

Monoclonal Antibodies in the Treatment of Systemic Lupus Erythematosus

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Abstract: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by B cell hyperactivity and defective T-cell function, with production of high titer autoantibodies. In the recent years, conceptual advances and the introduction of new therapies are yielding improvements in the management of this disease. In recent years, clinical studies have been undertaken with selected monoclonal antibodies (mAbs) in the treatment of SLE. The important role of B cells in the pathogenesis of autoimmune disorders has provided a strong rationale to target B cells in SLE. Selective therapeutic depletion of B-cells became possible with the availability of the anti-CD20 antibody rituximab and anti-CD22 antibody epratuzumab. Several clinical studies confirm high activity of rituximab in SLE patients especially with lupus nephritis and neuropsychiatric involvement. Recently, several new mAbs reacting with CD20 have been developed. New mAbs directed against CD20 include fully human mAb ofatumumab (HuMax CD20), IMMU-106 (hA20) which has a >90% humanized framework and GA-101, a novel third-generation fully humanized and optimized mAb. These agents are highly cytotoxic against B-cell lymphoid cells. Proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) play an important role in propagating the inflammatory process responsible for tissue damage. Blocking of these cytokines by mAbs can be also a successful therapy for patients with SLE. Finally, mAb eculizumab that specifically inhibits terminal complement activation has been recently developed and investigated in the phase I single dose study in SLE. In this review, new mAbs, potentially useful in SLE are presented.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the B cell hyperactivity and defective T-cell function, with production of high titer autoantibodies and involvement of multiple organ systems [1-3]. So far, the pathogenesis of this disease has not been definitely elucidated. However, based on recent knowledge, impairment of at least three main mechanisms seems to be involved in SLE development: apoptosis, antigen presentation and immune response [1,4].

SLE is a clinically heterogeneous disease typically affecting women in their childbearing age. Common manifestations include arthralgias and arthritis, malar and other skin rashes; pleuritis or pericarditis, renal or central nervous system involvement and hematologic cytopenia [1,2]. The disease has an extremely variable clinical picture characterized by a wide variety of active clinical manifestations, by an alternating course of flares and remissions and by the possible presence of chronic sequel of the disease itself and/or treatment received [1].

The diagnosis of disease requires evidence of 4 out of 11 clinical and/or laboratory symptoms proposed by the American College of Rheumatology [5]. For many years, attention has been drawn to the problem of finding a valid and sensitive tool for measuring SLE activity and more than 60 different methods have been proposed. Unfortunately, none of

them has been generally accepted. Amongst the most frequently used are SLEDAI scale and its modifications: BILAG and SLAM. The systems have been validated in prospective studies, and their reproductibility, validity and sensitivity have been compared. At present, they are widely used in clinical practice [6-8].

Over the last two decades, a dramatic improvement in the prognosis of SLE patients has been observed. It is a result of earlier diagnosis and treatment, better patient education, availability of corticosteroids and more potent immunosuppressive agents, and better supportive care for infective complications and renal replacement therapy [9]. After the introduction of immunosuppressive drugs, lupus patients now achieve a 10-year survival rate of 90-95% [10,11]. The decrease in the mortality caused by active disease manifestations has led to a longer survival of patients with SLE [10, 12]. In consequence, the causes of death observed in patients with SLE in recent years are different from those reported in the past. Currently, the most frequent causes of death in patients with SLE are infections, acute cardiovascular events caused by atherosclerosis, and cancer.

Antimalarias, antiinflammatories and immunosuppressive drugs have been the basis for SLE therapy over the past 30 years [13,14]. Patients with mild SLE can be usually maintained on a combination of nonsteroidal antiinflammatory drugs and antimalarias. However, when these agents have failed to control symptoms of arthralgia/arthritis or rash, sufficiently low dose corticosteroids are used [15]. Moreover, the onset of active SLE with major organ involvement requires prompt, aggressive therapy with cyclophosphamide. Azathioprine may also be used as a steroid sparing agent in

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patients in whom reducing the dose of prednisone results in a flare. Methotrexate may also have a place in the treatment of patients in whom cutaneous or articular manifestations have proven refractory to hydrochloroquine and low dose prednisone. Other traditional therapies occasionally used in SLE patients include plasma exchange and intravenous high dose immunoglobulins. Moreover, cyclosporin in low doses may offer reasonable disease control, especially when combined with low doses of steroids.

Recently, the advances in monoclonal antibody (mAb) technology and identification of factors and pathways initiating and maintaining autoimmune disorders allow to introduce a variety of monoclonal antibodies (mAbs), potentially useful in the treatment of SLE, into intensive preclinical investigation and early clinical trials [16, 17]. These mAbs are designed mainly to target different molecules on B-cell and cytokines involved in SLE pathogenesis, especially interferon- α (IFN- α), interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α). In this review, mAbs potentially useful in the treatment of SLE are presented.

MONOCLONAL ANTIBODIES AGAINST B-CELLS

The rationale for B-cell directed mAbs in SLE is multi-fold with a growing body of evidence strongly pointing to the importance of the central role of B lymphocytes in both murine and human SLE [17]. In SLE, dysregulated B cells contribute to the pathogenesis of the disease by an autoimmune mechanism mediated by autoantibodies [18]. B-cells may also participate in presenting autoantigens to T cells, and regulating and organizing inflammatory responses through cytokine and chemokine secretion. In B-cells obtained from SLE patients, several intrinsic defects have been observed, including increased expression of CD154, CD80, CD86 and IL-10 [19-21]. Moreover, enhanced mutational activity of *V κ* gene rearrangement is markedly enhanced, compared to normals but with no subsequent positive selection of mutations [22]. The enhanced mutational activity may play a role in the emergence of autoreactivity in SLE patients. In addition, B-cell receptor (BCR) signaling defects have been reported in SLE patients including molecular defects of Lun, HS1 and SHIP. In consequence, calcium mobilization and tyrosine phosphorylation are upregulated upon activation of BCR on SLE B-cells [23, 24]. In addition, a naive B-cell lymphopenia and an expansion of peripheral blood plasmablasts have been documented in patients with SLE [25, 26]. Therefore, mAbs that target B-cells directly and bind B-cell surface antigens such as CD19, CD20, CD21 and CD22 are of special interest in the treatment of this disease [27].

RITUXIMAB

Rituximab (IDEC C2B8) (Rituxan®; Genentech, Inc., San Francisco CA, and IDEC Pharmaceuticals, San Diego, CA; MabThera®, Roche, Basel, Switzerland) is a high affinity chimeric mouse anti-CD20 mAb currently used for the treatment of non-Hodgkin lymphoma (NHL). The antibody is an IgG1 kappa immunoglobulin containing murine light and heavy-chain variable region sequences and human constant region sequences [28, 29]. The Fab domain of rituximab binds specifically to the CD20 antigen expressed on

normal and malignant B-lymphocytes. The Fc domain recruits immune effect or functions to mediate B-cell lysis *in vitro* and *in vivo*. The precise mechanism of rituximab cytotoxicity remains unclear. However, several mechanisms by which rituximab may be cytotoxic are suggested. These include complement dependent cytotoxicity (CDC), which involves fixation of complement by the Fc portion of immunoglobulin and the subsequent activation of the complement cascade [30-32]. Moreover, rituximab induces antibody dependent cell mediated cytotoxicity (ADCC) *in vitro*. These two mechanisms are categorized into the "immunomobilizing" mechanism or direct effects. However, accumulating evidence suggests that rituximab can also directly induce apoptosis [33]. Rituximab rapidly eliminates most circulating B-cells, suggesting that it could be beneficial in autoantibody-mediated diseases by targeting the autoreactive B-cells [34]. Moreover, some studies on other autoimmune diseases did not show any correlation between the decline in autoantibody levels and response, suggesting that additional mechanisms involving antigen presentation and help to T cells are important [35]. However, CD20 is not expressed on all B-cell forms. In particular, progenitors, as well as plasma cells, may be devoid of CD20 and hence unresponsive to this agent. Thus, after rituximab treatment plasma cells still may continue to produce disease-causing autoantibodies for months or even years [36]. Moreover, it is unknown whether all CD20 B-cells are equally susceptible to rituximab mediated deletion. Until now, there have been little or no data regarding depletion of B-cells at sites other than blood. Moreover, there may be pro-survival factors acting in certain autoimmune diseases, or in lymphoproliferation-associated conditions, which may protect B-cells from the mechanisms of action of this agent. A recent study has shown that treatment with rituximab affects both the cellular and humoral arm of the immune system in patients with SLE [37]. Rituximab was the first MoAb approved in 1997 by the Food and Drug Administration (FDA) for the treatment of cancer [34]. This MoAb demonstrates significant activity in patients with various lymphoid malignancies, including indolent and aggressive forms of B-cell NHL, chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL) [38-41]. Rituximab is also used in the treatment of autoimmune disorders, especially in rheumatoid arthritis and autoimmune cytopenias [32, 42].

Rituximab is administered as an intravenous infusion with a recommended dosage of 375 mg/m² given once weekly for 4 weeks [38]. Treatment with this agent is usually well tolerated. However, infusion-related reactions occur in the majority of patients. These adverse events are typically fever, chills, rigors and rare hypertension and bronchospasm, although the incidence of these side effects decreases with subsequent rituximab infusion. Moreover, the prolonged impairment of antibody production causes the increased risk of viral and bacterial infections. It should be also remembered that rituximab is a human mouse chimeric antibody and hence treated patients may be susceptible to the development of human antichimeric antibodies, which can impact on responsiveness. These host responses could attenuate response to retreatments and potentially mediate allergic reactions.

A number of prospective open studies and several retrospective cohort studies of rituximab in the treatment of SLE have been reported and larger clinical trials are summarized in Table 1 [43-58]. In 2002, Leonardo *et al.* [46] described six female patients with SLE who were treated with combination of rituximab, CY and prednisolone. Each patient received two infusions of rituximab (500 mg/dose), two infusions of CY (750 mg/dose) and 60 mg prednisolone per day for five days. Five patients were analyzed and one patient was lost to follow up after 3 months. All five patients showed an improvement in the British Isles Lupus Assessment Group (BILAG) scores from a median of 14 at baseline to a median of 6 at six months. Recently, the same group reported the results of 24 patients with active SLE treated with a combination of rituximab, CY and high-dose corticosteroids [49]. All but one patient showed profound B-cell depletion in the peripheral blood with the period of B-cell depletion ranging from 3-8 months and clinical improvement in BILAG global scores. Moreover, decreases in serum DNA antibody levels were observed. Twenty-one patients completed at least 6 months of clinical follow-up and 13 of them remained off immunosuppressive therapy at a follow-up of 7-51 months.

Willems *et al.* [53] described the safety and efficacy of rituximab in 11 girls (mean age 13.9 years) with severe SLE including 8 girls with class IV or V lupus nephritis, 2 girls with severe autoimmune cytopenia and 1 girl with antithrombin antibody. Patients received 2 to 12 intravenous infusions of rituximab (350-450 mg/m²/infusion) with corticosteroids. Remission was achieved in 6 of 8 patients with lupus nephritis and in 2 patients with autoimmune cytopenia.

Looney *et al.* [55] performed a dose escalation trial of rituximab added to ongoing therapy in 18 patients with moderately active SLE. Six patients received a single infusion of 100 mg/m², six received one infusion of 375 mg/m² and six patients received four weekly doses of 375 mg/m². A significant improvement in the Systemic Lupus Activity Measures

(SLAM) scores was observed in those patients who depleted B cells to <5/ μ l. Most patients were able to decrease corticosteroid dose from 13 to 10 mg by the end of the study and three patients were able to discontinue concomitant immunosuppressives. The clinical response was most notable for rashes and arthritis.

Ng *et al.* [57] reported recently the long-term clinical outcome and safety profile of a combination protocol of rituximab and cyclophosphamide in a large group of 32 patients with refractory SLE. Patients were assessed with the BILAG activity index. In this group of patients 12 remained well after one cycle of therapy. Median global BILAG scores decreased from 13 to 5 at 6 months ($p=0.006$). The time to flare after treatment was 10 months and the median duration of B-cell depletion was 4 months. Interestingly, improvements of wide spectrum of clinical features were observed, but no identifiable features predicted a better response. However, there was a corresponding serological improvement with a decrease in median anti-dsDNA antibody level from 164 to 58 IU/ml and an increase in median serum C3 from 0.75 to 0.90 g/liter.

Rituximab is also an active treatment agent in patients with lupus nephritis and the CNS involvement. Sfrikakis *et al.* [47] reported clinical response in 80% and sustained complete response in 40% of patients with class III and IV nephritis treated with rituximab and moderate doses of corticosteroids. More recently, Vigna-Perez *et al.* [50] reported the results of their study of the clinical and immunological effects of rituximab therapy in 22 patients with active SLE and renal involvement, refractory to conventional therapy. Rituximab at a dose of 0.5 to 1.0g was added on days 1 and 15 to the immunosuppressive therapy. They found a significant reduction in disease activity and proteinuria on days 60 and 90 of rituximab therapy. However, no significant changes in creatinine clearance and erythrocyturia were detected. In the study of Ng *et al.* [57], 21 patients with renal involvement treated with rituximab and cyclophosphamide

Table 1. Monoclonal Antibodies Active in Systemic Lupus Erythematosus

Therapeutics	Characteristics	Doses	Side Effects	References
Rituximab	Anti-CD20 mAb	375mg/m ² i. v. weekly for 4 doses or 1000mg once weekly for 2 doses	Infusion related symptoms (fever, chills rigors, hypotension), infections	Leonardo <i>et al.</i> [46,49] Sfrikakis <i>et al.</i> [48]
Epratuzumab	Anti-CD22 mAb	360mg/m ² i. v. every 2 weeks for 4 doses	Infusion related events	Dorner <i>et al.</i> [66]
Infliximab	Anti-TNF α mAb	300 mg x 4	Infections	Aringer <i>et al.</i> [82,83]
B-N10	Anti-IL-10 mAb	20 mg/day i. v. for 21 days	Safe and well tolerated treatment	Llorente <i>et al.</i> [116]
IDEC 131	Anti-CD40L humanized mAb	0.5 mg/kg i. v. over 16 weeks	Mild infections	Kalunian <i>et al.</i> [137]
BG 9588	Anti-CD40L humanized mAb	20mg/kg Every 2-4 weeks	Thromboembolic events	Boumpas <i>et al.</i> [141]
Belimumab	BLYS inhibitor	1.0-20 mg/kg i.v. every 21d	Side effects similar to placebo group	Furie <i>et al.</i> [96]
Toclimuzumab	Anti-IL-6 receptor mAb	2-8mg/kg biweekly for 12-16 weeks	Well tolerated	Illei <i>et al.</i> ¹⁰
Eculizumab	Anti-C5 mAb	0.1-8 mg/kg	Well tolerated	Furie <i>et al.</i> ¹²

mAb – monoclonal antibody; TNF – tumor necrosis factor; IL-10 – interleukin 10.

¹²Furie R, Matis L, Rollins S *et al.* A single dose, placebo-controlled, double blind, phase I study of the humanized anti-C5 antibody hbG1.1 in patients with systemic lupus erythematosus. Abstract presented at the 64th Annual Scientific Meeting of the American College of Rheumatology Philadelphia PA 2004.

Table 2. Larger Clinical Studies of Rituximab in SLE Patients

Study	No of Patients	Doses of Rituximab	Other Concurrent Treatment	Clinical Response
Leonardo <i>et al.</i> [49]	24	1000mg x 2, weekly	i. v. CY	BILAG score, C3 levels and dsDNA improved
Looney <i>et al.</i> [55]	17	100 mg/m ² , 375mg /m ² x 1-4 weekly	AZA, MM or MTX in 59% of pts	SLAM improvement In 11 pts
Sfikakis <i>et al.</i> [47]	10	375mg/m ² x 4, weekly	-	CR – 5 pts; PR – 3 pts
Figna-Parez <i>et al.</i> [50]	22	500-1000/m ² x 2, weekly	AZA, MM or MTX in 86% of pts	SLEDAI index andproteinuria decreased in 90% pts
Smith <i>et al.</i> [54]	11	375 mg/m ² x 4 weekly	i. v. CY	CR – 6 pts; PR – 5 pts
Vallerskog <i>et al.</i> [51]	11	375mg/m ² x 4, weekly	i. v. CY	SLAM decreased In 7 pts
Tokunaga <i>et al.</i> [56]	10	375-1000 mg, weekly x 4	Immunosuppressive drugs before RIT	Reduced SLEDAI score in all pts
Willems <i>et al.</i> [53]	11	350-450 mg/m ² x 2 -12	GLU/CY (2 pts)	Remission in 8 pts
Ng <i>et al.</i> [57]	32	500 mg/m ² -1000 mg x 2	CY, GLU, HCQ (24pts)	BILAG scores decreased from 13 to 5 at 6m
Nwobi <i>et al.</i> [58]	18	188-375 mg/m ² 2-4 doses	GLU,HCQ	CR-7; PR-7
Pololskaya <i>et al.</i> [151]	19	750 mg/m ² x 2	CY before RIT	Decrease in BILAG scores from 14 to 6
Lindholm <i>et al.</i> [152]	31	375 mg/m ² weekly x4	---	CR or PR in 11/17 pts with lupus nephritis
Cambridge <i>et al.</i> [154]	25	500-1000 mg x 2	CY, GLU	All improved clinically
Tanaka <i>et al.</i> [153]	14	500-1000 mg x 2-4 every 1-2 weeks	GLU	9 major or partial clinical response
Jonsdottir <i>et al.</i> [155]	16	375mg/m ² weekly x 4	CY	Clinical improvement in 13 pts

Abbreviations: HCQ – hydroxychloroquine; CY – Cyclophosphamide; AZA – azathioprine; MM – mycophenolate mofetil; RIT – rituximab; ND – not determined; MTX – methotrexate; GLU – glucocorticosteroids; CR – complete response; PR – partial response; m – months; BILAG – British Isles Lupus Assessment Group; SLEDAI – SLE Disease Activity Index; SLAM – Systemic Lupus Activity Measure.

had a decrease in median urinary protein creatinine ratio (PCR) from 446 to 190 mg/mmol at 6 months. In a smaller study of Sfikakis *et al.*, 10 patients with refractory proliferative nephritis were treated with rituximab and oral steroids [47]. Four patients achieved complete response at one year. In addition, Nwobi *et al.* [58] performed retrospective analysis of 18 children with lupus nephritis treated with rituximab with initial dose of 188 mg/m². Subsequent doses were 375 mg/m² per dose, infused over 6h to 8h for at least one course of two to four doses. All patients received concurrent therapy with low doses corticosteroids and hydroxychloroquine. Sixteen patients had failed or had suffered toxic effects from intravenous therapy with cyclophosphamide and corticosteroids. Clinical activity scores, anti-dsDNA antibodies, renal function and proteinuria improved in 93% of the patients. Rituximab was well tolerated by the majority of patients.

The study performed by Tokunaga *et al.* [56] showed marked improvement following rituximab therapy in patients with neuropsychiatric SLE. A monoclonal antibody was administered at doses of 375 mg/m² once weekly for four weeks or 1000 mg once weekly for two weeks in 10 patients with refractory neuropsychiatric SLE. Treatment resulted in rapid improvement of CNS-related manifestations, particularly acute confusional state. Rituximab also improved cognitive dysfunction psychosis and seizure and reduced the SLE Disease Activity Index Score (SLEDAI) on day 28 in

all 10 patients. These effects lasted for more than a year in 5 patients. In another study, Smith *et al.* [54] evaluated prospectively the effects of rituximab treatment for refractory SLE and vasculitis. Patients received four weekly infusions of rituximab at a dose of 375 mg/m². Intravenous CY (500 mg) was administered along with the first infusion in an effort to achieve early disease control. Remission followed rapid B cell depletion was achieved in all 11 patients including 6 complete responses and 5 partial responses. Moreover, a renal response occurred in all 6 patients with lupus nephritis. Clinical improvement was accompanied by a significant reduction in the daily dose of prednisone. Seven of 11 patients experienced a relapse, a median of 12 months after treatment. After relapse, six patients with SLE were re-treated with rituximab and all achieved remission and did so more quickly than after the primary treatment.

Rituximab is generally well tolerated [43]. Even fewer adverse events have been observed in patients treated for SLE than in the lymphoma patients [43]. The most common adverse events during or following rituximab therapy are infusion related symptoms, typically fever, chills, rigors and hypotension. In patients who receive premedication consisting of antipyretic and antihistaminic drugs together with corticosteroids, infusion-related side effects are usually only mild or moderate and do not require discontinuation of rituximab administration. Occasionally, serious infections

were also reported [55]. However, these may have been related to the underlying disease and/or concomitant therapy with other immunosuppressive agents. In 2006, an FDA alert was reported after two SLE patients treated with rituximab had died from progressive, multifocal leukoencephalopathy [16]. However, both patients had received additional treatment with cyclophosphamide. At present, it is difficult to estimate the risk of this complication in SLE patients treated with rituximab. Careful consideration of potential risk and benefits is necessary before therapy with this agent is undertaken.

NEW MONOCLONAL ANTIBODIES TARGETED CD20

Recently, several new mAbs reacting with CD20 have been developed. New mAbs directed against CD20 include fully human mAb of ofatumumab (HuMax CD20), IMMU-106 (hA20) which has a >90% humanized framework and GA-101, a novel third-generation fully humanized and optimized mAb [59,60]. These agents are highly cytotoxic against B-cell lymphoid cells. They are evaluated in preclinical studies and in early clinical trials.

Ofatumumab

Ofatumumab (HuMax-CD20) (Gemab, Copenhagen, Denmark and GlaxoSmithKline) is a fully human monoclonal immunoglobulin (Ig)G1 κ antibody targeting a CD20 molecule on B cells. It recognizes a novel CD20 epitope localized in the second extra-cellular loop distinct from the site of the epitope recognized by rituximab [59]. Ofatumumab is produced by a murine cell line (NSO) which has been transfected with a GS vector carrying the genes obtained from the human anti-CD20 hybridoma cell line 2F2. Ofatumumab in comparison to rituximab shows the superiority in complement dependent cytotoxicity (CDC) of B-cells and does not induce cell death of tumor B-cells by apoptosis. The translocation of CD20 into lipid rafts seems to be important for induction of cell signaling and more effective complement activation. In contrast to rituximab, ofatumumab as a human mAb is anticipating very low immunogenicity and good tolerance [60, 61]. Ofatumumab in comparison to rituximab shows the superiority in complement dependent cytotoxicity of B-cells and does not induce cell death of tumor B-cells by apoptosis. In contrast to rituximab, ofatumumab as a human molecule is anticipating very low immunogenicity. Ofatumumab has been investigated in phase I/II study in CLL, follicular lymphoma and rheumatoid arthritis¹ [61, 62]. Objective responses were noted in all three studies. Ofatumumab was well tolerated and did not induce development of human antihuman antibodies. Infusion related symptoms (rigors, pyrexia, fatigue, rash, increased sweating) were frequently observed. However, the intensity and frequency of these symptoms usually decreased during subsequent infusions. These encouraging preliminary data support further studies on the role of ofatumumab in patients with lymphoid malignancies and autoimmune diseases, including SLE.

¹Ostergaard M, Wiell C, Sierakowski S, Wallace D, Kastberg H, Petersen J, Dawes PT. HuMax-CD20, a novel fully human monoclonal IgG1 antibody in the treatment of rheumatoid arthritis *Arthritis Rheum* 2006, 54, 9 (Abs 2122).

IMMU-106

IMMU-106 (hA20) (Immunomedics, Inc., Morris Plains, NJ, USA) is a new humanized anti-CD20 mAb evaluated to elucidate its action as an antilymphoma therapeutic [63, 64]. The mechanism of cytotoxicity of IMMU-106 is similar to rituximab and includes direct apoptosis, antibody dependent cellular cytotoxicity (ADCC) and CDC. It is also very similar to rituximab in terms of antigen binding, specificity binding avidity and dissociation constant. In preclinical study, median survivals of mice treated with IMMU-106 were 70 days and of mice treated with rituximab – 98 days.

A phase I/II dose escalation study performed in patients with relapsed/refractory NHL indicates that IMMU-106 is well tolerated and at least as effective as rituximab^{2,3}. Further studies in NHL and other disorders including autoimmune disorders are expected.

GA-101

GA-101 (Roche, Basel, Switzerland) is a novel third-generation fully humanized and optimized anti-CD20 IgG1 differing significantly from other anti-CD20 mAbs, like rituximab^{4,5}. Using GlycoMab technology the Fc-region of GA-101 was glycolengineered to contain bisected, afucosylated carbohydrates. It has the high affinity binding to the CD20 type II epitope induction. As a result, GA-101 mediated 5-50 fold enhanced induction of ADCC in comparison with rituximab and other non-glycoengineered standard anti-CD20 mAbs⁵. Moreover, it exhibits superior caspase-independent apoptosis induction in comparison with rituximab. However, reduction in CDC upon binding to CD20 was observed. In B-cell depletion assays with the whole blood from healthy donors GA-101 was significantly more potent and efficacious in depleting B-cells than rituximab⁶. In B-cell depletion assay in NHL xenograft models of different histological origin, GA-101 induced complete tumor regression and long-term survival⁶. The studies on the growth of aggressive NHL xenograft models in SCID beige mice demonstrated on outstanding dose-dependent antitumor activity of GA-101.

²Morschhauser, F.; Leonard, J.P.; Fayad, L.; Coiffier, B.; Schuster, S.J.; Dyer, M.J.S.; Petillon, M.O.; Coleman, M.; Bahkti, A.; Horne, M.; Xu, L.; Teoh, N.; Wegener, W.A.; Goldenberg, D.M. Rituximab-relapsing patients with non-Hodgkins lymphoma respond even at lower doses of humanized anti-CD20 antibody IMMU-106 (hA20): phase I/II results. *Blood*. **2006**, *108* (Suppl.1), Abstract 279.

³Morschhauser, F.; Leonard, J.P.; Coiffier, B.; Petillon, M.O.; Coleman, M.; Bahkti, A.; Sapra, P.; Teoh, N.; Wegner, W.A.; Morak, J.D.; Goldenberg, D.M. Initial safety and efficacy results of second-generation humanized anti-CD20 antibody IMMU-106 (hA20), in non-Hodgkin's lymphoma. *Blood*. **2005**, *10*, (Suppl. 1) Abstract 2428.

⁴Umana, P.; Moessner, E.; Bruenker, P.; Unsin, G.; Puentener, U.; Suter, T.; Grau, R.; Schmidt, C.; Gerdes, C.; Nopora, A.; Patre, M.; Moser, S.; Sondermann, P.; Wheat, L.; Dyer, J.S. M.; Poppema, S.; Bauer, S.; Strein, P.; Friess, T.; Klein, C. Novel, 3rd generation humanized type II CD20 antibody with glycoengineered Fc and modified elbow hinge for enhanced ADCC and superior apoptosis induction. *Blood* **2006**, *108* (Suppl. 1), Abstract 229.

⁵Friess, T.; Gerdes, C.; Nopora, A.; Patre, M.; Preiss, S.; van Puijenbroek, E.; Schuell, C.; Bauer, S.; Umana, P. GA101, a novel humanized type II CD20 antibody with glycoengineered Fc and enhanced cell death induction, mediates superior efficacy in a variety of NHL xenograft models in comparison to Rituximab. *Blood* **2007**, *110* (Suppl. 1), Abstract 2338.

⁶Umana P, Moessner E, Bruenker P, Klinger G, Puentener U, Suter T, Grau R, Schmidt C, Herter S, Gerdes C, Nopora A, Patre M, Moser S, Sondermann P, Wheat, L.; Dyer, J.S. M.; Poppema, S.; Bauer, S.; Kubbies, M.; Strein P, Fertig G, Friess T, Dabbagh K, DalPorto J, Klein C. GA101, a novel humanized type II CD20 antibody with glycoengineered Fc and enhanced cell death induction, exhibits superior anti-tumor efficacy and superior tissue B cell depletion *in vivo*. *Blood*. **2007**, *110* (Suppl. 1), 694a.

This mAb mediated superior antitumor efficacy in Z138 mantle cell lymphoma and SU-DHL4 diffuse large B-cell lymphoma xenograft models in direct comparison to rituximab⁷. In cynomolgus monkeys, the induction of B-cell depletion mediated by this antibody included complete, rapid and long-lasting B cell depletion both in peripheral blood and in lymphoid tissues⁶. It showed superior depletion of total B cells from lymph nodes compared to rituximab from day 9 to 35 with B-cell numbers decreased by over 95%. Based on this data GA-101 mAb is a promising therapeutic agent for CD20 positive B-cell lymphoid malignancies and autoimmune disorders.

Epratuzumab

Epratuzumab (Immunomedix, Inc, Moris Plains, NJ, USA) is a humanized monoclonal IgG antibody that specifically targets the CD22 antigen on B cells⁸ [65, 66]. It has been derived from the murine IgG2a MoAb, LL2 generated against Raji Burkitt lymphoma cells. This mAb is 90% to 95% of human origin, thus greatly reducing the potential for immunogenicity. Unconjugated anti-CD22 antibodies only partially deplete B cells, but might deliver a negative signal by binding to cell surface CD22, thereby recruiting the intracellular phosphatase, src-homology 2-domain-containing protein tyrosine phosphatase 1 (SHP1), and opposing protein kinase mediated signaling through the B-cell receptor [66, 67]. Epratuzumab is selectively active against normal and neoplastic B cells. This mAb acts as an immunomodulatory agent in contrast to rituximab, which is an actually cytotoxic therapeutic antibody [65, 68, 69]. Unlike rituximab, no ADCC was significant with epratuzumab. Epratuzumab is currently in clinical trials for treatment of NHL and autoimmune disorders⁷ [68-70]. In clinical trials over 400 patients with NHL or other B cell malignancies were treated with epratuzumab administered as 4 consecutive weekly infusions over about 60 minutes. Four consecutive weekly doses of 360 mg/m² of this antibody were selected for further clinical studies as a sufficiently safe and efficacious therapy [157]. Recently, Dorner *et al.* [65] reported the results of an open-label, single-center study of 14 patients with moderately active SLE. Patients received 360 mg/m² of epratuzumab intravenously every 2 weeks for 4 doses with analgesic antihistamine premedication prior to each dose. Total BILAG scores decreased by $\geq 50\%$ in all 14 patients at some point during the study with 92% having a decrease in various amounts continuing to at least 18 weeks. Epratuzumab toxicity consisted primarily of mild to moderate transient infusion-related events during the first infusion. These results support conducting multicenter controlled studies to examine the effects of epratuzumab in broader patient populations.

ANTICYTOKINE THERAPIES

Cytokines are low molecular weight mediators of cell-cell interactions with pro- and antiinflammatory effects [71]. In the course of SLE, a wide variety of cytokines is dysregu-

lated, many of which likely influence autoimmunity and lupus tissue inflammation [72-74]. Proinflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-1 and interferon- γ (IFN- γ) may play an important role in propagating the inflammatory process responsible for tissue damage [72]. IL-12, IL-15 and IL-18 are probably also involved in pathogenesis of SLE [75]. The possibility of blocking the proinflammatory cascade by selective inactivation of cytokines can be a successful therapy for patients with SLE. Among the proinflammatory cytokines, TNF- α has been found to be overexpressed in SLE [76]. IL-10 is overproduced by the B cells and monocytes of patients with SLE [17]. The level of this cytokine is increased in sera of the patients with this disease and is assorted with SLE activity [76,77]. Therefore, anticytokine therapy may be an interesting candidate approach for treating SLE.

Anti-TNF- α Therapy

TNF- α concentration is increased in sera of SLE patients and closely associated with disease activity [78,79]. However, because of the concern of antibody production with anti-TNF- α blocking agents, mAbs reacting with this cytokine have not been widely used in the treatment of SLE [80]. Moreover, most of the data are available from case reports or case series and the results of these are controversial.

The most widely used anti-TNF- α mAb is infliximab (Remicade[®], Centor). It is a chimeric genetically engineered mAb that is manufactured using cells containing human and mouse antibody genes. This agent, which is taken intravenously, binds and blocks the action of TNF- α [81]. Infliximab has been approved by the United States Food and Drug Administration (FDA) for the treatment of moderately to severely active rheumatoid arthritis (RA), Crohn's disease, psoriasis and psoriatic arthritis. Early results by Aringer *et al.* [82-84] in a small number of patients suggest that anti-TNF- α agents such as infliximab may be useful in SLE patients. In this study, six SLE patients with arthritis and/or nephritis were treated with four infusions of infliximab (300 mg each in combination with methotrexate or AZA). All patients were refractory to standard therapy. All patients completed the study at 52 weeks. In all patients, significant improvement in clinical manifestations was observed including amelioration or proteinuria. However, simultaneously a slight rise in anti-DNA and IgM cardiolipin antibody level was seen in four patients. Toxicity was restricted to infections, mainly of the urinary tract. None of the patients had an infusion reaction or an increase in SLE activity while receiving infliximab and or during the follow-up. All the patients with arthritis presented a significant but transient improvement with relapses at 8-11 weeks after the infusion. Moreover, in all patients with nephritis, proteinuria decreased and remained at a low level for at least 6 months after the last infusion of mAb. These results were confirmed in the longer follow-up⁸. Recently, Hayat *et al.* [85] reported on the safety and efficacy of infliximab given to a patient with active lupus with diffuse proliferative nephritis (WHO Class IV). The patient failed to remit with a combination of full-dose steroids, mycophenolate mofetil and cyclosporine. However, she went to sustained remission with the addition of infliximab infusions. Currently, a trial of infliximab in lupus membranous nephritis is in progress (<http://www.clinicaltrials.gov>).

⁷Kaufman J, Wegener WA, Horak ID, *et al.* Initial clinical study of immunotherapy in SLE using epratuzumab (humanized anti-CD22 antibody). *Arth Rheum.* 2004; 49: Abstract 1127.

⁸Houssian FA, Graminger WB, *et al.* Open-label infliximab for systemic lupus erythematosus: long-term follow-up of 11 patients. *Arthritis Rheum* 2006, 54, 5260-1.

It should be noted, however, that induction of autoantibodies and lupus-like syndrome in patients treated with infliximab was observed and the results reported in these small open label trials and case reports should be interpreted with caution [86-89].

ANTI-BLYS MONOCLONAL ANTIBODY

The protein B-cell activating factor (BLyS) is a member of the TNF family also known as BAF-T, TALL-I and THANK [90]. This protein plays an essential role in the homeostasis, activation and differentiation of B cells. The three known receptors for BLyS are differentially expressed at various stages of B-cell and plasma cell development indicating that the neutralization of this protein may lead to different consequences than B-cell depletion by anti-CD20 mAbs [91].

In patients with SLE, the serum levels of BLyS are elevated and its neutralization has suggested that higher levels of BLyS contribute to the generation of autoantibodies and is important in SLE pathogenesis [90, 92]. In consequence, neutralization of BLyS may play a role in the therapy of this disease.

Belimumab (LymphoStat-B™, Human Genome Sciences, Inc, Rockville, Maryland, USA) is a fully human IgG1 mAb that specifically binds and inhibits the biological activity of BLyS [93]. The antibody exerts its biological activity by preventing the binding of BLyS to its receptors⁹ [94]. Belimumab inhibits BLyS-induced proliferation of B-cells *in vitro* and prevents human BLyS-induced increases in splenic B-cell numbers and serum IgA titers in mice [94]. Belimumab reduced significantly the number of lymphoid tissue and peripheral blood CD20+ B cells [95].

The safety, tolerability, immunogenicity, and pharmacology of belimumab were investigated in a phase I, randomized, placebo controlled, double-blind study in patients with SLE [96]. Seventy patients with mild to moderate disease were enrolled in this trial. Fifty-seven patients were treated with mAb and 13 with placebo. The drug was administered at 4 different doses (1.0, 4.0, 10 and 20 mg/kg) as single infusions, 21 days apart. The incidence of adverse events and laboratory abnormalities was similar among the belimumab and placebo groups. Significant reduction in median percentage of CD20+ B-cells was noted with a single and two doses of belimumab versus placebo. However, SLE disease activity did not change after treatment with this mAb. In contrast, a subsequent phase II study has shown significant improvements in several SLE disease activity measures in serologically active patients, and two phase III trials in patients with active SLE are ongoing⁹ [85].

Anti-Interleukin-6 Monoclonal Antibodies

Interleukin-6 (IL-6) is one of the proinflammatory, pleiotropic cytokine that acts on a variety of cells, including immune-competent cells and hematopoietic cells, to cause

proliferation and differentiation [97]. IL-6 mediates its biological activity through binding to a receptor complex consisting of two glycoproteins: an 80000 molecular weight (MW) and a 130000 MW signal transmission element (gp130). This cytokine induces B-cell differentiation to plasma cells and T-cell proliferation and cytotoxic T-cell differentiation. IL-6 plays an important role in pathogenesis of several autoimmune conditions including rheumatoid arthritis (RA) and SLE [97]. IL-6 is induced by anti-dsDNA antibodies, TNF- α , IL-1 and interferon- γ [17]. In NZB/W mice IL-6 promotes the development of murine lupus and exacerbates glomerulonephritis [98, 99]. Moreover, IL-6 is highly expressed in SLE glomerulonephritis [100]. Chronic administration of anti-IL-6 antibody prevents the production of anti-dsDNA antibodies, significantly reduces proteinuria, prevents the development of severe kidney disease and prolongs survival of lupus prone NZB/NZW F1 mice [98, 101]. Moreover, the serum level of IL-6 is significantly higher in patients with SLE than in normal controls [102, 103]. In addition, positive correlation was found between the serum levels of IL-6 and SLE activity [103]. These effects of IL-6 suggest that this cytokine is an attractive target for mAbs therapy for SLE.

Tocilizumab is a humanized IgG1 mAb directed to human IL-6 receptor [104, 105]. It has been developed collaboratively by Osaka University and Chugai Pharmaceutical Company Ltd (Japan). Tocilizumab inhibits IL-6 signaling mediated by both membranous and soluble IL-6 receptor (IL-6R) and is expected to ameliorate the autoimmune inflammatory diseases with IL-6 overproduction. Tocilizumab is under development for the treatment of autoimmune diseases such as RA, systemic onset juvenile idiopathic arthritis (JIA), adult onset Still's disease, Castleman's disease, Crohn's disease and SLE (105-107). Its activity in RA and JIA has been recently confirmed in randomized double blind, placebo-controlled, multicenter trials [108, 109]. The results of an open-label, dose escalating phase I study of tocilizumab have been recently published in an abstract form¹⁰.

The drug was administered at three doses (2, 4 and 8 mg/kg) biweekly for 12 weeks to 16 SLE patients with mild to moderate disease activity. Tocilizumab was well tolerated and at 14 weeks SLEDAI and SLAM scores improved (P=0.001 and 0.0017, respectively). However, three patients required increased prednisone dosages for minor flares. In addition, there was a decrease in complement, IgG and anti-dsDNA antibody levels. In contrast, there were no significant changes in absolute lymphocyte counts or major lymphocyte subsets. These results warrant further studies of tocilizumab as treatment for SLE.

ANTI-IL-10 THERAPY

IL-10 is a regulatory cytokine, mainly produced by B-cells, which use this cytokine for their proliferation, and by myeloid cells, which use IL-10 to reduce proinflammatory responses [110]. Numerous studies have shown an increased

⁹Stohl W, Merrill JT, *et al.* Oranges in circulating B-cell counts, autoantibody levels and immunoglobulins that associate with therapeutic responsiveness in SLE to BLyS protein antagonism by belimumab. *Arthritis Rheum.*, 2006, 54(Suppl): S780.

¹⁰Illei G, Yarboro C, Shirota Y, Tackey E, Lapteva L, Fleisher T. Tocilizumab (Humanized Anti IL-6 Receptor Monoclonal Antibody) in patients with systemic lupus erythematosus (SLE): safety, tolerability, and preliminary efficacy. *Arthritis Rheum.* 2006, 54(Suppl): 4043 (Abstract).

IL-10 production by the peripheral blood B cells and monocytes from SLE patients [111]. In addition, disease severity correlates with an increased ratio of IL-10: IFN- γ - secreting cells in the peripheral blood. Moreover, mean serum concentration of IL-10 in SLE patients is significantly higher than in healthy persons [112, 113]. It has been also shown that inhibition of IL-10 results in decreased disease marker expression in SLE patients [114]. The indication of IL-10 in B-cell hyperactivity and autoantibody secretion in patients with SLE has led to use anti-IL-10 antibody in the treatment of this disease [27].

The anti-IL-10 mAb (B-N10) is a murine IgG1 mAb, which neutralizes human IL-10 [115, 116]. Llorente *et al.* evaluated the safety and clinical efficacy of this agent in six patients with active and steroid dependent disease [116]. Treatment consisted of 20 mg/day intravenous administration of B-N10 for 21 consecutive days. Subsequently, the patients were followed up monthly for 6 months. Treatment was safe and well tolerated. However, all patients developed antibodies against B-N10. Cutaneous lesions and joint symptoms improved during mAb administration in all patients. In all patients, the decrease in disease activity following initiation of anti-IL-10 mAb administration allowed a gradual tapering of corticosteroid treatment. The SLE Disease Activity Index decreased from in men from 8.83 on day 1 to 3.67 on day 21. At the end of the follow-up, the disease was clinically inactive in five of the six patients. These results indicate that the use of the anti-IL-10 mAb is safe and may be beneficial in the management of refractory SLE patients.

MONOCLONAL ANTIBODIES TARGETING INTERFERON- α

There is evidence that interferon- α (IFN- α) has a significant role in pathogenesis of SLE [117]. Long-standing data indicating elevated levels of this agent in the serum of patients with SLE have recently been supplemented by reports from gene expression data, and clinical practice [117, 118]. A potential role of IFN- α in this disease was suggested when patients with cancer or hepatitis treated with IFN- α developed SLE [119-121]. In addition, elevated serum level of this cytokine has been reported in serum obtained from SLE patients and there was a correlation between IFN- α levels and disease activity [122, 123]. Moreover, gene expression profiles from SLE peripheral blood mononuclear cells demonstrated a cluster of regulated genes that are modulated by IFN [124, 125]. In view of this evidence implicating IFN- α in SLE pathogenesis, therapeutic use of mAb neutralizing this protein may be useful for future therapies.

An antibody against human IFN- α (MEDI-545) has been developed and is currently in a phase I/II trial¹¹. This study called LISA (Lupus Interferon Skin Activity) involves a single intravenous dose of MEDI-545 in patients with mild SLE with lupus rash or skin lesion. Forty-five patients is planning to include at 20 centers in North America. MEDI-545, a fully human mAb targeting IFN- α , is evaluating as a single intravenous dose of 0.3 to 30 mg/kg in patients treated with

prednisone no more than 20 mg daily for at least 28 days. As the IFN- α has an important antiviral and antimicrobial activity, the effect of anti-IFN- α mAb on the risk of infection is an important issue, which can be solved only in clinical trials in SLE patients.

Data from several studies suggest that IL-6 plays an important role in the B-cell hyperactivity and immunopathology of SLE [98, 101]. This cytokine may have a direct influence on mediating tissue damage. Elevated levels of IL-6 were detected in serum, urine and renal glomeruli of patients with active SLE and in murine models of SLE [98, 99]. Intraregional administration of the antimurine IL-6 monoclonal antibody suppressed the production of anti-dsDNA autoantibody in New Zealand Black/White F1 mice with SLE-like disease [101]. Moreover, histological analysis showed that treatment with anti-IL-6 monoclonal antibody prevented the development of severe kidney disease.

Recently, humanized anti-IL-6 receptor antibody tocilizumab (MRA) has been developed as a therapeutic agent for inflammatory autoimmune diseases [106]. This antibody has improved the disease activity in rheumatoid arthritis, systemic onset juvenile idiopathic arthritis and Crohn's disease. An open-label phase I study of the humanized monoclonal antibody against the IL-6 receptor is currently underway in patients with moderately active SLE¹⁰.

Levels of IL-10 are also increased in the serum of patients with active SLE and correlate with disease activity. Alteration in IL-10 regulation may result in accelerated T-cell apoptosis and aberrant T-cell dependent B-cell function [114, 115]. The anti-IL-10 monoclonal antibody was administered to six patients with steroid resistant SLE in an open-label pilot study. It was given intravenously on a daily basis for 21 days. Therapy was well tolerated and marked improvement in skin lesions and joint symptoms was observed in all patients over the next 6 months. Furthermore, three times lower doses of prednisone were used.

MONOCLONAL ANTIBODIES DIRECTED AGAINST CD40L

CD40 is a number of the tumor necrosis factor receptor superfamily expressed on antigen-presenting (APC) cells [126,127]. It modulates the activity of CD4+T helper cells *via* CD40 ligand (CD40L or CD154) and other cell types, including normal and malignant B lymphocytes. In non-transformed B cells, CD40 ligation results in inhibition of apoptosis, differentiation and expression of activation antigens including B7, CD23 and CD95 (FAS) [128].

The CD40-CD40L interaction is one of the most important costimulatory signals resulting in activation, proliferation and Ig class switching in the germinal center environment [129]. The interaction of CD40 on B cells with CD40L on activated T cells provides a costimulatory signal that induces T dependent B cell proliferation antibody production.

CD40L is overexpressed in T helper cells of patients with active SLE [130-132]. It has been also shown that enhanced CD40-CD40L interaction in SLE patients contributes to increased Ig and autoantibody production [132]. Elevated levels of soluble CD40L in serum of patients with SLE have been also noted [133]. Therefore, Interference with this sig-

¹¹Medical News Today. Accessed April 24, 2007. MedImmune Begins Dosing of Lupus Patients in Phase I Clinical Trial (Press release). Available at: http://www.medicalnewstoday.com/medical_news.php?newsid=41611.

nal pathway may inhibit autoantibody production and immune complexes formation and deposition.

Studies of lupus prone mice showed that brief treatment with anti-CD40L therapy had a sustained beneficial effect on their spontaneous disease long after the antibody had been cleared from their system [134]. Further studies documented that prolonged therapy within anti-CD40L antibody reduced anti-DNA autoantibody production and renal disease and significantly prolonged survival compared with control mice [135]. Moreover, pathologic examination verified the significant renal damage or immune deposition in responding mice. In addition, Kalled *et al.* [136] demonstrated that long anti-CD40L treatment of mice with established nephritis prolonged survival and decreased incidence of severe nephritis. Following encouraging results in murine models with nephritis, two different anti-CD40L mAbs were developed IDEC-131 and GB2588/hu5c8.

IDEC-131 IDEC Pharmaceuticals is a humanized mAb against CD154, comprising human $\gamma 1$ heavy chains and human light chains with murine complementarity/determining regions [137]. This mAb does not activate T-cells, endothelial cells or platelets and does not upregulate tissue factor. There were three trials with IDEC-131 in patients with SLE, enrolling in global 110 patients [137-140].

A randomized, double-blind, placebo-controlled trial of IDEC-131 demonstrated its safety and good tolerance in 23 SLE patients [137]. Cohorts of 3 to 5 patients with symptomatic disease received 0.05, 0.25, 1.0, 5.0 or 15.0 mg/kg of mAb as a single intravenous infusion. No infusion-related cytokine-release syndrome was observed. However, all patients experienced at least one adverse event during a 3-month follow-up period. Mild infections not related to mAb administration were reported in 8 patients. No patients developed detectable antibody to IDEC-131. At doses between 1.0 and 15mg/kg the serum half-life ranged from 299 to 320h. Therapeutic activity of IDEC-131 was not evaluated in this single dose study. Subsequently, safety and tolerability of the IDEC-131 antibody was evaluated in a phase II double blind trial [140]. Eighty-five patients with mild to moderate active SLE were randomized to receive 6 infusions of IDEC-131 ranging from 2.5 mg/kg to 10 mg/kg or placebo over 16 weeks. Efficacy was assessed at week 20, primarily by the Systemic Lupus Erythematosus Disease Activity Index (SLE DAI). SLEDAI scores improved from the baseline levels of disease activity in all groups. However, these scores were not statistically different in mAb treated patients and placebo group at week 20. Moreover, the type and frequency of adverse events were similar between IDEC-131 and placebo groups.

BG9588 (Biogen, Inc., Cambridge, MA) is a humanized antihuman CD40L antibody which consists of the complementary-determining regions of the murine mAb 5c8 (anti-human CD40L antibody) with human variable region framework residues and IgG1 constant region [141]. This mAb is secreted from murine myeloma cells, which overexpress the protein. BG9588 blocks antigen-specific IgG responses in human and nonhuman primates. Boumpas *et al.* [141] evaluated the toxicity and efficacy of BG9588 in patients with proliferative lupus nephritis in a phase II multi-center open-level study [141]. Twenty-eight patients were

scheduled to receive 20 mg/kg of BG9588 at biweekly intervals for the first 3 doses and monthly intervals for 4 additional doses. The study was terminated prematurely because of the thromboembolic events in two patients and complications reported in other trials. However, of the 18 patients for whom efficacy could be evaluated, two had a 50% reduction in proteinuria without worsening of renal function. Moreover, hematuria disappeared in all 5 patients with significant hematuria at baseline. Immunologic effect of BG3588 was evaluated in 5 patients from this study [142]. Treatment with this agent markedly reduced the frequency of IgG and G anti-DNA antibody producing B cells and these changes persisted for several months after cessation of treatment. These results indicate that further studies with mAbs blocking CD40L are warranted.

MONOCLONAL ANTIBODIES AGAINST C5 COMPONENT OF THE COMPLEMENT SYSTEM

The complement system consists of 3 pathways and more than 30 proteins including those with biological activity that directly or indirectly mediate the effects of this system [143,144]. In addition, a set of regulatory proteins prevent injudicious complement activation. The complement system is activated in patients with SLE following immune complex deposition and it amplifies the immune and inflammatory responses contributing to the lupus nephritis pathogenesis [144]. A monoclonal antibody to the C5 component of the complement system inhibits the cleavage of C5 to C5a and C5b and blocks the following formation of the late membrane complex (MAC) [145].

In murine models of SLE the administration of anti-C5 mAb (BB5.1) given intraperitoneally, delayed the onset of proteinuria, improved renal histology and prolonged survival [146]. Furthermore, in mice with renal disease induced by human anti-ds DNA, antibody RU-14 anti C5 mAb significantly reduced proteinuria [147, 148]. Monoclonal antibody eculizumab that specifically inhibits terminal complement activation has been recently developed and investigated in phase I single dose study in humans [148, 149]. Eculizumab (Soliris; Alexion Pharmaceuticals, Inc., Cheshire CT) is a humanized IgG κ immunoglobulin comprised of human constant regions and murine complementarity determining regions grafted onto human framework light and heavy chain-variable regions [149, 150]. This mAb binds to the human C5 complement protein with high affinity, thereby inhibiting its cleavage to C5a and C5b and preventing the terminal complement complex C5b-9.

Eculizumab was evaluated in phase I study in patients with SLE¹¹ [148]. The patients received a single intravenous infusion of eculizumab or placebo in doses ranging from 0.1 to 8 mg/kg. Patients treated with 4 and 8 mg/kg maintained the longest duration of C5 blockade with 5 days and 10 days of femoral complement inhibition, respectively. The drug was well tolerated and no clinically significant side effects were observed. However, significant clinical improvement in SLE symptoms was not stated during assessments at day 28 and 56. On March 2007, eculizumab received accelerated approval by the US FDA for the treatment of patients with paroxysmal nocturnal hemoglobinuria to reduce hemolysis [149].

CONCLUSIONS

SLE is an incurable autoimmune multisystem disease of unknown etiology and a highly variable course and outcome. Newly developed biologic therapies, especially monoclonal antibodies are under investigation in this disease. Rituximab (anti-CD20 mAb), approved by the FDA in 1997 for the treatment of non-Hodgkin lymphoma is also active in SLE. Recently, several reports from several series of patients with SLE have shown good therapeutic results. However, larger controlled clinical trials are required to establish the safety and efficacy of this agent. More recently, several newer mAbs have been developed and are being evaluated in phase I/II clinical trials. These include anti-cytokine therapies anti-CD40L mAbs, anti-CD-22 mAb, anti-BLys mAbs and anti-C5 mAbs. These potentially useful agents should be further evaluated in well designed controlled trials.

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