The BAFF/APRIL system in SLE pathogenesis.

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Abstract

Systemic lupus erythematosus (SLE) is characterized by multisystem immune-mediated injury in the setting of autoimmunity to nuclear antigens. The clinical heterogeneity of SLE, the absence of universally agreed clinical trial end points, and the paucity of validated therapeutic targets have, historically, contributed to a lack of novel treatments for SLE. However, in 2011, a therapeutic monoclonal antibody that neutralizes the cytokine TNF ligand superfamily member 13B (also known as B-cell-activating factor of the TNF family [BAFF]), belimumab, became the first targeted therapy for SLE to have efficacy in a randomized clinical trial. Because of its specificity, the efficacy of belimumab provides an opportunity to increase understanding of SLE pathophysiology. Although belimumab depletes B cells, this effect is not as powerful as that of other B-cell-directed therapies that have not been proven efficacious in randomized clinical trials. In this article, therefore, we review results suggesting that neutralizing BAFF can have effects on the immune system other than depletion of B cells. We also identify aspects of the BAFF system for which data in relation to SLE are still missing, and we suggest studies to investigate the pathogenesis of SLE and ways to refine anti-BAFF therapies. The role of a related cytokine, TNF ligand superfamily member 13 (also known as a proliferation-inducing ligand [APRIL]) in SLE is much less well understood, and hence this review focuses on BAFF.

Keywords

A proliferation-inducing ligand (APRIL); autoimmunity; B cell activating factor of the tumour necrosis factor (TNF) family (BAFF); innate immunity; interferon (IFN); systemic lupus erythematosus (SLE).

Review criteria

We performed a PubMed search for relevant articles from 1999–2013, using key words, including "systemic lupus erythematosus", "BAFF", "BLyS", "THANK", "TALL-1", "zTNF4", "TNFSF13B", "APRIL", "TNFSF13", "hetero-trimer", "TACI", "BAFF-R", "BCMA", "Nogo-66 receptor", "B cell", "T cell", "natural killer", "dendritic cell", "neutrophils", "interferon", "toll-like receptor", "Belimumab", "GSK1550188", "HGS1006", "Blisibimod", "AMG623", "A-623", "Tabalumab", and "LY2127399". Articles in English were reviewed by title and abstract and selected if deemed important and relevant.

Key points

- TNF ligand superfamily member 13B (also known as B cell-activating factor of the TNF family [BAFF]) and TNF ligand superfamily member 13 (also known as a proliferation-inducing ligand [APRIL]) are important modulators of autoimmunity
- Data indicate that alteration of the BAFF/APRIL system affects the capacity of the innate immune system to regulate B-cell activation
- BAFF and type I interferons function together in systemic lupus erythematosus (SLE) pathogenesis in both a Toll-like receptor-dependent and independent manner
- Defining the clinical manifestations of disease related to alterations of the BAFF/APRIL system might help to stratify patients with SLE into subgroups that are more likely to benefit from anti-BAFF treatment
- Differences in the molecular forms of BAFF might affect the efficacy of BAFF-specific therapies

Introduction

Systemic lupus erythematosus (SLE) is an idiopathic, systemic autoimmune disease.[1] Although the cause of this disease is unclear, research over the past two decades has provided new insights into its pathogenesis. One breakthrough was the discovery, in 1999, of a crucial B-cell survival factor, TNF ligand super family member 13B (also known as B cell-activating factor of the TNF family [BAFF], B-lymphocyte stimulator [BLyS], zTNF4, THANK and TALL1),[2] which has an important role in autoimmunity, and in particular in SLE pathogenesis.[3,4] In 2011, belimumab, a mono clonal antibody targeting human BAFF, was shown in randomized clinical trials to be efficacious in a subset of patients with SLE and has now become the first approved targeted therapy for SLE.[5,6] As a consequence, clinical inhibition of BAFF has generated substantial interest and similar biologic agents are being tested in clinical trials. The efficacy of a specific anti-BAFF treatment demonstrated that SLE is not exclusively a T-cell mediated disease and has renewed the scientific community's interest in the pathogenesis of SLE.

Clinical trials and *post hoc* analysis of data obtained from belimumab treated patients with SLE have pro vided useful information about BAFF and a related cytokine, TNF ligand superfamily member 13 (also known as a proliferation inducing ligand [APRIL]). Data suggest that mechanisms other than B cell depletion are affected during responses to treatment with belimumab. In addition, the deregulation of BAFF and APRIL is found in specific subsets of patients with SLE.[7] The 'BAFF/APRIL system' and the innate immune system coordinate in regulating the innate activation of B cells, and seem to be defective in a mouse model of SLE in which BAFF is overexpressed.[8] Several receptors and various biochemical forms of the ligands exist in the BAFF/APRIL system,[9–11] but further work is required to elucidate their respective contributions to the pathogenesis and phenotype of SLE. In this article, we review the BAFF/APRIL system and highlight new discoveries that are relevant to the improvement of SLE management and that might lead to therapeutic innovation. In light of an ongoing debate about the role of APRIL in SLE, we have predominantly focused this Review on BAFF.

The BAFF/APRIL system

Two ligands, BAFF and APRIL, and three receptors, TNF receptor superfamily member 13C (also known as BAFF receptor [BAFFR] or BLyS receptor 3 [BR3]), TNF receptor superfamily member 17 (also known as B-cell maturation antigen [BCMA]) and TNF

receptor superfamily member 13B (also known as transmembrane activator and cyclophilin ligand interactor [TACI]) form the backbone of the BAFF/APRIL system. BAFF and APRIL can both interact with BCMA and TACI, whereas BAFF is the sole ligand for BAFFR (reviewed previously[12]; Figure 1). BAFFR is essential for both survival and maturation of immature B cells, whereas TACI is critical for T-cell-independent responses of B cells to type I and type II antigens, negative regulation of the B-cell compartment and class-switch recombination of B cells (reviewed previously[13]). BCMA is expressed by plasmablasts and plasma cells, and promotes plasma cell survival.

BAFF and APRIL are produced by myeloid cells, pre dominantly by macrophages, neutrophils and dendritic cells (DCs), and also by radiation-resistant stromal cells.[14] Lymphoid cells, including B cells and activated T cells, can also produce BAFF and APRIL. Finally, Toll like receptor 9 (TLR9)-activated plasmacytoid DCs and IL2-activated natural killer cells also produce BAFF (reviewed previously[12]).

BAFF and APRIL are type II transmembrane pro teins.[3,15,16] BAFF can also be processed into a soluble cytokine after cleavage at a furin protease site,[3,15] whereas APRIL is mainly produced in a soluble form.[16,17] Two exceptions, which are membrane bound, are the APRILδ isoform, first identified in malignant B-cell tumours,[18] and TWEPRIL, a hybrid protein of APRIL and TNF ligand superfamily member 12 (also known as TNF related weak inducer of apoptosis [TWEAK]).[19] The receptor-binding domain of BAFF is trimeric;[20] however, 20 BAFF trimers can assemble into a BAFF 60mer.[10,11] Furthermore, BAFF and APRIL can assemble as BAFF - APRIL heterotrimers of undefined stoichiometries, which have been detected in the serum of patients with rheumatic diseases (Figure 2). Recombinant BAFF–APRIL heterotrimers are biologically active, albeit less than BAFF or APRIL homotrimers.[21,22] Hetero-trimers of various compositions might be differentially susceptible to BAFF-targeting therapies.

Mice expressing BAFF with a mutated furin cleavage site have a phenotype similar to *Baff*–/– (*Tnfsf13b–/–)* mice, suggesting that membrane-bound BAFF cannot substitute for soluble BAFF.[23] However, when treated with soluble BAFF, B cells from the BAFF furin-mutant mice became more similar to B cells from wildtype mice than to B cells from *Baff*–/– mice that were also treated with soluble BAFF.[23] These results suggest a twostep process in BAFF-mediated B-cell maturation. First, soluble BAFF promotes B-cell survival by activating BAFFR, and then the B-cell phenotype is modulated by membrane-bound BAFF signals, possibly by activating TACI.[23]

Human BAFF is a 285 amino acid-long protein with two potential *N*-glycosylation sites.[24] Because some enzyme-linked immunosorbent assays (ELISAs) detect only unglycosylated BAFF, whereas other ELISAs detect both unglycosylated and glycosylated BAFF, post-translational *N*-glycosylation status could explain the failure to consistently associate disease activity with serum BAFF concentrations.[24] Alternative splice variants of BAFF, particularly ΔBAFF, might have an important role in SLE pathogenesis.[25–28] Mouse ΔBAFF decreases the bioactivity and release of full-length BAFF by associating with BAFF and forming inactive hetero trimers.[26] In mice, BAFF and ΔBAFF can both modulate B1 B cells.[25,26] BAFFφ is a nonfunctional isoform identified in human cell lines.[26] Although currently unknown, it is possible that anti-BAFF biologic agents might differ in their ability to bind and neutralize the various human BAFF isoforms or glycosylation variants.

The BAFF/APRIL system in SLE

BAFF is necessary for B-cell maturation and survival. Mice genetically deficient in BAFF lack mature B cells and are immunodeficient,[29] whereas mice that over produce BAFF have high numbers of mature B cells and antibodies, including autoantibodies, and develop an autoimmune disease similar to SLE in humans.[2] Together with the observation that BAFF blockade decreased symptoms of SLE in mouse models,[30] these findings promoted BAFF as a therapeutic target for the treatment of patients with SLE.

APRIL is important for antibody class-switching and plasma-cell survival (reviewed previously[13]). APRIL overexpressing transgenic mice develop B1 B-cell neoplasia, but do not develop SLE-like pathology.[31] Selective APRIL blockade can delay the development of disease in a lupus-prone mouse model (NZB/W F1 mice).[32] Using NZM.*April*–/– (NZM.*Tnfsf13*–/–) mice, Jacob *et al.*[33] showed that, in this model, APRIL was not necessary for SLE pathogenesis. Although NZM.*Baff* –/–*April*–/– (NZM. *Tnfsf13b*–/–*Tnfsf13*–/–) mice had fewer bone-marrow plasma cells and autoantibodies than NZM.*Baff* –/– (NZM.*Tnfsf13b*–/–) mice, both strains had mild kidney immunopathology,[33] suggesting that blocking both BAFF and APRIL in patients with SLE might not be beneficial and could even increase the risk of toxicity. Further supporting this idea, a phase II/III clinical trial of the chimeric recombinant fusion protein atacicept (combining TACI with an immunoglobulin constant region), which neutralizes BAFF, APRIL and BAFF–APRIL hetero-trimers in patients with active lupus nephritis, was stopped because of serum IgG depletion and an increased rate of infections.[34]

Belimumab

Belimumab is a fully human recombinant monoclonal IgG λ antibody that impairs Bcell survival by targeting soluble, not membrane-bound, human BAFF.[35] The addition of belimumab to standard therapy has been studied in two multi-centre, double-blind, placebo-controlled, randomized phase III clinical trials in patients with SLE, cumulatively examining 1,684 patients for up to 76 weeks.[5,6] Various scoring systems are used to evaluate disease activity in human SLE clinical trials, such as SLE disease activity index (SLEDAI), British isles lupus assessment group (BILAG) or SLE responder index (SRI).[36–38] These composite scores of clinical and laboratory features enable scaled assessment of disease activity at a single time point. Patients with active SLE who tested positive for SLE-associated antibodies benefited from belimumab compared with placebo with respect to disease activity (SRI) and health-related quality of life, with a satisfactory drug safety profile.[5,6,39,40] Belimumab therapy was associated with reduced autoantibody levels and normalized low complement levels,[41] and in *post hoc* analysis, patients with high disease activity (Safety Of Oestrogens In Lupus Erythematosus National Assessment [SELENA] SLEDAI), high anti-double-stranded DNA antibody and low complement levels at baseline had superior treatment responses to belimumab.[42] Belimumab therapy also reduced the number of circulating naive B cells, activated B cells and plasma cells, but did not reduce the number of circulating memory B cells or T cells.[41] No effects on antibody responses to previous pneumococcal, tetanus or influenza immunizations were detected after 1 year of treatment, consistent with a preserved memory B-cell compartment.[43]

BAFF and APRIL as biomarkers of SLE

In comparison with serum from healthy individuals, serum concentrations of BAFF and APRIL are higher in patients with autoimmune diseases, such as SLE, primary Sjögren's syndrome and rheumatoid arthritis (reviewed previously[13]). Whether serum BAFF or APRIL can be used as a biomarker in SLE has been a matter of debate over the past decade, with some studies reporting correlations between serum BAFF and APRIL concentrations and overall disease activity,[44–47] whereas others found no such correlations.[41,47–50] Petri *et al.*[51] showed, using multivariate analysis, that elevated baseline serum BAFF concentration (≥2 ng/ml) was predictive of moderate-to-severe SLE flares in patients receiving the standard therapy, prednisone with antimalarial (hydroxychloroquine) or immunosuppressive drugs

(methotrexate, mycophenolate mofetil and azathioprine). This association is supported by another study, in which a high serum BAFF concentration was associated with flares of SLE disease activity (assessed by the BILAG score) after rituximab treatment.52 However, in our study,[7] among others, no significant association between serum BAFF concentration and disease activity (assessed using SLEDAI2k) was found. Moreover, in the belimumab phase III clinical trials, baseline serum BAFF concentration was not found to be predictive of outcome in either anti-BAFF or control-treated patients.[41] These discrepancies might be caused by differences in assay sensitivity, disease activity scores or study populations. However, serum BAFF and APRIL concentrations might not accurately reflect total BAFF production or, in the case of APRIL (which binds proteoglycans), the concentration of the active cytokine in tissues. Urinary excretion of cytokines, for example, might reduce serum concentration in patients with renal disease, suggesting that the measurement of BAFF and APRIL in urine should be investigated by SLE researchers.[53]

Perhaps the failure to reliably show an association between disease activity and the concentration of a cytokine known to be involved in the pathogenesis of SLE means that the measurement of disease activity requires reexamination. Moreover, given that *post hoc* analysis of clinical trial data revealed that BAFF inhibition was most effective in patients with active disease and high serum autoantibody titres,[42] this subset of SLE patients might be best suited for analysis of clinical associations of BAFF and APRIL expression. We could, therefore, study SLE patient subsets, defined according to specific clinical or immunological phenotypes.

SLE clinical subsets

Some studies measuring cytokines in patients with SLE have focused on SLE subsets rather than over all disease activity measured with composite indices of disease activity.[54–60] We showed that serum BAFF and APRIL concentrations, although not correlating with a composite measure of disease activity (SLEDAI2k), were increased and decreased, respectively, in sub sets of patients with SLE who also had renal or central nervous system (CNS) pathology.[7] These data are sup ported by findings from mouse studies, in which separate pathways that regulate autoimmunity, inflammation and renal damage led to differentiated phenotypes of disease.[61,62] Combining different genetic loci such as *Sle3* and *Sle5*, with or without *Sle1*, leads to differentiated phenotypes in lupus-prone mice.[62] If distinct

immunological pathways are activated in humans with different disease manifestations, then general biomarkers for SLE might not exist. Some subsets of patients with SLE might, however, benefit from therapies targeting these pathways. The identification of reliable biomarkers for these subsets would be useful as a tool for the selection of therapy. Serum BAFF, for example, is increased in patients with CNS or renal pathology, therefore, these patients might benefit from anti-BAFF therapy.[7] Although patients with severe CNS manifestations or severe lupus nephritis were excluded from two phase III clinical trials of belimumab,[5,6] *post hoc* pooled analysis suggested that belimumab might be an effective treatment for patients with SLE and renal involvement.[63] Also, in the subset of SLE patients without renal disease activity at baseline, those treated with belimumab had less renal involvement than patients given placebo.[64] Further research is needed to investigate the accuracy of using serum BAFF and APRIL measurements to predict SLE phenotype, and therapeutic responses in patients within the different subgroups.

Ethnicity and polymorphisms

Substantial ethnic variation in the prevalence and characteristics of SLE exists.[65,66] Asian, Indigenous Australian and African patients with SLE suffer from more severe disease compared with white people.[7,65–68] In one report, serum BAFF or APRIL concentration was not associated with Asian ethnicity;7 however, African American patients with SLE had higher serum BAFF concentrations than white American patients with SLE, although not significantly after multivariate analysis.[69] By contrast, increased serum BAFF concentration was associated with an increase in the SLEDAI in white American patients, but not in African American patients.[69] Further research is needed to understand how particular ethnic subgroups respond to anti-BAFF therapies.

Future research could focus on BAFF and APRIL single nucleotide polymorphisms (SNPs). Several SNPs have been identified in the promoter, coding and untranslated regions of *TNFSF13B* (the human BAFF gene), but in most studies no significant association with SLE susceptibility has been found.[70,71] Only one study reported an association (in Egyptian patients with SLE) between disease susceptibility and 871C>T and 2701T>A SNPs in the BAFF promoter region.[72] *TNFSF13B* pro moter SNPs were not significantly associated with symptoms of SLE. Monocytes from healthy individuals with the 871T allele had a significant increase in BAFF mRNA compared with monocytes from healthy individuals without the 871T allele.[70]

SNPs have also been found in *TNFSF13* (the human APRIL gene), including Gly67Arg, which was significantly associated with SLE susceptibility in Japanese patients.[73] Another study showed a possible association between Gly67Arg and SLE susceptibility in African American and Hispanic people, but not in white American people.[74] Additionally, the c.199A-c.287G (67Arg96Ser) SNP was reported to be a protective haplotype whereas the c.199G-c.287A (67Gly96Asn) haplotype was associated with disease susceptibility in Japanese patients with SLE.[75] In line with its protective effect, the protective haplotype 67Arg96Ser decreased soluble APRIL secretion in transfection experiments.[76]

BAFF and APRIL receptors in SLE

Exacerbation of disease in lupus-prone BCMA-deficient mice (MRL*Faslpr*/J.*Tnfrsf17*–/– and Nba2.*Tnfrsf17*–/– mice), suggests that BCMA has a direct or indirect negative regulatory role.[77] In these mouse models, exacerbation of disease might occur by abnormal signalling of BAFF or APRIL through TACI or BAFFR in the absence of BCMA. Compared with wild-type and single-mutant control strains, BCMA-deficient lupus prone mice have more mature B cells and plasma cells in secondary lymphoid organs, more serum autoantibodies and BAFF, and more immune complex deposition in the kidneys.[77] An increase in the number of BAFF producing cells, such as DCs and macrophages, was also detected in these mice.[77] BCMA signalling might have an indirect regulatory role in the homeostasis of BAFF producing cells, because BCMA was not expressed by splenic DCs in all mouse strains in this study (MRL *Faslpr*/J, *Tnfrsf17*–/–, MRL*Faslpr*/J.*Tnfrsf17*–/– and wild type mice).[77] Jacob *et al.*[78] showed that the deletion of BAFFR alone, which leads to profound B-cell depletion, or of BCMA or TACI alone, did not protect against SLE-like disease in NZM lupus-prone mice. These data suggest that, unlike BAFF-over-expressing transgenic mice,[79] T cells might have an important role in the pathogenesis of NZM lupusprone mice.[78]

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BAFF, TLR cross-talk and IFN signatures

Type I interferons, and particularly IFNα, are thought to have a major role in SLE pathogenesis. SLE is characterized by increased expression of IFNα-induced genes, referred to as the IFNα signature, in peripheral blood mononuclear cells and in target organs, such as the kidneys.[54,82,83] In some studies, serum IFNα concentrations and IFN-induced genes positively correlate with SLE disease activity (measured by SLEDAI).[82,84] *IFNG* expression is also increased in SLE,[60] and higher serum IFNγ concentrations have been reported in patients with SLE compared with healthy individuals.[85] Plasmacytoid DCs are the major source of IFNα produced in response to viruses and bacteria (reviewed previously[86]). Activation of endosomal TLR7 and TLR9 by their respective agonists, single-stranded RNA and unmethylated CpG-rich DNA, transduces signals through myeloid differentiation primary response protein MyD88 (MyD88) and upregulates IFNα expression. In SLE, antibody–nucleic acid immune complexes, for example RNA or DNA from necrotic or apoptotic cells,[87] are suggested to be bound by immunoglobulin γ Fc region receptor IIa (FcγRIIa) and internalized by plasmacytoid DCs in the form of autoimmune complexes, subsequently activating endosomal TLRs and upregulating IFNα production (Figure 3).[86,88]

Several studies indicate that interferons induce BAFF expression: IFNα upregulates BAFF expression by mouse macrophages;[89] IFNα and IFNγ both upregulate BAFF and APRIL expression by human DCs;[90] and IFNα and IFNγ can upregulate BAFF expression by human monocytes.[90] In response to IFNγ, more BAFF was produced by monocytes from patients with SLE than by monocytes from healthy donors.[91] In a phase Ia clinical trial of patients with SLE treated with an anti-IFNα monoclonal antibody, BAFF mRNA expression in whole blood was suppressed.[92] Strikingly, in NZM lupus-prone mice, BAFF deficiency prevented glomerulonephritis and clinical disease in response to IFNα treatment.[93] The activity of IFNα in serum from patients with SLE positively correlated with serum BAFF concentrations.[69] Moreover, we demonstrated a regulatory feedback loop between TLR7 or TLR9 and TACI in BAFF transgenic mice,[79] and cooperation between TACI and TLR

signalling in Fas-induced apoptosis, a mechanism that is defective in these mice (Box 1).[8] Collectively, these mouse and human studies of SLE pathogenesis suggest that the roles of BAFF and IFNα are closely linked (Figure 3). One study showed that, through an IFNα-dependent pathway, monocytes from healthy individuals can differentiate into DCs when cultured with serum from patients with SLE (SLEDCs), and this induction of DC differentiation also correlated with the SLE disease activity (measured by SLEDAI).[94] SLEDCs can induce B-cell proliferation and plasma blast differentiation.[95] Interestingly, DCs differentiated from monocytes, from healthy individuals, and cultured with IFNα plus granulocyte macrophage colony stimulating factor (IFNDCs), phenotypically differ from SLEDCs. One of the main differences is that IgG secretion was enhanced (dependent on BAFF) by SLEDCs but not IFNDCs.[95]

Therapeutic innovation

Although known anti-BAFF biologic agents bind to soluble or membrane-bound BAFF, the specificity for homotrimer, hetero-trimer and 60-mer forms is unclear. Belimumab targets only soluble human BAFF.[35] Tabalumab (also known as LY2127399), a fully-human IgG4 monoclonal antibody, neutralizes both soluble and membrane-bound human BAFF.[96] Blisibimod (also known as A623) is a fusion polypeptide protein that targets human BAFF and binds to both soluble and membrane-bound BAFF in mouse models of SLE and rheumatoid arthritis.[97]

Major differences between BAFF trimer and 60mer forms have been investigated using mouse models; however, in humans, these forms of BAFF have not been studied in detail and the 60-mer has not been described. In primary B cells isolated from mice, TACI is activated by the BAFF 60-mer, but not the BAFF trimer.[20] TACI interacts with MyD88, a signalling adaptor critical for the development of BAFF-mediated autoimmunity in mice,[79] suggesting that TACI, and hence the BAFF 60-mer, might be important in regulating autoimmune disease. One of the major unanswered questions is whether human BAFF exists in both trimer and 60-mer forms, and what functions the different forms might have in disease. Indeed, if the BAFF 60-mer is the active form in humans, active-form-specific anti-BAFF therapies might be developed by neutralizing only the active BAFF 60-mer, or by therapeutically disrupting the active 60-mer into the less active trimer (Figure 2). The BAFF 60-mer is not an established molecular form of BAFF *in vivo* and has yet to be characterized in mice and humans.

If findings on the reduced bioactivity of recombinant BAFF–APRIL hetero-trimers apply to the native hetero trimer, hetero-trimer formation could have a role in determining BAFF or APRIL activity *in vivo*. Analysis performed on a small cohort of 36 patients with SLE showed a nonsignificant trend towards a positive correlation between serum BAFF–APRIL hetero-trimer con centration and disease activity (measured by SLEDAI).[21] If supported by larger longitudinal, prospective studies, these data could be interpreted to mean either that hetero-trimer formation drives specific pathogenic signals or that it reflects the action of a negative feedback loop without which the pathology is more severe (Figure 2). Whether hetero-trimer formation occurs through *de novo* assembly of monomers in a cell producing both BAFF and APRIL, or whether homotrimers equilibrate into hetero-trimers after synthesis and release is not known. However, hetero-trimers can be predicted not to form 60mers. BAFF contains a loop sequence (the 'flap') that protrudes out of the monomer and hooks to the flap of BAFF in adjacent trimers.[10] Size analyses of recombinant BAFF 60-mers, showed some BAFF trimers in addition to the 60-mer, but no 6-mer or 9-mer (etc.) intermediates,[11] suggesting that flap–flap interactions are weak and are probably only important when they act cooperatively in the 60-mer. APRIL has no flap sequence[98] and, therefore, theoretically cannot be incorporated into a BAFF 60mer; if APRIL were incorporated it would most likely lead to 60mer dissociation. A better understanding of the relative proportions and activity of BAFF–APRIL homotrimers and hetero-trimers might redirect therapeutic targeting of the BAFF/APRIL system.

Conclusion

The approval of BAFF inhibition as the first targeted therapy for SLE is a major advance in the treatment of this disease, and confirms a role for BAFF in the pathogenesis of SLE. However, the diversity of SLE manifestations and the monetary cost of biologic therapy might restrict the generalized use of anti-BAFF biologic agents in the treatment of SLE. The role of the BAFF/APRIL system in the pathogenesis of SLE needs to be more completely understood in order to improve stratification of patients with SLE for effective anti-BAFF therapy. Studies of clinical subsets of patients who overexpress BAFF, but who were previously excluded from clinical trials, are particularly needed. Novel data suggest the potential for various approaches to target the BAFF/APRIL system in patients with SLE, such as targeting BAFF 60mers, hetero-trimers or novel receptors. Further work on the biology of the BAFF/APRIL system, and its connection to type I interferons and innate

immunity (Box 2), together with rapid translation to clinical validation, is required. The role of current and future BAFF/APRIL system targeting therapies in SLE will be determined by careful application of experimental and clinical approaches.

Box 1 – Innate activation-induced cell death of marginal zone B cells

The BAFF/APRIL system is important in the regulation of innate B-cell activation. Nonspecific activation of innate-like marginal zone B cells by TLR4 causes an efficient and rapid antibody response that is usually short-lived. The mechanism of nonspecific activation of marginal zone B cells by TLR4 leads to increased expression of TACI. Simultaneous TLR4 activation and BAFF or APRIL signals promote expression of Fas and FasL by marginal zone B cells, and repress expression of antiapoptotic proteins. These events prepare marginal zone

B cells for apoptosis, ultimately terminate the response to TLR4 activation and possibly also maintain tolerance. Defective FasL upregulation on TLR4-activated marginal zone B cells has been observed in lupus-prone BAFF-overexpressing transgenic mice.[8]

Box 2 - BAFF and immunity

- Studies showed that BAFF (and APRIL) is involved in splenic neutrophil activation of marginal zone B cells, promotion of plasma cell differentiation and increased immunoglobulin production[99]
- Cytoplasmic anti-neutrophil antibodies stimulate BAFF production by neutrophils leading to enhanced B-cell survival[100]
- Neutrophils infiltrating the joints of patients with rheumatoid arthritis release BAFF after TNF activation[101]
- Human NK cells produce BAFF when stimulated with IL-2[102]
- An *in vitro* study of mouse splenocytes showed that soluble BAFF can indirectly enhance NK-cell activity through upregulation of IL-2 and IFN-γ production by CD4+ T cells[103]

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Figure 1. Soluble BAFF and APRIL signalling.

BAFF and APRIL are type II transmembrane proteins, but BAFF can be processed into a soluble cytokine after cleavage at a furin protease site. APRIL is soluble, having been cleaved intracellularly. BAFF only has weak affinity for BCMA. BAFF-R is essential for survival and maturation of immature B cells. TACI is critical for T-cell-independent responses of B cells to type I and type II antigens, negative regulation of the size of the B-cell compartment and class-switch recombination. BCMA promotes plasma-cell survival. Dashed line indicates that BAFF possibly binds NgR, which is expressed on neurons and astrocytes, and has been shown to mediate negative effects on neuron outgrowth. Abbreviations: APRIL, a proliferation-inducing ligand (also known as TNF ligand superfamily member 13); BAFF, B-cell-activating factor of the TNF family (also known as TNF ligand superfamily member 13B); BAFF-R, BAFF receptor (also known as TNF receptor superfamily member 13C); BCMA, B-cell maturation antigen (also known as TNF receptor superfamily member 17); NgR, Nogo-66 receptor (also known as reticulon 4 receptor); TACI, transmembrane activator and cyclophilin ligand interactor (also known as TNF receptor superfamily member 13B).

Figure 2. Structural variants of BAFF and APRIL.

Different BAFF–APRIL structures might be involved in the pathogenesis of SLE, or might counteract the effects of BAFF and APRIL homotrimers by sequestering monomeric components required for their formation, competing for receptor binding or inducing suboptimal signals. APRIL interacts with polysaccharide side chains of HSPG, and interactions

of TACI and HSPG regulate antibody class-switching. Solid lines indicate known interactions. Dashed lines indicate hypothetical interactions. Abbreviations: APRIL, a proliferation-inducing ligand (also known as TNF ligand superfamily member 13); BAFF, B-cell-activating factor of the TNF family (also known as TNF ligand superfamily member 13B); BAFF-R, BAFF receptor (also known as TNF receptor superfamily member 13C); BCMA, B-cell maturation antigen (also known as TNF receptor superfamily member 17); HSPG, heparan sulphate proteoglycans; SLE, systemic lupus erythematosus; TACI, transmembrane activator and cyclophilin ligand interactor (also known as TNF receptor superfamily member 13B); TWEAK, TNF-related weak inducer of apoptosis (also known as

TNF ligand superfamily member 12); TWE–PRIL; a hybrid of TWEAK and APRIL.

Figure 3. Role of BAFF in the pathogenesis of SLE.

n SLE, it is believed that antibody–nucleic acid immune complexes (1), for example ssRNA or DNA from dead cells, are bound by FcγRIIa, activating TLRs and IFN-α production (2). IFN-α increases BAFF production (3). BAFF interacts with receptors on B cells (4). Excess BAFF can increase autoreactive B-cell survival, driving autoimmunity (5). TLR4 and TACI signalling cooperate to commit MZ B cells to apoptosis via induction of Fas and FasL (6), possibly contributing to the mechanism that terminates the short-lived antibody response of activated innate B cells. This mechanism is defective in BAFF-overexpressing transgenic mice (7; Box 1). Abbreviations: BAFF, B-cell-activating factor of the TNF family (also known as TNF ligand superfamily member 13B); BAFF-R, BAFF receptor (also known as TNF receptor superfamily member 13C); BCMA, B-cell maturation antigen (also known as TNF receptor superfamily member 17); DC, dendritic cell; FasL, Fas ligand; FcγRIIa, immunoglobulin γ Fc region receptor IIa; LPS, lipopolysaccharide; MyD88, myeloid differentiation primary response protein MyD88; MZ, marginal zone; ssRNA, single-stranded RNA; TACI, transmembrane activator and cyclophilin ligand interactor (also known as TNF receptor superfamily member 13B); TLR4, Toll-like receptor 4.

Competing Interests

F.M. and E.F.M. declare that they have acted as consultants for Eli Lilly and GSK. P.S. declares that he has a research agreement with Merck-Serono. F.B.V. declares no competing interests.

Author contributions

All authors researched the data for the article. F.B.V. wrote the manuscript and all authors reviewed/edited the manuscript before submission.

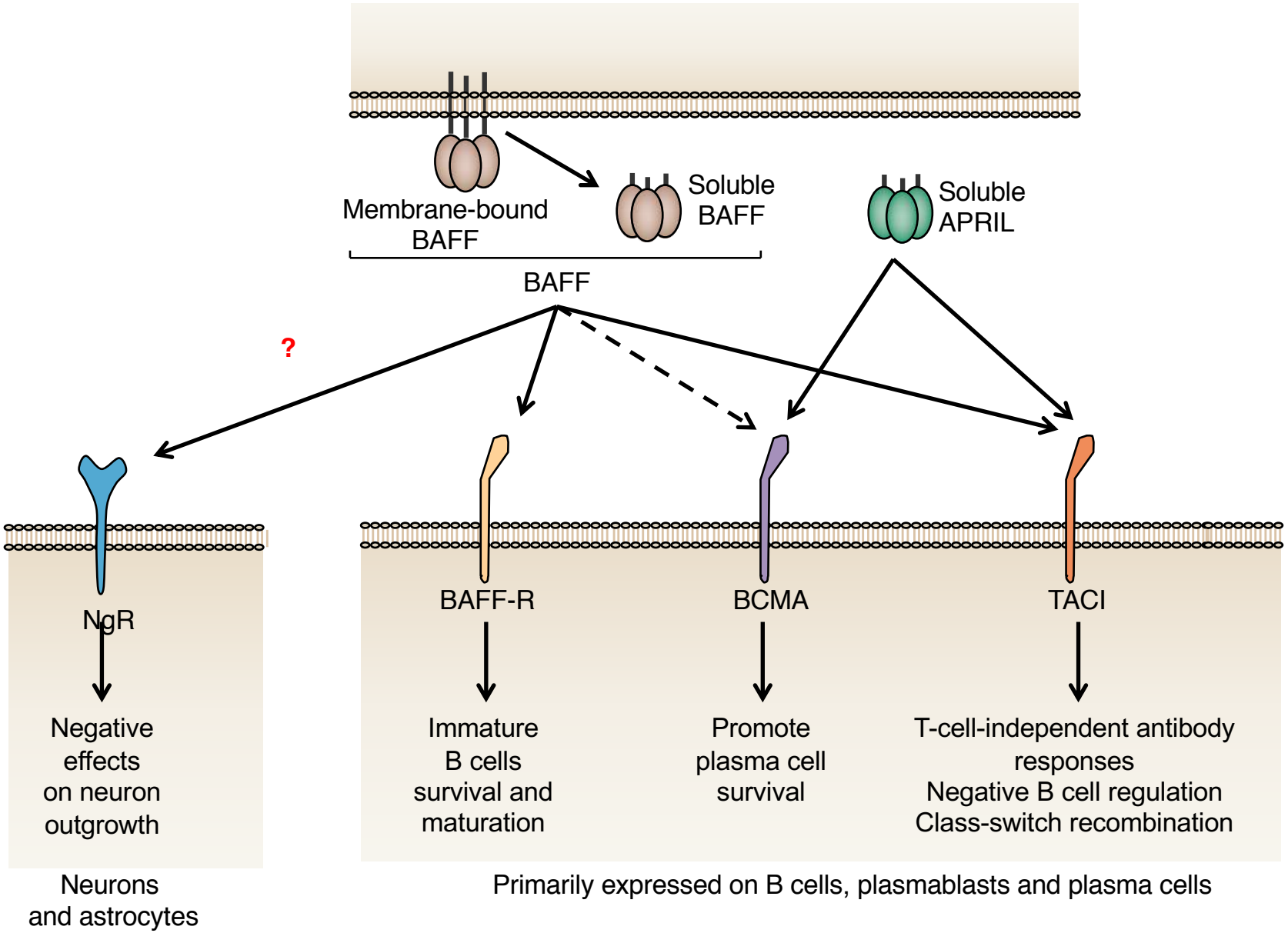
Membrane-bound
BAFF
Soluble
BAFF
Soluble
APRIL
BAFF
?
NgR
BAFF-R
BCMA
TACI
Negative
effects
on neuron
outgrowth
Immature
B cells
survival and
maturation
Promote
plasma cell
survival
T-cell-independent antibody
responses
Negative B cell regulation
Class-switch recombination
Neurons
and astrocytes
Primarily expressed on B cells, plasmablasts and plasma cells

Figure 1

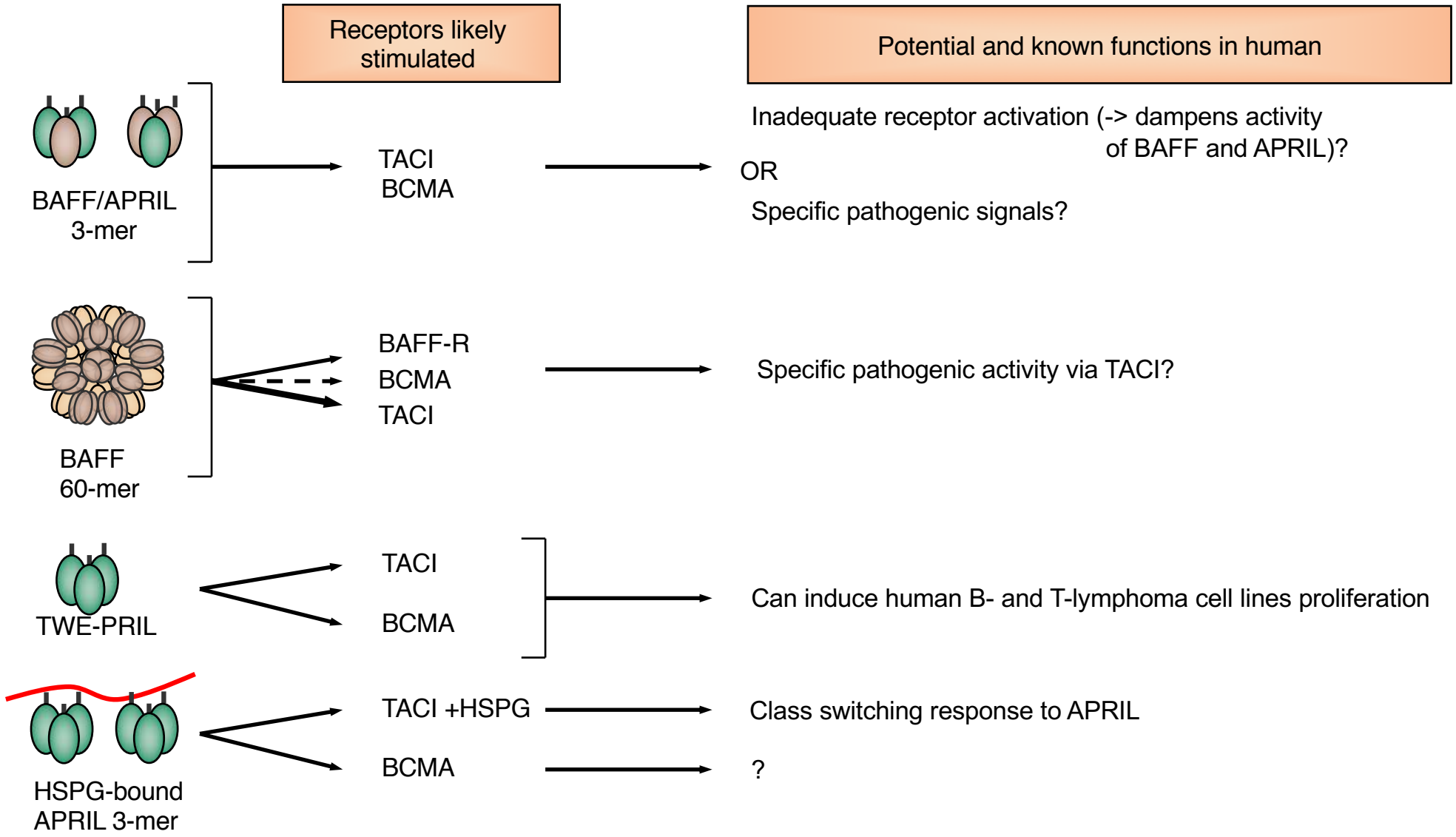
Receptors likely stimulated
Potential and known functions in human
BAFF/APRIL 3-mer
TACI
BCMA
Inadequate receptor activation (-> dampens activity of BAFF and APRIL)?
OR
Specific pathogenic signals?
BAFF 60-mer
BAFF-R
BCMA
TACI
Specific pathogenic activity via TACI?
TWE-PRIL
TACI
BCMA
Can induce human B- and T-lymphoma cell lines proliferation
HSPG-bound APRIL 3-mer
TACI +HSPG
Class switching response to APRIL
BCMA
?

Figure 2

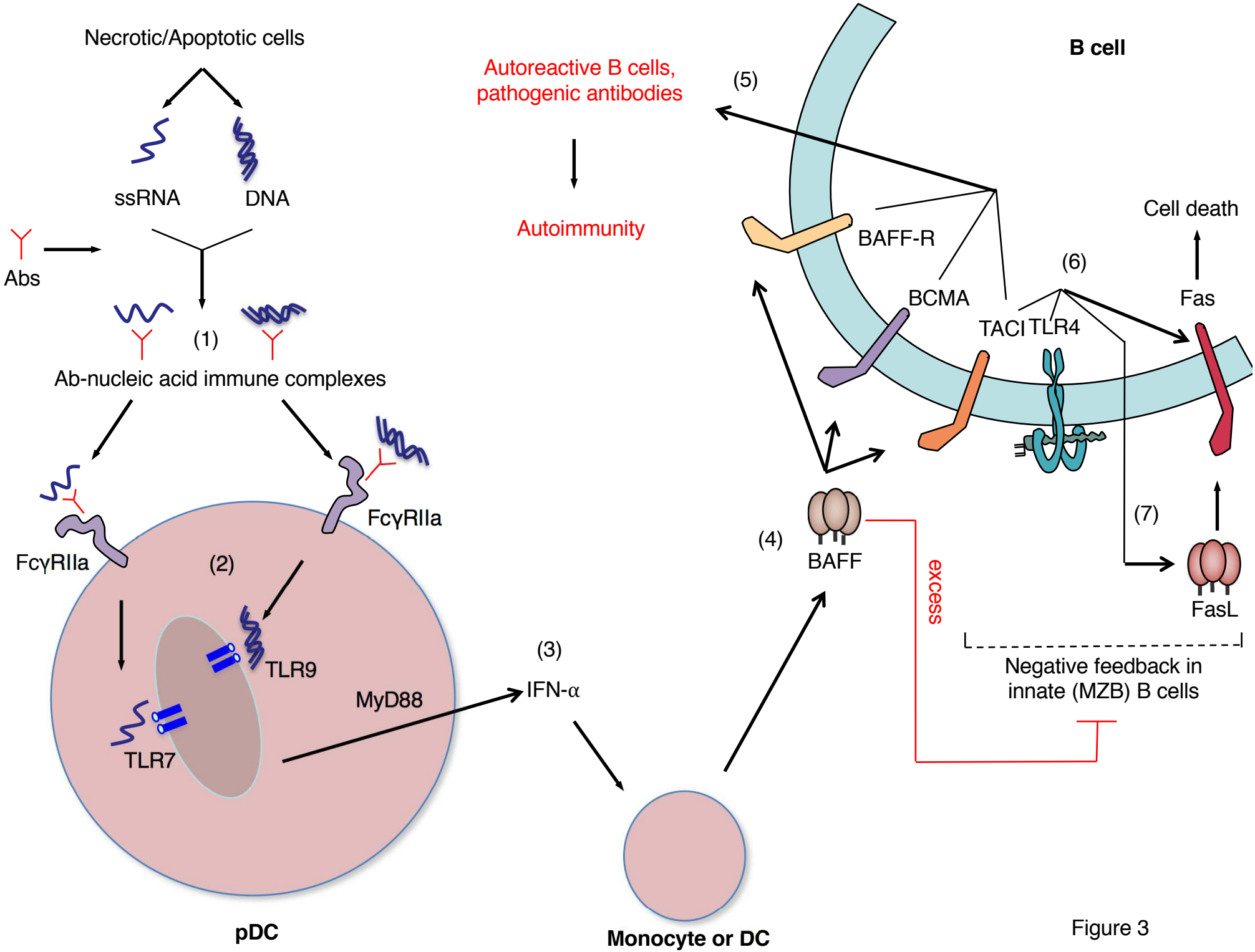
Necrotic/Apoptotic cells
ssRNA
DNA
Abs
(1)
Ab-nucleic acid immune complexes
FcγRIIa
FcγRIIa
(2)
TLR9
TLR7
MyD88
pDC
(3)
IFN-α
Monocyte or DC
Autoreactive B cells,
pathogenic antibodies
Autoimmunity
(5)
B cell
BAFF-R
BCMA
TACI
TLR4
(6)
Cell death
Fas
(4)
BAFF
(7)
FasL
excess
Negative feedback in
innate (MZB) B cells

Figure 3