BLyS/BAFF: A Potential Target in the Treatment of Active Systemic Lupus Erythematosus

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Many previous studies reported that B cell-deficient mice had a severe defect in T cell priming and in the development of delayed-type hypersensitivity when local antigens were injected. These mice were resistant to the development of certain autoimmune diseases such as experimental autoimmune encephalitis [1–3]. In addition to the complete absence of autoantibodies in B cell-deficient J_H-MRL lpr+ mice, the accumulation of T cells in lymphoid organs was attenuated. T lymphocytes from these mice were found to be defective in their ability to provide B cell help or produce interleukin-4 [4,5].

Dentritic cells from B cell-deficient mice enhance IL-12 production and preferentially induce T helper 1 cells. This phenotype was comparable to that of IL-10 knockout mice. Following IL-10 treatment of dentritic cells from these mice, T cells were driven to produce IL-4. Thus, B cells appear to have important immunoregulatory effects that are mediated through dentritic cells, perhaps due to B cell production of IL-10 [6].

Given that B cell survival and proliferation are important for the development of autoimmune diseases, we wished to explore how these cells survive and which factors could contribute to their polyclonal activation and, in some situations, to the excessive production of autoantibodies. In this regard, BLys (B lymphocyte stimulator) – also called BAFF (B cell-activating factor) – was recently introduced as a new important player whose role in maintaining B lymphocyte survival and proliferation turned out to be crucial [7]. This was followed by the demonstration that the overexpression of BLyS in transgenic mice led to the development of systemic lupus erythematosus-like disease [8]. When serum BLyS was investigated in SLE, rheumatoid arthritis and Sjogren's syndrome, it was found to be elevated and to correlate with titers of anti-ds DNA, rheumatoid factor, and anti-SSA/Ro antibodies [9,10]. These important findings unequivocally point to B cells as one of the potential targets in the treatment of autoimmune diseases. Following the reported success of B cell depletion by anti-CD20 (rituximab) in the treatment of rheumatoid arthritis, many investigators implemented this strategy and reported its beneficial effect in treating other autoimmune diseases such as active SLE [11,12].

In this review, we summarize the current knowledge on BLyS and discuss the possibility of implementing this knowledge by introducing anti-BLyS treatment.

BLyS/BAFF: a B cell survival factor

BAFF, a new survival factor for mature B lymphocytes, was discovered in 1999 [7]. BAFF, also known as TALL-1 and BLyS (B lymphocyte stimulator), is a member of the tumor necrosis factor ligand superfamily, which is produced by activated macrophages and dendritic cells. TNF family ligands are characterized by a C-terminal domain coined THD (for TNF homology domain). Within the THD, BLyS/BAFF shares about 20–30% similarity with other TNF family members such as FasL and CD40L. BAFF also shares up to 50% similarity with APRIL (a proliferation-inducing ligand), which shares several biological activities with BAFF [13–15]. Both BAFF and APRIL bind to two receptors called BCMA (B cell maturation antigen) and TACI (transmembrane activator and CAML interactor). Later, BAFF was found to selectively bind another receptor called BAFF-R, which APRIL cannot bind. Similar to their ligands, all these receptors belong to the TNF receptor superfamily.

Genetic analyses have shown that BAFF/BAFF-R interactions are the most dominant in controlling B cell survival [16–18]. Of all the identified receptors, BAFF-R is the only one required for the survival of most mature splenic B cells. Therefore, blocking either BAFF-R itself or BLyS/BAFF was proven in all experimental models to be therapeutically sufficient. The role of APRIL, BCMA and TACI in B cell activation is still under investigation [19,20]. Therefore, a more sophisticated approach would be to devise ways to use both the

IL = interleukin
BLyS = B lymphocyte stimulator
SLE = systemic lupus erythematosus

BAFF = B cell-activating factor
TNF = tumor necrosis factor

BLyS and APRIL systems to selectively eliminate cells responding to self antigens.

T cell-independent responses produce low affinity immunoglobulin M, but also IgG. BAFF and APRIL have been shown to stimulate class-switch in synergy with other factors such as IL-10 or transforming growth factor-beta. The participation of APRIL indicates that isotype switch does not require BAFF-R. In addition to its action on B cells, BAFF was shown to have co-stimulatory effects on T cells, and therefore BAFF antagonists such as TACI-Ig inhibited T cell activation [21]. CD4+ T cells are probably required for BAFF-dependent autoantibody production. High levels of serum BAFF were found to correlate with higher titers of antiphospholipid antibodies in patients with human immunodeficiency virus [22]. Recent data have shown that activated neutrophils express high levels of BAFF mRNA and also release biologically active BAFF, suggesting that they play a role in regulating B cell homeostasis [23].

Signal transduction pathways through which B cells escape or undergo enhanced apoptosis are not fully defined. Some of the suggested molecules involved in B cell homeostasis are TRAF proteins, which can activate NF-kappa B and MARK pathways, thus inducing anti-apoptotic activity that is consistent with the survival function of BAFF. A possible mechanism by which BAFF/BAFF-R interaction is followed by NFκB activation is the induction of NFκB-inducing kinase (NIK) which activates Ikka, or the induction by p50/RelB in resting primary B cells, promoting survival by the up-regulation of Bcl-2 family members [24–26]. Whether the processing of BAFF is required to maintain its activity or whether the membrane-bound form alone is sufficient is currently under investigation. Although membrane-bound BAFF has been detected on the surface of murine dentritic cells, human monocytes, and on cells infiltrating salivary glands of patients with Sjögren's syndrome, little is known about whether surface, uncleaved BAFF is biologically active and whether neutralization of only soluble BAFF is sufficient to attenuate the activation of B and T cells [27–29].

- *BLyS/BAFF, a survival factor for B cells that is found elevated in SLE in association with anti-dsDNA elevation*
- *BLyS/BAFF is a potential target in the treatment of SLE*
- *The administration of anti-BLyS antibodies is well tolerated and effective in ameliorating SLE disease activity*

Local production of BLyS/BAFF

Following the detection of BAFF in different tissues, it was important to evaluate its role both in serum and in tissues such as lymph nodes, synovia, and others. It was documented that the concentration of BAFF was higher in the synovial fluid than in the serum of patients with many types of arthritis, even in the absence of overt systemic inflammation, evidence for local production of BAFF. However, in the synovial fluid from patients with rheumatoid arthritis, surface expression of BLyS by monocytes was decreased as compared to the increase of soluble BLyS levels in these same synovial fluid samples. This suggests that the rate of cleavage of membrane to soluble BLyS is an important regulatory factor in the process of joint inflammation both in rheumatoid arthritis and SLE. Local production of BLyS/BAFF not only promotes the survival of B cell aggregates in tissues, but also induces class-switching in lymphoid organs. This may partially explain the local production of IgG rheumatoid factor antibodies in the synovium of rheumatoid arthritis [30]

BLyS/BAFF in disease

Recombinant BAFF treatment in mice boosted the primary *in vivo* immunoglobulin response to various antigens, e.g., pneumovax. In the first 4–5 days only IgM and IgA titers were increased, but longer treatment led to an increase in IgG levels as well. In general, IgG and IgM titers increased two to fourfold, but these results indicated that BAFF could have an adjuvant-like effect [31].

The cleavage and secretion of BAFF was demonstrated in BAFF-transgenic mice in which the full-length form was over-expressed in the liver, and soluble BAFF was detected in the blood in association with B cell hyperplasia, indicating the transfer of soluble BAFF from liver into organs where the transgene was not expressed. All three different lines of BAFF-Tg strains had enlarged spleens, lymph nodes, circulating immune complexes, rheumatoid factor and anti-ds-DNA autoantibodies. The kidneys of BAFF-Tg mice were involved, exhibiting abnormally enlarged glomeruli, high protein levels and evidence of vasculitis. The involvement of BAFF in the evolvement and progression of autoimmune diseases was demonstrated in mouse disease models such as the NZB/W. Treatment with BAFF blocker BCMA-Ig inhibited the development of collagen-induced arthritis and the rapid decrease in anticollagen IgG levels in these mice [24].

BLyS/BAFF in human disease

With regard to human autoimmune diseases, it was shown that BAFF levels were elevated in sera from patients with SLE and Sjögren's syndrome [9,10]. Whether elevated BAFF plays a direct role as a disease driver or is simply symptomatic of chronic inflammation must be studied further. The expression of BAFF by macrophages and dendritic cells is stimulated by interferon-gamma and IL-10, both produced during inflammation and/or infection. Chronic infection may lead to the increased release of BAFF and the emergence of autoreactivity, especially in those with susceptible genes.

In a recent longitudinal observation study, a considerable heterogeneity in serum BLyS phenotype was observed among SLE

Ig = immunoglobulin

Tg = transgenic

patients. Whereas serum BLyS remained persistently normal in half the patients, in 25% it remained persistently high over a period of one year [32]. In this study, as well in previous ones [9], serum BLyS levels correlated with anti-ds DNA antibody titers, however the association with SLE disease activity (according to the SLE Disease Activity Index) remains questionable. A few studies have pointed to the lack of association between BLyS levels and SLEDAI, mainly when observed in any given patient at any time point. However, data from a larger cohort of SLE patients suggested that serum BLyS levels do correlate with SLEDAI scores when analyzed as a whole group over time [9,10,33].

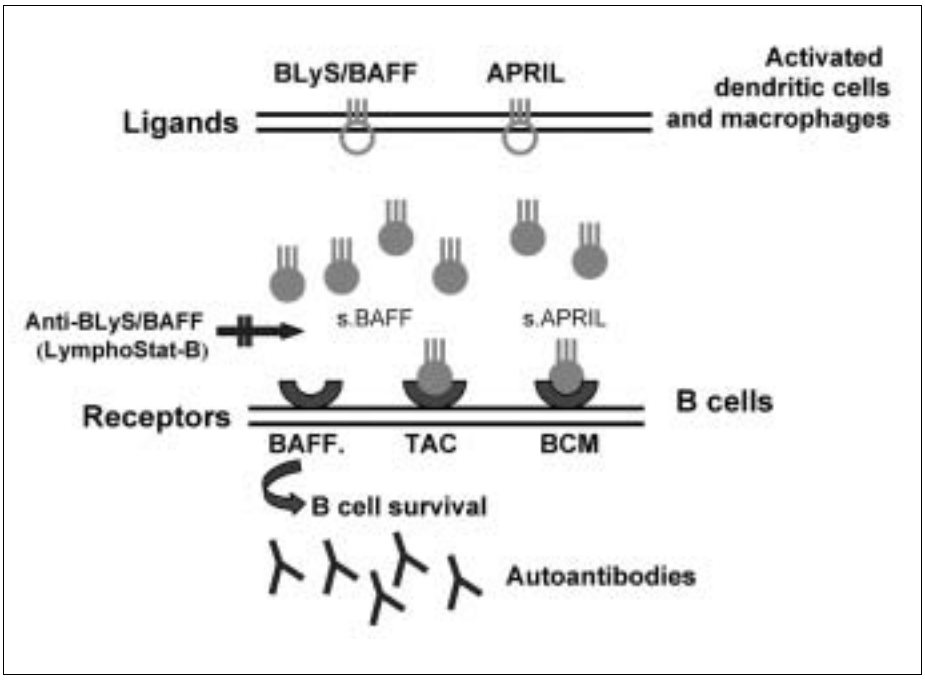

Figure 1. Both soluble BLyS and APRIL bind BCMA and TACI on B cells. However, BLyS/BAFF selectively binds BAFF-R, the most dominant pathway in controlling B cell survival.

The beneficial effect of targeting BLyS/BAFF

BAFF became a potential target in the treatment of autoimmune diseases as a key mediator of polyclonal autoantibody production and possibly also of the development of antigen-specific antibody response. Blocking the biological effects of BAFF with neutralizing antibodies may be an effective approach to ameliorate B cell hyperactivity and/or in the long-term decrease of autoantibody production.

Soluble BAFF receptors (TACI-Fc or BR3-Fc) were used as BAFF antagonists for treating the SLE-like NZB/WF1 mice. When administered early in the disease, they inhibited proteinuria and prolonged the survival of these mice [24,34,35].

A potent recombinant fully human IgG1 monoclonal antibody that binds human soluble BLyS/BAFF with high affinity and neutralizes its bioactivity was recently developed. This antibody (LymphoStat-B) was selected by affinity maturation of a parental antibody, which itself was derived from screening a phage display library for high affinity binding of BLyS/BAFF [36].

The administration of LymphoStat-B to mice and monkeys prevented human BLyS-induced increases in splenic B cell numbers and immunoglobulin titers. When injected at doses of 0.5, 1.5, or 5 mg/kg, it effectively neutralized BLyS bioactivity and inhibited the binding of BLyS to its three receptors, TACI, BCMA, and BLyS/BAFF-receptor [Figure 1]. Upon administration, BLyS antagonist was well tolerated and pharmacologically active (as evidenced by a reduction in B cells) in monkeys at dose levels up to 50 mg/kg injected weekly for 4 weeks or every other week for 26 weeks. In the 6 month monkey toxicology study there were no treatment-related infections [36]. BLyS antagonist is currently being evaluated in a phase I clinical trial in SLE patients [37].

The beneficial effect of BLyS antagonist therapy should be evaluated, taking into consideration the other therapeutic regimens administered to SLE patients. The initiation of high corticosteroid doses was shown to be followed by serum BLyS reductions. However, discontinuation or tapering of the treatment resulted in increases of serum BLyS levels [32]. The reducing effect of immunosuppressive drugs such as azathioprin on serum BLyS needs further evaluation.

In a recent preliminary study we showed that the attenuation of elevated serum BAFF in active SLE patients was achieved following add-on quinacrine to hydroxychloroquine and other therapeutic regimens. This attenuation was found to occur in association with the improvement of clinical and laboratory parameters such as SLEDAI and anticardiolipin antibodies, suggesting that the presence of BAFF is associated with SLE disease activity (E.T., unpublished data).

In this regard, future studies should be able to answer the question of when BLyS-antagonists are to be given as a monotherapy and when in combination with other therapeutic regimens. Also, should elevated serum BLyS levels be the only indicator for treatment, or should disease activity also be considered? The question regarding the extent of targeting BAFF that could ameliorate autoimmune diseases (especially in humans) has yet to be answered. However, one should remember that with an established disease such as SLE, there are already autoantigen-specific T cells. Therefore, even if B cell presentation of autoantigens could be attenuated by the neutralization of BAFF, once autoimmune memory T cells have been generated the presentation of autoantigens by other cells may be enough to maintain the autoimmune inflammation. At this stage, the role of B cells in maintaining the activation of autoimmune T cells in SLE is not fully clear. Thus, it is too early to predict the real potential efficacy of neutralizing BAFF in the treatment of SLE.

SLEDAI = SLE Disease Activity Index

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Do not value money for any more nor any less than its worth; it is a good servant but a bad master

Alexandre Dumas (1824-95), illegitimate son of the more famous French novelist and dramatist (The Count of Monte Cristo and The Three Musketeers). Known as Dumas fils, his best known work is the novel La Dame aux camelias, the basis of a play and Verdi's opera La Traviata.