New therapies for systemic lupus erythematosus

F. Goldblatt and D. A. Isenberg

Centre for Rheumatology, The Middlesex Hospital, University College London, London, UK

Summary

In the past 40 years, prognosis for patients with systemic lupus erythematosus (SLE) has improved, with 10-year survival now approximately 90%. This is due probably to a combination of earlier disease diagnosis and diagnosis of milder disease, due in part to availability of multiple serological tests for SLE, use of steroids and other immunosuppressive agents, and availability of renal dialysis and transplantation. Despite this, however, the potential for significant morbidity and mortality remains in the group of patients with partially responsive or treatment resistant disease. More recently, advancements in the understanding of molecular mechanisms involved in the pathogenesis of SLE have translated to the development of novel therapies, offering possible alternatives to this patient cohort. Discussion of these pharmacological options and ongoing research forms the basis of this review.

Keywords: systemic lupus erythematosus, new therapies

Introduction

Systemic lupus erythematosus (SLE) is a prototypical autoimmune rheumatic disease principally affecting women during childbearing years. Its prevalence has been estimated at between 40 and 200 per 100 000 in Caucasian and Afro-Caribbean populations, respectively [1]. The American College of Rheumatology (ACR) have proposed revised classification criteria for SLE [2]. Clinical disease manifestations are diverse and may range from non-specific symptoms, such as fatigue and musculoskeletal complaints (arthralgia, myalgia) to life-threatening renal or cerebral disease. SLE is characterized serologically by a variety of autoantibodies to deoxyribonucleic acid (DNA), ribonucleic acid (RNA), other nuclear antigens (e.g. Smith, Ro, La) and cytoplasmic antigens. The presence of anti-double-stranded DNA antibodies has been linked most closely to pathogenicity [3], in particular the renal histological activity score [4].

Although the exact aetiopathogenesis of SLE remains uncertain, there is consensus that its aetiology is dependent upon a combination of environmental, hormonal and genetic factors. It is generally agreed in SLE that autoreactive T cells are necessary to activate B cells, which are further stimulated to proliferate and produce autoantibodies by the elevated levels of proinflammatory cytokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-10

and interferon (IFN)-γ evident in patients with SLE [5,6]. Furthermore, the autoantibody production may be enhanced further by T and B cell interaction via co-stimulatory molecules that generate anti-apoptotic signals. It is considered that these autoantibodies are very likely to be related directly to the pathogenic effects on tissues in patients with SLE [7]. In addition, imbalance between IL-10 and IL-12 [8,9] results in further B cell activation and inhibition of T cell function [10]. IL-12 levels are down-regulated by IL-10, with lower levels correlating with increased disease activity and nephritis [9,11]. A more recent proposal supports the view that it is the failure to remove apoptotic cells efficiently that is the stimulus to autoantibody production [12,13].

In the past 40 years, prognosis for patients with SLE has improved, with 10-year survival now approximately 90% [14,15]. This is probably because a combination of earlier disease diagnosis and diagnosis of milder disease, and due in part to the availability of multiple serological tests for SLE, use of steroids and other immunosuppressive agents and availability of renal dialysis and transplantation. Despite this however, the potential for significant morbidity and mortality remains in the group of patients with partially responsive or treatment resistant disease. More recently, advancements in the understanding of molecular mechanisms involved in the pathogenesis of SLE have translated to the development of novel therapies, offering possible alternatives for this

patient cohort. Discussion of these pharmacological options and ongoing research forms the basis of this review.

General management of SLE

Treatment of SLE is multi-factorial and includes education, such as avoidance of ultraviolet light, general management of infections, cardiovascular risk factors and treatment complications including osteoporosis, in combination with pharmacological therapies tailored to the individual's disease. Initial therapy in the 1950s consisted of corticosteroids and antimalarials, with the introduction of immunosuppressives, including cyclosporin [16,17], to therapeutic regimens in the 1970s. Many other therapies have been trialled in patients with SLE over more recent years, including intravenous immunoglobulin and systemic or topical tacrolimus [18–21]. The efficacy of hydroxychloroquine for skin and joint manifestations of SLE has been well established [22,23] and long-term outcome studies suggest 200–400 mg/day of hydroxychloroquine protects against disease flares [24]. Thought to exert its therapeutic effect via interference with antigen processing, inhibition of phagocytosis, neutrophil migration and membrane phospholipid metabolism, hydroxychloroquine is a safe and well-tolerated medication [23]. Corticosteroids have both anti-inflammatory and immunosuppressive actions in SLE and their effectiveness in treating the disease has been recognized since the 1950s. In particular, their efficacy in treating active lupus nephritis and other SLE complications is well documented [25,26]. Used alone, however, corticosteroid effects are often transient and associated with multiple side effects. This often necessitates the introduction of medications such as azathioprine, cyclophosphamide or mycophenolate mofetil for long-term management, in an effort to control disease and minimize steroid requirements. The use of azathioprine has been studied extensively in patients with various manifestations of SLE, although most literature relates to its use in lupus nephritis where it has been shown to stabilize renal function and reduce proteinuria [27,28]. However, intravenous pulse cyclophosphamide has, until recently, been used more widely for more severe lupus nephritis [28]. Combination therapy with pulse intravenous cyclophosphamide (0·5–1·0 mg/kg/m^2 monthly for 6 months) and high-dose glucocorticoids, followed by a 2-year maintenance phase is the currently recognized gold standard for treatment of proliferative lupus nephritis [29–35]. Additionally, intravenous cyclophosphamide and prednisolone have been reported to be efficacious in other manifestations of severe disease, such as central nervous system lupus [36,37]. The principal limitations to cyclophosphamide are its adverse events, including cytopaenia, infections, gonadal failure and possibly malignancy [38]. Furthermore, this regimen is not universally successful and thus alternatives, including lower dosage regimens [39] and newer therapeutic options including mycophenolate mofetil, B cell depletion, biological agents

and haemotopoietic stem cell transplant are being considered.

New therapies in systemic lupus erythematosus

Mycophenolate mofetil

Mycophenolate mofetil selectively suppresses T and B lymphocyte proliferation by inhibiting inosine monophosphate dehydrogenase, the enzyme involved in *de novo* purine nucleotide synthesis. Consequent biological actions include suppression of antibody synthesis and glycosylation of adhesion molecules and cytokine antagonism [40]. Initially developed to prevent organ rejection, mycophenolate mofetil has been utilized more recently as a substitute for cyclophosphamide in the treatment of lupus nephritis, primarily in an effort to reduce serious adverse effects.

Use of mycophenolate mofetil in murine models of lupus demonstrated efficacy in reducing nephritis and reducing mortality [41,42]. Subsequent trials in renal and nonrenal patients have been supportive of mycophenolate mofetil as a viable alternative therapy for active lupus [43–45]. Chan *et al.* studied 42 patients with diffuse proliferative lupus nephritis, comparing 12 months treatment with prednisolone and 1·0 g twice daily mycophenolate mofetil, with a regimen of 6 months prednisolone and oral cyclophosphamide followed by 6 months of prednislone and azathioprine [46]. Remission rates were similar (81% *versus* 76%), as were relapse rates (15% and 11%, respectively). Infections occurred with similar frequencies between the treatment groups; however, all other side effects were present only in the cyclophoshamide/azathioprine-treated group, and included leukopenia (10%) and amenorrhoea. Another recent study compared various maintenance therapies for proliferative lupus nephritis following cyclophosphamide induction. It concluded that maintenance with mycophenolate mofetil or azathioprine was more efficacious and safer than long-term intravenous cyclophosphamide [47]. An abstract of a recent open-labelled clinical trial reported improved compliance and clinical outcomes with mycophenolate mofetil when compared with intravenous cyclophosphamide [48]. In addition, a distinct advantage has been the lack of reports of mutagenic effects with mycophenolate mofetil [40]. As a result of these clinical trials, mycophenolate mofetil is becoming increasingly regarded as both an appropriate alternative for treatment of lupus nephritis and as maintenance therapy after cyclophosphamide induction and its use is expected to increase.

B cell depletion therapy

While many facets of the immune system, including pathogenic T cells, cytokines and autoantibodies, may play a role in the pathogenesis of SLE, it has been generally agreed that B cell dysfunction is central to SLE pathogenesis, thus

providing a rationale for trials to further evaluate the anti-CD20 monoclonal antibody rituximab for treatment of SLE. B cell ontogeny is characterized by a series of changing surface phenotypes. CD20 is a surface marker expressed during intermediate stages of development and lost on terminal differentiation to the immunoglobulin producing plasma cell. Rituximab, a chimeric monoclonal antihuman CD20 antibody, rapidly depletes peripheral blood CD20 positive B cells via complement-mediated and antibody-dependent cell mediated cytotoxicity, induction of apoptosis and inhibition of cell growth [49]. Rituximab was licensed initially for treatment of relapsed low grade B cell follicular non-Hodgkin's lymphoma (NHL) [50]. Subsequently, experimental use in autoimmune disorders was instigated with initial promise shown in chronic idiopathic thrombocytopenic purpura (ITP) [51]. Published trials of rituximab in combination with various immunosuppressive agents have also been encouraging in patients with treatment resistant rheumatoid arthritis [52–54].

More recently, rituximab is being studied in patients with SLE unresponsive or poorly responsive to conventional therapies. The first published trial of rituximab for patients with mild to moderately active SLE reported tolerance and efficacy using a dose escalation protocol of between a single 100 mg/m^2 dose and 4 weekly 375 mg/m^2 doses in patients without severe organ involvement [55]. Higher dosage resulted in more prolonged and consistent B cell depletion. Interestingly, despite improvements as assessed by the Systemic Lupus Activity Measure (SLAM) score, no significant alterations in dsDNA or complement levels were identified in this study. A smaller open study of six patients with more severely active disease investigated a combination of rituximab, cyclophosphamide and high-dose oral corticosteroids. All patients improved clinically in their systemic, cutaneous and joint symptoms (as assessed by British Isles Lupus Assessment Group), and a proportion showed improvement in haematological parameters, C3 levels and antidsDNA titres [56]. Two of five patients continued disease-free and without immunosuppressive agents for 2 and 3 years post-B cell depletion. Relapse occurred in the remaining with or after B cell repopulation. Similar encouraging results were published by the same group, who used two doses of 1000 mg rituximab, two doses of 750 mg cyclophosphamide and high-dose corticosteroids over 2 weeks for 14 patients with treatment 'resistant' active renal lupus (WHO classes IV or V), including failure with intravenous cyclophosphamide [57]. Reponses of six of the most 'homogeneous' patients with lupus nephritis were analysed. Reduction in disease activity, improvements in renal function and immunological and haematological indices were reported. Apart from mild infusion reactions, adverse events were minimal. Outcomes of a recently published phase I/II study demonstrated improvements in B cell homeostasis and tolerance after B cell depletion with rituximab [58].

Although the exact mechanism of action of B cell depletion in SLE remains uncertain, rituximab therapy seems to offer an alternative option for lupus patients with active systemic disease, who have failed or are only partially responsive to conventional treatments. As with RA and several other autoimmune conditions, in SLE there is some variability in the degree of B cell depletion achieved with rituximab, and also in the association between B cell depletion, levels of circulating antibodies and patient response [52,58,59]. Further studies to address these questions, optimize dosing regimens, requirements for adjuvant therapies and to ensure long-term tolerability are in progress.

Autologous haematopoietic stem cell transplant

Immunoablation followed by autologous haematopoietic stem cell transplant (HSCT) has been explored in patients with severe systemic lupus who are unresponsive to conventional therapies or suffer intolerable side-effects. HSCT is most commonly applied for haematological diseases, allowing repopulation of the bone marrow with normal, healthy haematopoietic stem cells and peripheral blood after chemotherapeutic ablation of 'malignant' clones. HSCT has been used for a variety of autoimmune diseases [60]. The hypothesis that disease mediating lymphocytes in patients with SLE should be prone to such eradication resulted in the first HSCT being performed for SLE in 1997 [61]. Over the past few years a number of case reports and small series have been published [61–64]. The most often-used protocol consisted of mobilization with high-dose cyclophosphamide and granulocyte colony stimulating factor followed by cyclophosphamide plus antithymocyte globulin ± methylprednisolone as conditioning. HSCT in 15 patients with severe systemic lupus resulted in sustained improvements in disease activity and normalization of organ function [62]. No deaths were reported in this group, SLE Disease Activity Index (SLEDAI) scores decreased to ≤5 in 12 patients and complement and dsDNA levels normalized in all. Ten patients reviewed at 12 months had discontinued all immunosuppressive medication and two patients clinically relapsed. An analysis of the smaller studies reported an overall procedure mortality rate of 12% and remission rate of 66% (as assessed by reduction in SLEDAI to <3), although 33% of these later relapsed [60–64]. Disease control was reflected in improvements to serological and immunological parameters and reduction or withdrawal of corticosteroids. The studies demonstrated the effectiveness of HSCT in inducing remission, although it appears to be curative in less than 50% [63]. Autologous HSCT is associated with the possibility of severe adverse events, including infection and organ dysfunction, and longer-term toxicities are yet to be defined. In addition, development of new autoimmune diseases following HSCT has been reported. Further trials are required to assess whether response is related to the mobilizing and conditioning regimen alone without the HSCT. A phase III

randomised trial to directly compare HSCT and conventional therapies is also required. It may eventuate that HSCT is best used not with curative intent, but to alter severe disease towards a more treatment responsive type. Currently, however, it should be reserved only for those patients with persistence of organ-threatening SLE despite standard aggressive therapy.

Biological therapies: anti-tumour necrosis factor-α therapies

The role for anti-TNF-α agents in rheumatoid arthritis is now well established [65–67], although it remains less clear in SLE. TNF-α participates in the immune dysregulation evident in SLE by increasing production of other proinflammatory cytokines such as IL-1, IL-6 and IL-8, and furthermore may be altered by circulating immune complexes. High serum concentrations of the proinflammatory cytokine TNF-α have been reported in lupus patients [68–70]. Analogous to rheumatoid arthritis synovial tissue, TNF-α has been identified in renal biopsies of SLE patients, with expression and serum levels correlating closely with disease activity [71–73]. However, results from experimental animal models seem to convey a somewhat ambiguous role for TNF-α in SLE. Some trials report that a deficiency of TNF-α improved murine glomerulonephritis, anti-TNF-α agents reduced anti-dsDNA titres and low-dose TNF-α accelerated disease in lupus-prone NZB×NZW and lpr mice [74]. In contrast, other studies demonstrated that NZB×NZW TNF knockout mice still develop active lupus, suggesting that the effect of TNF-α on diseases activity is not straightforward. The development of anti-dsDNA antibodies in approximately 16% of RA patients treated with anti-TNF-α therapies, and a transient lupus-like syndrome that resolves on treatment cessation in 0·2% [75], further confuse the role of the cytokine in SLE pathogenesis and treatment. A small open-labelled study of patients with moderate disease activity refractory to standard therapy was performed with infliximab infusions given at 0, 2, 6 and 10 weeks. Resolution of arthritis and reduction in proteinuria and SLE disease activity were reported in a proportion of patients. In contrast to RA, disease relapsed following drug suspension, settling only after drug reintroduction. There were no consistent effects on anti-dsDNA titres or C3 and patients developed transient increases in anti-histone antibodies and anti-phospholipid antibodies [76]. Although some investigators are hopeful that TNF-α agents will prove beneficial in SLE, at present it seems the consensus is that anti-TNF-α receptor therapies are not clinically indicated for SLE.

Biological therapies: co-stimulatory molecules

Inhibition of several different pathways in lupus pathogenesis have been explored. Targeted immunosuppression of CD40 ligand/CD40 or CTLA-4/CD28/CD80/CD86 interactions results in the blockage of costimulatory signals required for antigen presenting cell activation and thus effective B cell autoantibody production. CD40 ligand on activated T cells (also known as CD154) is a member of the tumour necrosis superfamily of transmembrane proteins, and by binding to its receptor CD40 constitutively expressed on B cells, it facilitates normal immune function [77–80]. Murine experiments have demonstrated over-expression of CD40 ligand (CD40L) on T cells of SLE mice [81] and showed that early anti-CD40L therapy delayed disease onset by reducing B cell activation markers, autoantibody production and renal immune complex deposition [82,83]. In addition, anti-CD40L treatment was reported to reduce or normalize self-antigen presentation by apoptotic cells and limit dendritic cell proliferation and splenic migration [84]. While anti-CD40L immunotherapy in mice with established disease reduced nephritis severity and prolonged survival, results suggest that it is the prolonged early use that is most effective, specifically to reduce dsDNA antibodies and improve renal disease [83,85].

Unfortunately, these initially promising results have not been translated successfully to human trials [86]. Several groups have demonstrated increased CD40L expression and abnormal regulation on human SLE T cells [87–89], increased CD40 and CD40L on mononuclear cells in WHO classes III and IV glomerulonephritis [90] and elevated soluble CD40L levels in patients with SLE [90,91]. A phase I clinical trial with anti-CD40L monoclonal antibody demonstrated safety and tolerability in patients with SLE with reports of only minor adverse effects, including headache and nausea [92]. Further studies, however, have demonstrated contradictory results. Use in a small group of patients with active SLE revealed prompt reduction of dsDNA antibody levels and improvements in proteinuria and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [93]. In contrast, a 16-week phase II double-blind placebo-controlled trial incorporating patients with mild–moderate disease demonstrated no significant difference in disease activity after six infusions of 2·5–10·0 mg/kg anti-CD40L monoclonal antibody [94]. Furthermore, of concern was the increased incidence of thromboembolic complications reported with anti-CD40L monoclonal therapies, necessitating early termination of a recent trial studying patients with proliferative lupus nephritis [95]. Establishment of safety is required before larger studies to define utility of this novel agent can be considered.

The soluble recombinant molecule CTLA4-immunoglobulin, consisting of the extracellular domain of CTLA4 linked to an immunoglobulin Fc region, has been shown to inhibit co-stimulatory signals in SLE and transiently prevent or delay disease progression according to the animal model used [96]. Prolonged survival was evident in CTLA4-immunoglobulin plus cyclophosphamide treated mice *versus* controls, although single-agent treatment with CTLA4-immunoglobulin did not improve proteinuria [97,98]. Effect

Table 1. Biological therapies in development for systemic lupus erythematosus

Therapy	Mechanism of action	Trials
(1) Recombinant IL-1 receptor antagonist (Anakinra)	IL-1: potential role in development and maintenance of inflammation in SLE. IL-1RA; physiological antagonist to IL-1 Elevated IL-1RA in some patients [99] reduced IL-RA production by monocytes, granulocytes; lower levels in renal *versus* non-renal disease [102–104]. Aim with IL-1RA is to address imbalance with IL-1	Well tolerated. Principally effective (transient) for arthritic symptoms. Reduction in C3 and C4; need to monitor serological markers [100,101]
(2) Anti-IL-10 monoclonal antibody	IL-10; pleiotropic cytokine, induces B cell differentiation	Use in NZB/WF1 mice delayed disease onset and autoantibody production [105] Clinical trial with 20 mg/day murine monoclonal antibody improved joint and cutaneous symptoms, reduced SLEDAI. Well tolerated, all patients developed anti-chimeric antibodies [106]
(3) B cell toleragens (LJP 394)	Synthetic molecule composed of multiple B cell dsDNA epitopes attached to non immunogenic carrier. Bind to anti-dsDNA receptors; modulates B cell responses, precipitates cell death and anergy and thus cessation of autoantibody production	Randomized, DBPC Serological improvement but minimal reduction in renal flares [107]
(4) Monoclonal anti-B lymphocyte stimulator	BLys is member of TNF protein family; anti-BLys modulates B cell immune responses by (BLyS) reduction of apoptosis, interference in B cell development and differentiation	Phase I study: reduction in immunoglobulin and anti-dsDNA titres [108] Phase II trial under way

DBPC: double-blind placebo-controlled; IL-1: interleukin 1; IL-1RA: interleukin I receptor antagonist; C3: complement 3; C4: complement 4; IL-10: interleukin 10.

on survival was also significantly greater when CTLA4-immunoglobulin was combined with cyclophosphamide. It may be that CTLA4-immunoglobulin's primary role is as an adjuvant to cyclophosphamide, allowing dosage reduction and thus probability of adverse events. Further trials are imperative.

Biological therapies: other

Advances in monoclonal antibodies and recombinant DNA technology have resulted in development of therapies designed to selectively inhibit distinct cell subsets, surface molecules and secreted products. Some of these are designed to manipulate responses of autoreactive T cells and B cells, others to alter cytokine function in autoimmunity (Table 1). Such strategies have been explored in murine models of SLE and may soon be translated into new therapies for patients with SLE.

Conclusion

The coming years promise to be an exciting time for the development and trial of new pharmacological treatments and immunotherapies for patients with SLE as we benefit from improved understanding of disease pathogenesis and molecular mechanisms.

References

1 Johnson AE, Gordon C, Palmer RG, Bacon PA. The prevalence and incidence of systemic lupus erythematosus in Birmingham, England. Relationship to ethnicity and country of birth. Arthritis Rheum 1995; **38**:551–8.

2 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; **40**:1725.

3 Rahman A, Giles I, Haley J, Isenberg D. Systematic analysis of sequences of anti-DNA antibodies – relevance to theories of origin and pathogenicity. Lupus 2002; **11**:807–23.

4 Okamura M, Kanayama Y, Amastu K *et al.* Significance of enzyme linked immunosorbent assay (ELISA) for antibodies to double stranded and single stranded DNA in patients with lupus nephritis: correlation with severity of renal histology. Ann Rheum Dis 1993; **52**:14–20.

5 Davas EM, Tsirogianni A, Kappou I, Karamitsos D, Economidou I, Dantis PC. Serum IL-6, TNFalpha, p55 srTNFalpha, p75srTNFalpha, srIL-2alpha levels and disease activity in systemic lupus erythematosus. Clin Rheumatol 1999; **18**:17–22.

6 Tokano Y, Morimoto S, Kaneko H *et al.* Levels of IL-12 in the sera of patients with systemic lupus erythematosus (SLE) – relation to Th1- and Th2-derived cytokines. Clin Exp Immunol 1999; **116**:169–73.

7 Hagelberg S, Lee Y, Bargman J *et al.* Longterm followup of childhood lupus nephritis. J Rheumatol 2002; **29**:2635–42.

8 Houssiau FA, Lefebvre C, Vanden Berghe M, Lambert M, Devogelaer JP, Renauld JC. Serum interleukin 10 titers in systemic lupus erythematosus reflect disease activity. Lupus 1995; **4**:393–5.

9 Liu TF, Jones BM. Impaired production of IL-12 in systemic lupus erythematosus. I. Excessive production of IL-10 suppresses production of IL-12 by monocytes. Cytokine 1998; **10**:140–7.

10 Emilie D, Mariette X. Interleukin 10: a new therapeutic target in systemic lupus erythematosus? Joint Bone Spine 2001; **68**:4–5.

11 Min DJ, Cho ML, Cho CS *et al.* Decreased production of interleukin-12 and interferon-gamma is associated with renal involvement in systemic lupus erythematosus. Scand J Rheumatol 2001; **30**:159–63.

12 Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. Arthritis Rheum 1998; **41**:1241–50.

13 Taylor PR, Carugati A, Fadok VA *et al.* A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. J Exp Med 2000; **192**:359–66.

14 Peschken CA, Esdaile JM. Systemic lupus erythematosus in North American Indians: a population based study. J Rheumatol 2000; **27**:1884–91.

15 Moss KE. Isenberg DA. Comparison of renal disease severity and outcome in patients with primary antiphospholipid syndrome, antiphospholipid syndrome secondary to systemic lupus erythematosus (SLE) and SLE alone. Rheumatology (Oxford) 2001; **40**:863–7.

16 Dostal C, Tesar V, Rychlik I *et al.* Effect of 1 year cyclosporine A treatment on the activity and renal involvement of systemic lupus erythematosus: a pilot study. Lupus 1998; **7**:29–36.

17 Radhakrishnan J, Kunis CL, D'Agati V, Appel GB. Cyclosporine treatment of lupus membranous nephropathy. Clin Nephrol 1994; **42**:147–54.

18 Francioni C, Galeazzi M, Fioravanti A, Gelli R, Megale F, Marcolongo R. Long-term i.v. Ig treatment in systemic lupus erythematosus. Clin Exp Rheumatol 1994; **12**:163–8.

19 Schroeder JO, Zeuner RA, Euler HH, Loffler H. High dose intravenous immunoglobulins in systemic lupus erythematosus: clinical and serological results of a pilot study. J Rheumatol 1996; **23**:71–5.

20 Lampropoulos CE, Sangle S, Harrison P, Hughes GR, D'Cruz DP. Topical tacrolimus therapy of resistant cutaneous lesions in lupus erythematosus: a possible alternative. Rheumatology (Oxford) 2004; **43**:1383–5.

21 Duddridge M, Powell RJ. Treatment of severe and difficult cases of systemic lupus erythematosus with tacrolimus. A report of three cases. Ann Rheum Dis 1997; **56**:690–2.

22 Morand EF, McCloud PI, Littlejohn GO. Continuation of long term treatment with hydroxychloroquine in systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis 1992; **51**:1318–21.

23 Fox RI. Mechanism of action of hydroxychloroquine as an antirheumatic drug. Semin Arthritis Rheum 1993; **23**:82–91.

24 Tsakonas E, Joseph L, Esdaile JM *et al.* A long-term study of hydroxychloroquine withdrawal on exacerbations in systemic lupus erythematosus. The Canadian Hydroxychloroquine Study Group. Lupus 1998; **7**:80–5.

25 Hoch S, Schur PH. Methylprednisolone pulse therapy for lupus nephritis: a followup study. Clin Exp Rheumatol 1984; **2**:313–20.

26 Kimberly RP. Pulse methylprednisolone in SLE. Clin Rheum Dis 1982; **8**:261–78.

27 Felson DT, Anderson J. Evidence for the superiority of immunosuppressive drugs and prednisone over prednisone alone in lupus nephritis. Results of a pooled analysis. N Engl J Med 1984; **311**:1528–33.

28 Chan TM, Li FK, Wong RW, Wong KL, Chan KW, Cheng IK. Sequential therapy for diffuse proliferative and membranous lupus nephritis: cyclophosphamide and prednisolone followed by azathioprine and prednisolone. Nephron 1995; **71**:321–7.

29 Takada K, Illei GG, Boumpas DT. Cyclophosphamide for the treatment of systemic lupus erythematosus. Lupus 2001; **10**:154–61.

30 Austin HA III, Klippel JH, Balow JE *et al.* Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. N Engl J Med 1986; **314**:614–9.

31 Mok CC, Ho CT, Siu YP *et al.* Treatment of diffuse proliferative lupus glomerulonephritis: a comparison of two cyclophosphamide-containing regimens. Am J Kidney Dis 2001; **38**:256–64.

32 Bansal VK, Beto JA. Treatment of lupus nephritis: a meta-analysis of clinical trials. Am J Kidney Dis 1997; **29**:193–9.

33 Gourley MF, Austin HA III, Scott D *et al.* Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. Ann Intern Med 1996; **125**:549–57.

34 Illei GG, Austin HA, Crane M *et al.* Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. Ann Intern Med 2001; **135**:248–57.

35 Dooley MA, Hogan S, Jennette C, Falk R. Cyclophosphamide therapy for lupus nephritis: poor renal survival in black Americans. Glomerular Disease Collaborative Network. Kidney Int 1997; **51**:1188–95.

36 Neuwelt CM, Lacks S, Kaye BR, Ellman JB, Borenstein DG. Role of intravenous cyclophosphamide in the treatment of severe neuropsychiatric systemic lupus erythematosus. Am J Med 1995; **98**:32–41.

37 Eiser AR, Shanies HM. Treatment of lupus interstitial lung disease with intravenous cyclophosphamide. Arthritis Rheum 1994; **37**:428–31.

38 Boumpas DT, Austin HA III, Vaughan EM, Yarboro CH, Klippel JH, Balow JE. Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide therapy. Ann Intern Med 1993; **119**:366–9.

39 Houssiau FA, Vasconcelos C, D'Cruz D *et al.* Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. Arthritis Rheum 2002; **46**:2121–31.

40 Allison AC, Eugui EM. Purine metabolism and immunosuppressive effects of mycophenolate mofetil (MMF). Clin Transplant 1996; **10**:77–84.

41 Van Bruggen MC, Walgreen B, Rijke TP, Berden JH. Attenuation of murine lupus nephritis by mycophenolate mofetil. J Am Soc Nephrol 1998; **9**:1407–15.

42 Corna D, Morigi M, Facchinetti D, Bertani T, Zoja C, Remuzzi G. Mycophenolate mofetil limits renal damage and prolongs life in murine lupus autoimmune disease. Kidney Int 1997; **51**:1583–9.

43 Karim MY, Alba P, Cuadrado MJ et al. Mycophenolate mofetil for systemic lupus erythematosus refractory to other immunosuppressive agents. Rheumatology (Oxford) 2002; **41**:876–82.

44 Kingdon EJ, McLean AG, Psimenou E et al. The safety and efficacy of MMF in lupus nephritis: a pilot study. Lupus 2001; **10**:606–11.

45 Briggs WA, Choi MJ, Scheel PJ Jr. Successful mycophenolate mofetil treatment of glomerular disease. Am J Kidney Dis 1998; **31**:213–7.

46 Chan TM, Li FK, Tang CS et al. Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrol Study Group. N Engl J Med 2000; **343**:1156–62.

47 Contreras G, Pardo V, Leclercq B et al. Sequential therapies for proliferative lupus nephritis. N Engl J Med 2004; **350**:971–80.

48 Ginzler E, Aranow C, Buyon J et al. A multicenter study of mycophenolate mofetil (MMF) vs. intravenous cyclophosphamide (IVC) as induction therapy for severe lupus nephritis (LN): preliminary results. Arthritis Rheum 2003; **48**(Suppl.): S467.

49 Reff ME, Carner K, Chambers KS et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994; **83**:435–45.

50 Hainsworth JD, Burris HA III, Morrissey LH et al. Rituximab monoclonal antibody as initial systemic therapy for patients with low-grade non-Hodgkin lymphoma. Blood 2000; **95**:3052–6.

51 Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001; **98**:952–7.

52 Edwards JC, Cambridge G. Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. Rheumatology (Oxford) 2001; **40**:205–11.

53 Leandro MJ, Edwards JC, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. Ann Rheum Dis 2002; **61**:883–8.

54 Edwards JC, Szczepanski L, Szechinski J et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004; **350**:2572–81.

55 Anolik JH. B lymphocyte depletion in the treatment of systemic lupus erythematosus. Arthritis Rheum 2002; **46**(Suppl 9):S717.

56 Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR. Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. Arthritis Rheum 2002; **46**:2673–7.

57 Leandro M. Treatment of refractory lupus nephritis with B lymphocyte depletion. Arthritis Rheum 2003; **48**(Suppl 9):S924.

58 Anolik JH, Barnard J, Cappione A et al. Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. Arthritis Rheum 2004, 2003; **50**:3580–90.

59 Specks U, Fervenza FC, McDonald TJ, Hogan MC. Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy. Arthritis Rheum 2001; **44**:2836–40.

60 Jayne D, Tyndall A. Autologous stem cell transplantation for systemic lupus erythematosus. Lupus 2004; **13**:359–65.

61 Marmont AM, van Lint MT, Gualandi F, Bacigalupo A. Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. Lupus 1997; **6**:545–8.

62 Traynor AE, Barr WG, Rosa RM et al. Hematopoietic stem cell transplantation for severe and refractory lupus. Analysis after five years and fifteen patients. Arthritis Rheum 2002; **46**:2917–23.

63 Rosen O, Hiepe F, Massenkeil G, Thiel A, Arnold R. Relapse of systemic lupus erythematosus. Lancet 2001; **357**:807–8.

64 Brunner M, Greinix HT, Redlich K et al. Autologous blood stem cell transplantation in refractory systemic lupus erythematosus with severe pulmonary impairment: a case report. Arthritis Rheum 2002; **46**:1580–4.

65 Lipsky PE, van der Heijde DM, St Clair EW et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000; **343**:1594–602.

66 Bathon JM, Martin RW, Fleischmann RM et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000; **343**:1586–93.

67 Weinblatt ME, Keystone EC, Furst DE et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003; **48**:35–45.

68 Maury CP, Teppo AM. Tumor necrosis factor in the serum of patients with systemic lupus erythematosus. Arthritis Rheum 1989; **32**:146–50.

69 Studnicka-Benke A, Steiner G, Petera P, Smolen JS. Tumour necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. Br J Rheumatol 1996; **35**:1067–74.

70 Gabay C, Cakir N, Moral F et al. Circulating levels of tumor necrosis soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. J Rheumatol 1997; **24**:303–8.

71 Takemura T, Yoshioka K, Murakami K et al. Cellular localization of inflammatory cytokines in human glomerulonephritis. Virchows Arch 1994; **424**:459–64.

72 Malide D, Russo P, Bendayan M. Presence of tumor necrosis factor alpha and interleukin-6 in renal mesangial cells of lupus nephritis patients. Hum Pathol 1995; **26**:558–64.

73 Herrera-Esparza R, Barbosa-Cisneros O, Villalobos-Hurtado R, Avalos-Diaz E. Renal expression of IL-6 and TNFalpha genes in lupus nephritis. Lupus 1998; **7**:154–8.

74 Aringer M, Smolen JS. Tumour necrosis factor and other proinflammatory cytokines in systemic lupus erythematosus: a rationale for therapeutic intervention. Lupus 2004; **13**:344–7.

75 Charles PJ, Smeenk RJ, De Jong J, Feldmann M, Maini RN. Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. Arthritis Rheum 2000; **43**:2383–90.

76 Aringer M, Graninger WB, Steiner G, Smolen JS. Safety and efficacy of tumor necrosis factor alpha blockade in systemic lupus erythematosus: an open-label study. Arthritis Rheum 2004; **50**:3161–9.

77 Saeland S, Duvert V, Moreau I, Banchereau J. Human B cell precursors proliferate and express CD23 after CD40 ligation. J Exp Med 1993; **178**:113–20.

78 Renshaw BR, Fanslow WC III, Armitage RJ et al. Humoral immune

responses in CD40 ligand-deficient mice. J Exp Med 1994; **180**:1889–900.

79 Lederman S, Yellin MJ, Cleary AM *et al*. T-BAM/CD40-L on helper T lymphocytes augments lymphokine-induced B cell Ig isotype switch recombination and rescues B cells from programmed cell death. J Immunol 1994; **152**:2163–71.

80 Barrett TB, Shu G, Clark EA. CD40 signaling activates CD11a/CD18 (LFA-1) -mediated adhesion in B cells. J Immunol 1991; **146**:1722–9.

81 Blossom S, Chu EB, Weigle WO, Gilbert KM. CD40 ligand expressed on B cells in the BXSB mouse model of systemic lupus erythematosus. J Immunol 1997; **159**:4580–6.

82 Mohan C, Shi Y, Laman JD, Datta SK. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. J Immunol 1995; **154**:1470–80.

83 Quezada SA, Eckert M, Adeyi OA, Schned AR, Noelle RJ, Burns CM. Distinct mechanisms of action of anti-CD154 in early versus late treatment of murine lupus nephritis. Arthritis Rheum 2003; **48**:2541–54.

84 Kalled SL, Cutler AH, Burkly LC. Apoptosis and altered dendritic cell homeostasis in lupus nephritis are limited by anti-CD154 treatment. J Immunol 2001; **167**:1740–7.

85 Kalled SL, Cutler AH, Datta SK, Thomas DW. Anti-CD40 ligand antibody treatment of SNF1 mice with established nephritis: preservation of kidney function. J Immunol 1998; **160**:2158–65.

86 Yazdany J, Davis J. The role of CD40 ligand in systemic lupus erythematosus. Lupus 2004; **13**:377–80.

87 Koshy M, Berger D, Crow MK. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. J Clin Invest 1996; **98**:826–37.

88 Desai-Mehta A, Lu L, Ramsey-Goldman R, Datta SK. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. J Clin Invest 1996; **97**:2063–73.

89 Devi BS, Van Noordin S, Krausz T, Davies KA. Peripheral blood lymphocytes in SLE – hyperexpression of CD154 on T and B lymphocytes and increased number of double negative T cells. J Autoimmun 1998; **11**:471–5.

90 Yellin MJ, D'Agati V, Parkinson G *et al*. Immunohistologic analysis of renal CD40 and CD40L expression in lupus nephritis and other glomerulonephritides. Arthritis Rheum 1997; **40**:124–34.

91 Kato K, Santana-Sahagun E, Rassenti LZ *et al*. The soluble CD40 ligand sCD154 in systemic lupus erythematosus. J Clin Invest 1999; **104**:947–55.

92 Davis JC Jr, Totoritis MC, Rosenberg J, Sklenar TA, Wofsy D. Phase I clinical trial of a monoclonal antibody against CD40-ligand (IDEC-131) in patients with systemic lupus erythematosus. J Rheumatol 2001; **28**:95–101.

93 Grammer AC, Slota R, Fischer R *et al*. Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154–CD40 interactions. J Clin Invest 2003; **112**:1506–20.

94 Kalunian KC, Davis JC Jr, Merrill JT, Totoritis MC, Wofsy D. Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002; **46**:3251–8.

95 Boumpas DT, Furie R, Manzi S *et al*. A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. Arthritis Rheum 2003; **48**:719–27.

96 Mariani SM. Conference report – lupus nephritis: diagnosis, therapy and outcomes. Med Gen Med 2004; **6**:23–9.

97 Cunnane G, Chan OT, Cassafer G *et al*. Prevention of renal damage in murine lupus nephritis by CTLA-4Ig and cyclophosphamide. Arthritis Rheum 2004; **50**:1539–48.

98 Daikh DI, Wofsy D. Cutting edge: reversal of murine lupus nephritis with CTLA4Ig and cyclophosphamide. J Immunol 2001; **166**:2913–6.

99 Chang DM. Interleukin-1 and interleukin-1 receptor antagonist in systemic lupus erythematosus. Immunol Invest 1997; **26**:649–59.

100 Moosig F, Zeuner R, Renk C, Schroder JO. IL-1RA in refractory systemic lupus erythematosus. Lupus 2004; **13**:605–6.

101 Ostendorf B, Iking-Konert C, Kurz K, Jung G, Sander O, Schneider M. Preliminary results of safety and efficacy of the interleukin-1 receptor antagonist anakinra in patients with severe lupus arthritis. Ann Rheum Dis 2004; **64**:630–3.

102 Andersen LS, Petersen J, Svenson M, Bendtzen K. Production of IL-1beta, IL-1 receptor antagonist and IL-10 by mononuclear cells from patients with SLE. Autoimmunity 1999; **30**:235–42.

103 Scuderi F, Convertino R, Molino N *et al*. Effect of pro-inflammatory/anti-inflammatory agents on cytokine secretion by peripheral blood mononuclear cells in rheumatoid arthritis and systemic lupus erythematosus. Autoimmunity 2003; **36**:71–7.

104 Sturfelt G, Roux-Lombard P, Wollheim FA, Dayer JM. Low levels of interleukin-1 receptor antagonist coincide with kidney involvement in systemic lupus erythematosus. Br J Rheumatol 1997; **36**:1283–9.

105 Ishida H, Muchamuel T, Sakaguchi S, Andrade S, Menon S, Howard M. Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. J Exp Med 1994; **179**:305–10.

106 Llorente L, Zou W, Levy Y *et al*. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. J Exp Med 1995; **181**:839–44.

107 Alarcon-Segovia D, Tumlin JA, Furie RA *et al*. LJP 394 for the prevention of renal flare in patients with systemic lupus erythematosus: results from a randomized, double-blind, placebo-controlled study. Arthritis Rheum 2003; **48**:442–54.

108 Furie R. Safety, pharmacokinetic and pharmacodynamic results of a phase I single and double dose-escalation study of LymphoStat-B (human monoclonal antibody to BLyS) in SLE patients. Arthritis Rheum 2003; **48**(Suppl.):S377.