMA, B cell maturation antigen; BAFF-R, BAFF receptor; HSPG, heparan sulphate proteoglycan

Figure 2.

Amplification pathways mediated by BAFF during immune and inflammatory responses: A: During antigen activation BAFF upregulates TLR expression, promotes B cell survival and, in collaboration with cytokines, costimulatory signals or TLR signals promotes Ig class switching. Activation of intracellular TLRs in B cells upregulates expression of TACI and increases both BCR signaling and BAFF/APRIL sensitivity. (Loop 1). Immune complexes induce release of Type I IFN from plasmacytoid dendritic cells that contributes to activation of myeloid dendritic cells. Activated monocytes and dendritic cells express TACI and produce BAFF. BAFF supports survival and differentiation of monocytes and enhances cytokine and chemokine production from macrophages and dendritic cells (Loop 2). T cells may be directly stimulated by BAFF to produce inflammatory cytokines including IFN γ or, if IL-6 is also present, IL-17 (Loop 3). BAFF can also be produced by cytokine activated neutrophils and by basophils that have been activated by immune complexes (Loop 4). B: inflammatory cytokines can collaborate with BAFF in the induction of memory B cell differentiation to plasma cells thus generating more immune complexes.

N, neutrophils; Mon, monocytes; Ba, basophils; TLR, toll like receptor; IFN, interferon; BCR, B-cell receptor;