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BAFF and selection of autoreactive B cells

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Abstract

BAFF is a critical survival factor for transitional and mature B cells and is a promising therapeutic target for SLE. A BAFF inhibitor, belimumab, is the first new drug in 50 years to be approved for the treatment of SLE. However, the mechanism of action of this drug is not entirely clear. In this review we will focus on the role of the BAFF–APRIL signaling pathway in the selection of autoreactive B cells and discuss whether altered selection is the mechanism for the therapeutic efficacy of BAFF inhibition in SLE.

Belimumab, a new drug for SLE

Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease in which the loss of tolerance to nucleic acids and their binding proteins, results in the generation of autoantibodies that initiate tissue-damaging inflammation. Current treatments for SLE have both insufficient efficacy and significant toxicities. Recently, biologics targeting immune cells, costimulatory pathways, or important cytokines have been developed and tested in a variety of autoimmune diseases, sometimes with astonishing success, but results have been almost universally disappointing in lupus[1]. Therefore, it is with great excitement that patients and physicians alike have greeted the recent FDA approval of belimumab, a human antibody targeting the B cell survival cytokine B cell activating factor (BAFF). Clinical efficacy of belimumab as evaluated by the SLE responder index was demonstrated at week 52 in two large phase III clinical trials (BLISS-52 and BLISS-76), as well as a decrease in severe flares and steroid sparing effects that persisted over time[2]. Nevertheless, enthusiasm has been tempered by the modest difference in primary outcome between standard of care and standard of care plus belimumab at 52 weeks, the failure of the primary efficacy outcome to be sustained at 76 weeks, the limited efficacy data in patients of African-American ethnicity, who often have poor outcomes, and the high cost of the drug. Furthermore, the mechanism by which belimumab benefits lupus patients is not entirely clear, making it difficult to define immunologic parameters of response or to predict which patients will respond best. In this review, we focus on the evidence supporting a role for BAFF, and its homologue APRIL, in regulating the selection and survival of autoreactive B cells at naïve and antigen-induced stages of B cell development and discuss how inhibitors of these cytokines might mediate their therapeutic effects.

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B cell selection

Autoreactive BCRs are generated through random rearrangement of immunoglobulin genes in the bone marrow (BM) but are usually removed from the repertoire by the time B cells have reached the mature B cell stage to ensure self-tolerance of the naïve repertoire. In the BM this regulation depends predominantly on the strength of signaling induced when self-antigen crosslinks the BCR[3]. A strong signal results in B cell removal through apoptosis, a process known as clonal deletion. Alternatively, re-expression of RAG proteins allows replacement of self reactive receptors with non-self reactive ones, a process known as receptor editing. Weaker signals may render the cell unresponsive to antigen stimulation, a state known as anergy. Anergic cells fail to activate NF- κ B upon BCR engagement and are susceptible to early death[4].

Once immature B cells exit the BM, their fate, should they encounter autoantigen, depends not only on the strength of the BCR signal they receive, but also on competition with non self-reactive cells for BAFF[5], as discussed in more detail later. The autoreactive B cells that escape this checkpoint and become mature cells still need additional signals to differentiate into effector cells. For instance, TLR activation promotes T-independent class switching and differentiation[6]. In normal individuals, apoptotic cells, the main source of endogenous TLR ligands, are rapidly removed from circulation by macrophages. The limited availability of such signals at steady-state protects against autoreactivity. Chronic BCR engagement by self-antigen also blocks autoreactive plasma cell differentiation by inducing activation of Erk thus preventing the expression of BLIMP1-1[4]. Autoreactive B cells are also usually excluded from participating in the germinal center (GC) reaction[7] and are therefore unlikely to undergo class switching and somatic hypermutation that may yield pathogenic high affinity self-reactive receptors. B cells that newly acquire self reactivity within the GC are removed from the effector repertoire by engagement with soluble self-antigen, by failure to obtain cognate help from T cells, by other unidentified checkpoints within the GC, or by post-GC receptor editing[3, 8]. FcRIIB is upregulated on antigen-exposed B cells and limits both differentiation and reactivation of memory B cells and survival of newly formed plasma cells[9–10]. A final tolerance checkpoint may prevent autoreactive CD138+ pre-plasma cells from differentiating into antibody-secreting plasma cells[11]. The relative importance of each checkpoint in the maintenance of self-tolerance is not entirely clear and whether clinical autoimmunity requires the breach of single or multiple checkpoints remains to be addressed.

Defects in B cell tolerance have been identified in SLE. Repertoire analysis of human B cells using single cell PCR identified two early tolerance checkpoints that are defective in SLE, one at the transition from the early immature to the immature stage and the other at the transitional to mature stage[12]. By tracking a self reactive heavy chain gene throughout B cell differentiation, a GC entry checkpoint was identified in normal individuals[13] that is defective in SLE, allowing autoreactive cells to differentiate into memory and plasma cells. Another study using a synthetic peptide to track anti-DNA B cells, revealed a tolerance checkpoint between naïve and antigen-experienced B cells that is compromised in active SLE[14]. SLE patients fail to upregulate FcRIIB on their memory cells, increasing the chance for survival of autoreactive B cells[10]. Taken together, these studies show that B cell tolerance is compromised at several checkpoints in lupus patients; defects may vary among patients, reflecting both genetic heterogeneity, and clinical and medication differences among patients[15].

There is abundant evidence that loss of B cell tolerance can result from either increased or diminished BCR signaling. Over-expression of proteins that enhance BCR signaling such as CD19, or dysfunction of inhibitors such as FcRIIB leads to autoimmunity, most likely as a

result of excessive B cell activation after antigen stimulation[16]. Conversely, diminished BCR signaling can also impair tolerance by allowing autoreactive B cells to escape negative selection. In humans, polymorphisms in genes encoding modulators of BCR signaling such as PTPN22 and BLK are to SLE susceptibility; both risk alleles are associated with diminished BCR signaling[17–18]. In mice, estradiol-induced upregulation of inhibitory SHP-1 and CD22 impair negative selection, leading to autoimmunity[16]. Taken together, these studies demonstrate the need to maintain BCR signal strength within a certain range to sustain tolerance (Figure 1).

The role of BAFF (BLyS) in promoting survival and selection of autoreactive B cells

BAFF and its homolog APRIL are members of the trimeric TNF family and are expressed by multiple cell types. BAFF binds to 3 receptors, BAFF-R, TACI Transmembrane activator and calcium modulator ligand interactor (TACI) and B cell maturation antigen (BCMA) that are expressed by B cells at various times during their ontogeny. In mice, BAFF-R is expressed on transitional and mature B cells whereas TACI is expressed by all peripheral B cells including MZ and B1 B cells, and BCMA is only expressed by antibody-secreting cells, that also downregulate BAFF-R [19]. In humans, BAFF-R is widely expressed by all B cells except for bone marrow plasma cells. TACI is expressed by CD27+ memory B cells, by plasma cells, and by certain subsets of naïve and activated B cells. BCMA is expressed by tonsillar memory B cells, GC B cells, and plasma cells [19]. BAFF-R is specific for BAFF, whereas TACI and BCMA also bind APRIL (Figure 2). BAFF-R signaling activates the alternative NF- κ B pathway, and Akt/mTOR and Pim2, and also weakly stimulates the classic NF- κ B pathway, enhancing B cell survival, growth and metabolic fitness[19]. TACI and BCMA signal through the classic NF- κ B pathway, as well as through other pathways to counteract apoptosis and to drive class switching[20].

BAFF does not play a role in central B cell selection in the BM because immature B cells have extremely low expression of BAFF-R[19]. BAFF-deficient mice have comparable numbers of immature BM B cells as wild type controls [19] and overexpression of BAFF does not rescue high affinity autoreactive B cells from deletion in the BM[21–23].

After their exit from the BM, B cells that encounter self-antigens in the periphery face a stringent tolerance checkpoint at the transitional stage[3]. A strong BCR signal programs the cells for deletion or anergy at the early transitional stage but enhances the survival of late transitional and mature follicular B cells; at the same time, these cells also compete for survival signals delivered by the interaction of BAFF with BAFF-R. BCR and BAFF-R mediated signals cooperate in several ways. First, BCR crosslinking in naïve cells triggers the expression of BAFF-R through the PI3K signaling pathway[24]. Second, the alternate NF- κ B pathway activated by BAFF-R requires a substrate p100, which is transcribed after BCR-mediated activation of the classical NF- κ B pathway[25]. BAFF-R engagement upregulates CD19 expression by modulating the transcription factor Pax5, thus enhancing BCR signaling and presumably increasing p100 production[19]. Finally, BCR and BAFF-R additively inhibit apoptotic pathways by altering the expression of different pro-survival and pro-apoptotic proteins[20].

Evidence that BAFF regulates B cell repertoire selection was first provided by studies of BAFF transgenic mice which develop anti-DNA antibodies and autoimmune disease[26]. The effect of BAFF on survival of naïve B cells might start as early as the immature to T1 transition as the amount of BCR expressed regulates BAFF-R expression in these cells[27]. Table 1 lists the experimental evidence, all demonstrated in transgenic mice, that BAFF levels regulate the stringency of selection of the naïve B cell repertoire after the T1 stage

[21–23, 28–30]. These studies in sum show that supraphysiologic BAFF excess does not alter negative selection that occurs before or at the T1 stage but rescues autoreactive cells that are anergized after the T1 stage and promotes their maturation into follicular and or marginal zone cells. Conversely, BAFF inhibition preferentially depletes anergic autoreactive transitional B cells compared with non-autoreactive cells and self reactive B cells with higher affinity receptors are more readily eliminated than ones with lower affinity BCRs. Importantly, BAFF excess has much less effect on B cell selection if competition is provided by non-autoreactive B cells. Because autoreactive and non-autoreactive B cells have the same access to BAFF, why do the latter show a distinct survival advantage? One possible explanation is that chronic BCR signaling by self antigen leads to elevated levels of pro-apoptotic molecules thus increasing the dependence on BAFF signaling to inhibit apoptosis[28]. Alternatively, partly anergized self-reactive B cells downregulate their expression of both BCR and BAFF-R and thus might require more BAFF to support their survival[31].

These findings have clinical implications. First, they suggest that the major effect of either BAFF excess or inhibition will be to alter the fate of B cells tolerized after the T1 stage in the periphery. Second, in SLE patients that have B cell lymphopenia, either due to the disease or induced by immunosuppressive therapy, excess BAFF may synergize with a decrease in competition to perpetuate the loss of naïve B cell tolerance. This idea is supported by studies in anti-dsDNA IgG heavy chain transgenic R4A mice showing that B cell depletion induces a homeostatic increase in serum BAFF concentrations and loss of tolerance of B cells that produce anti-dsDNA antibodies which deposit in the kidneys; this defect can be corrected by BAFF inhibition[32]. Third, these studies suggest that BAFF inhibition may correct only some of the early tolerance defects that have been described in SLE patients.

The role of BAFF in regulating tolerance checkpoints after the naïve B cell stage is less clear. In SLE, class switching of autoreactive B cells from IgM to more pathogenic IgG is a critical step in the initiation of clinical disease. Autoreactive B cells in SLE internalize nucleic acid-containing immune complexes or apoptotic material that activate TLRs thereby inducing the expression of BAFF receptors, particularly TACI[33–34]; this is associated with increased B cell survival. Recruitment of MyD88 by TACI may further amplify the effects of TLR engagement in B cells[35]. High serum concentrations of BAFF or APRIL may therefore preferentially support the survival and class switching of B cells that recognize nuclear antigens. In support of this notion, marginal zone and B1 B cells undergo T-independent class switching in BAFF transgenic mice and secrete anti-dsDNA autoantibodies of sufficient affinity to cause mild SLE[36]. It is not yet clear whether BAFF–APRIL inhibition can prevent TLR-activated B cells from producing IgG autoantibodies in SLE. Also puzzling is the relevance to B cell selection of the inhibitory role of TACI. Although TACI-deficient mice display defective T-independent antibody responses and impaired class switching to IgA[37], they develop B cell hyperplasia and autoimmunity. Similarly, in humans, TACI mutations or deficiency are risk factors for common variable immunodeficiency and for concomitant autoimmunity [38]. It is to be determined whether TACI regulates the autoimmune response directly through negative signals[37], regulates TLR expression or signaling[35], or acts indirectly by competing with BAFF-R for BAFF.

The GC reaction produces IgG autoantibodies, which can have improved affinity for autoantigens or which have acquired autoreactivity *de novo* as a result of somatic mutation. Follicular exclusion of anergic cells is an important precaution to limit the access of autoreactive B cells to the GC; excess BAFF permits follicular inclusion in several models (Table 1). Little is known about the role of BAFF in either positive or negative selection of

GC or post-GC autoreactive B cells. BAFF is essential for the formation of a mature FDC network and for survival of late GC B cells[39]. However although both primary and secondary IgG responses are diminished by BAFF deficiency[39–40], class-switched and somatically mutated antibodies still arise after immunization[39]. Thus pathogenic IgG autoantibodies could arise as a result of somatic mutation and class switching in germinal centers even in BAFF is absent. It is not surprising therefore that BAFF inhibition does not prevent the spontaneous formation of GCs or the generation of somatically mutated pathogenic IgG anti-dsDNA antibodies in several lupus-prone mouse strains[41] although the amount of tissue damage is limited. Even complete BAFF deficiency in a lupus prone strain does not prevent the eventual emergence or tissue deposition of anti-dsDNA antibodies. These autoantibodies are however skewed towards the IgG1 isotype and renal damage is attenuated[42]. Whether the decrease in renal damage observed after BAFF inhibition or in BAFF deficient SLE-prone mice is due to secondary effects of B cell depletion on T cell expansion and inflammatory mediator production, or whether this is a direct effect of BAFF deficiency on selection of the antigen activated repertoire or on class switching has not yet been determined.

B cells that clonally expand in the GCs develop into memory B cells or long-lived plasma cells. Although memory B cells express BAFF-R and TACI[19], survival and reactivation of class switched B cell memory is BAFF-independent in mice under normal physiologic circumstances[43–44]; accordingly, belimumab did not decrease class switched memory B cells in humans even after treatment for several years[45]. Recent *in vitro* studies have indicated however, that BAFF collaborates with inflammatory cytokines such as IL-21 and IL-17 in the reactivation of human memory B cells and their differentiation to plasma cells[46–47]; whether this occurs *in vivo* is not yet known. In addition, FcRIIB engagement decreases signaling through the BCR and this in turn prevents upregulation of BAFF-R, thus potentially decreasing B cell viability[48]. This function may be impaired in SLE patients due to their failure to upregulate FcRIIB on antigen activated B cells[10]. Surprisingly, BAFF inhibition with either belimumab or atacicept, which is a fusion protein of TACI with Fc that acts as an inhibitor of both BAFF and APRIL, induces rapid expansion of memory B cells in humans[38, 49–50]. Whether this is due to homeostatic proliferation, preferential expansion of autoreactive memory B cells or just mobilization into the circulation is not yet known. Further work will be needed to reconcile the findings from *in vitro* studies that suggest that BAFF may play a role in memory B cell reactivation and differentiation, with those of *in vivo* studies that have as yet failed to demonstrate an effect of BAFF inhibition on functional B cell memory.

Plasma cells express TACI and/or BCMA and their survival is therefore supported by either BAFF or APRIL; blockade of both cytokines is required to deplete plasma cells in normal mice[43]. However, in diseased NZB/W SLE-prone mice TACI-Ig has no effect on serum levels of IgG, suggesting that during inflammatory states, other bone marrow factors are sufficient to support plasma cell survival[51]. Studies in humans have similarly shown that BAFF–APRIL blockade has little effect on antibody titers to recall antigens, indicating that fully differentiated bone marrow plasma cells may no longer be dependent on BAFF and APRIL[49, 52]. Furthermore, BAFF–APRIL inhibition with TACI-Ig is associated with a preferential decrease in IgM and IgA compared with IgG producing plasma cells[49, 52], [53–54]. Similarly, in humans treated with belimumab the observed decrease in circulating autoreactive plasmablasts preferentially involves the IgM subset[45]. The decreased sensitivity of IgG-producing plasma cells to BAFF–APRIL inhibition could be due to differences in signaling through the IgG cytoplasmic tail or to B cell epigenetic changes that accompany class switching[55–56]. Regardless of the cause, these findings raise concerns about the prospect of dual BAFF–APRIL inhibition in treating SLE patients as serum IgM appears to be protective in SLE[57].

What is the mechanism for the therapeutic efficacy of targeting BAFF?

As predicted from the murine studies showing that BAFF is an important gatekeeper for naïve B cell selection, belimumab preferentially depletes transitional and naïve B cells in SLE patients[45]. However it is not known whether this is in fact the mechanism for the efficacy of belimumab in SLE. Some SLE patients have abnormal selection at the immature to early transitional stage, presumably due to intrinsic B cell defects[58], and this defect may not be corrected by BAFF inhibition. Furthermore, it is not clear whether the GC and post-GC checkpoints that are defective during active flares of SLE are susceptible to modulation by BAFF inhibition. It is possible that chronic B cell depletion that reduces B cell-mediated immune functions such as antibody production, cytokine and chemokine production, antigen transport, lymphoid organization and antigen presentation to T cells is responsible for the efficacy of BAFF inhibition⁵⁴. The lack of efficacy of B cell depletion with Rituxan in SLE[59] suggests that this is not the entire answer; it is possible however, that there are differences between the two approaches. For example, the chronic BAFF inhibition could allow for shrinkage of secondary lymphoid organs[60] and therefore a decrease in the total number of inflammatory cells and the overall burden of inflammatory mediators[41, 61]. In addition the high BAFF levels that result from Rituxan-mediated B cell depletion may synergize with cytokines or TLR stimulation to activate remaining and reconstituting B cells. Other differences between the two reagents in their effects on particular B cell subsets such as regulatory B cells remain to be explored. Finally, we need to consider that efficacy of BAFF inhibitors could be due to direct effects on immune cells other than B cells.

In contrast to the many studies of the role of BAFF in B cell functions, little effort has been devoted to the role of BAFF in other cell types, even though BAFF receptors can be expressed both by T cells[62] and mononuclear phagocytes, including activated monocytes in humans[63]. Several studies have provided evidence that BAFF has a role beyond that of regulating B cell responses. For instance, BAFF enhances T cell proliferation in the absence of APCs; this effect is not seen in T cells bearing a mutant BAFF-R[64]. Furthermore, in Lyn-deficient mice BAFF blockade directly inhibits the activation of CD4 T cells and decreases their secretion of IFN γ [62]. TACI-BAFF interactions also appear to be critical for DCs to acquire the ability to prime CD8 T cells[65]. In a rheumatoid arthritis model, local BAFF silencing inhibits DC maturation and production of IL-6 and therefore inhibits their ability to drive Th17 differentiation, thus improving joint pathology[66]. In vitro, BAFF treatment strongly promotes the survival and activation of human monocytes which secrete proinflammatory cytokines and upregulate costimulatory molecule expression[63]. Collectively, these studies suggest that regulation of non-B cell functions may contribute to the overall therapeutic effects of BAFF inhibition in SLE.

Concluding remarks

While precise mechanisms for the efficacy of BAFF inhibition remain to be elucidated, the development of new pharmacologic agents that target BAFF pathways is moving full steam ahead with three new drugs in clinical trials for SLE (Figure 2, Table 2 (<http://clinicaltrials.gov/>)). Application of these agents to other autoimmune diseases will need to be approached with caution given the lack-luster response of patients with RA to either belimumab or atacicept and the apparent worsening of disease in patients with MS following treatment with TACI-Ig. How each isoform of the BAFF family contributes to disease and whether differential targeting will be useful remaining open questions. For example, it is not known whether the receptor fusion proteins block membrane bound forms of BAFF and APRIL or whether blocking membrane BAFF, including Δ BAFF, will have different therapeutic effects than blocking only soluble BAFF. Studies in mice have shown that the robust effects of BAFF inhibition on naïve B cell selection may be offset by the

continued progression of class switching and somatic mutation of the antigen activated repertoire resulting in continued generation of autoreactive effector B cells. This could help account for the modest effects on the autoantibody response and the perceived attenuation of clinical benefit of BAFF inhibition over time. Non-B cell effects need to be further investigated as potential mechanisms of action of BAFF inhibitors. Important clinical questions remain to be resolved, including the effects of race on therapeutic responses, the definition of a responder and, given the observed decrease in severe flares, the long-term effects of BAFF inhibition on disease damage indices. Further investigation should improve our ability to use BAFF targeting therapies, to identify synergistic therapies and to determine which patients will most benefit from this intervention.

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Figure 1.

The effects of various factors on the negative selection of autoreactive B cells: The fate of autoreactive B cells is determined by the strength of the BCR signal induced by self antigens and is different for early transitional and antigen activated mature B cells.

A. In immature bone marrow cells and early transitional B cells, if the strength of the BCR signal reaches a certain threshold (purple dashed line), autoreactive B cells are eliminated through deletion or anergy (grey area); otherwise they continue to differentiate into mature cells. Diminished BCR signals associated with genetic variants of PTPN22 or BLK increase the threshold for elimination (red dashed line), allowing more autoreactive B cells to escape immune tolerance. Conversely, enhanced BCR signals conferred by upregulation of CD19, or augmented TLR signals, may lower the threshold for elimination (green dashed line), leading to more stringent regulation of autoreactive B cells. *BAFF appears to have little role in the bone marrow but regulates the threshold for selection of early transitional B cells in the directions shown.

B. In antigen activated mature B cells the threshold for deletion of autoreactive B cells is increased (red dashed line) by a number of factors such as increased T cell help and costimulation, enhanced BCR signals via CD19 upregulation, increased TLR signals, or impaired negative signals mediated through FcγRIIB and CD22, leading to differentiation of autoreactive effector cells. Further studies are needed to investigate the role of BAFF in tolerance of antigen activated autoreactive B cells.

Figure 2.

The BAFF–APRIL family and their receptors: BAFF can be expressed by a wide range of cells including B and T cells, neutrophils, macrophages, other myeloid cells, stroma l cells as a type II transmembrane protein that is cleaved by a furin protease to yield soluble homotrimers. BAFF is also expressed on the cell membrane as an alternatively spliced form missing 57 bp (Δ BAFF) that is inefficiently cleaved from the cell surface, does not bind receptors and limits BAFF availability by forming heterotrimers with full length BAFF [67]. BAFF and APRIL can heterotrimerize; small amounts of heterotrimers are found in the sera of patients with autoimmune diseases [68]. APRIL, like BAFF, is expressed by multiple cell types and even some tumor cells. APRIL is expressed on the cell membrane if it is fused to the transmembrane and cytoplasmic portion of TWEAK (TWE-PRIL) [69]. Other splice variants of various family members and their receptors have been identified [70–72]. Soluble BAFF can multimerize into a 20 trimer structure that is the preferential activating ligand for TACI and BMCA [73]. Similarly, APRIL is multimerized by binding to proteoglycans [19] and only the oligomeric form activates TACI [73]. TACI can also bind to proteoglycans such as syndecan [19]. Inhibitors block either BAFF alone or both BAFF and APRIL (see Table 2). 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 sulfate proteoglycan.

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 sulfate proteoglycan.

Table 1

Systems for analyzing the effect of BAFF excess or deficiency on naïve B cell tolerance

Expt	Mice/System	BAFF	Fate of self reactive B cells	Competition	Reference
1	Soluble HEL ^a /anti-HEL double Tg	BAFF Tg ^b	High affinity cells are usually anergized at the T2 stage and excluded from the FO ^c and MZ ^d subsets. Excess BAFF rescues cells from anergy and allows them access to the FO but not the MZ.	No	21
2	Anti-HEL Tg + WT ^e mixed BM ^f transfer into HEL Tg	BAFF Tg	High affinity cells are usually anergized at the T1 stage. Excess BAFF has no effect.	Yes	21
3	Anti-HEL (Heavy chain only Tg)/HEL double Tg	BAFF Tg	High affinity cells are deleted and are not rescued by BAFF. Intermediate affinity cells are not tolerized and are directed to the FO compartment. Excess BAFF rescues these cells to the MZ.	Yes	21
4	Anti-HEL Tg BM transfer into membrane HEL Tg	BAFF Tg	High affinity cells recognizing the high avidity antigen are deleted in the BM and are not rescued by excess BAFF	No	21
5	Adoptive transfer of soluble HEL/anti-HEL double Tg spleen B cells or non-Tg B cells into soluble HEL/anti-HEL double Tg	BCMA-Fc	Autoreactive B cells from the donor are usually anergic (See Expt 1). Rapid elimination of /75% of transferred anergic transgenic B cells with BCMA-Ig treatment. Autoreactive B cells are much more sensitive to deletion by low dose BCMA-Ig than transferred control non-transgenic B cells.	No	28
7	IgK-reactive macroself Ag Tg	BAFF Tg	Normally transgenic cells are arrested in the transitional state. BAFF excess rescues self reactive B cells into lymph nodes and spleen	No	23
8	IgK-reactive macro-self Ag/human Ck +/- double Tg	BAFF Tg	Self reactive B cells are no longer rescued by BAFF excess once competition is provided	Yes	23
9	3H9 anti-chromatin heavy chain Tg 3H9K ^{-/-} Tg	Exogenous BAFF	Autoreactive B cells are usually anergic and excluded from the follicle. Excess BAFF increases number and maturity of autoreactive B cells but they are still excluded from the follicle and ANA is not induced	Yes No	30
10	R4A heavy chain Tg anti-dsDNA IgM	BAFF Tg	BAFF does not change the total number of transgenic B cells in the spleen but promotes their maturation into T2, FO and MZ compartments and induces anti-DNA IgM	Yes	22
11	3H9 heavy chain Tg	BAFF Tg	Increased Tg B cells with increased usage of V _κ genes associated with self reactivity	Intermediate	29
12	3H9 heavy chain Tg	Δ BAFF Tg	Decreased Tg B cells with decreased usage of V _κ genes associated with self reactivity	Intermediate	29

^a hen egg lysozyme

^b transgenic

^c marginal zone

^d follicular

^e wild type

^f bone marrow

Table 2

Drugs in development for SLE that target BAFF/APRIL

Drug	Target	Current status for SLE
Belimumab <i>HGS/Glaxo</i>	Human antibody to soluble BAFF <i>Blocks soluble BAFF</i>	FDA approved
Atacicept <i>Merck-Serono</i>	Fusion protein of TACI with Fc* <i>Blocks both BAFF and APRIL</i>	Phase II/III in general SLE Phase II/III in SLE nephritis high dose arm stopped (toxicity)
A-623 <i>Anthera</i>	Fusion protein of BAFF-R derived peptide with Fc* <i>Blocks BAFF</i>	Completed Phase 1b Phase IIb suspended (manufacturing problem)
LY2127399 <i>Eli-Lilly</i>	Human antibody to soluble and membrane BAFF <i>Blocks soluble and membrane BAFF</i>	Phase III
Briobacept <i>Biogen-IDEC/Genentech</i>	Fusion protein of BAFF-R with Fc* <i>Blocks BAFF</i>	Completed Phase I for RA
Anti-BR3 <i>Genentech</i>	Antibody to BAFF-R <i>Blocks BAFF-R and depletes BAFF-R expressing cells</i>	Under development

* It is not known whether the fusion proteins also block membrane Δ BAFF or BAFF or whether blocking membrane BAFF will have different therapeutic effects than blocking only soluble BAFF