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The Role of B-Cells and B-Cell Targeted Therapies in Lupus Nephritis

Irene Blanco, Saakshi Khattri and Chaim Putterman
Albert Einstein College of Medicine
Division of Rheumatology
Bronx, NY
USA

1. Introduction

Systemic Lupus Erythematosus (SLE) is a multi-systemic autoimmune disorder that can have severe and potentially life-threatening manifestations. One such manifestation is lupus nephritis. While survival rates have improved significantly, SLE, and in particular lupus nephritis, continues to be associated with significant morbidity and mortality.

The incidence of SLE varies greatly among different populations. As a whole, rates are highest in non-Caucasians. Estimated incidence rates of SLE are similar in the US and in Europe where Caucasians have rates of 3.5-4 per 100,000 and those of African descent have significantly higher rates of 9.2-11.4 per 100,000.[1,2] While the disease is present to a certain degree in all populations, SLE affects primarily women in their child-bearing years with a ratio of 9:1 compared to men.[3,4] When men do present with SLE there is evidence that they have worse disease outcomes including increased mortality.[5,6] Regardless of race or gender, mortality in SLE has improved significantly since the 1950's, where the 5-year survival rate was approximately 50%.[7]

Currently, 10-year survival rates range between 85% and 95%.[7] Despite improved overall survival, lupus nephritis (seen in upwards of 60% of all SLE patients) continues to significantly affect morbidity and mortality.[8] While 5-year survival for those without nephritis is approximately 92%, those with this complication have a much lower rate of 82%.[8] Both in the US and in Europe, non-Caucasians have increased rates of nephritis, where not only is the condition more prevalent in these groups, it also tends to be more severe.[5,9] In the US, risk factors for progression to end-stage renal disease include: being African American, Hispanic, male, less than 24 years old and having high activity and chronicity on renal biopsy.[10] Risk factors notwithstanding, it has been shown that patients that receive early treatment have better outcomes.[11,12]

However, even with early and aggressive treatment, one study has found no change in the incidence of end-stage renal disease from lupus nephritis.[13] Therefore, it appears that while medications are usually effective in controlling renal inflammation, a substantial amount of patients fail treatment. In addition, conventional therapies are not target-specific and can lead to significant toxicity. Therefore, given that B cells play such an integral role in the pathogenesis of SLE and lupus nephritis, these cells are potential targets for more specific therapies.

2. Pathogenesis of lupus

The etiology of SLE and specifically lupus nephritis is multi-factorial involving environmental, genetic and hormonal factors to name a few. Loss of tolerance and interactions between the innate and adaptive immune system, as well as T cells and B cells, all play a role in the development of SLE and lupus nephritis.

2.1 Genetics

SLE is a genetically complex disease that generally does not exhibit straightforward mendelian modes of inheritance. In several cases, SLE is associated with rare but highly penetrant mutations: homozygous deficiencies of the complement components C1q, C2 or C4, complete FcγRIIIb deficiency, and mutations in the DNA exonuclease *TREX1*.[14-17] Genes in the human leukocyte antigen (HLA) region of the short arm of chromosome 6 exhibit the strongest association with the risk of developing SLE. Graham et al. identified HLA class II haplotypes containing DRB1 and DQB1 alleles as strong risk factors for human disease.[18]

In a majority of cases, however, SLE genetic susceptibility is probably determined by relatively common variants that are found throughout the population. Each only contributes modestly to the risk of disease; hence the finding of 34% concordance rate in monozygotic twins and 3% in dizygotic twins.[19,20] Genome-wide association studies have found hundreds of single nucleotide polymorphisms associated with SLE. A recent meta-analysis shows a total of 17 well-validated common SLE risk variants including: HLA-DR3, DR2, PTPN22 and STAT4. These variants account for a fraction of the total genetic contribution to SLE. Initial pathway analyses of these risk alleles indicate an important role for B cell development and signalling, signaling through toll-like receptors 7 and 9, and neutrophil function.[21]

2.2 Role of B-cells in SLE

B cells play a major role in the development of SLE, where loss of B cell tolerance is presumed to be the basis of disease. The most common alteration seen is B cell hyperactivity and subsequent autoantibody production. The presence of autoantibodies, particularly anti-nuclear antibodies, anti-Smith and anti-double stranded DNA (anti-DNA) antibodies, form part of the diagnostic criteria for SLE.

Immunoglobulin genes undergo rearrangement during B cell development in the bone marrow, where many autoreactive B cell receptors (BCR) are generated. In lupus patients, many, if not all of the checkpoints that eliminate autoreactive B cells are breached.[22] SLE patients also exhibit high levels of B cell activating factor (BAFF), a survival factor that contributes to the survival of these autoreactive cells. There is also overexpression of CD40L on B cells which results in excessive T cell co-stimulation, another mechanism for the survival of autoreactive B cells.[23,24] However, they not only generate autoantibodies; B cells also act as antigen presenting cells (APC's) providing costimulatory signals necessary for T cell activation, differentiation and expansion. In addition, B cells also produce cytokines like IL-10, IL-16, TNF-α and INF-γ that influence other cells in the immune system.[25] (Figure 1.)

2.3 Toll like receptors in SLE

Toll-like receptors (TLRs) play a key role in innate responses to infections. When bound by endogenous or exogenous ligands, they are involved in acute and chronic inflammatory

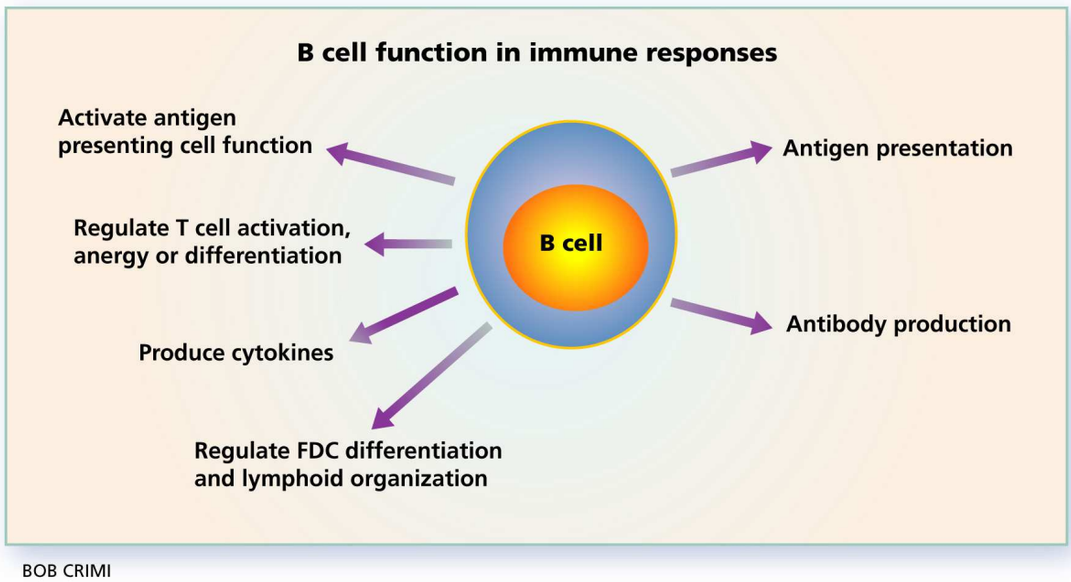

Fig. 1. B cell Function in Immune Responses.
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processes. Numerous *in vitro* studies have established that TLR-7 and TLR-9 are involved in immune complex recognition. It is hypothesized that nucleic acid-containing immune complexes are engaged simultaneously by both the BCR and TLR.[27] This engagement of TLR-7 or TLR-9 by an immune complex induces a MYD88-dependent pathway that activates inflammatory transcription factors, including IRF-7, NF-κB and AP-1. This leads to B cell and plasmacytoid dendritic cell (PDC) activation. The activation of PDC's stimulates the inappropriate production of many cytokines, particularly type I IFN which is intimately involved in the pathogenesis of SLE.[26]

How TLRs gain access to nuclear antigens in SLE can be explained by two mechanisms. The first suggests that pre-existing anti-Smith and anti-DNA antibodies form immune complexes with endogenous RNA and DNA, that are then taken up by APCs via Fc receptors. In endosomes, these complexes are degraded into nucleic acid components. These exposed nucleic acids then interact with TLR-7 or TLR-9 resulting in type I IFN production from PDC's and B cell activation. Another model hypothesizes that type I IFN production is induced by TLR recognition of nucleic acids from aberrant degradation of apoptotic cells.[29] Regardless of the mechanism of upregulation of type I IFN, once increased it induces upregulation of TLRs in B cells and subsequent maturation into IgG-secreting plasma cells.[30,31]

3. Pathogenesis of lupus nephrtitis

The serologic hallmark of SLE is the presence of autoantibodies against nuclear antigens.[3] Several antibodies, such as anti-DNA and anti-nucleosome antibodies, are highly specific for the disease.[3,32] There is evidence to suggest that they play a significant role in the pathogenesis of lupus nephritis.

While there are several different classes of lupus nephritis, what all have in common is renal deposition of antibodies, immune complexes and complement.[10] Immunofloresence typically shows staining for various immunoglobulins, where IgG is predominant, in addition to C3 and C1q.[33] Several mechanisms have been proposed to explain the presence of immune complex disposition in lupus nephritis. The first is that extra-renal immune complexes form and are subsequently deposited in the kidney. The second theory postulates that there is direct binding of autoantibodies to renal targets. Finally, there is also the possibility that autoantibodies bind to autoantigens that have previously been bound to the glomerulus. It is likely that all three of these mechanisms explain the pathogenesis of lupus renal disease to some degree.[34]

The antibody that has most consistently been associated with lupus nephritis is anti-DNA antibody. These antibodies were the first to be found in both humans with lupus nephritis and animal models of the disease.[35,36] They are implicated in all postulated mechanisms of disease; however the true extent of the pathogenicity of anti-DNA antibodies is unclear. SLE patients have a dysregulation in apoptosis with decreased clearance of apoptotic bodies that are released in the serum.[37,38] These circulating apoptotic bodies, double-stranded DNA fragments and nucleosomes likely serve as autoantigen for autoreactive B cells.[39] Exposure to these antigens, subsequently causes the generation of anti-nuclear antibodies such as anti-DNA.[40] It is possible that anti-DNA antibodies and the above antigens form complexes in the serum that then deposit in the kidney.

While interesting, several researchers have not been able to confirm the presence of substantial amounts of DNA-anti-DNA complexes in the serum or that these complexes are formed extra-renally and are then significantly deposited in the kidneys.[41,42] Immune complexes injected into normal mice can cause transient depositions in the glomeruli resulting in the activation of mesangial cells, but this is unlikely to cause disease.[43] If extra-renal immune complexes are involved in lupus nephritis, they likely amplify inflammation as opposed to initiating it.

What may in fact happen is that DNA fragments and nucleosomes bind to proteins in the glomerular basement membrane. They then act as "planted antigens" to which previously formed anti-nuclear antibodies can bind.[43,44] Collagen, fibronectin and laminin all have binding sites for DNA. In addition, DNA may bind to previously captured immune complexes and nucleosomes in the basement membrane. Anti-DNA antibodies can then bind, leading to *in situ* immune complex formation.[33,39]

Finally, there is significant evidence to show that anti-DNA antibodies bind directly to glomerular basement membrane proteins. Alpha-actinin, laminin, collagen and heparan sulfate can all directly bind anti-DNA antibodies.[45] The pathogenicity of these antibodies that bind to planted antigens on the basement membrane and/or directly to glomerular antigens is likely the same. Both can cause direct renal toxicity via complement fixation and via FcR expression on infiltrating leukocytes.[43] Neutrophils, macrophages and renal parenchymal cells are subsequently activated releasing inflammatory mediators and upregulating adhesion molecules, subsequently recruiting more leukocytes into the kidney.[46]

Despite the role that B cells play in lupus nephritis through the generation of auto-antibodies, they also exert their influence through immunoglobulin independent mechanisms. Several experiments using MRL-*lpr/lpr* (MRL-*lpr*) mice show that in the setting of B cell deficiency, mice do not develop nephritis while those with altered antibody production continue on to develop disease. Also B cell deficient lupus mice fail to activate CD4 T cells.[47] Therefore, B cells can participate in the pathogenesis of nephritis

by acting as highly-efficient APC's thereby activating T cells.[48] CD4 T cells are activated through the MHC II molecule/T cell receptor interaction as well as through CD40/CD40 ligand co-stimulation. When these interactions are blocked, significant improvement in SLE disease activity is observed.[49] B/T cell interactions lead to an amplification of the immune response where activated CD4 T cells activate macrophages and drive autoreactive B cells to undergo somatic hypermutation and affinity maturation.[50]
With the increased understanding of the role of B cells in SLE, new therapies have been developed to specifically target this cell type. Over the course of the next few sections we will discuss the current, less selective, standard of care for the treatment of lupus nephritis and move on to novel, more specific B cell targeted therapies.

4. Current immunosuppressive therapy in lupus nephritis

Treating lupus nephritis involves an induction period of intensive immunosuppressive therapy aimed at halting disease progression. This is followed by a period of maintenance therapy to maintain the response. The treatment of lupus nephritis has undergone a dramatic change from steroids as initial therapy to immunosuppressive therapy like cyclophosphamide.
Steroids were the mainstay of treatment of lupus and lupus nephritis in the early 1960's but such an approach was often unable to control the progression to renal failure and was associated with significant morbidity and mortality.[51] Felson et al. conducted a pooled analysis of all published clinical trials where prednisone alone, or prednisone plus cyclophosphamide or azathioprine was used. They showed that patients receiving immunosuppressive therapy had less renal deterioration, were less likely to have end-stage renal disease, and had better survival rates compared to patients receiving steroids alone. When cyclophosphamide and azathioprine were considered separately, both were associated with a 40% reduction in the rates of adverse renal outcomes.[52]

4.1 Cyclophosphamide

Cyclophosphamide (CYP), an alkylating and cytotoxic agent, depletes both T and B cells reducing the production of pathogenic autoantibodies. Since the publication of studies by Austin et al. and Boumpas et al., CYP has become a mainstay for the treatment of lupus nephritis.
Patients with lupus nephritis enrolled in trials at the NIH between 1969 and 1981 were randomized into one of five treatment protocols: (1) high-dose prednisone; (2) azathioprine + low-dose prednisone; (3) oral CYP + low-dose prednisone; (4) combined oral azathioprine and oral CYP; and (5) intravenous CYP + low-dose oral prednisone. This study showed that renal function was better preserved in patients receiving immunosuppressive therapy, but the difference was statistically significant only for the intravenous CYP plus low-dose prednisone group as compared to the high-dose prednisone group.[53] Boumpas et al. later showed that an extended course of pulse CYP is more effective than 6 months of pulse methylprednisolone in preserving renal function in patients with severe nephritis. The addition of a quarterly intravenous CYP maintenance regimen to monthly pulses also reduced exacerbation rates.[54] These and several other studies established the NIH protocol for the treatment of lupus nephritis. However, this protocol can lead to significant morbidity with complications such infertility, and an increased risk of severe infections.

In response to increased morbidity rates attributable to CYP, the Euro-Lupus Nephritis Trial was developed to test lower doses of intravenous CYP (500 mg every 2 weeks for a total of 6 doses) against the typical NIH protocol for induction. Houssiau et al. found that there was no significant difference between the high dose and low dose patients in renal remission rates, or in developing recurrent renal flares or renal failure. Severe infection was more than twice as frequent in the high-dose group, though the difference was not statistically significant.[55] Ten year follow up data shows that death, sustained doubling of serum creatinine and end-stage renal disease rates did not differ between the low-dose and high-dose group nor did mean serum creatinine, 24 h proteinuria and damage score at last follow-up.[56]

4.2 Mycophenolate mofetil

Mycophenolate mofetil (MMF), the prodrug of mycophenolic acid, inhibits inosine monophosphate dehydrogenase which then suppresses DNA synthesis and the proliferation of T and B cells. Inspired by MMF's efficacy in preventing renal transplant rejection, investigators began to evaluate the drug's use in lupus nephritis. Several small trials showed that MMF was comparable to CYP in the treatment of proliferative LN.[57,58] Ginzler et al. conducted a 24-week randomized, open-label, non-inferiority trial comparing oral MMF with monthly intravenous CYP as induction therapy. They showed that MMF was more effective in inducing remission of lupus nephritis and had a better safety profile.[59]

A study by Chan et al. showed that for the treatment of diffuse proliferative lupus nephritis, one year of MMF therapy was as effective as a six months of CYP followed by six months of azathioprine. The MMF regimen was also found to be less toxic.[60] In the long-term extension trial, serum creatinine in both groups remained stable and comparable. Creatinine clearance increased in the MMF group, but the difference was not statistically significant. MMF treatment was also associated with fewer severe infections. This study showed that MMF constitutes an effective continuous induction and maintenance therapy for lupus nephritis.[61]

Given the reassuring data for the use of MMF, the Aspreva Lupus Management Study (ALMS), a large multicenter, randomized clinical trial, was developed to determine if MMF was in fact better than CYP therapy. They found no difference in response rates when comparing MMF to CYP pulse + azathioprine for maintenance. However, MMF was noted to be better in Hispanic and African American patients. Over the course of the maintenance phase, there was a higher failure rate in the azathioprine group versus in the MMF group (32% v 16%) at 3 years.[62]

Induction therapy with MMF is associated with fewer side effects than with CYP. MMF may be particularly suitable as induction therapy in women of child bearing age where there are less concerns for infertility as compared to CYP treatment. It also may work particularly well in African American and Hispanic patients, two groups with a large burden of disease. Nevertheless, while often better tolerated than CYP, MMF can cause significant morbidity. Many patients experience gastro-intestinal side effects such as diarrhea, nausea and vomiting. Patients treated with MMF are at risk for severe and possibly lethal infections, and are at a higher risk for the development of lymphoma and skin malignancies.Also, while MMF may not affect fertility, the drug had been associated with an increased risk of birth defects.

4.3 Azathioprine

Azathioprine (AZA) is used as a steroid sparing agent in patients with active SLE and as an alternative to CYP for maintenance therapy in lupus nephritis. It is a purine analog and inhibits nucleic acid synthesis thereby affecting both cellular and humoral immune function.

In 1975, Hahn et al. found that there was no significant effect of the addition of AZA to steroids for the early treatment of lupus nephritis.[63] In 2006, Grootscholten et al. showed that CYP pulse therapy was superior to AZA plus methylprednisolone with regards to relapses and short-term infections but renal function at the last visit did not differ between the 2 groups.[64] Although the MAINTAIN nephritis trial showed that MMF was not inferior to AZA in maintaining remission, there were fewer renal flares in patients on MMF.[65] Therefore AZA remains an option for therapy, particularly during pregnancy when other medications are contraindicated. Nevertheless, CYP and MMF continue to be the preferred treatment modalities for lupus nephritis.

5. B-cell targeted therapies

5.1 Rituximab

Rituximab (RTX) is a chimeric antibody directed against CD20, a cell surface protein expressed on certain B cell subsets but not plasma cells. The mechanisms by which it induces B cell depletion is likely through the induction of apoptosis as well as through cell mediated toxicity.[66] Initially used in non-Hodgkin's lymphoma, off-label use in lupus has shown potential efficacy in SLE and lupus nephritis.[67, 68]

Looney et al. conducted one of the first trials investigating the role of RTX in SLE. Eighteen SLE patients were recruited into a phase I/II dose escalation trial, where 7 of 18 patients had nephritis. Although those on CYP at baseline were excluded, the cohort overall had moderately active disease at baseline. Of the 10 patients that successfully depleted their B cells, all experienced some disease improvement. The SLE manifestations that experienced the most improvement were: rashes, mucositis, arthritis and alopecia. The authors do not comment on the overall efficacy in the nephritis patients but do mention one patient with class IV nephritis that entered remission, with remission, with resolution of proliferative changes on repeat biopsy.[69]

An open label trial was then conducted looking at the combination of CYP and RTX in the treatment of refractory SLE. Although 21 of 32 patients had lupus nephritis, the authors do not comment on these patients specifically. Overall, 12 remained disease free after one cycle of RTX. Global British Isles Lupus Assessment Group (BILAG) scores significantly improved at 6 months. Therefore we assume that the nephritis patients did well after RTX, given the entire group did well post treatment. However we do not know if they responded to the same magnitude as the non-nephritis patients.[70]

Jonsdottir et al. showed decreased disease activity scores at 6 months in 16 patients treated with RTX combined with CYP and steroids for refractory SLE. Of these 16 patients, 9 had nephritis, where 8 of 9 had failed treatment with CYP prior to starting RTX. All of the nephritis patients had BILAG scores of A before being given RTX. Response in these 16 patients was good. Five of 9 nephritis patients had BILAG renal scores of C or D at 6 months.[71] Other small trials showed similar efficacy in both treating lupus as well as lupus nephritis.[72-74]

Nevertheless, the results from recent randomized control trials were not as promising. The 52-week Exploratory Phase II/III SLE Evaluation of Rituximab trial, (EXPLORER), tested the efficacy and safety of RTX plus immunosuppressive therapy versus placebo plus immunosuppressive therapy in patients with moderate to severe extra-renal SLE.[75] This study showed that overall, there was no difference between the two groups in terms of both the primary or secondary efficacy endpoints. In subgroup analyses, Africans

Americans and Hispanics (approximately one-third of the study population), who received RTX had significantly better rates of both major and partial responses compared to placebo.

A second large trial, the LUpus Nephritis Assessment with Rituximab trial (LUNAR) was conducted to specifically look at the role RTX therapy in the treatment of class III/IV lupus nephritis. In this trial 144 patients on MMF and corticosteroids were randomized to either the addition of RTX or placebo. Although there were more renal responders in the RTX group (57% v 45.9%), the difference was not statistically significant. Again, though under-powered to detect a difference, the African American group had a higher proportion of patients that responded to RTX versus placebo (70% v 45%).[76] While there may be some benefit to the addition of RTX in African Americans, the medication overall does not seem to grant any additional benefit to current regimens.

Despite the failure of RTX for SLE as well as lupus nephritis in randomized clinical trials, data from the French Autoimmunity and Rituximab registry shows that overall 80 of 113 patients in the cohort responded to RTX therapy. Of those patients with renal disease, 74% showed at least a partial, if not complete response.[67] At the Hôpital Necker in Paris, good results were reported at 22 months of follow-up in the treatment of nephritis patients with concomitant RTX where 60% had a renal response.[77] Recently, Roccatello et al. found that a regimen of 4 doses of RTX with 2 intravenous pulses of CYP in eight patients with severe multi-organ SLE involvement, including nephritis, led to significant improvements in proteinuria at 3,6, and 12 months of follow-up.[78] While the use of RTX as first-line treatment or in patients with a mild form of the disease is not recommended, its off-label use in severe, refractory SLE appears to be sufficiently positive to warrant its use.

5.2 Epratuzumab

Another potential B cell depleting agent is epratuzumab (EPR), a humanized recombinant monoclonal antibody that targets CD22. CD22 is a co-receptor of the BCR and is present throughout B cell maturation, disappearing from the cell surface once it develops into a plasma cell.[79] CD22 has two functions on B cells: it is a negative regulator of BCR signaling and it also acts as an adhesion molecule for the homing of IgD B cells to the bone marrow.[80, 81] Although EPR appears to be cytotoxic to B cells, particularly CD27 negative cells, the depletion is not as pronounced as with RTX.[82,83] Therefore, the mechanism of action may be through the modification of action of CD22.[83,84]

Fourteen moderately active SLE patients were included in the initial clinical trial of EPR. It was infused intravenously every other week for 4 doses, while the patients continued on their baseline immunosuppression. Patients did well; where most decreased their BILAG score ≥50% at 6 weeks, and 38% had a sustained decrease at 18 weeks.[82] Although only four nephritis patients were included in this initial study, 3 of the 4 did show some improvement in BILAG scores.

Data from the phase IIb, EMBLEM trial, shows that EPR continues to be a promising regimen for moderate to severe SLE. A group of 227 patients were randomized to either placebo or one of several doses of EPR where the primary endpoint was the reduction of baseline BILAG scores. A cumulative dose of 2400 mg, either given as 600 mg weekly or 1200 mg every other week, was associated with significant improvements in general BILAG scores over placebo.[85] It remains to be seen what effect the drug will have on lupus nephritis given that the phase III, EMBODY trial excludes patients with active renal disease.[86]

5.3 Belimumab

B cell activating factor (BAFF), also known as B-lymphocyte stimulator (BLyS), and its homologue APRIL (a proliferation-inducing ligand), are members of the TNF-superfamily critical in the development of B cells. They are both widely expressed by neutrophils, macrophages, monocytes and dendritic cells as well as B and T cells.[87] BAFF binds to three receptors: BAFF-R, transmembrane activator and calcium modulator ligand interactor (TACI), and B cell maturation antigen (BCMA). Where BAFF-R only binds BAFF, APRIL can bind to both TACI and BCMA.[87] These receptors are expressed at different times of B cell development. BAFF-R is expressed by all B cells with the exception of plasma cells in the bone marrow. TACI is expressed by naïve and activated B cells, as well as CD27-positive memory B cells, and plasma cells. BCMA is found on tonsillar memory cells, germinal center B cells and plasma cells.[87] Binding of BAFF to its receptor promotes B cell survival and development, allowing for progression past the T1 stage into follicular and marginal zone cells.[88,89] Binding of BAFF and APRIL to TACI and BCMA allows for further survival and progression to class switching and somatic hypermutation, although BAFF is not necessary for this to occur.[90] For further details regarding the effects of BAFF on B cell biology, please refer to a recent review by Liu and Davidson.[88]

BAFF as well as BAFF-R and TACI have been implicated in murine models of SLE and nephritis. NZB/NZW mice have increased levels of circulating BAFF with increased B and T cell activation as well as short and long lived plasma cells.[91] Early inhibition of BAFF-R and TACI in these mice leads to improved mortality, decreased renal inflammation and decreased glomerular immune complex deposition.[92] Young MRL-*lpr* mice treated early with anti-TACI-Ig did not develop anti-DNA antibodies while older mice treated after the onset of disease had improved anti-DNA antibody levels.[93]

BAFF levels have been found to be elevated in patients with SLE compared to normal controls.[94] Whereas serum protein levels may be variable in patients, serum BAFF mRNA levels are less so and correlate better with disease activity.[95] Nevertheless, in a group of 245 patients followed over the course of 2 years, serum BAFF levels were significantly correlated with both disease activity scores and anti-DNA antibodies. In multivariate analyses, changes in disease activity were associated with changes in BAFF levels.[96]

There is data indicating that targeting B cell survival factors may be a potential treatment in lupus nephritis. Neusser et al. recently found on microdissected renal biopsies of lupus nephritis patients that BAFF and APRIL mRNA levels were significantly increased in the glomeruli of patients with class III and IV nephritis when compared to pre-transplant living donor kidneys. There was also increased tubulointerstitial expression of BAFF, APRIL, BCMA and TACI.[97]

Since BAFF levels correlate with disease activity, belimumab was designed as a recombinant fully human antibody, with a high affinity to soluble BAFF. In a phase II, 52 week placebo-controlled trial, 449 SLE patients were randomized to several doses of belimumab versus placebo. Although patients had moderate disease activity at entry, active nephritis patients were excluded from this trial. The medication was well tolerated; however, this study failed to meet its primary end points of reduction of disease activity scores and decreased time to flare at 24 weeks.

However a significant amount of these patients (38.5%) were ANA negative at enrollment. In subgroups analyses, seropositive patients performed significantly better on belimumab than on placebo with regards to disease activity, physician's global assessment (PGA), and

Short Form-36 (SF-36) physical component score.[98] There were also decreases in IgM levels as well as anti-DNA antibody titers, in addition to increased C4 levels in those on belimumab.[92]

Two multi-national phase III trials were conducted in ANA positive, SLE patients with a similar study design to the phase II study. One trial followed patients for 52 weeks (BLISS-52) while the other for 76 weeks (BLISS-76). In both studies, all patients were seropositive for anti-nuclear antibodies and had moderate to severe disease activity. Patients were randomized to either standard of care + placebo, standard of care + belimumab 1 mg/kg, or standard of care + belimumab 10 mg/kg. Again, patients with active nephritis were excluded, and less than 15% of the patients randomized had a history of nephritis. The primary end point was the response rate at 52 weeks, as measured by the SLE Responder Index (SRI).[89,99]

In both studies a total of 1684 patients were randomized to the 3 groups. In the combined results for both BLISS-52 and BLISS-76, SRI was significantly improved by week 52; however, the difference when compared to placebo was modest: belimumab 1 mg/kg v placebo: 46.2% v 38.8%, belimumab 10 mg/kg v placebo: 50.6% v 38.8%. In both belimumab groups, there was significant improvement in the PGA compared to placebo, and time to flare was prolonged in both treatment groups. Moreover, belimumab-treated patients were able to modestly reduce their prednisone dose as compared to placebo. Further details are given in Table 1.

Although there was significant improvement in the SRI in both treatment groups at 52 weeks, the effect appeared to wane. In BLISS-76, the SRI at 76 weeks was no longer significantly different in the treatment groups compared to placebo.[89,99,100] In subgroup analyses by organ system, there was improvement and/or less worsening in several systems; however, renal parameters did not show any significant changes. This was likely due to the fact that lupus nephritis patients were under-represented in these studies.[101]

Despite the modest improvement in disease activity, belimumab is the first medication for SLE approved by the Food and Drug Administration in 50 years. It remains to be seen though what role this drug will play in the treatment of lupus nephritis given that active nephritis patients were excluded from these studies. There is also a possibility that patients of African descent do not respond as well to belimumab. These patients, in both BLISS 52 and 76, had less efficacy as measured by SRI compared to placebo. It is possible though that these are spurious results given the small amount of patients of African descent that were recruited into the BLISS trials. Yet, these results are worrisome given that this population tends to have a worse prognosis with regards to SLE and lupus nephritis.

5.4 Atacicept

As noted, both BAFF and APRIL bind to the TACI receptor. To inhibit the action of both, a fully human fusion protein of the extracellular domain of TACI and the Fc portion of IgG was created as a potential therapy for SLE.[102] Binding of BAFF and APRIL to TACI contributes to short-lived plasma cell survival, resulting in decreased IgM and IgA levels.[103,104] In a phase I trial of atacicept in SLE patients, subcutaneous administration of the medication was well tolerated. As expected, IgM and IgA levels were reduced by approximately 50% and 33% respectively. Both mature and total B cells counts were also reduced.[105] Pena-Rossi et al. found similar findings in their phase I trial investigating the safety of intravenous and subcutaneous preparations of atacicept versus placebo.[106] In these phase I trials, atacicept was well tolerated and appeared to be effective in suppressing B cell function. Two trials are

Endpoints	SOC+ Placebo (N=562)	SOC+ Belimumab 1mg/kg (N=559)	p-value	SOC+ Belimumab 10mg/kg (N=563)	p-value
SRI at Week 52, n(%)	218 (38.8)	258 (46.2)	<0.01	285 (50.6)	<0.0001
• SS≥4-point reduction (%)	230 (40.9)	269 (48.1)	<0.01	297 (52.8)	<0.05
• ≤0.3-point PGA worsening (%)	372 (66.2)	424 (75.8)	<0.001	420 (74.6)	<0.01
• No new 1A/2B BILAG (%)	389 (69.2)	429 (76.7)	<0.01	425 (75.5)	<0.05
Mean % PGA improvement at week 24	24.3	28.8	ns	32.3	<0.01
Mean % PGA improvement at week 52	27.1	36.7	<0.01	37.8	<0.001
SFI flare %(HR)/median time to first flare, days	81.5/84	74.6 (0.82)/110	<0.01	74.6 (0.84)/110	<0.05
Severe SFI flare, %(HR)	23.7	17.0 (0.71)	<0.05	15.6 (0.64)	<0.01
New 1A/2B BILAG, % (HR)	32.0	27.2 (0.83)	ns	24.9 (0.75)	<0.05
New 1A BILAG, % (HR)	23.1	19.0 (0.81)	ns	16.2 (0.67)	<0.01
Prednisone dose reduction from >7.5mg/day baseline by 25% or to ≤7.5mg/day, during weeks 40-52, n (%)	39 (12.3)	67 (20.1)	<0.01	58 (17.9)	<0.05
Prednisone dose increase from ≤7.5 to >7.5mg/day at week 52, n (%)	82 (33.6)	58 (25.8)	ns	62 (25.9)	ns
SF-36 PCS change from baseline at week 52, mean (SD)	+2.9 (0.3)	+4.3 (0.4)	<0.01	+3.8 (0.3)	<0.05
FACIT-fatigue score change from baseline at week 52, mean (SD)	+2.5 (0.4)	+4.8 (0.4)	<0.001	+4.7 (0.4)	<0.001

Table 1. Combined Efficacy Results Of Bliss-52 And Bliss-76**
Abbreviations: BLISS: Belimumab International SLE Study: SRI: Systemic Response Index: SS: SELENA-SLEDAI; PGA, Physician's Global Assessment; HR, Hazard Ratio; SD: Standard Deviation; SFI: SS Flare Index; SF-36, Short Form-36; PCS, Physical Component Score; SOC: Standard of Care.
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currently underway to study the potential efficacy in the treatment of SLE. The first is a phase II/III trial in SLE patients with moderate disease activity; active nephritis patients are excluded from this study. However, a phase Ib trial in active lupus nephritis patients failing MMF treatment is currently recruiting patients.[86]

6. Possible future therapies

6.1 Abatacept

Binding of CD28 on T cells to its ligand CD80/86 on APCs and activated B cells provides the major costimulatory activation signal to T cells. Endogenous CTLA-4 is an inhibitory molecule on previously activated T cells that binds CD80/86 with higher affinity than CD28. Abatacept, a fusion protein of the extracellular portion of CTLA-4 with the Fc portion of IgG, takes advantage of this and blocks the interaction of CD28 with CD80/86, blocking T cell activation.[107]

Abatacept has been studied extensively in mouse models of lupus. Administration of CTLA-4 Ig to female NZB/NZW lupus-prone mice prevented the development of autoantibody production and kidney disease resulting in prolonged survival.[108] Administration of CTLA-4 Ig also prevented the onset of disease in SLE-prone NZB/NZW F1 mice.[109] Schiffer et al. showed that short-term administration of CTLA-4 Ig and CYP induced remission of nephritis in NZW/W mice with the resolution of proteinuria.[110] Treatment with abatacept increased lifespan of mice treated with CTLA-4 Ig plus CYP compared with those treated with CYP alone.

A phase IIb, multicenter, randomized, placebo controlled trial was conducted to evaluate the efficacy and safety of abatacept versus placebo for the treatment of SLE. This study included only patients with non-life threatening manifestations where the primary endpoint was a new BILAG flare A or B over 12 months. 118 patients were randomized to receive abatacept and 57 to receive placebo. The percentage of new BILAG A/B flares over 12 months did not different significantly between the groups (79.7% in the abatacept group and 82.5% in the placebo group). Consequently, this study failed to meet its primary endpoints. However, improvements in certain measures were noted in the study. At each visit throughout the trial, investigators were asked to state if the patient was experiencing a disease flare. By this measure, a difference in flare rates between the abatacept group (63.6%) and placebo group (82.5%) was noted.[111] Adverse events were comparable in the abatacept and placebo groups but serious adverse events were higher in the abatacept group.

Abatacept potentially has some efficacy in SLE and is being studied in two ongoing trials for the treatment of lupus nephritis, one in conjunction with MMF and the other in conjunction with CYP.[86]

6.2 TLR inhibitors

As previously mentioned, there are several studies that point to Toll-like receptors as possible contributors to the pathogenesis of SLE. While there are multiple TLRs, there are two that are of particular interest: TLR-7 that recognizes single stranded viral RNA and TLR-9 that recognizes bacterial hypomethylated DNA.[112] These two TLRs are implicated in both the activation of B cells in murine models and SLE patients, as well as in the generation of type I IFN, which can in turn also upregulate B cells.[112]

TLR-9 recognizes hypomethylated CpG-oligodeoxynucleotides (CpG-ODN), a motif present in bacterial DNA which has been found to induce T cell independent activation of B cells as well as the activation of other APCs such as dendritic cells.[113] Humans recognize foreign DNA as foreign because it is not methylated as is human DNA. SLE patients though have abnormalities in DNA methylation leading to segments of hypomethylated DNA.[112] Theoretically, when SLE patients release apoptotic bodies and create immune complexes with chromatin, they are exposing this hypomethylated DNA to TLR-9.[112] This has been shown *in vitro* where immune complexes containing DNA and IgG were bound to autoreactive B cells and induced the activation of the cell through TLR-9.[114]

Administration of CpG-ODN in several murine models of SLE is associated with worse disease activity. In MRL-*lpr* mice, CpG-ODN precipitated the progression to cresenteric nephritis and an increase in anti-DNA antibodies where TLR-9 localized to glomerular, tubulointerstitial and perivascular infiltrates.[115,116] TLR-9 localizes to the proximal tubules in NZB x NZW mice and is associated with increased proteinuria and tubular fibrosis when stimulated with CpG DNA and serum from lupus patients.[117]

In mononuclear cells of SLE patients with moderate disease activity, TLR-9 expression is higher than in those with inactive disease or healthy controls. The percentage of memory B cells and plasma cells expressing TLR-9 is higher in those with active disease and correlates with anti-DNA antibody titers.[118, 119] When B cells from active SLE patients were cultured with CpG-ODN, not only was there increased expression of TLR-9 but there was also increased anti-DNA antibody production.

In patients with lupus nephritis, TLR-9 and the other TLRs that bind nucleic acids (TLR-3, -7, and -8) are expressed throughout the kidney: in the glomeruli, proximal and distal tubules as well as in the capillaries.[120] In proliferative disease, TLR-9 expression co-localizes to areas with immune complex deposition, and is increased in those with higher disease activity on pathology and with increased serum anti-DNA antibodies.[26]

Autoimmunity via TLR-7 seems to function in a manner analogous to that of TLR-9. The RNA associated Smith and RNP proteins are common targets for auto-antibodies in SLE patients. RNA bound to these proteins are bound to immune complexes by anti-RNP antibodies causing activation of B cells through BCR as well as TLR signaling.[121] Activation by late apoptotic thymocytes generates anti-DNA antibodies via TLR-7 in B6 mice. In the Yaa murine lupus model, where autoimmunity is caused by a duplication of TLR-7 gene, when the receptor is deleted there is an improvement in auto-antibody levels and glomerulonephritis.[122] Although TLR-7 has not been as extensively studied in human disease, there is increased TLR-7 mRNA expression relative to healthy controls and it is correlated with type I IFN levels.[119] In patients with lupus nephritis there is moderate TLR-7 expression in the distal tubules and in Bowman's capsule.[120]

Given the apparent role that TLRs play in SLE and the likely role they play in nephritis, TLR inhibitors are now being investigated as possible treatments for SLE. This is especially important in light of recent findings that glucocorticoids in murine lupus models fail to inhibit TLR signaling in plasmacytoid dendritic cells.[123] This may partially explain why certain patients do not respond sufficiently to steroid therapy.

Several synthetic inhibitory oligonucleotides (INH-ODN) have been generated with the goal of blocking the activation of TLR-9 and TLR-7.[124,125] These INH-ODNs have proven to be effective in various mouse models. In NZB/NZW mice, treatment with an INH-ODN

expressing either a TTAGGG motif or GpG not only delays the signs of nephritis but leads to increased survival.[126,127] Treatment of MRL-*lpr* mice with INH-ODNs that are specific for TLR-7 (IRS 661) and TLR-7 and -9 (IRS 954) leads to improvement of nephritis and in the case of IRS 661, reduced levels of anti-dsDNA antibodies, and anti-Smith antibodies.[116,128] NZBxNZW mice injected twice weekly with IRS 954 from age 4-9 months showed lower levels of autoantibodies as compared to controls as well reduced proteinuria and nephritis on pathology.[129]

Lenert et al. recently developed restricted activity-INH-ODNs (R-INH-ODN). They modified several INH-ODN constructs so that they no longer formed configurations, such as the G4 stack, that may have non-specific immune properties. *In vitro*, R-INH-ODNs were found to be 10 to 30 fold less potent in murine and human B cells. However, when encountering autoreactive B cells in vivo, R-INH-ODN were more potent than broad acting-INH-ODN. MRL-*lpr* mice treated with R-INH-ODN showed less proteinuria, improved renal score, and decreased renal IgG deposits as well as decreased anti-DNA and anti-Smith antibodies. Therefore R-INH-ODN are potentially more effective than other INH-ODNs when the BCR is activated in conjunction with either TLR-7 or TLR-9.[130]

Although promising, there are currently no clinical trials looking at the safety and efficacy of INH-ODNs in the treatment of SLE. It is unknown what role these potential medications will play with regards to current regimens, given that blocking TLRs may cause a significantly increased risk of infection. Only time will tell if these molecules will be developed further for potential use in humans.

7. Conclusion

Lupus nephritis causes significant morbidity and mortality. While current regimens have reduced the burden of disease, many patients continue on to end-stage renal disease despite treatment with aggressive immunosuppression. Given that B cells play such an integral role in nephritis through the generation of pathogenic autoantibodies and by acting as APCs, they are a natural target for therapy. Despite the fact that rituximab causes profound B cell depletion, two phase III clinical trials have failed to show increased efficacy in SLE. Epratuzumab, on the other hand, also causes some level of B cell depletion and may possibly modify the actions of CD22 on B cells. So far early trials are positive for this medication, however lacking nephritis specific data, it is difficult to say if epratuzumab will add significantly to current nephritis regimens.

Belimumab, while showing modest efficacy for the treatment of SLE, has also not been tested in patients with active lupus nephritis. Now that it has been approved by the FDA, it will be important to follow reports of its use in active nephritis patients, and to conduct nephritis specific trials to evaluate its use in this population. Nevertheless, belimumab is an exciting addition to our anti-B cell armamentarium.

Abatacept failed to meet primary endpoints in a phase IIb clinical trial. Despite this there is sufficient positive data that clinical trials in lupus nephritis are currently being conducted. Hopefully this medication proves to have significant efficacy in the nephritis population. Lastly, TLR inhibitors such as INH-ODNs, may eventually become an option for the treatment of lupus nephritis. Currently data is limited to mouse lupus models; however, studies are showing promise in that they limit the progression of renal disease in these animals.

Figure 2 summarizes some of the possible B cell directed therapies that can be used in lupus nephritis. All in all, while B cells seem to be a logical target for the treatment of lupus nephritis, the best way to target them remains elusive. Further studies and medication development will hopefully lead to an optimal treatment regimen where end-stage renal disease will become a rarely seen complication of nephritis.

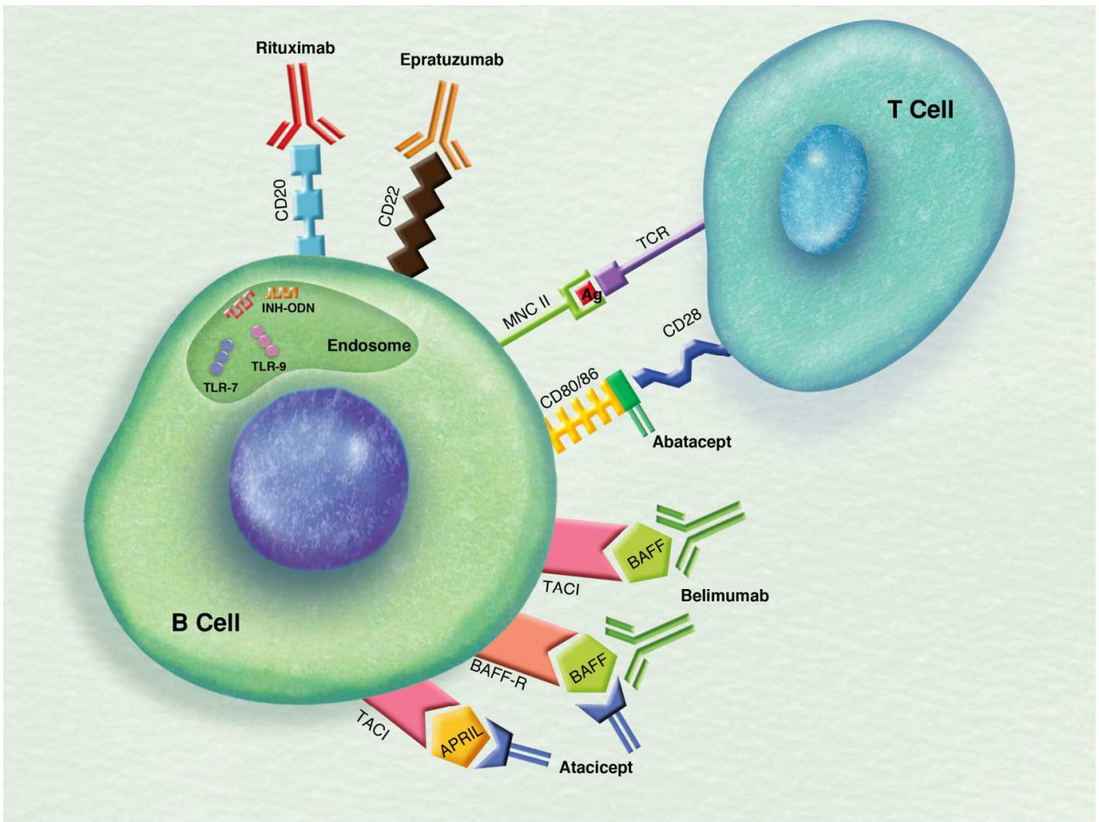

Fig. 2. Potential B cell Targeted Therapies for SLE and Lupus Nephritis.*
Rituximab: Chimeric, anti-CD20 monoclonal antibody; **Epratuzumab**: Humanized anti-CD 22 monoclonal antibody; **Belimumab**: Humanized monoclonal antibody targeting soluble BAFF; **Atacicept**: Recombinant fusion protein of the extracellular portion of the TACI receptor to the Fc portion of IgG targeting both soluble BAFF and soluble APRIL; **Abatacept**: Recombinant fusion protein of the extracellular portion of the CTLA-4 inhibitory molecule to the Fc portion of IgG targeting interaction of CD80/86 with CD28, inhibiting T cell co-stimulation; **INH-ODN**: Synthetic oligonucleotides that inhibit Toll-like Receptors, particularly TLR-7 and TLR-9.
*For the sake of simplicity BCMA, a receptor that also binds to both BAFF and APRIL, has been left off of the schematic.

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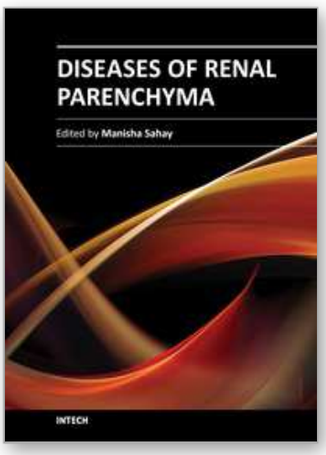

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Clinical nephrology is an evolving speciality in which the amount of information is growing daily. This book gives quick access to some important clinical conditions encountered in nephrology including the diseases of glomeruli, tubules and interstitium. It presents the latest information on pathophysiology, diagnosis and management of important diseases of renal parenchyma. The information is presented in a very user friendly and accessible manner while the treatment algorithms enable the reader to quickly access expert advice on arriving at the most appropriate treatment regimen. The book discusses the renal involvement in various systemic diseases including diabetes and autoimmune diseases. Diabetic nephropathy is fast becoming the commonest cause of end stage renal disease all over the globe and is discussed in this book. The editors believe that this book will be a valuable addition to the reader's library.

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