

New therapies for systemic lupus erythematosus: has the future arrived?

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Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by defects in many parts of the immune cascade. Current therapies can be criticized for being too wide-ranging in their actions, but newer biological therapies described in this article might, in the future, enhance efficacy and reduce toxicity by targeting specific aspects of B-cell, T-cell and cytokine function. Some evidence for these newer agents is encouraging but unwanted and unexpected effects have been encountered suggesting their usefulness might be questioned. While minimizing unwanted effects and maximizing the effectiveness of treatment is the current aim of therapy, permanent remission or cure must be the gold standard aim in the future. The extent to which we are approaching this exciting objective is reviewed in this article.

Systemic lupus erythematosus (SLE) is a chronic multisystem, nonorgan-specific autoimmune disorder, typically affecting women of childbearing age. Experience with established immunosuppressive therapeutic agents, particularly corticosteroids and cyclophosphamide, has demonstrated that these agents are toxic with widespread actions, many of which are detrimental. Newer immunosuppressive agents may provide one answer. Mycophenolate mofetil is an immunosuppressive agent that inhibits T-cell proliferation and T-cell-dependent antibody responses. Recent evidence suggests it is more effective than intravenous cyclophosphamide in induction therapy for lupus nephritis as shown in a randomized, open-label trial in 140 SLE patients, with less toxicity than cyclophosphamide [1]. Immunosuppressive therapy will undoubtedly continue to be used in daily practice, perhaps as the basis for combination therapy, but our understanding of the pathogenesis of SLE has advanced and in the future will allow the introduction of newer therapeutic agents aimed at more specific targets in the immune cascade. This article will review these specific targets and new therapeutic strategies aimed at exploiting their role in this complex disease.

New therapies in SLE

T cells, B cells and humoral mediators have been implicated in the etiology of SLE (Figure 1). At the cellular level, defective apoptosis and abnormal clearance of apoptotic debris by macrophages allow the persistence of apoptotic material and prolonged exposure of nuclear autoantigens [2]. Modification of autoantigens may render them immunogenic and allow

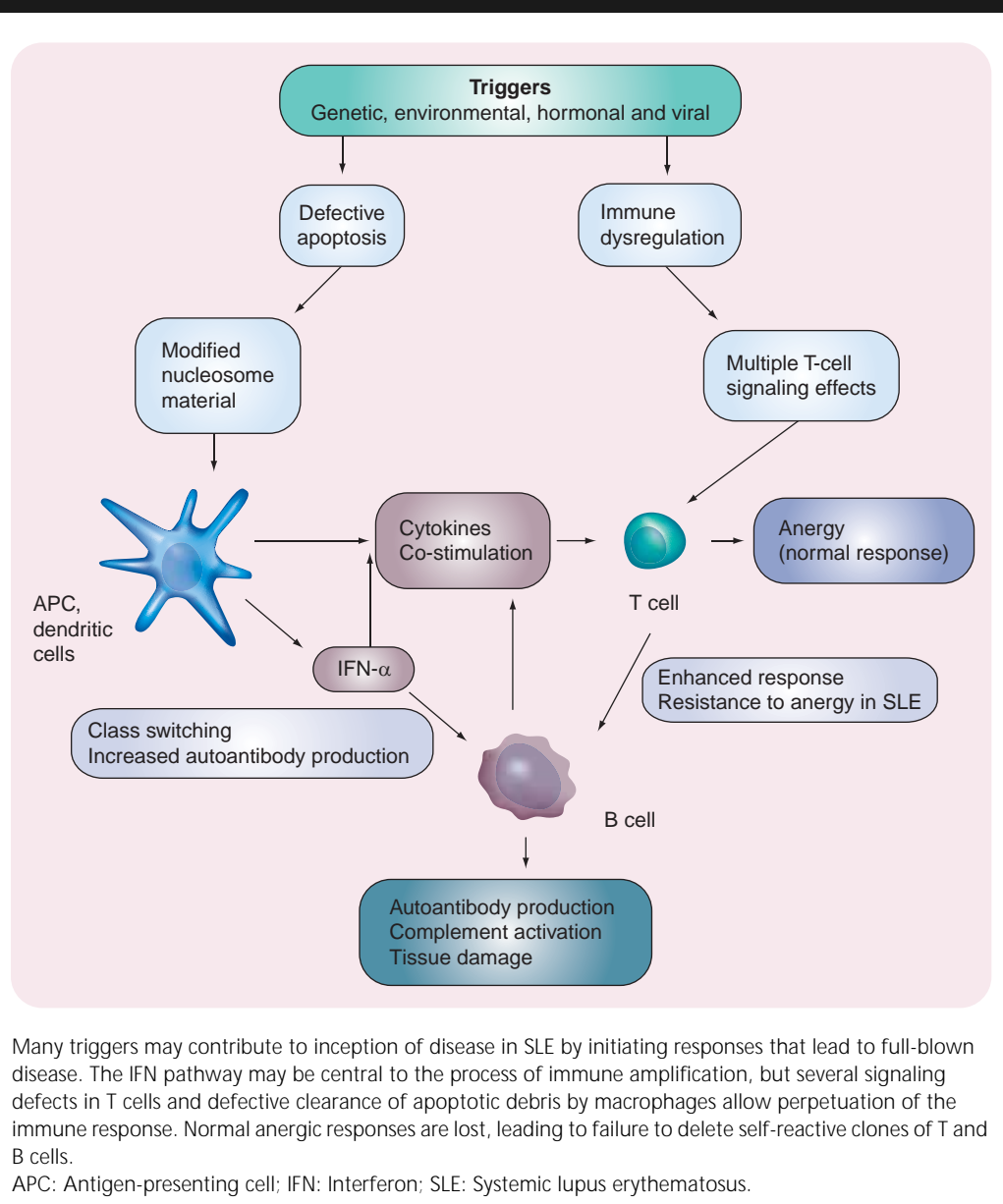
breakage of tolerance [3]. Impaired T-cell regulation, with increased spontaneous apoptosis, increased responsiveness to activation signals [4] and resistance to anergy [5] might drive dysfunctional B-cell regulation, leading to autoantibody production, hyper- γ -globulinemia and immune complex formation typical of SLE. Immune complexes may also directly stimulate B cells, bypassing T-cell control [6].

Many of these cellular interactions are mediated by cytokines, whose role in SLE is complex. Interferon (IFN)- α released from antigen-presenting cells (APCs) in response to immune complexes might be an initial trigger for immune amplification [7], while the balance of T helper (Th)1 and Th2 cytokines might influence subsequent disease progression. The Th1 cytokine tumor necrosis factor (TNF)- α may in part be protective because it induces the release of C-reactive protein and serum amyloid P from the liver. These molecules bind to DNA, rendering it nonimmunogenic and enhancing tolerance to apoptotic fragments [8]. This seemingly protective action is seen in patients given anti-TNF- α treatment for autoimmune diseases where treatment is associated with production of double-stranded (ds)DNA antibodies, although frank lupus is rare [9]. The levels of other Th1 cytokines, including interleukin (IL)-12 and IL-18, are also raised in SLE sera and correlate with disease activity. However, levels of IL-10, a Th2 cytokine, are also raised in SLE sera and associated with SLE disease activity, while levels of the Th2 cytokine, IL-6, are increased in the cerebrospinal fluid (CSF) of SLE patients with neurological lupus, as are those of the Th1 cytokine IL-1 [8].

Keywords: B cells, cellular interactions, complement, cytokines, gene therapy, stem cells, T cells

**future
medicine**

Figure 1. Factors contributing to disease inception in SLE.



In order to make sense of the actions of the large array of cytokines implicated in SLE, it is important to understand that, in the complex autoimmune milieu, which may be typical of SLE *in vivo*, cytokine combinations may produce very different actions from those seen in individual cytokine assays *in vitro*. For example, macrophage production of TNF- α in response to lipopolysaccharide is inhibited by IL-10 unless the macrophages are pre-primed with IFN- α , in which case it is increased [6]. IL-10 may influence proliferation and inhibit IL-12 gene expression in peripheral blood mononuclear cells from SLE patients. Proliferation is

restored by the addition of exogenous IL-12 [10]. Furthermore, the presence of soluble receptors can influence the activity of many cytokines [8]. These results suggest the balance of cytokines and soluble receptors is likely to be more important than any single cytokine in reflecting disease activity and phenotype *in vivo*.

The numerous abnormalities of immune regulation that appear to be involved in the pathogenesis of SLE, together with the heterogeneity of the disease and difficulty in validating clinical outcome measures, have made lupus a challenging disease to study. However, the many abnormalities of immune regulation mean therapeutic

intervention is possible at several sites and these novel targets and respective therapeutic agents will be reviewed in this article (Figure 2).

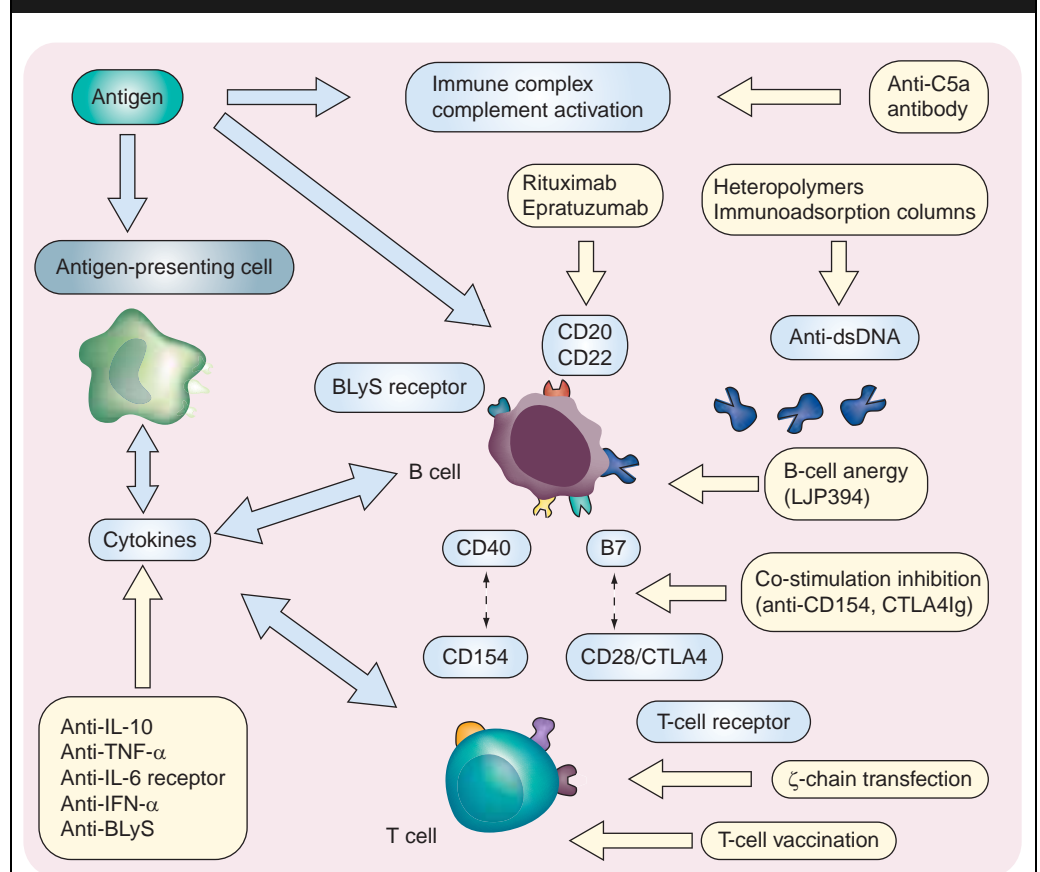
Targeting T cells

T cells exhibit hyper-responsiveness and resistance to anergy in SLE patients and murine lupus models. Several possible targets for therapy might exist based on the available data. CD137 is a TNF superfamily member inducible on CD4⁺ and CD8⁺ T cells, activated natural killer (NK) cells and dendritic cells, but not B cells. Anti-CD137 monoclonal antibody (mAb) suppresses CD4⁺ T-cell help during T-cell-dependent humoral immune responses, probably by an action on regulatory T cells, making anti-CD137 a potentially useful target for therapy. A study of lupus-prone New Zealand black (NZB) × New Zealand white (NZW) F₁ mice treated with anti-CD137 mAbs found a profound suppression of the disease

with extension of lifespan, compared with that of normal mice. The ability of anti-CD137 to induce anergy in CD4⁺ Th populations led to the inability to produce pathogenic immunoglobulin (Ig)G autoantibodies, but without immunosuppression [11]. As breakage of tolerance seems central to the pathogenesis of lupus, a recent study used a consensus peptide based on amino acid sequences from the variable region of the Ig heavy chain (V_H) region of murine anti-DNA antibodies likely to stimulate T cells from NZB × NZB F₁ mice in an attempt to induce tolerance [12]. Delayed onset of nephritis, reduced cytokine production and prolonged survival compared with control mice were noted.

Methylation of deoxycytosine bases in guanine–cytosine (GC) pairs in DNA may suppress gene expression and treatment of CD4⁺ T cells with methylation inhibitors induces autoreactivity and promotes antibody formation. DNA

Figure 2. Possible targets and targeted therapies for SLE management.



The multiple targets (blue boxes and arrows) and targeted therapies (yellow boxes and arrows) which are likely to become established in the treatment of SLE in the future.
 BLyS: B-lymphocyte stimulator; CTLA: Cytotoxic T-lymphocyte antigen; ds: Double-stranded;
 IFN: Interferon; Ig: Immunoglobulin; IL: Interleukin; LJP: La Jolla Pharmaceuticals; TNF: Tumor necrosis factor.

hypomethylation might explain aberrant T-cell behavior in SLE, and one study has found that treating T-cell lines with the methylation inhibitor 5-azacytidine caused increases in CD70, a co-stimulatory ligand for B-cell CD27 and associated T-cell-dependent B-cell stimulation. Furthermore, CD70 is overexpressed in T cells from patients with active lupus, compared with healthy controls, and expression correlates with disease activity measured by SLE disease activity index (SLEDAI) [13]. The overexpressing CD70⁺CD4⁺ T cells overstimulate B-cell immunoglobulin production, which is reversed by anti-CD70 mAbs, suggesting that anti-CD70 could be useful as a future therapeutic agent.

Trials of therapies aimed at modifying specific T-cell responses in humans are as yet limited. Immunization with inactivated autoreactive T cells (T-cell vaccination) induces idio-type-anti-idiotypic reactions, depleting specific autoreactive T-cell subsets involved in SLE. One study of six lupus patients refractory to standard immunosuppressive therapy used inoculated inactivated autoreactive T cells and found an improvement in SLEDAI scores, induction of clinical remission and decreased levels of autoantibodies, including dsDNA, without significant side effects [14]. The results of this small study need to be interpreted with caution and larger trials are needed to determine whether the results can be reproduced. Another potential avenue for therapy is the finding that most SLE patients have a decreased expression of the T-cell receptor- ζ chain with reduced IL-2 production owing to limited IL-2 promoter transcriptional activity [15]. Forced expression of this aberrant chain by transfection in T cells from SLE patients can correct many signaling defects and increase IL-2 production [16].

The potential to target T cells is an exciting one. However, clinical therapies are at a very early stage in their development and much larger, longer-term trials are required to assess their efficacy.

Targeting cellular interactions

Blockade of APC-T-cell B7-CD28 co-stimulatory interaction by cytotoxic T lymphocyte antigen 4 Ig

After interaction with antigen, APCs and activated B cells express surface B7-1 (CD80) and B7-2 (CD86). Indeed, SLE patients with active disease exhibit enhanced levels of B7-2 on B cells [17]. B7 interacts with T-cell CD28 and

cytotoxic T-lymphocyte antigen (CTLA)4 (CD152), providing a co-stimulatory signal for T-cell activation with a consequent expression of both IL-2, a cytokine critical for T-cell growth, and Bcl-X_L, an anti-apoptotic protein [18–20].

CTLA4 is expressed on activated T cells and binds to B7 with a higher affinity than CD28. Although the CTLA4–B7 interaction may be inhibitory, there is some evidence that it is associated with clonal expansion of T cells [18]. This knowledge has led to the development of CTLA4Ig, a fusion of the extracellular domain of CTLA4 and the murine or human Ig constant chain, which allows the blockade of CTLA4/B7–CD28 interaction, preventing T-cell activation. There have been encouraging reports of efficacy in rheumatoid arthritis (RA) [21], and one study in a murine lupus model suggested comparable efficacy in mild lupus nephritis to cyclophosphamide [22]. Furthermore, combination treatment with cyclophosphamide and CTLA4Ig in murine models is more effective in reducing renal disease and prolonging survival than with either drug alone, suggesting synergy [22]. CTLA4Ig may be an effective treatment for SLE, but the results of the Phase I/II multicenter clinical trials with combination cyclophosphamide, which are underway in humans, are awaited.

T cell-B cell (CD40-CD154) interactions

The high affinity autoantibodies typical of lupus are encoded by mutated immunoglobulin genes produced by B cells that have participated in a germinal center reaction [23]. This reaction depends on the engagement of inducible CD154 on T cells with constitutive CD40 on B cells, and leads to the expression on B cells of early activation markers (CD69 and CD154) and differentiation markers (CD38, CD5 and CD27), after which B cells proliferate rapidly into secondary follicles, the germinal centers (GC). CD154 is also found on platelets, while CD40 is found on endothelial cells, renal parenchymal cells and epithelial cells.

Release of CD154⁺ T and B cells is abnormally increased in active SLE patients, suggesting overactivity of GC reactions. In an attempt to block this reaction, a trial of anti-CD154 mAb was conducted on four SLE patients with active proliferative lupus nephritis [24]. Significant reductions in dsDNA levels, circulating numbers of Ig-secreting (CD38⁺) B cells and proteinuria were seen. Disease activity scores measured by SLEDAI remained suppressed for 20 months

post-treatment. In another Phase II open-label trial of biweekly anti-CD154, reductions in dsDNA levels and hematuria were seen in some SLE patients with proliferative lupus nephritis [25].

The results of these trials are tempered by two problems. First, the high rate of thromboembolic complications that ensued, including myocardial infarction, fatal pulmonary embolism and fetal death, forced the studies to conclude prematurely. The prothrombotic effect may occur because platelet CD154 stabilizes thrombi, so its blockade could allow a thrombus to disseminate. Second, a Phase II, double-blind, placebo-controlled trial of inflammatory dendritic epidermal cell (IDEC)-131, a humanized mAb against CD154, did not show efficacy compared with placebo in 85 SLE patients, although safety and tolerability were demonstrated [26]. Surmounting these significant obstacles remains the future aim before therapies targeting CD40–CD154 interactions can be accepted as treatment options for lupus.

Targeting B cells

Therapy with anti-CD20 (rituximab) and anti-CD22 (epratuzumab)

The importance of B cells in immune responses makes them a viable therapeutic target. CD20 is a cell surface membrane nonglycosylated antigenic phosphoprotein, important in B-lymphocyte cell-cycle initiation and differentiation. It is expressed on pre-B and mature B cells and is neither internalized nor shed, providing a stable therapeutic target.

Rituximab is a chimeric anti-CD20 mAb. It comprises human IgG₁ Fc and κ constant regions and small variable light and heavy chain regions from the murine anti-CD20 antibody fragment IDEC-2B8. Rituximab depletes B cells by three mechanisms: antibody-dependent cell-mediated cytotoxicity, complement-mediated cytotoxicity and promotion of B-cell apoptosis by cross linkage of Fc-γ receptor-expressing cells. It improves peripheral B-cell abnormalities, with circulating plasmablast numbers and naïve lymphopenia resolving after the treatment of SLE [27].

Rituximab was licensed originally for the treatment of non-Hodgkin's lymphoma in 1997 and, to date, published reports have described its use in over 90 SLE patients. An open-label study initially showed improvement in disease activity measured by British Isles Lupus Assessment Group (BILAG) scores in six female patients with active SLE [28]. Improvements in fatigue, arthralgia/arthritis and serositis were

particularly impressive, but rises in hemoglobin and C3 levels, as well as reductions in urinary protein–creatinine ratios, were also noticed.

These results were largely confirmed in a dose-escalation trial of rituximab and moderate-dose corticosteroid in 16 SLE patients, including seven with nephritis (three with WHO class III, three with class IV and one not classified). In all those who achieved complete B-cell depletion, disease activity improved, in contrast to six whose B cells did not deplete and who did not experience a clinical benefit. One patient showed complete histological resolution of class IV nephritis with major clinical improvement [29]. Sustained improvements were seen up to 12 months after rituximab treatment, with some patients requiring little or no steroid therapy or other immunosuppressants. The side-effect profile of rituximab is good, with infusion reactions in 10% of patients but few serious infections. Studies from RA suggest combinations of rituximab with cyclophosphamide or methotrexate are more effective than rituximab alone and such combinations have formed the basis for rituximab therapy in SLE, although data on which combination is best are lacking at present [30].

These studies confirm that patients can remain in remission even though B-cell counts normalize between 3 and 12 months after treatment. The reason for this may be repopulation by naïve B cells, but downregulation of co-stimulatory molecules CD40 and CD80, which has the effect of attenuating T-cell activation, may also be important [31].

The use of rituximab has been extended with corticosteroids to treat a patient with primary antiphospholipid syndrome (APS) already on warfarin with poor symptom and international normalized ratio (INR) control [Unpublished Data]. INR values stabilized with symptom improvement and similar results have been reported in APS secondary to SLE, with reductions in antiphospholipid antibody levels [32].

Phase I/II studies using humanized anti-CD22 mAb (epratuzumab) are also underway after initial encouraging reports of its use in a heterogeneous group of 14 SLE patients, some of whom were refractory to conventional therapy [33]. Epratuzumab (360 mg/m²) was administered every 2 weeks for four doses. It was well tolerated and disease activity measured by BILAG and American College of Rheumatology scores improved in all patients. Epratuzumab might not fully deplete B-cell numbers and may work by modulating B-cell function.

The reported cases of SLE treated with rituximab and epratuzumab are heterogeneous with variations in disease severity, organ involvement and dosing regimens. Despite this, both agents show promise in the treatment of severe lupus, with long-term remission demonstrated in some patients on rituximab.

Tolerizing B cells (LJP394)

Anti-dsDNA is found with high sensitivity and specificity in lupus. There is a good correlation between dsDNA levels and lupus disease flares in many settings [34], so reducing dsDNA levels might have therapeutic potential. La Jolla Pharmaceuticals (LJP), CA, USA, LJP394 (abetimus sodium, riqent) consists of four 20-mer dsDNA epitopes conjugated to a nonimmunogenic polythene glycol platform. It tolerizes B cells by cross-linking anti-dsDNA surface Ig receptor on B cells, initiating a signal transduction pathway that leads to B-cell anergy or apoptosis [35]. In BXSB mouse models, treatment with LJP394 led to a reduction in dsDNA levels and proteinuria with improved survival [36]. In one human trial of 230 SLE patients, subgroup analysis of patients with high affinity IgG to the DNA epitopes of LJP394 had fewer renal lupus flares, longer time to institution of cyclophosphamide with high-dose steroids and fewer treatments than high-affinity patients given placebo. However, there was no difference in time to renal flare in the intention-to-treat analysis [37].

The reasons for the largely disappointing results may be owing to patient selection, with more low-affinity patients having worse renal function and perhaps suboptimal dosing. The results also suggest the precise role of dsDNA antibodies in SLE requires clarification. TNF- α blockade is associated with raised dsDNA levels, but few of these patients develop lupus or nephritis [9] and not all patients with renal lupus have dsDNA antibodies. Antibody affinity and patient selection may, therefore, be the key to any future use of LJP394.

Targeting cytokines

Anti-TNF- α therapy

TNF- α probably exerts two actions in SLE, one proinflammatory and the other protective, preventing autoantibody formation. Timing of exposure, dosage and soluble receptor levels may all influence its action. TNF- α administered late in the disease to NZB/NZW lupus-prone mice caused a deterioration of nephritis, but this effect was not seen if administration occurred later or at

higher doses. However, high levels of TNF- α are found in the kidneys of MRL/lpr lupus-prone mice and levels are also raised in SLE and are associated with disease activity [8], providing a rationale for anti-TNF- α therapy for SLE. This treatment is an established and highly efficacious treatment for RA [38]. An open-label study of six patients with moderately active SLE (four with nephritis, three with refractory arthritis) who were given infliximab found remission of arthritis during the treatment period, with relapse after the infliximab was discontinued. However, significant reduction of proteinuria was seen despite increases in dsDNA levels and this improvement persisted after the end of the treatment period [39]. Further randomized, controlled trials in SLE are needed to clarify these effects and ensure long-term use is safe and effective.

B-lymphocyte stimulator

B-lymphocyte stimulator (BLyS), a 285 amino acid peptide ligand is essential for B-cell development. BLyS-deficient mice exhibit normal bone marrow B-cell and spleen T1 B-cell development, but lack all other peripheral B-cell subsets [40]. A profound reduction of mature B cells and plasma immunoglobulin levels ensues. Conversely, some BLyS-transgenic mice exhibit elevated circulating B-cell numbers and lupus-like features, including polyclonal hyper- γ -globulinemia, increased autoantibody levels (including dsDNA) and renal immunoglobulin deposits [41,42]. Raised levels of BLyS are found in MRL-1pr/1pr and NZB/NZW F₁ autoimmune-prone mice [43] and increased plasma levels of BLyS have been demonstrated in SLE patients [44].

Anti-BLyS mAb has undergone formal testing in Phase I trials. A total of 57 stable SLE patients were enrolled into one study and followed up for 84–105 days. The results suggest anti-BLyS treatment is safe but no clinical improvement was noted in a short follow-up period, despite significant reduction in peripheral B-cell counts [45]. Other BLyS antagonists are being evaluated, but the results of clinical trials are awaited.

A proliferation-inducing ligand

A proliferation-inducing ligand (APRIL) is a Type II membrane-binding protein of 250 amino acids and a close homolog of BLyS. APRIL-transgenic mice exhibit enhanced T-cell survival and antigen-specific responses but do not exhibit either B-cell abnormalities or autoimmune markers serologically or clinically. Serum APRIL levels are significantly higher in

patients with SLE than in healthy controls or RA patients. In one study, APRIL levels correlated with BILAG musculoskeletal score but not other organ damage indices or dsDNA levels [46], while another study found APRIL inversely correlated to SLEDAI scores and dsDNA levels [47]. There are no reports to date of targeted therapy against APRIL, but these findings suggest subtle changes to lupus markers may be possible with such therapy in the future.

Anti-interleukin-1

Anti-IL-1 receptor therapy has been used with some success in RA. Anti-IL-1 receptor mAb anakinra (100 mg/day subcutaneously) has been used in a limited study of arthritis refractory to conventional agents, including methotrexate and steroids, in four SLE patients over 12 weeks [48]. Although tender joint counts and European consensus lupus activity measurement reduced after 4 weeks, the tendency thereafter was for these measures to plateau or worsen. One patient withdrew owing to active arthritis while on treatment. No firm conclusions can be drawn from this small, short-term trial until longer-term trials are carried out.

Anti-interleukin-6

Levels of IL-6, a proinflammatory cytokine important in the differentiation of B cells into antibody-producing cells and T cells into effector cells, are raised in SLE. Blockade of IL-6 in murine lupus models reduces disease activity and trials of anti-IL-6 receptor mAb, are underway currently in humans [49]. Good results and limited toxicity have been reported in the treatment of RA [50].

Anti-interleukin-10

IL-10 is a pleiotropic cytokine with an important role in the regulation of the immune response and is a potent *in vitro* inducer of B-cell differentiation. It is released spontaneously by peripheral blood mononuclear cells of SLE patients and raised levels correlate with disease activity [51,52]. Animal studies with severe combined immune deficient mice show that anti-IL-10 mAb significantly reduces renal impairment with enhanced benefit when used with anti-C5 anticomplement antibody [53]. In a heterogeneous group of SLE patients, the murine anti-IL-10 antibody B-N10, given daily for 21 days intravenously, improved cutaneous and joint symptoms in six SLE patients

and was well tolerated. Disease activity measured by SLEDAI decreased significantly, and five out of the six patients had inactive disease at 6 months. However, treatment additions were allowed during the trial and little or no change was seen in serological markers, making the interpretation of results without a control group difficult [54].

Other interleukins

Supernatants of cultured mononuclear cells from SLE patients inhibit T-cell allogeneic responses and this effect is reversed by the addition of IL-12 [9], suggesting that IL-12 has potential as a therapeutic agent. Levels of IL-15 are raised in some SLE patients and might drive polyclonal B-cell activation [55]. IL-18 is a cytokine with potent proinflammatory properties. Levels are raised in SLE sera, which correlate with disease activity and may enhance leukocyte migration and production of inflammatory chemokines [56]. All of these interleukins are potential targets for future therapy, but no trials have yet been carried out.

Interferon- α & - γ

The IFNs were the first cytokine family to be discovered. IFN- α treatment is associated with induction of SLE [57], and levels correlate with SLE disease activity and organ involvement in some studies [9]. IFN- α has many actions that could be central to the initiation and perpetuation of SLE. It is induced by the action of immune complexes on APCs and lupus-prone mice deficient in the α -chain of the IFN- α β -receptor have markedly attenuated disease [58]. IFN- α can modify the action of other cytokines, including IL-10, effectively enhancing TNF- α release [59].

IFN- γ , a cytokine important in macrophage activation, Th1 differentiation and adhesion molecule expression, may be important in the pathogenesis of proliferative glomerulonephritis via the upregulation of CD40 on mesangial cells [60]. Animal studies show that blocking the effect of IFN- γ with an anti-IFN- γ antibody or soluble receptor can inhibit the onset of glomerulonephritis and, in humans, genetically determined IFN- γ production might influence the histological phenotype of lupus nephritis [61].

Based on these observations, it makes sense to target the IFN system, but thought must be given to the possibility that blockade of IFN- α may compromise normal defenses against viral infection; human trials are awaited.

Targeting dsDNA

The reduction of anti-dsDNA levels has been accomplished by the use of heteropolymers. These are two cross-linked mAbs, one directed at the complement receptor CR1 on red cells, the other to the target antigens, such as anti-dsDNA. Binding to dsDNA allows its clearance via erythrocytes and tissue macrophages. A recent study has shown that out of six patients with mild SLE and no major organ involvement, three exhibited significant reductions in anti-dsDNA antibodies after 28 days with a single dose of the heteropolymer Elusys Therapeutics Inc.-104, but no change in dsDNA levels was noted in three other patients [62]. No adverse events were noted during this proof-of-principle trial. Reduction of anti-dsDNA can also be achieved by IgG immunoabsorption columns that bind IgG and allow the clearance of immune complexes. Using this technique, improvement in proteinuria, lupus activity and anti-dsDNA levels has been reported [63]. Treatment is labor intensive and costly and larger, controlled trials are needed to assess efficacy fully.

Targeting complement: anti-C5

Complement activation, with C5 as the final common pathway, follows immune complex formation in SLE, leading to the formation of the membrane attack complex (MAC) comprising C5b–C9, and target tissue damage. Lupus-prone NZB/NZW mice treated with anti-C5 mAbs exhibit attenuation of renal disease and increased survival [64]. These promising results have been followed up by a Phase I clinical trial of a chimeric anti-C5 mAb, hbG1, which was safe at doses of up to 8 mg/kg with a trend to improve disease activity at higher doses [65].

Stem cell therapy

The disadvantage of strategies that target one specific pathway in the immune response is that dysfunctional regulation occurs at several points in SLE. Replacing the global autoimmune repertoire in SLE with nonautoimmune cells may bypass this problem and case reports of cure of RA after bone marrow transplantation [66] have led to further research. Strategies employed in clinical oncology, using immunoablation and high-dose cyclophosphamide, with or without reconstitution with autologous hematopoietic stem cell transplants (HSCTs) have been used in autoimmune disorders with some success. Although cyclophosphamide is not truly myeloablative as stem cells are resistant to its effects,

high doses (50 mg/kg per day for 4 days) in conjunction with granulocyte colony-stimulating factor are useful in refractory SLE without HSCT [67]. Registry data from the European Group for Blood and Marrow Transplantation (EBMT) and the European League Against Rheumatism (EULAR) show that, of 50 patients who underwent autologous HSCT, eight (16%) died within 6 months of the procedure. However, more promisingly, 80% were in partial or complete remission at 6 months, although 32% of these subsequently relapsed [68].

Immunoablation/HSCT does not appear to be a cure, but further refinements to the procedure may help reduce the mortality rate and make the procedure more acceptable.

Gene therapy

Gene therapy can alter the expression of regulatory proteins or genes. Single gene targeting is unlikely to work for all SLE patients, except in rare causes, such as C4 deficiency, because several mechanisms account for the disease process. However, constructs have targeted IFN- γ , with lupus-prone mice exhibiting delayed disease onset and milder disease [69]. Good results in similar murine models have also been obtained with DNA transfection with cytokine targets transforming growth factor- β and IL-2, as well as co-stimulation blockade with gene transfection of CTLA4Ig [70]. A model of how well gene transfer can work has been demonstrated in NZB/NZW lupus-prone mice using adeno-associated virus (AAV)-mediated gene transfer of CTLA4Ig or CD40Ig. A single injection of AAV serotype 8-CTLA4Ig reduced autoantibody production, proteinuria and prolonged lifespan, with a synergistic effect shown when AAV-8-CD40Ig was co-injected [71]. Many ethical and legal aspects of human gene therapy need to be resolved before human trials are possible, however, gene therapy remains an exciting area for further research.

Future perspective

The future for lupus therapy looks bright indeed, with the developments outlined in this review. The future will undoubtedly usher in a new era of biologic therapeutic agents more targeted in their actions. These therapies may allow better control of lupus, but longer-term use and well-designed clinical trials are needed to determine efficacy and toxicity. Treatment may even be tailored to individuals as patient subgroups with distinct clinical and serological features are

identified [72]. Synergistic actions between targeted therapeutic agents may mean co-therapy or the construction of novel bispecific proteins might be more useful than monotherapy in the future [73]. With stem cell and gene therapy, the prospect of replacing a dysfunctional immune system and reconstituting it with a normal one is also real if issues surrounding toxicity can be addressed.

The challenge for the future is to clarify the key mechanisms that initiate and perpetuate the disease. Only by doing so can we hope to achieve what clinicians, scientists and patients all want – a cure and freedom from a disease that causes significant morbidity and mortality.

Conclusion

The complexities of SLE are still being unravelled. Multiple problems in the immune cascade alter the normal immune phenotype into

one expressing SLE. Current approaches to management use agents with actions at multiple targets and with significant toxicities. Newer, less toxic immunosuppressives, such as mycophenolate mofetil, may provide the basis for combination therapy in the future. However, the advent of targeted therapies has enabled a more focused approach, which has been successful in controlling the clinical manifestations of SLE in some cases. Some unexpected effects have resulted from this cutting-edge therapy, exemplified by the high rate of thromboses that occurred during trials of CD40/CD154 blockade. However, these problems allow us a glimpse into an immune response interconnected with many other physiological systems. The next few years of lupus research therefore promise to be exciting and will advance our knowledge and illuminate our understanding significantly.

Executive summary

Setting the scene for targeted therapies

- Abnormalities in systemic lupus erythematosus (SLE) have been found in all parts of the immune cascade.
- Apoptosis and the clearance of apoptotic debris are abnormal and result in the persistence of nucleosomal antigen, which, if modified, might allow breakage of self-tolerance.
- The dysfunctional immune response is perpetuated by abnormal T-cell regulation, heightened activation and resistance to anergy.
- Perhaps driven by these abnormalities, autoreactive B cells produce a variety of autoantibodies.
- Cytokines including tumor necrosis factor (TNF)- α , interferon (IFN)- α , interleukin (IL)-10 and B-lymphocyte stimulator (BLyS), are all elevated in active SLE and may provide novel therapeutic targets.
- Cytokine combinations may act very differently *in vivo* to single cytokine assays and trials *in vitro*.

Therapeutic targets

T cells

- T cells from SLE patients exhibit many signaling pathway abnormalities.
- Animal studies suggest several possible targets for therapeutic manipulation, including anti-CD137 to suppress T-cell help for humoral responses, use of consensus peptides to induce tolerance and anti-CD70 to prevent overstimulation of B-cell immunoglobulin production.
- Forced expression of the deficient ζ -chain of the T-cell receptor can lead to the correction of many signaling defects and increase IL-2 production *in vitro*.
- Depletion of autoreactive T cells by T-cell vaccination – injecting inactive autoreactive T cells – showed promise in one small trial.

B cells

- Rituximab is a chimeric monoclonal antibody to the B-cell specific surface marker, CD20. It depletes B cells by antibody-dependent cellular cytotoxicity, complement-mediated lysis and the promotion of B-cell apoptosis and improves peripheral B-cell abnormalities after treatment. Trials in humans have shown good benefit in controlling refractory SLE with few serious side effects. Remission after therapy persists in some patients.
- Epratuzumab (anti-CD22) has shown benefit in a small trial, improving disease activity in 14 SLE patients. The results of larger trials are awaited.
- La Jolla Pharmaceuticals (LJP), CA, USA, LJP394 comprises four 20-mer double-stranded (ds)DNA epitopes conjugated to an immunogenic polythene glycol platform. It crosslinks the dsDNA surface immunoglobulin on B cells leading to anergy. Subgroups of SLE patients with high affinity dsDNA might benefit from LJP394 but trial data show no improvement in the intention-to-treat analysis.

Executive summary (cont.)*Cellular interactions*

- Interactions between antigen-presenting cells and T cells, vital for perpetuating the immune response, have been blocked with cytotoxic T-lymphocyte antigen (CTLA)4-immunoglobulin (Ig), with good results in rheumatoid arthritis (RA). Trials in lupus-prone mice have shown efficacy and synergy with cyclophosphamide.
- B cell–T cell interactions may be attenuated with anti-CD154 (CD40 ligand) monoclonal antibody (mAb). *In vivo* trials have shown variable results, with two showing improvement and one showing none. Trials have been terminated early owing to concerns regarding thromboembolic complications, including myocardial infarction, probably due to interaction with platelet CD154.

Cytokines

- Anti-TNF- α therapy has shown some benefit in arthritis and nephritis in one open-label trial.
- BlyS is important for B-cell development. A Phase I trial of anti-BlyS mAb did not show improvement in clinical indices of SLE, despite reductions in B-cell numbers.
- A proliferation-inducing ligand (APRIL), a closely related homolog of BlyS, may also be a future target for therapy as serum APRIL levels are raised in active SLE.
- IL-1 receptor blockade in four SLE patients showed some early improvement that was not sustained through the 12-week study period.
- Anti-IL-6 receptor mAb is beneficial in RA but trials in SLE are awaited.
- Anti-IL-10 improved disease activity in one small human trial of blocking therapy, but the lack of a control group and failure of lymphopenia or complement levels to normalize means interpretation of the study is difficult.
- Many other interleukins might be targeted in the future with anti-IL-12, anti-IL-15 or anti-IL-18 the most likely to be useful based on current data.
- Targeting IFN- α and IFN- γ might prove useful if concerns regarding host defenses can be overcome.

Reducing dsDNA levels

- Levels of dsDNA antibodies correlate with SLE disease activity, particularly nephritis. Cross-linked mAbs (heteropolymers) reduce dsDNA levels in SLE patients.
- IgG immunoadsorption columns also reduce dsDNA levels as well as disease activity.
- Not all patients have detectable dsDNA levels and, therefore, larger trials are needed to examine more fully the effectiveness of these novel methods.

Targeting complement

- Activation of the complement pathway can lead to tissue damage.
- Murine lupus models show attenuation of disease with anti-C5 mAb.
- Phase I trials in humans also demonstrated safety; larger trials are needed to confirm this.

Stem cell therapy

- Replacing the dysfunctional immune repertoire with a normal one in SLE might lead to cure.
- Trials of immunoablation, with or without autologous stem cell transplants, show encouraging results in refractory SLE, but high mortality rates are problematical and many patients relapse.

Gene therapy

- Altering the SLE genotype has become possible through the use of vectors to deliver new genes into cells.
- The results from murine lupus models are encouraging, with targets including cytokine genes IL-2 and transforming growth factor- β and the co-stimulatory molecule CTLA4.
- Future human trials are awaited.

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