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New therapies for systemic lupus erythematosus

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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.

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