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B-Cell-Targeted Therapy for Systemic Lupus Erythematosus

An Update

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Abstract

Systemic lupus erythematosus (SLE) is a classic autoimmune disease characterized by a myriad of immune system aberrations, most likely resulting from pathogenic autoantibody production, immune complex deposition, and subsequent end-organ damage. B cells play a key role in the pathogenesis; therefore, B-cell-targeted therapies, including B-cell depletion and blockage of B-cell survival factors such as B-lymphocyte stimulator (BLyS), are potential therapeutic targets for SLE. In uncontrolled clinical trials from approximately 20 studies, rituximab – a mouse-human chimeric anti-CD20 monoclonal antibody that effectively depletes B cells – has been demonstrated to reduce disease activity and decrease serum autoantibodies, with a clinical response of 86% in a case series of approximately 400 SLE patients with refractory disease, with or without concomitant use of cyclophosphamide. Epratuzumab, a humanized anti-CD22 monoclonal antibody that partially depletes B cells, has also been shown to reduce disease activity but not to decrease autoantibody levels in patients with moderately active SLE. Randomized controlled phase I/II trials in patients with active SLE have documented that belimumab, a humanized anti-BLyS monoclonal antibody, reduces B-cell numbers, inhibits disease activity and decreases anti-double-stranded DNA autoantibody in SLE patients. All these therapies are well tolerated, but accompanying infectious complications have been observed. Other B-cell-targeted therapies such as 'humanized' monoclonal antibodies to CD20 (e.g. ocrelizumab) and agents that interrupt B-cell/T-cell interactions also have potential, and the efficacy of these, along with rituximab, belimumab and epratuzumab, needs to be determined by randomized controlled trials.

Systemic lupus erythematosus (SLE) is a classic autoimmune disease characterized by a myriad of immune system aberrations that involve B cells, T cells and cells of the monocytic lineage, resulting from pathogenic autoantibody production, immune complex deposition, hyperglobulinemia, vasculitis, and end-organ damage.[1] Although active SLE is often associated with

lymphopenia, especially B-cell lymphopenia,[2,3] certain B-cell subsets (autoantibody-secreting B cells, early plasma cells and pregerminal center cells) are expanded[3,4] in the peripheral blood, and B cells in SLE patients are more sensitive to the stimulatory effects of cytokines such as interleukin (IL)-6 than non-SLE B cells.[5] These B-cell abnormalities precede the development of SLE,[1] as does a positive antinuclear antibody (ANA), often by many years. B-lymphocyte stimulator (BLyS; also known as TNF ligand superfamily member 13B [TNFSF13B] and B-cell activating factor [BAFF]), a member of the tumor necrosis factor (TNF) ligand superfamily, has been considered one of the major factors determining the size and repertoire of the B-cell compartment, and thus has emerged as a potential driver of B-cell survival and autoantibody production in human autoimmune diseases.[6] Indeed, elevated serum BLyS levels correlate with anti-double-stranded DNA (anti-dsDNA) titers in SLE patients.[7] B cells have a central role in the pathogenesis of SLE;[8,9] therefore, B-cell-targeted therapies, including B-cell depletion and neutralization of BLyS activity, are most likely to be therapeutically beneficial for SLE. Currently, a combination of corticosteroids, antimalarials and cyclophosphamide-based regimens (the latter in lupus nephritis)[10] are the mainstays of treatment but have significant potential for toxicity and often result in incomplete disease control.

1. B-Cell Development, Survival, and Roles in Systemic Lupus Erythematosus (SLE)

B cells develop from hematopoietic stem cells (HSCs) to early-stage B cells (pro-B and pre-B) in the bone marrow. The cells move out to the peripheral lymphoid organs and differentiate from immature B cells to transitional B cells and then to activated mature B cells, and finally to memory B cells or plasma cells (short- and long-lived), which produce immunoglobulins (some immunoglobulins are autoantibodies that recognize self-targets and cause self-harm or autoimmune diseases) [figure 1].

During B-cell development, specific cell membrane determinants are expressed at different stages. CD20, a tetraspan cell surface molecule of about 33–37 kDa molecular weight and a rather limited predicted extracellular loop of 44 amino acids, is expressed at low levels on late pre-B cells. CD20 is up-regulated to ≈90 000 copies/cell on most normal and malignant B cells, with no expression on plasma cells[8] (figure 1), and is not present on other cells, including T cells.[11] CD20 is phosphorylated and may signal phosphorylation or serve as a calcium channel,[12] but the exact function of CD20 in B cells remains unknown. It is expressed at high levels, does not exist in a soluble form, and does not shed or internalize when exposed to antibody, so it is an effective target for therapy directed at B-cell abnormalities.[13]

CD22, a 135 kDa B-cell-specific transmembrane glycoprotein known to function as a co-receptor for the B-cell receptor, is variably expressed at different stages from low levels on pre-B cells to higher levels on mature B cells, and is absent on plasma cells and memory B cells[14] (figure 1). CD 22 regulates B-cell interaction with T cells and appears to play a key role in B-cell development and survival. CD22 deficiency induces reduction of mature B-cell numbers in the bone marrow and circulation, and a shorter life span and enhanced apoptosis of B cells.[15] CD22 is a target for therapy because it is only expressed on B cells; it is not exposed on embryonic stem cells or pre-B cells, nor is it normally shed from the surface of antigen-bearing cells. It is internalized by B cells and is therefore a relatively poor target for cell surface binding antibodies.[16]

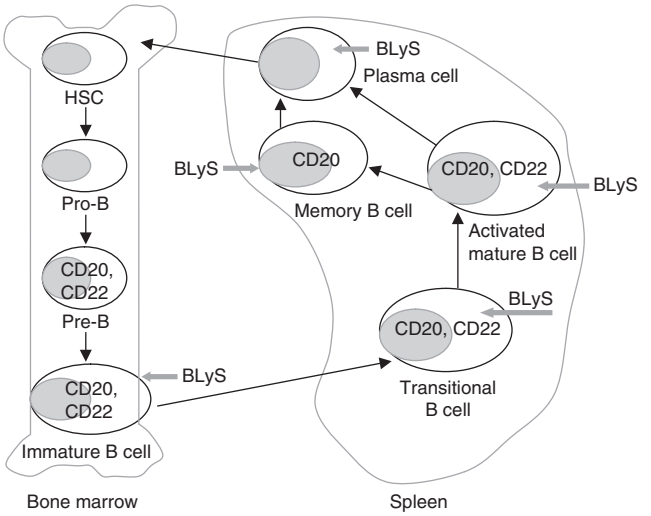

Fig. 1. B-cell development from bone marrow to peripheral lymphoid organs. Specific cell membrane determinants (CD20, CD22 and CD19) are expressed at different stages of B-cell maturation, and B cells can respond to B-lymphocyte stimulator (BLyS) from the immature B-cell stage through to differentiation into plasma cells. **HSC** = hematopoietic stem cell; **Pre-B** = pre-B cell; **Pro-B** = pro-B cell.

CD19 is also a cell surface glycoprotein expressed on B cells. Although CD19 is expressed from pro-B cells to plasma cells, it is partially internalized by B cells, hence it is a poor target for antibodies without a conjugated toxin[16] but can be used to monitor B-cell-targeted therapies.

BLyS is produced by macrophages, monocytes, dendritic cells, neutrophils, T cells and a few stromal cells, and is up-regulated in response to pro-inflammatory cytokines.[6] It has receptors on B-lineage cells: BLyS receptor 3 (BR-3, also known as TNF receptor superfamily member 13C [TNFRSF13C], and BAFFR), and, to a lesser extent, transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI, also known as TNF receptor superfamily member 13B [TNFRSF13B]) and B-cell maturation antigen (BCMA, also known as TNF receptor superfamily member 17 [TNFRSF17]). It triggers intracellular signaling to maintain the survival of B cells from immature B cells to plasma cells and promotes antibody production. Autoreactive B cells can be rescued from peripheral deletion by excess BLyS.[17] Over-produc-

tion of BLyS can result in elevated titers of multiple autoantibodies (including anti-dsDNA), circulating immune complexes, and deposition of immune complexes in the kidney leading to glomerulonephritis.[6] Blockage of BLyS in murine models of lupus inhibits both B- and secondary T-cell activation, and reduces autoantibody titers, thus improving survival.[8]

B cells were first implicated in SLE pathogenesis because plasma cells (short- and long-lived) produce autoantibodies. The breakdown of B-cell tolerance may play an initiating role in the production of autoantibodies, which contributes to disease pathogenesis in SLE through a number of classic effector roles, including immune complex formation, a direct detrimental action on chromatin, and a variety of other self-antigens.[8,18] Other mechanisms that are independent of autoantibodies also play important roles. For example, B cells can effectively present autoantigens (with an overall efficiency that is ≥1000-fold greater than for a traditional antigen-presenting cell) and provide co-stimulation signals for T cells that lead to T-cell activation.[19] B cells can also produce cytokines and chemokines (such as IL-10, IL-6, interferon-γ and lymphotoxin-α), which enhance inflammation and immunologic involvement.[20]

2. Rituximab: An Anti-CD20 Monoclonal Antibody to Deplete B Cells

Rituximab is a mouse-human chimeric monoclonal antibody consisting of human IgG1 heavy chain and κ-light chain constant regions, and mouse variable regions from a hybridoma directed at human CD20.[21] Rituximab can deplete B cells from the pre-B-cell stage to the pre-plasma-cell stage. Blood B cells are depleted most rapidly (>90% depletion in minutes) as compared with B cells from the lymph node and spleen (60–70% at day 1) and from the peritoneal cavity (significant deletion only after day 7).[8] Germinal-center and marginal-zone B cells are relatively resistant to killing; in contrast, follicular B cells are depleted efficiently.[22] The exact mechanisms of B-cell depletion in SLE are unclear but, from animal studies, rituximab is believed to deplete B cells by the following:

- antibody-dependent cell-mediated cytotoxicity via binding of the IgG1 constant regions to B cells to generate decoy sacrificial cellular immune complexes that efficiently attract and bind Fcγ receptor-expressing effector cells (monocytes, macrophages and neutrophils), which diminishes recruitment of these effector cells at sites of immune complex deposition and, therefore, reduces inflammation and tissue damage;
- complement-dependent cytotoxicity;
- apoptosis induced by hyper-crosslinking of membrane-associated CD20 molecules.[23-25]

Rituximab was first approved by the US FDA in 1997 for the treatment of B-cell lymphoma, and in 2006 it was approved for the treatment of patients with rheumatoid arthritis with inadequate response to TNF antagonists.

2.1 Effectiveness

Uncontrolled studies totalling 256 patients have strongly suggested that rituximab is effective for the treatment of SLE (including CNS disorders and nephritis), with a combined clinical response of 90%. Phase III randomized controlled trials are currently underway to evaluate its efficacy.

Six recent studies[26-31] have reported that rituximab was effective for the treatment of adult and childhood-onset SLE in about 140 patients, with a combined clinical response of >80%.

2.1.1 Effect on Global Disease Activity

An early open-label phase I/II trial (n = 17) with a long-term follow up period[32-34] showed that rituximab in varying doses (from one infusion of 100 mg/m^2 to four weekly infusions of 375 mg/m^2) without cyclophosphamide or bolus glucocorticoid treatment (other immunosuppressive medications such as azathioprine were allowed) normalized the abnormalities in B-cell homeostasis (naive B-cell lymphopenia, expansion of early plasma cells and expansion of autoreactive memory B-cell populations)[32] and significantly decreased the systemic lupus activity measure (SLAM) score over 1 year in 65% of patients.[33] Although serum anti-dsDNA and complement levels in the whole group did not change significantly,[33] a subset with effective B-cell depletion and reconstitution did have a decrease in serum autoantibodies (serologic responders, n = 4) over a 1-year period.[34] The reduction in anti-dsDNA antibodies expressing the 9G4 idiotope (indicating VH4-34 germline gene origin) was associated with a significant decrease in 9G4-specific memory B cells at 12 months, and this was maintained up to 3–5 years post-treatment. The clinical responses in these serologic responders were maintained at 1 year post-treatment, but the serologic nonresponders had only a transient clinical response (up to 6 months). Only one patient had long-term clinical remission (3 years) with complete normalization of all autoantibodies.[34]

Rituximab (two infusions of 500 or 1000 mg 2 weeks apart) with concomitant cyclophosphamide and methylprednisolone infusion may be a useful way of treating patients with SLE (n = 24) who have failed conventional immunosuppression.[35] All except one treated patient achieved B-lymphocyte depletion (<5/μL) at 3–8 months, and one patient remained depleted at >4 years. The mean British Isles Lupus Assessment Group (BILAG) global score decreased from a mean of 13.9 at baseline to 6.8 at 3 months and 5.0 at 6 months. The mean daily prednisolone dose reduced from 13.8 mg at baseline to 10 mg after 6 months. Serum anti-dsDNA decreased and C3 (serum complement component) increased significantly after 6 months.[35] After one cycle of rituximab treatment (median follow-up 39 months, range 3–78

months), 12 of the 32 patients (the original cohort and an additional eight patients) remained in clinical remission with no disease flare. Patients with baseline positive anti-extractable nuclear antigens (anti-ENA) and low serum C3 levels were more likely to flare at any time after rituximab treatment.[36] Seven of these 24 patients received re-treatment with rituximab (two infusions of 1000 mg, 2 weeks apart) because of disease relapse.[37] Four of the patients improved clinically at 4–6 months after the first re-treatment with decreased anti-dsDNA and increased C3 levels. The mean BILAG scores for all patients dropped from 15 to 6 after 5–7 months, and the median duration of clinical response was 13 months, which is longer than the initial 7 months.[35] These four patients received the second re-treatment, and two of them improved clinically, with a beneficial duration of 12 months.[37]

The rate of remission, the frequency of disease relapse after B-cell depletion and the effectiveness of re-treatment were further addressed in 11 patients by a prospective study with a long-term observational period (median follow-up of 24 months, up to 3 years).[38] A full rituximab dose (375 mg/m^2, once weekly for 4 weeks) was given with one concurrent cyclophosphamide infusion at the start, and a two-dose regimen (1 g, 2 weeks apart) was re-administered if disease relapse occurred. Peripheral blood B cells were depleted completely (<2/μL) at 4 weeks in all SLE patients (n = 11), followed by 100% remission (six complete and five partial) within a median of 4 months, a reduction in the dose or withdrawal of concomitant immunosuppressive agents, and a decrease in proteinuria in six patients with active lupus nephritis. Disease relapses occurred within a median of 12 months in 64% of patients on or after the return of circulating B cells. Re-treatment of rituximab in six patients achieved 100% remission again rapidly (within a median of 2 months), and one patient was treated a fourth time, with further remission. In contrast, anti-dsDNA levels did not decrease significantly even in seven patients in whom remission lasted longer than 20 months.

2.1.2 Effectiveness in Lupus Nephritis

The therapeutic effects of rituximab on proliferative lupus nephritis, the most severe manifestation of SLE, have been the subject of three studies.[39-41] In a total of 39 treated patients who received four weekly infusions of rituximab 375 mg/m^2, or 0.5–1 g on days 1 and 15 (in addition to immunosuppressive therapy), significant clinical improvement was observed, with a reduction in SLE disease activity index (SLEDAI) scores, anti-dsDNA antibody levels, proteinuria and/or anti-C1q (anti-complement) antibody levels, and an increase in complement level. In addition, improvement in the histopathologic findings was observed, and the renal activity index decreased significantly between the first and the repeat biopsy (within 12 months). The renal chronicity index remained largely unchanged (p = 0.77), whereas a significant increase in the renal chronicity index was observed (p = 0.015) between the biopsy obtained at the time nephritis was diagnosed and the pre-rituximab biopsy,[41] suggesting that rituximab may stop progression of lupus nephritis.

2.1.3 Effectiveness in Neuropsychiatric SLE

Neuropsychiatric SLE is a serious intractable phenotype. In a recent study,[42] six patients were treated with rituximab 375 mg/m^2 once weekly for 2 weeks, and one patient received a single administration of the same dose. Two patients received rituximab 500 mg once weekly for 4 weeks, while one patient was treated with 1000 mg once every 2 weeks for a total of 4 weeks. Treatment with rituximab resulted in rapid improvement of CNS-related manifestations, particularly acute confusional state. It also reduced the SLEDAI score at day 28 in all 10 patients and improved cognitive dysfunction, psychosis, seizure, cerebral blood flow and abnormal MRI findings. These effects lasted for >1 year in five patients. Rituximab may not act on the CNS directly because it cannot cross the blood-brain barrier due to its large molecular weight. Its effects on the CNS may be mediated by its down-regulation of CD40 and CD80 on B cells, and CD40 ligand (CD40L), CD69 and inducible co-stimulator (ICOS) on CD4+ T cells.

2.1.4 Effectiveness in Childhood-Onset SLE

Childhood-onset SLE has significant morbidity despite aggressive immunosuppressive therapy, and tends to have more severe hematologic and renal involvement than adult-onset disease. Rituximab has been reported in the treatment of childhood-onset SLE in more than 50 patients,[43-47] with a clinical response of 75%. In seven patients (aged 7.7–16.1 years) with active SLE that was resistant to standard immunosuppressive agents, intravenous infusions of rituximab (750 mg/m^2 on days 1 and 15) significantly improved the BILAG score from a median score of 22 at baseline to a median score of 6 at follow-up (p = 0.002) over a period of 6–18 months.[43] In two patients with severe multi-system and life-threatening disease unresponsive to standard therapy, renal replacement therapy was successfully withdrawn following rituximab therapy, and renal function and proteinuria subsequently improved.[43] There was a trend toward an increase in complement C3 and C4 levels, with a reduction in anti-dsDNA antibodies.[43,45]

2.1.5 The Role of Concomitant Medications

In most of these non-controlled studies, concomitant use of immunosuppressive agents such as cyclophosphamide and glucocorticoids was allowed (tables I and II), which may confound the effectiveness of rituximab – although it makes little difference in rheumatoid arthritis.[48] Because the patients had active disease despite intensive immunosuppressive treatments at the time of study entry, this indicates that the concomitant drugs were unlikely to have played a major role in the induction of disease remission. Concomitant cyclophosphamide may have a higher rate of effectiveness[35] than no concomitant cyclophosphamide infusion;[33] however, two rituximab-treated patients without concomitant

Table I. Early clinical data (to 2005) of rituximab in refractory systemic lupus erythematosus (SLE)

Study design	Study period	Effectiveness	Safety	References
Open study: n = 32; 500 (n = 6) or 1000 mg, × 2 [2 wk apart]; IV cyclophosphamide (750 mg × 2 [2 wk apart]) and glucocorticoids	3–78 mo	B-cell depletion in 30/32 pts (94%) within 3–8 mo; BILAG ↓, C3 ↑, anti-dsDNA ↓, anti-nucleosome antibodies ↓, 9G4+ anti-dsDNA ↓	1 severe reaction with thrombocytopenia, 1 urinary tract infection, 1 thoracic shingles, 1 pneumococcal pneumonia, 2 serum sickness, 1 HACAs, 1 grand mal seizure; Igs ↓ but normal	35,36,49,50
Phase I/II trial: n = 17; 100 mg/m^2 × 1 (n = 6), 375 mg/ m^2 × 1 (n = 7), and 375 mg/m^2 [once weekly for 4 wk]; no IV cyclophosphamide or glucocorticoids	1 y	B-cell abnormalities normalized in 11/17 pts (65%), SLAM ↓ in 16 pts (p = 0.046) at 2 mo, anti-dsDNA and C ~ but ↓ in autoantibodies in 4/17 pts over 1 y	Well tolerated; 1 mild infusion reaction, 3 unrelated serious adverse events, 6 (35%) HACAs; IgG and IgM ↓ but normal	32,33
Phase I trial: n = 8; 375 mg/m^2 [once weekly for 4 wk]; IV glucocorticoids	1 y	Full B-cell depletion in 5/8 pts (63%) for 6–10 mo, and in 2/8 for <5 mo, SLEDAI ↓ in 6/8 (75%), long-term remission (>6 mo) in 2/8 (25%); autoantibody C ~	Well tolerated; 1 transient bradycardiac episode, HACAs 3 (38%), Ig ~	51,52
Open study: n = 5 (CNS disorders); 375 mg/m^2 [once weekly for 2 wk];	6–20 mo	B cells ↓, CD40 and CD80 on B cells ↓; clinical conditions all improved; WBC ↑, ESR ↓, C ↑, anti-dsDNA ↓	Well tolerated; 1 infection (herpes zoster) with IgG ↓, 1 HACAs	53
Open study: n = 10 (lupus nephritis); 375 mg/m^2 [once weekly for 4 wk]; oral prednisolone	12 mo	B-cell depletion in 8/10 pts for 1–7 mo; regulatory T cells ↑; partial remission in 8/10, complete remission in 5/10 with 4/10 sustained for 12 mo; C ↑, anti-dsDNA ↓, antinuclear antibodies ↓	1 hypersensitivity reaction, 3 mild infections, 1 pneumococcal meningitis, Igs ↓ but only IgM significantly	39,54
Open study: n = 15; 375 mg/m^2 [once weekly for 4 wk]; IV cyclophosphamide (0.5 mg/m^2 × 2) plus glucocorticoids	2–50 mo	BILAG and SLEDAI all ↓, dosage of prednisolone ↓	No report	55
Open study: n = 7 (children); 750 mg/m^2 on days 1 and 15; IV cyclophosphamide (750 mg on days 2 and 16) and glucocorticoids	1 y	B-cell depletion in 5/7 pts at study end, reappearance in 2/7; BILAG all ↓, C ↑, anti-dsDNA ↓, hemoglobin ↑, renal function ↑ in 2/7	No significant infusion-related or later adverse events	43
Open study: n = 8 (children); 375 mg/m^2 [once weekly for 4 wk]	3–13 mo	Partial response in 7/8 pts (88%), C3/C4 ↑, fatigue ↓, proteinuria ↓, anemia ↓	1 zoster, 1 infusion reaction, 1 hypogammaglobulinemia	44
Open studies; n = 102 adults or children; mostly full dosage (375 mg/m^2)	>2 mo	Clinical response in 93/102 pts (91%)	Infections: 9/102 (9%); HACAs: 11/102 (11%)	Above studies combined

BILAG = British Isles Lupus Assessment Group; **C** = complement components; **CNS** = central nervous system; **dsDNA** = double-stranded DNA; **ESR** = erythrocyte sedimentation rate; **HACAs** = human antichimeric antibodies; **Igs** = immunoglobulins; **IV** = intravenous; **pts** = patients; **SLAM** = systemic lupus activity measure; **SLEDAI** = SLE disease activity index; **WBC** = white blood cells; ↑ indicates increased; ↓ indicates decreased; ~ indicates inconsistent or unchanged.

Table II. Recent open studies (from 2006) of rituximab in refractory systemic lupus erythematosus (SLE)

Study design	Study period	Effectiveness	Safety	References
Open study: n = 11; 375 mg/m^2, × 4 [weekly], further 2 g 2 wk apart for disease relapse; IV cyclophosphamide 500 mg × 1; no IV glucocorticoids	Median 24 mo	B-cell depletion in all pts within 4 wk, re-depletion after re-treatment; BILAG ↓, 100% remission (6 complete); ESR ↓, proteinuria ↓; re-treatment after relapse: all quick remission; anti-dsDNA ~	Infusion reaction: common, 1 severe; infection: 23% (maybe unrelated); IgG and IgM in normal range; HACAs in 3 of 6 tested pts	38
Open study: n = 22 (lupus nephritis); 0.5–1 g at days 1 and 15 added to the immunosuppressive therapy	90 days	B-cell depletion in 20/22 pts (91%); disease activity (MEX-SLEDAI index) ↓ in 90% of pts at 60 days ($p < 0.05$); proteinuria ↓, regulatory T cells ↑	1 death due to invasive histoplasmosis; no important adverse events in other pts	40
Open study: n = 7 (nephritis); 375 mg/m^2 on days 2, 9, 16 and 23; IV cyclophosphamide 0.5 g/m^2 on days 1 and 23 + glucocorticoids	12 mo	SLEDAI all ↓; prednisolone dosage ↓ in 6/7 pts, serum creatinine ↓, anti-dsDNA ↓, anti-C1q ↓, C3 and C4 ↑, renal histopathology: improved	1 photosensitive eruption, 1 herpes zoster infection, 1 neutropenic fever, 1 urinary tract infection	41
Open study: n = 11; 375 mg/m^2 once weekly for 4 wk; IV cyclophosphamide 0.5 g/m^2 at weeks 1 and 4 + glucocorticoids	6–30 mo	B-cell depletion 100%; SLAM all ↓; anti-dsDNA ↓, anti-C1q ↓, regulatory T cells ↑	IgG ~, IgM ↓, IgE ↓	56
Neuropsychiatric SLE (n = 10): 375 mg/m^2 once weekly for 2 wk (n = 6), 375 mg/m^2 × 1 (n = 1), 500 mg/m^2 × 4 (n = 2), 1 g once every 2 wk for 4 wk (n = 1)	7–45 mo	B cells ↓, clinical conditions (CNS disorders, particularly acute confusional state) all improved, SLEDAI all ↓, MRI lesions ↓, cerebral blood flow ↑	2 pneumonia, 1 herpes zoster, 1 chickenpox, 1 intractable infection of decubitus ulceration	42
Open study: n = 15; 1 g, × 2 every 2 wk (n = 10) or 500 mg once weekly for 4 wk; no cyclophosphamide or glucocorticoids	28 wk	B-cell depletion in all pts, BILAG ↓, major and partial clinical response in 9/14 (64.3%), C3 ↑, anti-dsDNA ~, prednisolone dose ↓	2 severe infections (pneumonia and enteritis), 6 moderate infections, 1 mild infusion reaction, 4 (27%) HACAs	57
Open study: n = 6; 375 mg/m^2 once weekly for 4 wk or 1 g, × 2 on days 3 and 18; IV or oral steroids	8–21 mo	B cells ↓ in 4/5 pts, SLEDAI ↓, improvement of 1 or multiple manifestations in 5/6, prednisone dose ↓ in 5/6, C3 ↑, anti-dsDNA ~	1 herpes zoster infection, 1 urinary tract infection, Igs ~	58
Open study: n = 11 (girls); 350–450 mg/m^2 × 2–12 with corticosteroids; IV cyclophosphamide in 2 pts	6–26 mo	B-cell depletion in 7 of 8 evaluated pts, remission in 8/11 (73%), anti-dsDNA ↓ in 6/11, C normalized in 4	5 severe adverse events including septicemia in 2 and hematologic toxicities in 4; 3 non-severe infections, IgG and IgM ↓ in 3 and 5 pts, respectively	45
Open study: n = 14 (children); 375 mg/m^2 × 1 with methylprednisolone 100 mg	Up to 29 mo	Partial remission in 8/14 pts (57%), complete remission in 4/14 (29%), ESR ↓, C ↓, anti-dsDNA ↓	3 fevers	46
Open study: n = 12 (childhood onset) 600 mg/m^2 6 mo apart; IV cyclophosphamide	0.3–1.8 y	SLEDAI all ↓, prednisolone dose ↓, C3 ↑, ESR ↓	IgG ~, IgM ~	47
3 recent open studies: total n = 35		Clinical response in 34/35 pts (97%), C3 ↑, anti-dsDNA ↓, proteinuria ↓	4 mild infections (11%)	59-61
n = 154 adults or children; mostly full dosage (375 mg/m^2)	>6 mo	Clinical response in 137/154 pts (89%)	Infections 19%, HACAs 7/149 (5%)	Above studies combined

BILAG = British Isles Lupus Assessment Group; **C (C1q, C3, C4)** = complement components; **CNS** = central nervous system; **dsDNA** = double-stranded DNA; **ESR** = erythrocyte sedimentation rate; **HACAs** = human antichimeric antibodies; **Igs** = immunoglobulins; **IV** = intravenous; **MEX** = Mexican; **pts** = patients; **SLAM** = systemic lupus activity measure; **SLEDAI** = SLE disease activity index; ↑ indicates increased; ↓ indicates decreased; **~** indicates inconsistent or unchanged.

cyclophosphamide also achieved a significant clinical response.[35,49] The correlations between B-cell return and disease relapses implicate rituximab as being largely responsible for long-term remission.[38] It is unclear whether there are interactions between rituximab and any of the immunosuppressive agents, and this needs to be determined in future studies.

2.1.6 Effectiveness and Autoantibody Reduction

The mechanisms of rituximab therapy for SLE are unclear, but the autoantibody-independent mechanisms such as up-regulation of regulatory T cells[40,54,56] and down-regulation of CD40 and/or CD80 on T or B cells to decrease T-cell activation[39,53] may play roles. Ideally, B-cell depletion in SLE should reduce or eventually wipe out the autoimmune antibody clones, as has been shown to occur in the treatment of rheumatoid arthritis.[48] While six studies reported that disease-specific autoantibodies remained relatively stable during rituximab therapy, nine studies reported that anti-dsDNA antibody and/or other autoantibodies decreased significantly after treatment (tables I and II). The reasons for this discrepancy are unknown but may reflect the differences in disease severity, treatment duration, treatment doses and genetic susceptibilities in different studies, and effects on different plasma cells (short- and long- lived). In 16 of the 24 patients from a recent report,[49] both ANA and anti-dsDNA antibodies decreased to 64% and 38% of baseline values, respectively, 6–8 months after rituximab treatment. Interestingly, there was a trend toward a greater decrease in anti-dsDNA antibodies in patients with the longer clinical response (n = 9) compared with those experiencing a disease flare within 1 year (to 37% vs 83% of baseline values, respectively, at 10–14 months), and flares in two patients were accompanied by a rise in 9G4+ anti-dsDNA antibodies (9G4 expression was positive in four of six patients tested). This study suggests that a sustained clinical response to rituximab is associated with a trend toward a greater decrease in anti-dsDNA and antichromatin antibodies. Consistent with this, Anolik et al.[34] reported that serologic responders (who had a decrease in anti-dsDNA antibodies) had maintained a much longer clinical response than serologic nonresponders, and Jonsdottir et al.[62] reported that greater reductions in serum IgA anti-DNA were strongly associated with, and preceded, major clinical response after rituximab therapy, suggesting that reduction in autoantibodies is an important mechanism of action.

2.2 Adverse Events

Most studies suggest that rituximab is well tolerated in the treatment of SLE (tables I and II). As with other immunosuppressive therapies, the possibility of infectious complications remains a concern for B-cell depletion therapy but may also reflect concomitant medication use. A total of 40 subjects (16%) had infectious events during rituximab treatment. Although some studies found that serum IgM, IgG and/or IgE levels decreased significantly,[39,45,56] most studies reported that Ig levels were not significantly affected in rituximab-treated patients, which may in part be responsible for the safety profile (low infection rate) of rituximab. The infectious events included urinary tract infection, pneumococcal pneumonia and meningitis, thoracic shingles, herpes zoster, histoplasmosis, decubitus ulceration, enteritis and septicemia, most of which were treated successfully with antimicrobials. One patient died from invasive histoplasmosis, massive pulmonary hemorrhage and mucormycosis; the investigators suggested that the inhibitory effect of rituximab on the immune system may have contributed to the fatal opportunistic infection, although this patient was diabetic and was receiving three immunosuppressive drugs plus high doses of glucocorticoids.[40] In 22 patients (11 SLE and 11 vasculitis) undergoing re-treatment (2–3 cycles), there were four cases with severe bacterial infections (two pneumonia, one urinary tract infection, and one subcutaneous abscess) and one with cutaneous herpes zoster infection, which the authors suggested may not have been directly attributable to rituximab.[38] In 11 girls with severe SLE, two patients had septicemia, which was caused by *Streptococcus* species and then *Streptococcus bovis* in one patient, and by *Escherichia coli* in the other patient, within 2 months after rituximab infusion.[45] In December 2006, the FDA warned of safety concerns regarding rituximab in the treatment of SLE after two rituximab-treated SLE patients developed progressive multifocal leukoencephalopathy, a fatal viral infection of the CNS.[63] It should be noted that concomitant immunosuppressive medications, severe disease and significant leukopenia may confound the observations; therefore, randomized controlled trials with appropriate placebo arms and sufficient power will be required to determine the true infection risks in SLE.

Another safety-related concern is the development of human antichimeric antibodies (HACAs). The frequency of HACAs in all SLE studies is 7.4% (tables I and II). While four studies (n = 17, 8, 6, and 15, respectively) reported high frequencies (35%, 38%, 50% and 27%, respectively),[33,38,52,57] other studies reported very low or even no occurrence (≈1% in total), which is consistent with the studies in lymphoma (<1%)[64] and rheumatoid arthritis (4%).[48] The reasons for the high frequencies of HACAs in these four studies are unclear. Although Looney et al.[33] suggested that the low dose of rituximab used in the majority of patients in their study may have contributed to the increased frequency of HACAs, other studies[38,57] that used full doses of rituximab also had a high frequency of HACAs, suggesting that the dose of rituximab may be irrelevant. In contrast to other studies with low occurrence of HACAs, intravenous corticosteroids were not used concurrently in three of these four studies,[33,38,57] suggesting that such treatment may prevent the development of HACAs. HACAs might be associated with infusion reactions, reduced drug efficacy or even serum sickness. Smith et al.[38] documented that while there were

no clear differences in the rate or duration of remission between HACA-positive and HACA-negative patients, relapse was more common in HACA-positive patients (five of five) than in HACA-negative patients (four of nine). There was also one severe infusion reaction and one failure to achieve complete B-cell depletion with rituximab re-treatment in two patients with the highest HACA titers, suggesting that HACAs may be clinically significant.

Six recent studies[26-31] have suggested that rituximab treatment was well tolerated, and the adverse events included infections, infusion reactions, HACAs, and serum sickness. Six patients (four from one study[28] on SLE patients with severe disease) died, most likely because of disease progression.

3. Epratuzumab: an Anti-CD22 Monoclonal Antibody

Epratuzumab is a humanized anti-CD22 IgG1 monoclonal antibody that binds to the CD22 extracellular domain, with an affinity of Kd = 0.7 nmol/L. Binding of epratuzumab to B cells results in rapid internalization of the CD22/antibody complex and modest but significant CD22 phosphorylation.[65] Epratuzumab has a very distinct mode of action, acting as an immunomodulatory agent, while rituximab is a cytotoxic therapeutic antibody. Epratuzumab shows no complement-dependent cytotoxicity, but does demonstrate modest yet significant antibody-dependent cellular cytotoxicity. It does not totally deplete circulating B cells, but reduces this population by 30–45%.[66]

The effectiveness and safety profiles of epratuzumab in the treatment of SLE have been reported recently.[67] In an open-label, single-center study of 14 patients with moderately active SLE, patients received intravenous epratuzumab 360 mg/m^2 every 2 weeks for four doses, with analgesic/antihistamine premedication (but no corticosteroids) prior to each dose. Total BILAG scores were decreased by ≥50% in all 14 patients (including 77% with a ≥50% decrease at 6 weeks). At the final 32-week evaluation, 15% of the patients achieved ≥50% improvement. B-cell levels decreased by an average of 35% at 18 weeks and remained reduced at 6 months post-treatment. CD27$^-$ B cells and CD22 surface expression on CD27$^-$ B cells (mainly naive and transitional B cells) were profoundly suppressed.[68] There was no evidence of immunogenicity or significant changes in T cells, Ig or autoantibody levels. A total of ten patients reported adverse events, including six mild and transient infusion reactions, five infections (herpes zoster, otitis media, *Helicobacter pylori*-associated gastritis, vaginitis/vaginal candidiasis, cystitis and tonsillitis), and one spinal contusion from a traffic accident. This study suggests that anti-CD22 immunotherapy with epratuzumab is well tolerated and may be effective in the treatment of SLE with moderate disease activity. However, it seems to have a short duration of effect (achieving ≥50% improvement in the total BILAG score, from 77% at week 6 to 15% at week 32[67]), possibly due to its inability to deplete B cells completely. Combining rituximab and epratuzumab did not decrease the ability of rituximab to induce apoptosis, complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, and may have enhanced its clinical efficacy.[66]

4. Belimumab: a Humanized Anti-B-Lymphocyte Stimulator Monoclonal Antibody

Belimumab, the first in a series of human monoclonal antibodies that specifically recognizes and inhibits the biologic activity of BLyS, is currently undergoing phase III clinical trials for the potential treatment of autoimmune diseases, including SLE. Belimumab is specific in its binding to BLyS and does not bind to a number of other TNF ligand family members. Belimumab binds BLyS in both a solid-phase capture assay and in solution, but does not recognize membrane-bound BLyS. It inhibits the binding of BLyS to extracellular fusion protein forms of all three receptors (BR-3, TACI and BCMA) with equivalent potency *in vitro*. In individuals with stable mild and moderate SLE, the half-life of belimumab is reportedly 13–17 days.[6]

In a randomized, double-blind, phase I study, patients (91% female; mean age 41 years) with stable mild-to-moderate SLE disease activity had received a stable standard-of-care SLE treatment regimen for 2 months prior to enrollment. Belimumab (1, 4, 10 and 20 mg/kg; n = 57) or placebo (n = 13) was administered intravenously as a single infusion or two infusions 21 days apart. Patients were followed for 84–105 days to assess disease activity, using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) SLEDAI and measures of peripheral B-cell concentrations and serology. Compared with placebo, all belimumab doses significantly inhibited CD20$^+$ cells (B cells) by 12–47% at one or more assessments between days 42 and 105. Reductions in anti-dsDNA or immunoglobulin levels were observed in some belimumab groups. SLE disease activity was not changed over this treatment period.[6]

In a multicenter, placebo-controlled, double-blind, phase II trial,[6,69-72] patients with active SLE (n = 449) were randomized to receive belimumab (1, 4 or 10 mg/kg) or placebo intravenously, together with standard-of-care therapy, on days 0, 14 and 28, then every 28 days for 76 weeks. Placebo patients switched to belimumab at week 52. Belimumab did not meet the overall primary efficacy endpoints of reducing the signs and symptoms of SLE, as measured by the SELENA-SLEDAI instrument score, or increasing the time to the first SLE flare over 52 weeks. However, the treatment significantly reduced both the SELENA SLEDAI score (p = 0.044) in seropositive patients (ANA ≥1 **:** 80 or anti-dsDNA ≥30 IU; 71.5% of total participants) and the physician's global disease assessment in the whole population (p = 0.002) and the seropositive population (p = 0.001) at week 52. It also significantly improved health-related quality of life in seropositive patients.

Analysis of BILAG organ system scores demonstrated that belimumab slowed disease progression, reflected by increases in BILAG score across five of eight systems. A lesser requirement for prednisone therapy was also seen in patients treated with belimumab than with placebo (7% vs 15%; $p < 0.05$). Furthermore, treatment induced a 33% and 46% increase in the C4 complement at weeks 52 and 76, respectively, among patients with low baseline C4. IgG, IgA, IgE and IgM were reduced or normalized. Belimumab significantly reduced levels of circulating B cells (total CD20+ B cells, CD20+/CD138+ plasmacytoids and CD20+/CD27+ activated B cells) by 54–84% and anti-dsDNA autoantibodies by 30% at week 52, and further decreased B cells and anti-dsDNA autoantibodies at week 76. At week 52, 15% of anti-dsDNA+ subjects on belimumab treatment seroconverted to negative, versus 3% on placebo treatment. A ≥50% reduction in anti-dsDNA was associated with a greater reduction in the modified SELENA SLEDAI score ($p = 0.009$).

In the phase I study,[6] the overall incidence of adverse events was similar between the belimumab and placebo groups. There was no increased incidence of infections in the belimumab-treated groups, and none of the infections reported were related to this treatment. Serious adverse events were experienced by six patients, with similar frequencies observed in the placebo and treatment groups, and were not considered to be related to the study agent. One patient experienced an infusion reaction at the highest single dose, and one patient developed neutralizing antibodies to belimumab.[6] In the phase II study, no clinically significant differences were noted in safety between the belimumab and placebo groups. The patients with belimumab treatment had fewer cases of pleurisy (3% vs 8%; $p < 0.05$) but more urticaria (4% vs 0; $p < 0.05$). Infusion reactions were rare, while immunogenicity to belimumab was observed in one subject (1 mg/kg group).[69]

5. Conclusions

B cells not only secrete antibodies to eliminate foreign antigens but also are able to ingest and present antigens, express co-stimulatory molecules, and secrete chemokines and cytokines. They also regulate T-cell activation, the formation of lymphoid tissue, and the functions of dendritic cells. On the basis of these characteristics, the B cell has become an exciting new target for the management of autoimmune diseases, including SLE.

Uncontrolled studies and preliminary randomized controlled trials have suggested that current therapies targeting B cells, either via specific cell membrane-associated determinants such as CD20 and CD22 or by blockage of B-cell survival factors such as BLyS, are well tolerated and may be effective in the treatment of SLE. Rituximab, an anti-CD20 monoclonal antibody that effectively depletes circulating B cells, achieved a clinical response of 86% for refractory SLE in a total of approximately 400 patients with or without concomitant use of cyclophosphamide. Its efficacy for induction of remission versus maintenance of remission, and its potential for the rescue of disease that is refractory to other modalities, should be determined by future randomized controlled trials. In contrast, epratuzumab (an anti-CD22 monoclonal antibody) and belimumab (an anti-BLyS monoclonal antibody), both of which partially deplete B cells, appear to have modest effects on SLE. The combination of rituximab with belimumab or epratuzumab for the treatment of refractory SLE could improve clinical benefits because they have quite distinct mechanisms of action but may also have greater toxicity. Recent studies[73,74] reported that BLyS levels increased significantly after B-cell depletion and decreased upon B-cell repopulation in SLE patients, suggesting that complementary anti-BLyS therapy is essential to improve the clinical response in rituximab-treated patients. These combinations may also significantly decrease serum immunoglobulin levels (belimumab was demonstrated to reduce immunoglobulins in SLE[71]), which could subsequently increase susceptibility to opportunistic infections. Further randomized controlled trials will be required to determine the benefits and risks of combination therapies for SLE.

Other B cell-targeted therapies for potential treatment of SLE are currently under investigation. For example, ocrelizumab and ofatumumab, two humanized monoclonal antibodies to CD20, have demonstrated encouraging clinical benefits for the treatment of rheumatoid arthritis[75,76] and may have less immunogenicity than rituximab. TRU-015, a CD20-directed small modular immunopharmaceutical (SMIP™) [single chain polypeptide] drug candidate that effectively depletes B lymphocytes in a dose-dependent manner, has demonstrated meaningful clinical benefit in subjects with active rheumatoid arthritis despite methotrexate therapy[77] and may also have potential for SLE treatment. Atacicept (TACI-Ig), a soluble form of TACI and a recombinant fusion protein that blocks not only the activity of BLyS but also APRIL ('a proliferation-inducing ligand', the family member with greatest homology to BLyS), has demonstrated biologic activity in the treatment of SLE.[78] Abatacept (CTLA4-Ig), a recombinant fusion protein that interrupts B-T cell collaboration through inhibition of the CD40:CD40L pathway and CD28:B7 co-stimulatory interaction, has been demonstrated to be effective in mouse models of lupus.[79] Further clinical trials are needed to determine the beneficial effects of these new agents compared with rituximab or belimumab in the treatment of SLE.

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