The role of effector T-cells in the pathogenesis of lupus nephritis

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Regulators of B-cell activity in SLE: a better target for treatment than B-cell depletion?

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Abstract

B-cells play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE) being a source of characteristic antinuclear autoantibodies. There is increasing evidence that alterations in B-cell regulation are responsible for B-cell hyperactivity as seen in SLE. T-cells, soluble factors and even B-cells themselves regulate effector B-cell functions. The latter, so-called regulatory B-cells possess regulatory function via production of the cytokine IL-10 that can damp humoral immune responses. This review will focus on B-cell regulation in the pathogenesis of SLE as a target for intervention. In particular, the regulatory impact of T-cells via costimulation, soluble factors such as BLyS, and the characteristics of IL-10 producing regulatory B-cells will be discussed. Therapies targeting B-cells as well as B-cell regulation seem promising but the precise mechanisms involved in these interventions are not completely understood. More insight in B-cell regulation in SLE, and particularly in regulatory B-cells, could lead to novel therapeutic strategies.
Introduction
Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterised by autoantibody production associated with a wide range of clinical manifestations. The inflammatory response triggered by in situ formation and/or passive deposition of immune complexes is thought to be responsible for various clinical manifestations such as vasculitis, nephritis and skin involvement.

However, the precise etiopathogenesis of the disease remains unclear. It is widely accepted that B and T-cells play a pivotal role in the pathogenesis of SLE. Alterations of cell numbers, activation status and function in these lymphocyte subsets have been demonstrated. At first glance, B-cells, being a source of autoantibodies, are the protagonist in the immunological cascade ending in organ injury. There is growing evidence that, besides autoantibody secretion, other functions of B-cells such as antigen presenting capacity and cytokine production contribute to the development of SLE as well.

Regulation of B-cell activity is an essential part of immune homeostasis. T-cells play a major role in B-cell regulation. First, interaction between B and T-cells is necessary to induce T-cell dependent autoantibody production. The best characterised molecular interactions between T and B-cells occur via CD28–CD80/CD86 and CD154/CD40, respectively. Furthermore, several T-cell derived soluble factors play a role in B-cell homeostasis. Beside these T-cell dependent stimuli and interactions in B-cell activation current studies reveal the existence of a subset of B-cells with regulatory capacities. This regulatory subset is characterised by the secretion of IL-10 and TGF-β, and has been shown to be involved in the pathogenesis of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE). The existence and hypothetical impact of these regulatory B-cells in systemic lupus erythematosus remains unclear until now. However, the role of this B-cell population in different murine models reflects a potential target in the pathogenesis of SLE. The present review will shortly discuss the role of B-cells in the pathogenesis of systemic lupus erythematosus with special attention given to their regulation.
This set the stage for exploring new therapeutic modalities directed at B-cell regulation in this disease.

**B-cells as effector cells in SLE**

The hallmark of immune dysregulation in SLE is B-cell hyperactivity. B-cells produce a wide range of autoantibodies against soluble and cellular constituents. The most characteristic autoantibodies in SLE are targeting constituents of the nucleus, the antinuclear antibodies (ANA). Anti-double stranded DNA (ds-DNA) or anti-Sm antibodies are present in more than 90% of patients with SLE.

Animal models and clinical observations provide convincing evidence that autoantibodies play a key role in the pathogenesis of SLE. Especially anti-dsDNA antibodies are thought to play a major role in the pathogenesis of lupus nephritis. Vlahakos et al. demonstrated that transfer of monoclonal anti-DNA antibodies (Ab) derived from MRL-1pr/lpr and (NZBxSWR)F1 mice to normal mice resulted in intranuclear deposits of anti-DNA Ab within glomeruli, associated with hypercellularity and proteinuria. There is also increasing evidence that anti-DNA antibodies have the potential to cross-react with normal glomerular constituents such as surface antigens on endothelial cells, phospholipids, laminin, type IV collagen and α-actinin which could promote in situ immune complex deposition and inflammatory processes. Furthermore, the effects of anti-dsDNA antibodies on the expression of different cytokines have drawn attention. The observation by Sun et al. that normal human PBMCs incubated with antibodies to DNA from patients with active SLE showed diminished proliferation and enhanced secretion of IL-1β, IL-6, IL-8, IL-10 and TNF-α reflects the importance of autoantibodies in this inflammatory condition beyond immune complex formation.

However, B-cells are more than a source of pathogenic autoantibodies alone. This is supported by several murine models. Chan et al. created a JpI-D-MRL/MpJ-Fas1 mouse expressing B-cells but lacking antibody secretion. Despite the absence of circulating Ig these mice developed interstitial nephritis.
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The same authors could also demonstrate in another JnD-MRL/+ murine model that B-cells are required for the development of lupus nephritis. Crossing MRL/+ mice with JnD-MRL/pr mice resulted in lupus prone mice without B-cells. These animals did not develop glomerulonephritis and had, additionally, reduced amounts of activated T-cells. These data demonstrate a role for B-cells in the development of SLE apart from the secretion of autoantibodies. Obviously, B-cells can be autoreactive in the absence of autoantibody production, possibly by their antigen-presenting capacity. Antigen-presenting capacity of B-cells contributing to prolonged inflammatory responses in autoimmunity has also been suggested in studies on renal infiltrates of patients with lupus and ANCA-associated nephritis. Analysis of intrarenal B-cell clusters clearly showed an antigen presenting phenotype. Interestingly, these B-cell clusters expressed the lymphoid chemokines BCA-1/CXCL13 suggesting a B-cell attracting mechanism in inflamed tissue similar to secondary lymphoid organs. These findings suggest that B-cells in systemic lupus, besides being a source of pathogenic autoantibody production, also have other pathogenic potentials in this autoimmune disease.

Regulatory B-cells in SLE

The hypothesis that also B-cells could regulate the autoimmune response has further contributed to our understanding of B-cell physiology. These regulatory B-cells are characterised by the secretion of IL-10 and TGF-β. In an elegant mouse model Fillatreau et al. demonstrated that the course of T-cell dependent experimental autoimmune encephalomyelitis (EAE) was strongly influenced by IL-10. IL-10 deficient mice failed to recover from induced EAE whereas the transfer of purified splenic IL-10 producing B-cells reduced EAE severity. Remarkably, induction of IL-10 secretion by B-cells required the concurrent ligation of CD40. In a similar mouse model Matsushita et al. reported more recently that depletion of IL-10 producing B-cells by rituximab before EAE induction contributes to a more severe disease and adoptive transfer of B10 cells (IL-10–producing CD1dhi CD5+ regulatory B-cells) before EAE induction
restored this effect. In a model of chronic intestinal inflammation B-cells appear to suppress the progress of inflammation by downregulating inflammatory cascades which are associated with IL-1 upregulation and STAT3 activation. Singh et al. demonstrated in a murine model of allergic airway disease that B-cells from local lymph nodes are able to induce the conversion of CD4+CD25+ effector T-cells in Foxp3+ regulatory T-cells via TGF-β. In this model the suppressive B-cell function is TGF-β dependent and contributes indirectly to disease remission via regulatory T-cells. In human immune homeostasis IL-10 producing B-cells have been shown to be involved as well. This rises the question whether regulatory B-cells might also play a role in SLE. There are controversial data regarding the role of IL-10 in lupus like animal models and in human SLE. Yin et al. reported an increased Th1 response and autoantibody production in IL-10-deficient MRL-Faslpr mice as compared to IL-10+/+ control mice. Besides, these mice developed a more severe glomerulonephritis. These data suggest that IL-10 may down-modulate autoantibody production and end-organ disease in lupus via inhibition of Th1 cytokine production. On the other hand, continuous administration of anti-interleukin-10 antibodies in NZB/W F1 mice had a protective effect at the level of proteinuria and glomerulonephritis, and substantially delayed onset of autoimmunity. Similar beneficial effects of antagonistic IL-10 treatment were observed in a clinical study including six SLE patients. The source of IL-10 secretion, whether T-cell or B-cell derived, needs further investigation. Nevertheless, IL-10 seems to possess both immunosuppressive and immunostimulatory properties which may explain these contradictory results. The fact that SLE patients often benefit from B-cell depletion could suggest a minor role of regulatory B-cells in established disease. Unfortunately, there are no phenotypic markers for so called regulatory B-cells available in animal models and humans. Nevertheless, the previously mentioned findings suggest a pivotal role of this B-cell subset in the induction of autoimmune diseases such as SLE.
Interventions in B-cell homeostasis by B-cell depletion

Data demonstrating the crucial role of B-cells in the pathogenesis of SLE support the idea that B-cell depletion might be an attractive goal for treatment of SLE (Figure 1).

Figure 1. Regulation of B-cell activity in SLE: This figure illustrates different stages of B-cell regulation including potential therapeutic targets in systemic lupus erythematosus. The B-cell is regulated by B - T cell interaction (costimulation), soluble factors such as B lymphocyte stimulator (BLyS) and regulatory cells like regulatory T-cells and regulatory B-cells via IL-10 secretion. The figure shows promising therapeutic targets in B-cell regulation in SLE beside B-cell depletion with monoclonal chimeric antibody anti-CD20 (1). Therapeutic interventions in B-cell regulation can be achieved by blocking costimulation at the level of CD28–CD80/CD86 interaction, the CD154/CD40 pathway and preventing the CD134/CD134L ligation (2,3 and 4). Additionally, inhibition of binding BLyS to its receptors by monoclonal antibody could be a therapeutical target in human SLE as well. Regulatory B-cells (Bregs) regulate effector B-cells via IL-10 secretion (5). Possible future approaches could address Bregs as an important therapeutic goal to restore the balance between regulation and hyperactive B-cells.
The most widely used monoclonal chimeric antibody for B-cell depletion is Rituximab®, which is directed against the B-cell specific surface marker CD20. Initially, Rituximab® was successfully used in Non Hodgkin Lymphoma and over the last years data have become available regarding anti-CD20 therapy in the course of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. In open studies the clinical response in SLE patients, as assessed by SLAM, to B-cell depletion was very promising. Gunnarsson et al. demonstrated a clinical benefit for cyclophosphamide resistant lupus nephritis. Both urinary sediment abnormalities and histopathological features improved. On the other hand SLE patients without renal involvement do not seem to improve in disease activity as assessed by SLEDAI or BILAG after rituximab infusion as concluded from the Explorer trial, although this may, at least in part, be due to the design of that study. 

Because treatment with Rituximab® targets immature, naive and memory B-cells expressing CD20 but not plasma cells which are lacking this marker, additional strategies may be needed for long term efficacy. Another limitation of Rituximab® is its chimeric structure. A recent study investigated the variability in response to B-cell depleting therapy due to human anti-chimeric antibodies. These antibodies are evidently frequently present in SLE patients and associated with a far lower extent of B-cell depletion as compared to patients without these antibodies. Humanized monoclonal antibodies to CD20 (Ocrelizumab®) are currently also evaluated in SLE patients to assess their promised improvement in tolerance and efficacy. However, also humanized anti-CD20 antibodies fail to deplete long lasting plasma B-cells. Indeed, anti-dsDNA autoantibodies are decreased but persist after B-cell depletion in almost all SLE patients.

Apart from depletion of CD20+ B-cells this treatment indirectly affects also the T-cell compartment. Previous studies have shown that there are less activated but increased numbers of FoxP3+ regulatory T-cells in the peripheral blood of SLE patients following B-cell depleting therapy. In another study the suppressive capacity of regulatory T-cells improved significantly. The effect of
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B-cell depletion on regulatory B-cells in lupus models or patients has not been evaluated so far. From the experimental studies in EAE mice one would conclude that B-cell depletion in patients in remission of lupus disease could, possibly, be contraindicated but there are no clinical hints in human trials available supporting this hypothesis.²

An unresolved problem is the lack of specific surface markers identifying these regulatory B-cells which makes it difficult to monitor these cells in clinical practice. Identification of a specific regulatory B-cell marker would be helpful for therapeutic modulation of this population. So far one has to assume that anti-CD20 therapy also depletes the regulatory B-cell subset. Taken together B-cell depletion seems to improve the clinical course of some SLE patients but controlled studies have not confirmed this suggestion until now. Apart from B-cell depletion, B-T cell interaction and B-cell stimulating factors are other attractive goals to modulate B-cell activity.

Interventions in B-T cell interaction

The impact of T-cells on B-cells via costimulation is well known. The relevance of co-stimulatory molecules for immune-mediated disease was investigated in several murine models resembling human SLE. Blockade of the CD28–CD80/CD86 interaction and the CD154/CD40 pathway resulted in amelioration of disease in these mouse models.³⁰;³¹ A delay in disease onset as assessed by anti-DNA antibody titer, development of fixed proteinuria, histological evidence of renal damage, and survival could also be observed in NZB/NZW F1 mice after a single infusion of CTLA4Ig.³² CTLA4Ig is homologue to CD28 and binds to CD80/CD86 with high affinity. Infusion of CTLA4Ig obviously results in downregulation of B-cell activity by blocking the CD28–CD80/CD86 pathway. In human trials CTLA4Ig (Abatacept®) has been successfully evaluated in rheumatoid arthritis.³³ Controlled studies in patients with SLE are currently not available. A beneficial effect can be expected as high levels of co-stimulatory molecules, especially CD80 and CD86 on B-cells, were also found in human SLE.³⁴ Expression of these markers correlated with disease activity as
assessed by the SLEDAI score. Surprisingly, CD80 and CD86, which are usually found on APCs, were also found on T-cells of patients with SLE. However, the significance of this finding for disease development and activity remained unclear in this study. Furthermore, expression of CD134L has been shown to be up-regulated in proliferative lupus nephritis, suggesting a role for the CD134-CD134L pathway in its pathogenesis. Additionally, CD134 expression was correlated with disease activity and associated with renal involvement in human SLE. A current study in BXBS mice demonstrated that blocking this interaction could be an effective alternative target to attenuate lupus nephritis. A co-stimulatory antagonist against CD154 (CD40L) has also been examined as a possible therapeutic approach in human (renal) SLE but this was not fruitful, as short-term administration of the anti-CD154 was associated with life-threatening prothrombotic activity despite initial encouraging data in serology and renal function of the patients.

**Interventions in B-cell stimulation by targeting soluble factors**

An alternative approach to targeting costimulatory signals is to block cytokines that are required for B-cell function. The TNF-like molecule B lymphocyte stimator (BLYS), also known as BAFF (B-cell activating factor from the tumor necrosis factor family) is essential for B-cell survival and development and is mainly produced by monocytes and macrophages. The biologically active soluble form of BLYS binds to its 3 receptors TACI (transmembrane activator and calcium modulator ligand interactor), BCMA (B lymphocyte maturation antigen) and BAFF-R (BAFF receptor) expressed on B lymphocytes with highest binding intensity among mature B-cells. The homologous molecule APRIL (a proliferation-inducing ligand) mediates similar effects to those of BLYS after binding to TACI and BCMA. Elevated serum levels of BLYS have been detected in murine SLE models (MRL/Mp lpr/lpr). In this lupus model serum levels of BLYS seem to be associated with kidney damage whereas treatment with soluble BLYS receptor significantly prolonged survival of lupus mice. Zhang et al. demonstrated an association of soluble BLYS levels with increased production
of anti-dsDNA antibodies studying 150 patients' sera. More recently, a correlation between plasma BLyS levels and disease activity as assessed by SELENA-SLEDAI in SLE patients has been shown. These findings indicate that increased BLyS levels may contribute to the development of human SLE and suggest BLyS to be a crucial factor in B-cell regulation being a potential novel therapeutic target. Currently, belimumab (LymphoStat-B), a human monoclonal antibody that binds to soluble BLyS preventing binding to its receptors, is evaluated regarding activity and safety in a multicenter phase I trial in SLE patients. The authors showed that belimumab is safe and biologically active but significant improvement of disease activity in these patients was not achieved. Furthermore, a decrease of CD20+ B-cells occurred after treatment with belimumab. Interestingly, BLyS levels increase after B-cell depletion with rituximab and decrease with repopulation of B-cells in SLE and RA patients. Taken together, these data show an important role of BLyS in the regulation of B-cells and B-cell survival and might suggest an additive effect of belimumab to rituximab.

**Discussion**

The provided data demonstrate that activated B-cells have a multifunctional role in the pathogenesis of systemic lupus erythematosus. Besides their diverse properties promoting pathogenic events in lupus recent data suggest that an altered regulation of so-called regulatory B-cells contributes to hyperactivation of B-cells. Regulatory B-cells characterised by IL-10 secretion could, in addition to other factors, play a role in orchestrating the B - T-cell homeostasis in SLE as has been shown for other autoimmune diseases. However, the regulatory role of IL-10 in SLE has to be explored further. Functional studies on IL-10 producing B-cells in human SLE are, however, lacking. The balance between regulatory B- and T-cells should be investigated in order to get further insight in B-cell hyperactivity in SLE. Future efforts should first concentrate on the detection of the phenotype of regulatory B-cells. Without this information
monitoring and specific therapeutic modulation of this population remains difficult.

As mentioned before, B-cell depleting treatment appears effective in open series and case reports in SLE, but controlled studies are not conclusive until now. Although this may be due to study design, a further analysis of immunopathological changes following B-cell depleting therapy is necessary, including an analysis of changes in regulatory B-cells as well as T-cells. Furthermore, the immunopathological effects of intervention in B-T cell interaction and B-cell activating factors should be studied. Insights from these studies may not only help to understand the efficacy or lack of efficiency of different ways of B-cell targeting, but will also increase our understanding of pathogenesis of lupus in general.
References


