B-cell-targeted therapies: promising new treatments for systemic lupus erythematosus

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There is surely great satisfaction in the discovery that careful analytical assessment of the pathogenesis of the rheumatic diseases has resulted in the major therapeutic advances we use in practice today. The study of both rheumatoid arthritis and systemic lupus erythematosus (SLE) has highlighted the importance of B cells and specific proinflammatory cytokines such as TNF-α, IL-6 and IL-1 in the pathogenesis of these diseases. This information, coupled with rapid advances in molecular biology, has led to the development of B-cell-depletion and cytokine-blocking therapies. These treatments have already led to novel approaches to managing patients with the most refractory rheumatoid arthritis, and B-cell-depletion therapy has formed an important role in the contemporary management of SLE. This article aims to clarify the reasoning behind targeting B cells in SLE, the development of B-cell-depletion therapy and its current and potential roles in the management of this devastating connective tissue disease.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune condition driven by both antibody-dependent and -independent processes with resultant organ damage through inflammation. There is clinical and serological overlap with the other autoimmune rheumatic diseases and, historically, treatments have also overlapped. The overall goal of therapy is to achieve remission through reduction of autoimmunity and suppression of inflammation or prevention of progression of organ dysfunction.

Historically, therapies were derived from oncologic drugs, which have broad mechanisms of action with consequences on many cells, including the hemopoeitic lineages. Within a rheumatological context, this has meant a reduction in immune cell function but at the cost of significant side effects in other organs and systems.

The means through which disease control is achieved in most of the rheumatic diseases has changed dramatically in recent years with the advent of biologic-targeted therapies. The development of these more specific treatments stems from a better understanding of the pathogenesis of the conditions they are designed to treat. This increased understanding should lead to less bystander immunosuppressive effect and, furthermore, fewer side effects in other organs.

The immunopathogenesis of SLE is complex but involves genetic, hormonal and environmental factors. B cells and, more specifically, the loss of B-cell tolerance, play key roles that are central to the development of this disease. The biologic therapies first used to target specific areas of the inflammatory response were introduced in clinical practice in the early 1990s, but these, notably anti-TNF treatments, were initially thought to induce rather than control a lupus-like illness, due to the production of dsDNA antibodies in some patients [1,2]. Developments in the area of biologic therapies have since mushroomed, and include an IL-1 receptor antagonist, B-cell-targeted therapies and T-cell co-receptor antagonists within current use for rheumatology alone. Outside the field of rheumatology, hundreds of antibody-based therapies are now emerging.

Rationale for B-cell involvement in the pathogenesis of SLE

The pathogenesis of SLE is multifactorial and mirrors the diversity of both clinical presentation and the severity of the condition. It has environmental, genetic and hormonal influences. Therefore, is the concept of targeting B cells alone a rational therapeutic strategy?

In the appropriate, balanced humoral immune response, B cells are not only responsible for the production of memory cells, plasma cells and antibodies, resulting in immune complex formation, opsonization and complement activation, but are also involved in the presentation of antigen to T cells, and initiate or perpetuate inflammatory processes with the production of proinflammatory cytokines such as TNF-α, IL-12, IL-6 and IFN-γ. B-cell-mediated costimulation of the T cell results in a heightened T-cell response and increased presentation of B-cell adhesion molecules, promoting leukocyte recruitment and further T-cell activation (Figure 1).

Keywords: abetimus, atacicept, B-cell depletion, belimumab, epratuzumab, lupus, ocrelizumab, ofatumumab, rituximab, systemic lupus erythematosus

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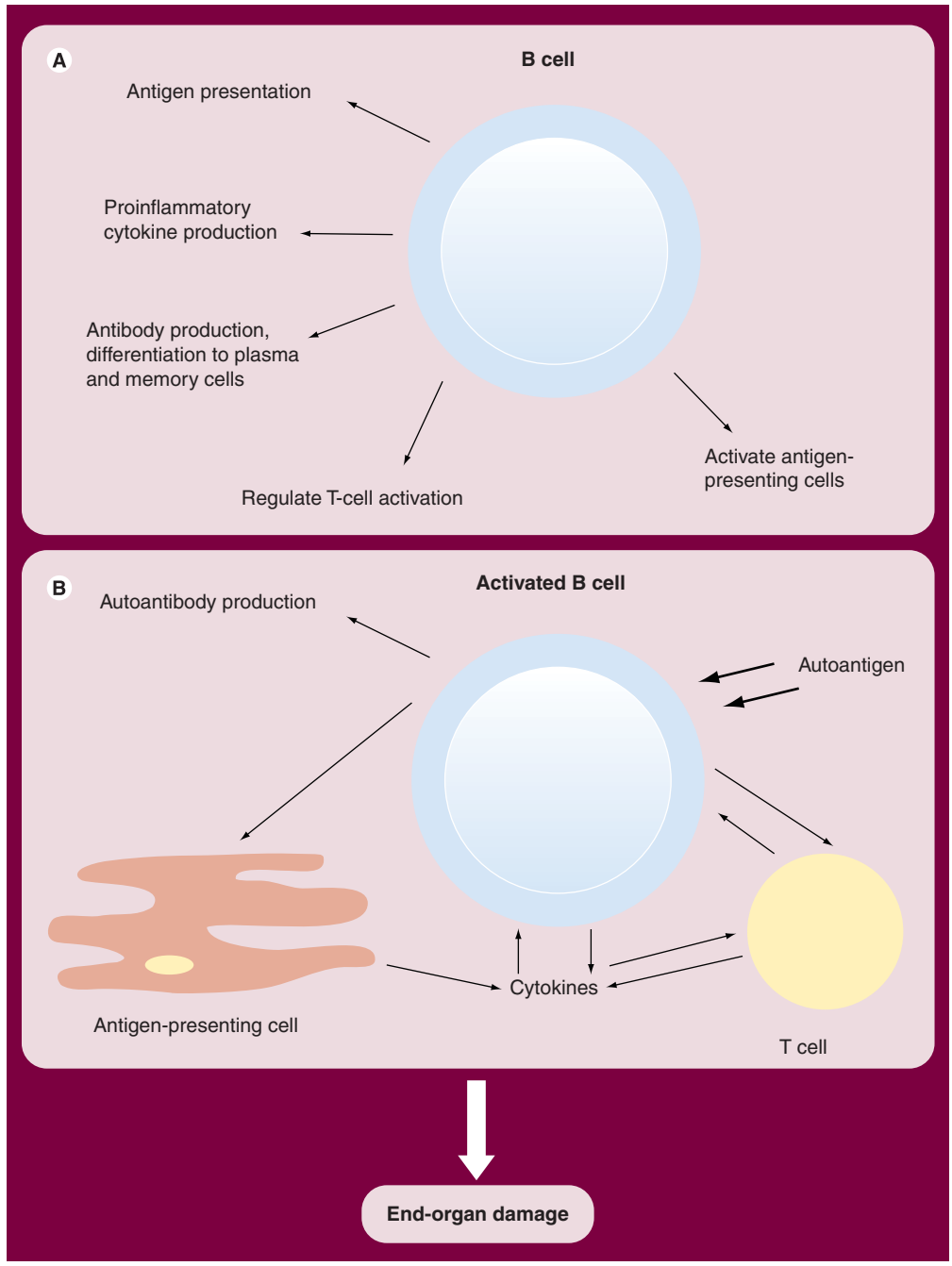
Figure 1. Normal and altered B-cell function.



(A) Functions of B cells in the normal adaptive immune response and **(B)** B-cell overactivation results in end-organ damage through several mechanisms.
Modified from [46,47].

Experimental models

The study of autoimmunity has been aided enormously by the availability of useful murine and other animal models of human SLE [3,4]. Animal studies on B-cell autoreactivity in SLE initially focused on the relative contributions of clonal autoantibody production, polyclonal activation and nonantibody-mediated actions of B cells. The ongoing detailed etiological analysis of organ-based disease in animal models of SLE is beyond the scope of this article, but major advances in the understanding of the interaction between B and T cells in SLE have emerged [5–8].

The presence of autoimmunity in NZB, MRL lpr/lpr and BXSB mouse models was found to arise from the polyclonal activation of B cells, with somatic mutation, affinity maturation and antigenic drive, rather than clonal proliferation of autoantibody-producing plasma cells [9]. However, murine studies of SLE have also shown that B cells are critical to the development of the disease, even when they are rendered incapable of secreting autoantibodies [10]. The contribution of B cells to lupus pathogenesis in animal models seems to be far more complex than pathogenic antibody formation alone.

Human studies

In both humans and mice with an SLE-like disease, the sera carry antibodies to a variety of nuclear, cytoplasmic and cell membrane antigens, as well as the diagnostic anti-dsDNA response. It is clear that some dsDNA antibodies, and the closely-related antinucleosome antibodies, have pathogenic properties, particularly in lupus nephritis, but these are not universally present or elevated serologically, and even when present, do not always lead to disease manifestations [11–13].

The eventual outcome of B-cell overactivation is, of course, autoantibody production, immune complex deposition, complement activation and depletion, proinflammatory cytokine production and general inflammation resulting in end-organ damage.

It is well-reported that the peripheral blood of patients with active SLE contains low lymphocyte numbers, and certain B-cell subsets are expanded. For instance, the frequency of $CD27^{high}$ plasma cells is significantly correlated with SLE disease activity and anti-dsDNA levels. CD27 is a B-cell costimulatory molecule of the TNF receptor family that binds CD70 as its ligand and effects the activation of T cells [14,15]. Targeting B cells in active SLE is thus a rational therapeutic strategy.

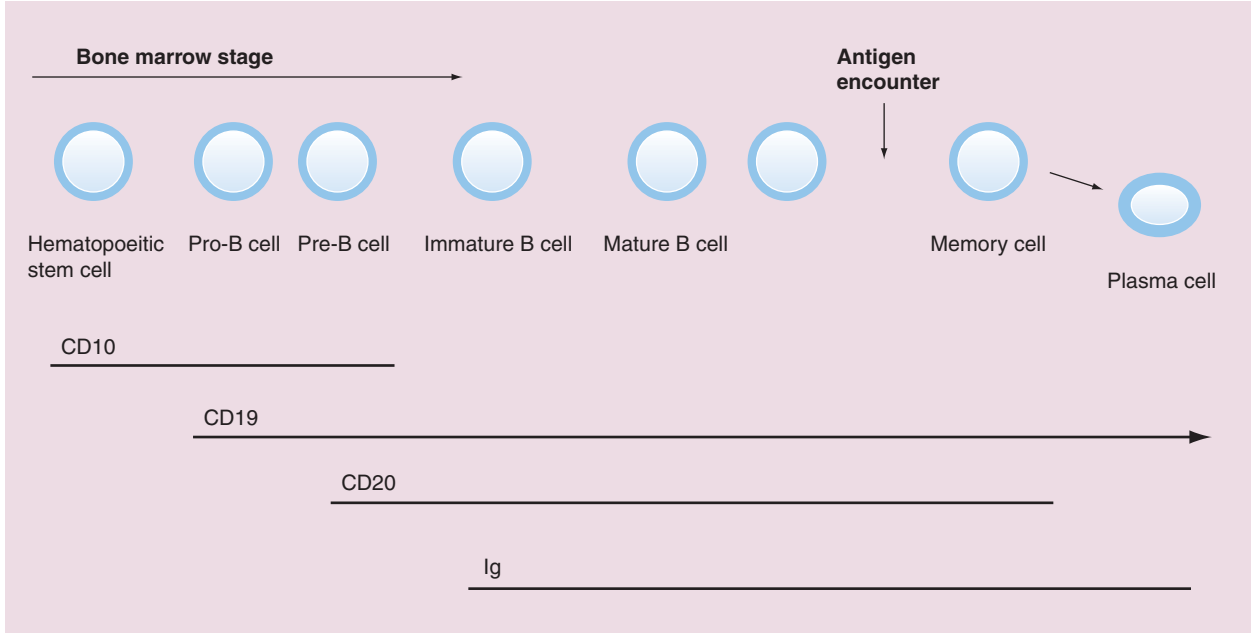
B-cell targeting methods

CD20

CD20 appears on the surface of the pre-B lymphocyte, between the time of light-chain rearrangement and expression of intact surface immunoglobulin, and is lost just before terminal B cells differentiate into plasma cells. Surface expression of CD20 on activated B cells is approximately fourfold greater than that found on resting B cells (Figure 2).

CD20 is almost specific for B cells. Weak expression was initially demonstrated in a subpopulation of T cells [16], but this may have been nonspecific binding and, furthermore, does not seem to have led to a biologically significant reduction in T cells after treatment with CD20-directed therapy. CD20 has not been found in any other cell type. It may function as a calcium-permeable channel and currently has no

Figure 2. B-cell lineage and expression of CD19 and CD20.



known ligand. Genetically engineered mice that lack CD20 (CD20 knockout mice) have not shown any clear defects in their B cells [17].

Rituximab is a chimeric antibody with human γ-1 and κ-constant regions and murine variable regions, which targets CD20. It mediates B-cell death by induction of complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity and CD20-mediated apoptosis [18,19]. It was approved by the US FDA for the treatment of non-Hodgkin's lymphoma in 1997. A decade of postmarketing surveillance in this patient group of close to 1 million is available and thus provides a well-described safety and side-effect profile. The mean duration of B-cell depletion is 6–9 months.

Rituximab has shown great potential in the management of refractory SLE with large-scale clinical trials, mainly recruiting for lupus nephritis, now underway [101]. The first observational study of patients who had failed routine immunosuppression showed significant improvement in their British Isles Lupus Assessment Group (BILAG) score [20]. A later and larger extension used higher doses of rituximab with similar results [21]. Many larger scale series and trials have now addressed specific questions related to efficacy and complications, using well-recognized activity scores such as BILAG, Systemic Lupus Activity Measure or Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Specific serological and biochemical evidence of improvement includes reduction in dsDNA antibodies, a rise in C3 complement and fall in urine protein:creatinine ratio. A recent study has shown histologic improvement on repeat renal biopsy of patients with severe lupus nephritis after combination rituximab and cyclophosphamide therapy [22], and even the notoriously resistant neuropsychiatric lupus appears to be responsive [23].

In our practice, rituximab is used in two doses of 1 g administered a fortnight apart in combination with cyclophosphamide infusion and methylprednisolone in patients who have failed conventional therapy. All three agents are known to suppress B-cell activity and provide a safe method of extending B-cell depletion. This protocol usually allows the patient to stop all other immunosuppressive agents, with a mean duration of B-cell depletion of 7 months. Interestingly, it appears that retreatment extends the duration of B-cell depletion [24]. Longer term follow-up at University College London (UK) over a mean of 39 months suggests a relatively safe therapy with four serious adverse events in that cohort, two of which were ascribed to rituximab therapy. One patient developed pneumococcal septicemia while B-cell depleted, 5 months after therapy. A second had a serum sickness-like reaction that resolved after treatment with intravenous steroids. The third died from pancarditis related to SLE 5 months after therapy, but had repopulated B cells a month earlier, and the fourth had a hyponatremia-induced grand mal seizure after infusion of cyclophosphamide [25]. In December 2006, the FDA reported two cases of fatal progressive multifocal leucencephalopathy in patients with end-stage systemic lupus, and case reports do suggest an increased likelihood of viral infections or reactivation, including JC virus. However, this report pointed out that around 8000 patients with SLE had been treated by this time. It also detailed over 20 lupus patients who had not been treated with rituximab but who had developed progressive multifocal leucencephalopathy.

It is becoming more obvious that there are demonstrable differences in response to rituximab in SLE patients. This is unsurprising considering the clinical diversity of the disease. Not all treatments will have equal efficacy for each complication. Autoantibody profile at baseline is a predictor of response, with those lacking Sm and ENAs likely to have a more favorable outcome. In addition, those with low C3 initially flare earlier [25]. There are concerns that patients who have prolonged B-cell depletion have impaired responsiveness to antigenic challenges or immunization [26]. However, levels of antimicrobial antibodies such as antitetanus and antipneumococcal IgG, which are already present, do not drop appreciably.

Human antichimeric antibodies (HACAs) will be expected to develop, given that rituximab is approximately 20% murine protein. The detection rate was significantly higher in SLE than in lymphoma patients. Studies suggest that human–mouse chimeric antibodies may be more immunogenic in autoimmune disease, especially in SLE, because of the highly activated state of B lymphocytes. An alternative possibility is that the lower dose regimens used in autoimmune disease compared with non-Hodgkins lymphoma result in increased HACA formation. Specifically, high-titer HACAs were detected in six out of 17 SLE patients, but in only one out of 166 lymphoma patients. This response was associated with African–American ancestry and high titers of HACA in SLE were reported to be associated

with disease activity, reduced B-cell depletion, low levels of rituximab and loss of efficacy of rituximab at 2 months after the initial infusion. Fortunately, levels of HACA do not routinely cause clinical concern at current doses [27,28]. One patient who did develop HACAs was successfully treated with humanized anti-CD20 [29].

In general, rituximab may provide a well-tolerated and effective therapy for refractory SLE. Large-scale randomized, controlled trials are underway. Results currently available suggest response rates of approximately 60% using rituximab monotherapy [27], and higher when used in combination with cyclophosphamide (21 out of 22 patients and two out of two patients [21,30]). Most flare between 6 and 12 months after therapy. In one study, 13 out of 24 patients remained well enough not to require further treatment for at least 7 months; the longest time when the study was published was 51 months. Antibody profiles help to determine outcome and whether retreatment is available.

The more humanized anti-CD20 alternative to rituximab mentioned above, used when a patient had developed HACA to rituximab, is currently in Phase I trials for non-Hodgkin's lymphoma. hCD20 provides a closely related alternative to rituximab. Ocrelizumab and ofatumumab are also humanized CD20 monoclonal antibodies and 'third-generation' antibodies are in development, offering 'improved CD20 binding, antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity activities' (Genentech, CA, USA) compared with earlier antibodies.

Small modular immunopharmaceuticals (SMIPs) are single-chain polypeptides that bind to specific antigen targets on a cell's surface with the full effector function of a monoclonal antibody, but are approximately a third to half the size, which is said to allow improved tissue penetration. Their development is more rapid than that of a monoclonal antibody. TRU 015 is a SMIP that binds CD20. There are ongoing Phase IIb trials in rheumatoid arthritis, although trials are yet to start in SLE.

CD20 was the first B-cell antigen to be targeted for large-scale clinical use in lymphoma. The choice of antigen, perhaps serendipitously, is difficult to improve upon. The aim is now to further the use of B-cell antigens or toleragens for therapeutic benefit in SLE by identifying targets with improved specificity for the exaggerated B-cell activation or enhanced proliferation or, additionally, that reduce side-effect profiles or immune suppression. For instance, one clinical concern with B-cell depletion is the irreversibility of the process. The approaches described below target the B-cell-mediated immune response in different ways in order to achieve these aims (Table 1 & Figure 3).

CD22

CD22 is another B-cell-specific transmembrane protein of the IgG superfamily that is associated with B-cell signaling and interaction with T cells. It is expressed in the late pro-B cell until terminal differentiation into plasma cells and is lost from the surface on activation. Animal studies have shown that CD22 plays a role in B-cell development and survival, with CD22-deficient mice having reduced mature B cells in the bone marrow and circulation, and with the B cells also having a shorter lifespan and enhanced apoptosis. Their B cells have hyper-responsiveness to B-cell receptor cross-linking, but with a paradoxical deficiency in response to T-cell-independent antigens. In the presence of other genetic risks, CD22 deficiency heightens propensity to autoimmunity. There is also some evidence in humans that CD22 polymorphisms are linked to SLE [31].

The anti-CD22 antibody epratuzumab is approximately 90–95% humanized and is currently undergoing controlled trials in SLE. This antibody yields approximately 30% of the B-cell depletion seen with rituximab [32], and may preferentially modulate the exaggerated activation of B cells in SLE, known to be a hallmark of the disease, compared with the acutely cytotoxic action of rituximab. It does not block CD22–CD22 ligand interactions, but does initiate signaling through CD22. The possible consequences of this signaling would be difficult to predict in individual patients but this antibody, attractive because of its action on a molecule with more restricted expression than CD20, was developed as a further therapy for non-Hodgkins lymphoma. In SLE, the initial Phase I clinical trial was a single-center uncontrolled study of 14 moderately active patients, as measured using BILAG scores. Total scores reduced by at least 50 and 93% of patients experienced improvement in at least one B or C-level disease activity. The infusions, 360 mg/m^2 every 2 weeks for four doses, were generally well-tolerated. Larger scale clinical trials have not yet been reported, but the evidence looks promising. It may be that the CD22 molecule, which has several functions, such as B-cell survival, relevant to the pathogenesis of autoimmunity, provides a target for

Table 1. Summary of drug therapies available or in development for systemic lupus erythematosus.

Drug name	Mode of action	Dosing regimen	Example study
Rituximab	Anti-CD20 antibody	1 g iv., two doses, 2 weeks apart, with or without cyclophosphamide and steroid for SLE	[21]
Ocrelizumab	Anti-CD20 antibody (humanized)	10–1000 mg iv. dose, Phase I/II dose escalation trial in RA	[101]
Ofatumumab (HuMax®)	Anti-CD20 antibody (humanized)	700 mg iv., two doses, 2 weeks apart, in RA	[101]
hCD20 (veltuzumab)	Anti-CD20 antibody (humanized)	sc. dose escalation trial in idiopathic thrombocytopenic purpura ongoing, 80–120 mg/m^2 iv. weekly dosing for 4 weeks in non-Hodgkins lymphoma	[44,101]
TRU015	SMIP binding CD20	2.5–15 mg/kg iv., single dose or two divided doses in RA	[45]
Epratuzumab	Anti-CD22 antibody	360 mg/m^2 iv., four doses, 2 weeks apart in SLE	[31]
Belimumab	Monoclonal antibody, inhibits soluble BLyS	1–10 mg/kg iv., three doses, 2 weeks apart, then monthly ongoing in SLE	[37]
AMG-623	Binds and neutralizes BLyS	sc. or iv., dose range 0.1–6 mg/kg, up to three injections in SLE	Phase I trials only
BR3-Fc	Binds and neutralizes BLyS	sc. or iv., dose-ranging study	Early Phase I trials only
Atacicept	Binds and neutralizes BLyS and APRIL	sc. 0–9 mg/kg dose escalation Phase Ib in SLE	[41]
Abetimus	Cross-links pathogenic dsDNA	iv. 300 or 900 mg, weekly, in SLE	[43]

iv.: Intravenous; RA: Rheumatoid arthritis; sc.: Subcutaneous; SLE: Systemic lupus erythematosus; SMIP: Small modular immunopharmaceutical.

more specific control of SLE with a lesser degree of B-cell depletion and, hence, bystander immunosuppression [31,33].

Targeting B-cell survival

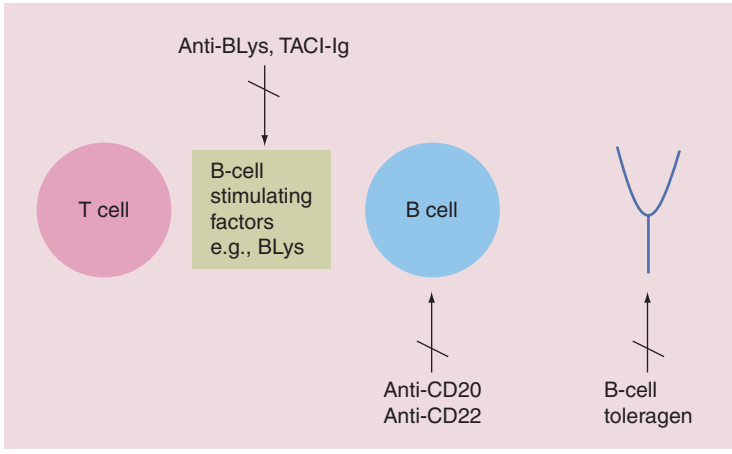
B cells, similarly to other cells, require survival and maturation factors. B-cell production, differentiation, activation and lifespan in the follicle, marginal zone and memory subsets are modulated by two proteins, BLyS and APRIL, which sometimes act additively, sometimes in opposition, and at least three receptors. The B-cell activating protein BLyS, or BAFF, has complex roles in the regulation of B-cell immunity, maturation and survival. It achieves this, in part, by upregulation of the anti-apoptotic bcl-2 family of proteins. IFN-γ and IL-10 both upregulate expression of BLyS. APRIL does not seem to be a required protein in murine development, suggesting that its primary function is immunomodulatory, but BLyS and APRIL can form active heterotrimers when coexpressed that are likely to contribute to a complex regulatory environment [34]. Their receptors include transmembrane activator, calcium modulator and cyclophilin ligand interactor (TACI), B-cell maturation antigen (BCMA) and BR3 (or BAFF-R). TACI plays a role in both positive and negative signaling, BCMA regulates plasma cell survival and BR3, to which APRIL does not bind, controls a survival signal for generation and maintenance of mature B cells [35].

Hence, the regulation of B-cell function is complicated and not yet fully understood, and a delicate homeostasis undoubtedly exists *in vivo*. The buffering actions between these molecules may prevent an excessive response, leading to autoimmunity. Constitutive overexpression of BLyS in mice leads to an autoimmune phenotype. In animal models of autoimmunity, BLyS antagonists reduce disease severity and delay progression [35].

In humans, BLyS levels are variable, and attempts to correlate levels with clinical lupus activity are inconsistent. In general, serum BLyS and BLyS mRNA levels are raised in SLE compared with healthy controls [36]. The temporal relationship between a rise in levels and a flare of disease is, again, unknown. These factors need to be taken into account when considering drug development.

Several BLyS antagonists are in development for the treatment of human SLE. Belimumab is a fully human monoclonal antibody that selectively inhibits soluble BLyS and its interaction with all three receptors. There are currently two Phase III

Figure 3. B -cell targets for therapy in systemic lupus erythematosus.



TACI: Transmembrane activator, calcium modulator and cyclophilin ligand interactor.

trials underway, but the Phase II results showed good tolerance and acceptable side-effect profiles in 449 patients with moderately active lupus with three different dose regimens. A combined end point was used encompassing SELENA SLEDAI, physician's global assessment and BILAG. The results showed statistically significant improvement for multiple thresholds, but overall SELENA SLEDAI and BILAG scores did not show improvement and hence did not meet primary efficacy end points [37]. There were, however, flaws in study design and, in particular, patient selection. There was a significant proportion (>25%) of ANA-negative patients. Subgroup analysis of the ANA-positive patients in that study yielded statistically significant results, so future trials, with more focused patient selection, should yield statistical significance for the same primary end point.

Less advanced in clinical trials are AMG-623 and BR3-Fc, the former an Fc-peptide fusion protein that blocks BLys and has completed two Phase I trials for SLE and the latter, as the name implies, a human BR3 fusion protein that has completed one Phase I trial.

The relationship between presence of *APRIL* gene polymorphisms and clinical evidence of SLE has recently been published and may provide even more B-cell immunotherapeutic targets [38].

Atacicept, or TACI-Ig, targets the TACI receptor and hence prevents binding of BLyS and APRIL. Transgenic mice engineered to overexpress a soluble form of the TACI receptor in the blood produce fewer mature B cells and show reduced levels of circulating antibody. Similar results were observed in normal mice treated with atacicept protein.

In animal models of SLE, treatment with atacicept inhibited the development of proteinuria and prolonged the survival of the animal [39]. Similarly, in a mouse model of collagen-induced arthritis, soluble atacicept was able to inhibit the development of collagen-specific antibodies and reduce both the incidence of inflammation and the rate of occurrence of disease [40]. These data suggest that atacicept may provide a novel approach to the treatment of autoimmune disease.

Phase I clinical studies with atacicept are complete in patients with SLE, rheumatoid arthritis and advanced B-cell malignancies [41]. Phase II trials were initiated in December 2006 in patients with rheumatoid arthritis whose disease had failed to respond to TNF inhibitors. Phase II/III studies for SLE are planned.

B-cell toleragens

The most studied B-cell 'toleragen' is abetimus sodium, which was introduced in the 1990s and was designed to target and reduce pathogenic dsDNA antibody levels. It is known that changes in dsDNA antibody levels correlate with the risk of renal flare, and correlate inversely with changes in C3 level [42]. It is therefore logical that a soluble, synthetic dsDNA tetramer that is capable of cross-linking circulating DNA antibodies, as well as those bound to B cells, could remove pathogenic antibodies and result in lower dsDNA levels and higher C3 levels. The initial studies looking at the use of abetimus in lupus nephritis did not reach primary efficacy end points, but when used in more defined patient groups, namely those with lupus nephritis associated with high-affinity anti-dsDNA antibodies, the time to renal flare was prolonged, the number of flares reduced and fewer treatments with high-dose corticosteroids and cyclophosphamide were required compared with placebo [43]. There may be a defined role for this well-tolerated therapy in the management of a subgroup of patients with lupus nephritis, although weekly intravenous dosing regimens could make this a less popular choice in practice.

Conclusion

The use of biological agents in patients with SLE, especially those that block B lymphocytes, is a fast-moving and exciting area of therapeutics. It has provided the rheumatologist with the first new drug choices for this prototypical autoimmune

disorder for 10 years. Scientific research coupled with clinical experience has created a more logical choice of therapy in this devastating disease. Investment by pharmaceutical companies has moved the field forward, accelerating the production of new monoclonal antibodies and initiating new biological methods of cell control.

The therapies discussed above target only B-cell function. This restriction must be seen in context, as there is equal interest in targeting other parts of the perturbed immune response in this condition, most notably complement, T-cell activation and cytokines such as IFN-α and IL-6.

Future perspective

The research field is moving at such a rate that new, specific, efficacious and safe therapies could become available that would dramatically change the management of not just refractory, but all patients with SLE. With more experience and newer production methods, we could be able to focus our care with much improved outcomes for patients.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

Rationale for B-cell involvement in systemic lupus erythematosus

- Systemic lupus erythematosus (SLE) is an autoimmune condition with genetic, hormonal and environmental factors implicated in its pathogenesis.
- Activated B cells and loss of B-cell tolerance are central to disease development.

B-cell-targeting methods

- The most widely used drug to target B cells is rituximab, for which there is good evidence of efficacy in rheumatoid arthritis. Outcomes in open-label trials in SLE are promising and large-scale, randomized, controlled trials are underway. The drug targets CD20.
- Newer agents have been produced that also target CD20, and other B-cell surface molecules have been targeted, such as CD22.
- B-cell function, maturation and survival depends on a complex interaction between two survival factors, BLyS and APRIL, through three receptors. These molecules and receptors have also been targeted for drug development.
- Inducing B-cell tolerance to dsDNA antibodies results in modest beneficial effects in lupus nephritis.

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