New treatments for SLE: cell-depleting and anti-cytokine therapies

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Although systemic lupus erythematosus (SLE) is indeed a complex autoimmune disease, recent advances in our understanding of lupus pathogenesis have suggested new, targeted approaches to therapy. The purpose of this review is to discuss the underlying scientific rationale and results of first clinical studies of new treatment approaches to SLE, with a focus on cell-depleting therapies and cytokine blockade. It has become clear that the B lymphocyte plays a key role in disease pathogenesis by both autoantibody-dependent and autoantibody-independent mechanisms. Additionally, aberrant interactions between B and T cells are critical to disease emergence and progression. New agents that directly target immune cells abnormal in SLE include the B-cell depleting or modulating antibodies, rituximab (anti-CD20) and epratuzumab (anti-CD22) and the anti-dsDNA tolerogen LJP394. Another promising approach has been to block co-stimulatory interactions between T and B cells, for example by inhibiting the CD40-CD40 ligand pathway with anti-CD40 ligand monoclonal antibody or the B7 pathway with CTLA-4Ig. Immune cells can also be manipulated indirectly through cytokine effects. For B cells, anti-BAFF (B-cell activation factor of the tumor necrosis family) provides an example of this approach. Other, more pleiotropic cytokines can likewise be blocked in SLE. In addition to the blockade of interleukin-10 (IL-10), the first anti-cytokine approach examined, it is mainly anti-tumor necrosis factor therapy that has come into focus, holding promise for some patients with lupus nephritis. The majority of the available data on these new treatment approaches stems from open-label trials, but controlled trials are under way. Moreover, many additional cytokines, such as interleukin (IL)-6, IL-18, and the type I interferons, represent interesting future targets.

Key words: B lymphocytes; monoclonal antibodies; receptor constructs; BAFF; B7-CTLA4; CD20; CD22; CD40–CD40L; IL-6; IL-10; IL-18; LJP394; TNF.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with considerable heterogeneity in clinical manifestations and disease course, characterized by dysregulation in multiple arms of the immune system and the development of anti-nuclear antibodies. The current treatment approach includes antimalarials, an expanding armamentarium of immunosuppressive drugs, and steroidal and non-steroidal anti-inflammatory agents. The expanded therapeutic options in SLE, earlier diagnosis, and improved management of disease and treatment complications have all contributed to a dramatic improvement in prognosis for SLE patients, with 5-year and 10-year survival rates as high as 96 and 85%, respectively. However, significant morbidity and mortality are still associated with the disease, to a substantial extent because of the toxicity of standard immunosuppressive therapies. Moreover, patients with active disease refractory to traditional therapies represent a unique management challenge. Thus, despite improved prognosis, patients with SLE still have a 3–5-fold increased mortality compared to the general population.

Fortunately, advances in our understanding of disease pathogenesis and drug development have converged toward new therapeutic strategies specifically designed to interrupt pathways involved in disease evolution and/or tissue damage. The pathogenesis of SLE involves a complex interplay of genetic and environmental factors and the adaptive and innate immune systems. Abnormalities in the function, regulation, and interactions of immune cells, with T and B lymphocytes central, result in immune-complex-mediated deposition and inflammatory organ damage. With the growing understanding of the pathogenesis of the disease and novel therapeutic agents available, new treatment approaches have focused on interfering with defined phases of the abnormal immune response. This chapter reviews published information regarding strategies that target cytokine pathways and/or immune cells critical to disease pathogenesis. Focus will be given to targets for which specific drugs exist that have been studied in clinical trials in humans.

IMMUNE-CELL-DIRECTED THERAPIES

Targeting the B-cell compartment in SLE

The current experience with therapies that target the B-cell compartment includes antibodies to B-cell surface antigens, tolerogens, blocking of co-stimulatory molecules, and inhibition of cytokines with direct B-cell effects (Figure 1). The rationale for B-cell-directed therapies in SLE is multi-fold, with a growing body of evidence regarding the importance of B lymphocytes in both murine and human SLE. In addition to secretion of autoantibodies, B cells can take up and present autoantigens, via specific cell-surface immunoglobulins, to T cells, as well as help regulate and organize inflammatory responses through cytokine secretion and regulation of other immune cells. The importance of these latter functions has been demonstrated in murine SLE, where B cells have been found to be critical to the development of disease even when they are unable to secrete autoantibodies.
Role of B cells in human SLE

Because of experimental limitations, the actual pathogenic mechanisms of B cells in human SLE have been more difficult to elucidate. As with murine SLE, it is postulated that autoantibodies have a direct pathogenic role in the disease process, as exemplified by anti-double stranded DNA (anti-dsDNA) in glomerular kidney disease and autoantibody-mediated cytopenias. Additionally, a large body of evidence indicates that B cells are abnormal in human SLE, with an increased number of spontaneous immunoglobulin-secreting peripheral B cells, increased calcium flux on signaling through the B-cell receptor, and expression of high levels of co-stimulatory molecules such as CD80, CD86, and CD40 ligand on B cells (reviewed in Ref. 13).

In concordance with data in mice, recent evidence suggests a role in human SLE for high serum levels of B lymphocyte stimulator (BlyS; also known as BAFF or B-cell activation factor of the tumor necrosis family), a cytokine that promotes B-cell maturation and survival and plasma cell differentiation. BlyS, along with other cytokines and intrinsic B-cell defects, might contribute to the abnormalities in peripheral B-cell subpopulations that have been observed in human SLE. For example, active SLE is associated with B-cell lymphopenia, particularly of naïve B cells, as well as...
the abnormal expansion of certain B-cell subsets in peripheral blood.\textsuperscript{13} The frequency and absolute number of early plasma cells in the peripheral blood is increased in SLE patients with increased overall disease activity as measured by the SLEDAI.\textsuperscript{16} Abnormal expansion of a second B-cell subset with a pre-germinal center (GC) phenotype has also been reported and may indicate either exuberant or abnormally regulated GC reactions in SLE.

**Antibodies that deplete B cells or modulate the function of B cells**

Given the large body of evidence implicating abnormalities in the B-cell compartment in both murine and human SLE, a novel and rational approach to treatment involves depleting B cells and/or modulating their function. The two drugs that have utilized this approach by targeting B-cell-specific antigens are the monoclonal antibodies rituximab (anti-CD20) and epratuzumab (anti-CD22) (see Figure 1—target 1).

**Anti-CD20**

The development of rituximab has raised the hope of a new therapeutic approach for autoimmune diseases such as SLE that are at least in part B cell mediated. Rituximab is a chimeric mouse/human monoclonal antibody against the B-cell-specific antigen CD20\textsuperscript{17}, which efficiently depletes B lymphocytes in vivo from the pre-B stage in the bone marrow (when CD20 is first expressed) to the mature B-cell stage. It was approved by the American Food and Drug Administration (FDA) for the treatment of relapsed or refractory B-cell lymphoma in 1997, and has since been used to treat over 300,000 patients with B-cell malignancies worldwide.\textsuperscript{18}

Using rituximab to deplete B cells in SLE has several major advantages. First, there is a large safety database in lymphoma indicating that rituximab is generally well tolerated, with only minor effects on immunoglobulin levels and no increase in the frequency of infections.\textsuperscript{18} Second, CD20 expression is restricted to B cells so that the effects of anti-CD20 should be directly on the B-cell compartment, with relative sparing of T cells and plasma cells (which are CD20 negative). This approach might thus have the advantage of being less immunosuppressive. Third, there is accumulating positive data on the use of rituximab in a variety of other autoimmune diseases, including IgM-antibody-associated polyneuropathy, idiopathic thrombocytopenic purpura (ITP), and autoimmune hemolytic anemia.\textsuperscript{13} Although these were open studies, there has been a recently reported double blind, placebo-controlled trial demonstrating the effectiveness and safety of rituximab in rheumatoid arthritis.\textsuperscript{19}

A number of open studies of rituximab in the treatment of SLE have now been reported, and phase III randomized controlled trials are in the planning stages. The first study began in 2000 at the University of Rochester as an open-label phase I/II trial to determine the safety, efficacy, and dose response of rituximab added to current therapy in the treatment of SLE.\textsuperscript{20–22} Seventeen patients with clinically active disease (Systemic Lupus Activity Measure [SLAM] \textsuperscript{O} \textsuperscript{6}) were treated in three dose-escalating groups: single doses of 100 mg/m\textsuperscript{2} (low) or 375 mg/m\textsuperscript{2} (intermediate) or four weekly doses of 375 mg/m\textsuperscript{2} (the latter high dose representing the typical lymphoma regimen). Rituximab was safe and well-tolerated in this patient population at all three treatment doses, with no infusion reactions. In the majority of patients with effective B-cell depletion (11/17), the SLAM score was significantly improved at 2 and 3 months (\textit{P} = 0.0016 and 0.0022, respectively), an improvement that persisted for 12 months.\textsuperscript{22} Six patients in this study had incomplete B-cell depletion (including one of the four
patients in the high-dose group) and no clinical improvement. Incomplete B-cell depletion was associated with certain Fc receptor genotypes, African–American ancestry, and lower serum rituximab levels, the latter suggesting that B-cell depletion might be more consistent if all patients are treated with high-dose rituximab. Additionally, 6 of the 17 patients developed elevated human anti-chimeric antibodies (HACAs), which were also associated with African–American ancestry, higher baseline SLAM scores, and reduced B-cell depletion. This is the only reported study of rituximab in SLE that comprehensively evaluated HACA development. The results raise the concern that rituximab might be more immunogenic in active SLE, although the low doses of rituximab used in the majority of patients might have contributed to the high frequency of HACAs.

In a subset of patients (3/11) anti-dsDNA and complement levels also normalized, and all medications were eventually tapered and discontinued, i.e. clinical remission was obtained (27% ‘remission’ in patients with effective B-cell depletion). However, in the study overall the serologic response was quite variable and seemingly independent of the clinical response. This disconnect between B-cell depletion and reductions in autoantibody levels is in accord with results in other autoimmune diseases and the fact that plasma cells do not express CD20. Of note, the finding that clinical responses were rapid and preceded autoantibody decline supports the purported autoantibody-independent role of B cells in the disease process. Moreover, the fact that long-term reductions in autoantibody titers were variable and incomplete suggests that autoreactive plasma cells might have heterogeneous life-spans.

Of note, in the University of Rochester SLE study, B-cell depletion effectively normalized a number of the disturbances in peripheral B cells characteristic of active disease, including naïve B-cell lymphopenia, expansion of early plasma cells, and expansion of populations of autoreactive memory B cells. Based on their experience, the authors conclude that rituximab is safe and promising in the treatment of SLE, but that high-dose rituximab is necessary, possibly with the addition of other immunosuppressive agents to ensure consistent B-cell depletion, prevent the development of HACAs, induce serologic response, and enhance clinical efficacy.

In accord with the concept that combination therapy might be more efficacious, Leandro et al., at University College London, reported the treatment of six active SLE patients with a combination of moderate dose rituximab (2×500 mg, 2 weeks apart), high-dose glucocorticoids, and cyclophosphamide. All subjects experienced profound B-cell depletion and a clinical improvement with a decrease in the BILAG global score from a median of 14 at baseline to 6 at 6 months, although again with variable response in anti-dsDNA antibodies in this first report. The University College group also reported an additional six patients treated with a similar protocol for proliferative nephritis. At 3 months there was a marked improvement in the BILAG global score (median of 19–6), and creatinine, proteinuria, C3 and anti-dsDNA were also improved. Recently, this same group reported an extension of these earlier studies, now including a total of 21 treated patients. The majority of patients (13/21) received full-dose rituximab (2×1000 mg, 2 weeks apart) in combination with IV cyclophosphamide and steroids. A total of 20/21 patients experienced effective B-cell depletion, with the period of B-cell depletion ranging from 3 to 8 months. With the exception of one infusion reaction, no serious adverse events were noted.

Most patients had a clinical response, with nine patients remaining off immunosuppressive therapy at a follow-up of 12–46 months (~45% ‘remission’). In a subset of patients, effects on autoantibody and antimicrobial antibody profiles were reported. Protective titers against tetanus and pneumococcus and anti-ENAs (Sm, RNP, ...
SSA) were relatively insensitive to B-cell depletion but anti-dsDNA levels decreased by a mean of 53% at 3 months. In line with the reasoning already discussed, the authors postulate that such heterogeneity in serum antibody responses may be due to differences in plasma cell longevity.²⁹

Another report of nine patients with class III and IV nephritis treated with rituximab and a prolonged course of moderate-dose steroids (0.5 mg/kg for 10 weeks followed by slow taper) also described good efficacy, with partial remission achieved in 80%, complete remission in 50%, and sustained complete remission (at 12 months) in 40%.³⁰ Interestingly, clinical response was associated with a decrease in T-helper-cell activation based on reduced expression of CD40-ligand co-stimulatory molecule and activation markers (CD69 and HLA-DR), again supporting an autoantibody-independent role for B cells in promoting disease.

The University of Pennsylvania is conducting an ongoing, phase I, open trial of full-dose rituximab as monotherapy in SLE patients with active visceral disease. Results for eight patients were reported recently, with all but one subject achieving greater than 99% B-cell depletion. Six patients demonstrated clinical responses defined by improvement in SLEDAI, with 2/6 having long-term remission (6–14 months) (~25% ‘remission’) and 4/6 having short-term remission (4 weeks to 6 months). Even in the short-term responders, disease control was better post rituximab. Interestingly, as in the University of Rochester study, serologic response in anti-dsDNA titer was inconsistent. A number of additional smaller cohorts of SLE patients treated in an uncontrolled fashion with rituximab have been reported recently (total of 18 additional patients treated with heterogeneous protocols).³²–³⁴ Additionally, fully humanized anti-CD20 monoclonal antibodies are under evaluation.³⁵,³⁶

Anti-CD22 (epratuzumab)

An open-label, pilot study of anti-CD22 in the treatment of 14 active SLE patients was reported recently.³⁷ This treatment was associated with some depletion of B cells (~60% at 4 and 12 weeks) but might also function by signaling through the inhibitory CD22 membrane molecule, causing down-modulation of B-cell receptor signaling. The drug was well tolerated, with the majority of patients experiencing a > 50% improvement in the BILAG. Limited analyses of autoantibodies revealed no consistent changes.

B-cell tolerogens

An approach related to B-cell depletion is specifically to target autoreactive B cells. Tolerogens are synthetic molecules that bind to and extensively cross-link autoantibodies, thereby causing either anergy (functional inactivation) or deletion of B cells expressing the autoreactive B-cell receptors. LJP394 was the first such B-cell tolerogen developed and studied in human trials (see Figure 1—target 2). It consists of four double-stranded oligonucleotides attached to a polyethylene glycol platform and binds avidly to anti-dsDNA antibodies. A phase II/III randomized, placebo-controlled trial in 230 patients with a history of lupus nephritis demonstrated significant but temporary decreases in anti-dsDNA levels (P < 0.0001) but without clinical benefit.³⁸ A post hoc analysis found a decrease in renal flare (67% fewer) and SLE flare in the patients with high-affinity antibodies and sustained reduction in anti-dsDNA. Although titers of anti-dsDNA antibodies were again decreased, significant clinical benefits were not confirmed in a second study.³⁹ A phase III trial of 317 randomized patients was recently completed (PEARL: Program Enabling Antibody Reduction in Lupus), and preliminary
results indicate that sustained reductions in anti-dsDNA antibodies lead to improvement in health-related quality of life. Overall, this novel tolerogen might have a role for induction or maintenance therapy in the subset of SLE patients with elevated high-affinity anti-dsDNA and active disease; additional studies are ongoing.

Inhibition of co-stimulation

As an alternative to selective B-cell depletion, there has been interest in targeting co-stimulatory signaling pathways. CD40 binding to CD40 ligand is one of the most important co-stimulatory signals on B cells inducing activation, proliferation, and class-switching in germinal center reactions. Direct inhibition of collaboration between B and T cells through inhibition of the CD40–CD40L pathway has been demonstrated to be effective in mouse models of lupus. Two well-designed studies of anti-CD40L antibodies in SLE have been reported (see Figure 1—target 3). The first open-label study (Biogen Hu5c9 antibody) focused on 30 patients with lupus nephritis and showed improvement in serology and hematuria. Unfortunately, this study was halted because of unexpected thromboembolic events.

The second double-blind, placebo-controlled trial (IDEC 131) of 85 patients with mild to moderate SLE failed to show clinical efficacy over placebo. Moreover, use of this anti-CD40L antibody in a separate study in Crohn’s disease was associated with thrombotic events. Interestingly, two small mechanistic studies attached to the first trial demonstrated beneficial immune effects (n = 5 for each), including a marked reduction in autoreactive anti-dsDNA-producing B cells and substantial reductions in abnormal B-cell populations, including pre-GC cells and IgD+ plasmacytes.

These reports suggest that anti-CD40L can interfere with aberrant germinal center reactions in SLE and translate into clinical benefit, if administered with the proper pharmacokinetics for adequate co-stimulatory blockade. The latter is a critical point as insufficient blockade could explain the lack of clinical efficacy in the second study. Additional controlled studies would be warranted to understand the mechanism of action of this therapy, which might take place at different points during B-cell differentiation. Moreover, the potential for untoward immunosuppression due to dendritic cell (DC) expression of CD40L (see Figure 1) requires further exploration. Unfortunately, such studies will probably be hampered by the thromboembolic side effects described. However, alternative approaches to blocking the CD40–CD40L pathway merit investigation.

Alternative co-stimulatory targets in SLE includes the CD28 and CTLA4 receptors and their B-cell co-ligands B7-1 and B7-2 (see Figure 1—target 4). Blockade of B7 stimulation on B cells with a fusion protein of the extracellular domain of CTLA and immunoglobulin constant regions (abatacept) has yielded promising results in murine SLE and demonstrated safety in human clinical trials in RA and psoriasis. This drug has yet to be used in human SLE, but clinical trials are in the planning stages.

ANTI-CYTOKINE THERAPIES

Background

The alternative to directly targeting immune cells is to interfere with their messengers. Immune cells exert many of their effector and immunoregulatory functions by cytokine
release (Figure 2). In fact, most cytokines investigated have been found to be
dysregulated in SLE. Although there are still many controversial issues regarding
cytokine regulation and cytokine effects in SLE, the consecutive development of a
variety of anti-cytokine agents has made insight into this regulatory process much more
relevant. These medications could also be useful in SLE, and at least two approaches
have been tested in SLE patients and found successful. We will therefore concentrate
on: (i) what is known currently regarding anti-cytokine therapy in patients with SLE; and
(ii) the potential therapeutic effects of anti-cytokine drugs, developed for other diseases
and not yet used in SLE patients, on the autoimmunity and organ manifestations of SLE.

Inhibition of cytokines with B-cell effects

BLyS or BAFF is a secreted cytokine of the TNF family that binds to three different
membrane receptors (BCMA, BAFFR, and TACI) expressed on B cells (see Figure 1—
target 5). It has profound effects on B-cell survival, stimulates plasma-cell
differentiation, and might have differential effects on autoreactive B cells, which show
greater dependence on BAFF signaling in mouse models. Mice over-expressing BAFF
develop an SLE-like phenotype. Moreover, lupus-prone mice have elevated levels of
circulating BAFF and administration of soluble BAFF receptors ameliorates disease progression and improves survival. Finally, elevated BAFF levels are evident in the serum of SLE patients and positively correlated with serum IgG and autoantibody levels.

Lympho-Stat-B is a fully human monoclonal antibody that specifically binds to and neutralizes soluble human BAFF. A phase I dose-escalation trial in mild to moderate SLE \((n = 70)\) demonstrated safety and biological activity with significant reductions \((12–47\%)\) in peripheral blood B cells. In this short-term study, there was no change in anti-dsDNA levels or disease activity. A phase II double-blind, placebo-controlled trial of the safety and efficacy of three different doses administered in addition to standard therapy is underway \((n = 350)\). Alternative approaches to inhibiting BAFF, including use of BAFF-R-Ig and TACI-Ig, are also under development.

### Interleukin-10 (IL-10) and anti-IL-10 therapy

Although this therapeutic approach is still being hampered by the absence of a therapeutic agent suitable for long-time application in human patients, IL-10 was the first cytokine successfully blocked in SLE. IL-10 is over-produced by the B cells and monocytes of patients with SLE, increased in SLE sera, and associated with disease activity. However, the specificity of these findings is unclear, as increased numbers of IL-10-producing cells were also found in first degree relatives as well as healthy spouses.

These findings would, in addition to environmental factors, suggest a combination between genetic and disease-induced events. In fact, IL-10 genotypes were linked to SLE and an IL-10 promoter polymorphism has been described and linked to IL-10 over-production, although a larger study including patient family members with increased IL-10 production has not confirmed this association.

In line with the association between IL-10 and disease activity, immune complexes from SLE sera, as well as monoclonal anti-dsDNA antibodies, induced IL-10 production in healthy monocytes and the probable removal of such immune complexes by immunoadsorption reduced the number of IL-10 producing cells. B-cell secretion of IL-10 might regulate DC- and T-cell function, promoting Th2 deviation of the immune response. In turn, IL-10 might contribute to a number of the earlier described peripheral B-cell abnormalities in SLE, including plasma cell expansion (reviewed in Ref. 13).

Murine models of SLE are characterized by IL-10 over-production and continuous early-onset therapy with an anti-IL-10 antibody delayed autoimmunity in NZB/W mice. Moreover, the continuous administration of recombinant IL-10 increased disease activity, while being well tolerated in normal mice. It is interesting that the protective effect of anti-IL-10 antibodies was abolished by the concomitant administration of blocking anti-TNF antibodies, suggesting that in the NZB/W mouse an immunoregulatory balance exists between these two cytokines. A neutralizing anti-IL-10 antibody also reduced serum immunoglobulin and renal immune complex deposition in SCID mice implanted with a hybridoma secreting a pathogenic anti-dsDNA antibody, but was not very effective in reducing proteinuria in these mice.

In the absence of a human or humanized antibody to IL-10, an anti-IL-10 murine monoclonal antibody (MoAb) was administered daily \((20 \text{ mg IV})\) to six SLE patients for a total of 21 days. All patients had skin and joint involvement and constitutional
symptoms despite corticosteroid therapy and therapy with chloroquine, azathioprine, or methotrexate. As was to be expected, all patients developed antibodies against the murine MoAb. Nevertheless, this therapy was well tolerated and rapid clinical improvement was seen in all six patients. Despite the cessation of therapy after 3 weeks, therapeutic benefits were stable at the end of observation (6 months). Although this was a small, uncontrolled, open-label study in patients with relatively mild disease, these findings suggest that anti-IL-10 therapy with an agent suitable for use in humans would probably benefit some patients with SLE. Such an agent might soon be available.

**Tumor necrosis factor (TNF) and anti-TNF therapy**

In contrast to the situation with regard to IL-10, therapeutic agents blocking TNF are readily available and widely used in other rheumatic diseases. However, TNF is a pleiotropic cytokine that exerts several functions in the immune system and can either promote or relieve autoimmunity.74,75

An example of the protective function of TNF in autoimmunity is provided by the NZB/W murine model of SLE.76 NZB/W mice produce much less TNF than other mice, a defect that stems from the NZW parent and fosters lupus-like autoimmunity, and the development of lupus nephritis can be delayed by TNF administration in these mice.76 In line with this finding, NZB mice rendered TNF-deficient showed a phenotype similar to NZB/W. However, low TNF is not required for the induction of lupus in NZB/W mice, since the same mice without the TNF defect still get severe disease, albeit later78, and the administration of TNF to NZB/W mice does not prevent development of the disease.76,79 Nevertheless, from this murine model of SLE it is clear that TNF has an important immunoregulatory function.

Given these immunoregulatory properties, it is perhaps not surprising that therapeutic TNF blockade in patients with autoimmune diseases, such as rheumatoid arthritis (RA) or Crohn’s disease, is associated with the development of ANA, anti-dsDNA, and anti-cardiolipin antibodies, as well as with rare cases of drug-induced lupus-like syndromes, all of which disappeared after therapy was stopped (reviewed in Ref. 80).

**Proinflammatory TNF effects in SLE**

Although one might thus argue that TNF is beneficial in SLE and that, accordingly, TNF blockade in SLE would be detrimental, in vivo data from SLE patients speak to the contrary. TNF concentrations are actually increased in sera of SLE patients and closely associated with disease activity (reviewed in Ref. 81). As both soluble TNF receptors are likewise increased and correlate with disease activity,82–84, it was postulated that they would block the biological activity of the increased serum TNF. In fact, however, the increased TNF in SLE sera is bioactive85, suggesting that these receptors might dilute the local concentration of TNF but not remove it.

Moreover, TNF was found in the inflamed kidneys of patients with lupus glomerulonephritis and correlated with histological disease activity (reviewed in Ref. 86). This is well explained by the fact that TNF, like IL-10, can be induced by anti-dsDNA antibodies and by immune complexes69,87, (and our unpublished data), the deposition of which is a major pathogenic event in lupus nephritis. In addition, renal cells express TNF receptors88 and might therefore bind some of the abundant circulating TNF.
As TNF is well-known for its pro-inflammatory properties\textsuperscript{75,89}, these findings therefore argue for a pathogenic role in the local inflammatory disease processes in SLE (see Figure 2).

Similar findings that support the local proinflammatory role of TNF in SLE kidney disease were also made in lupus mouse models (reviewed in Refs. \textsuperscript{74,81}). Sera as well as inflamed kidney tissue samples from MRL/lpr lupus mice contain significant amounts of TNF, again associated with disease activity. Moreover, even NZB/W inflamed kidney samples contained increased levels of TNF, and the additional administration of recombinant TNF later in life accelerated the nephritis of NZB/W mice. Finally, inhibition or blockade of TNF improved organ disease in several murine models of SLE (reviewed in Ref. \textsuperscript{81}).

**Therapeutic TNF blockade in SLE patients**

This background provided the rationale for using anti-TNF in SLE patients.\textsuperscript{81,90,91} In an open-label safety trial of infliximab in patients with mild to moderate SLE, a total of four infusions (300 mg each) were administered to patients with refractory lupus nephritis or lupus arthritis, on an immunosuppressive background medication of azathioprine or methotrexate and low-dose corticosteroids.\textsuperscript{80} It is important that infections were limited to urinary tract infections and non-specific viral disease, and that no infusion reactions occurred under this protocol. In contrast, in the absence of azathioprine or methotrexate, others commonly found severe infusion reactions\textsuperscript{92}, suggesting that the combination with immunomodulators is essential.

Both major findings of this trial are in accord with the background outlined above. Two-thirds of the patients experienced an increase in anti-dsDNA antibodies. Interestingly, in contrast to the situation in infliximab-treated RA or Crohn’s disease\textsuperscript{93}, all of these antibodies were of the IgG isotype.\textsuperscript{80} However, the increase in autoantibodies proved to be transient and did not lead to a fall in serum complement or to any lupus flares. By contrast, the inflammatory organ disease improved rapidly in all patients.\textsuperscript{80} Lupus arthritis remitted within days and only relapsed 8–11 weeks after the last infusion. Even more exciting, a significant renal response was observed. Specifically, the one patient with overt nephrotic syndrome experienced complete resolution of peripheral edema within days of starting infliximab. Moreover, proteinuria decreased to less than 50% within weeks in all four of the nephritic patients treated, and remained at this low level for at least 1 year thereafter.

Similarly, other groups found beneficial effects of TNF blockers with regard to lupus arthritis and refractory skin disease.\textsuperscript{94,95} Taken together, these clinical results suggest that a limited time period of TNF blockade, when combined with azathioprine or methotrexate, might be safe and effective in a subset of SLE patients, particularly those with lupus nephritis. It is imperative now to study TNF blockade in SLE in a double-blind, placebo-controlled fashion in order to appropriately evaluate the role of anti-TNF agents in SLE therapy.

**Anti-IL-1**

IL-1 can both be increased by TNF and by autoantibodies to dsDNA.\textsuperscript{69,96} In SLE glomerulonephritis, IL-1 (both α and β) was clearly detectable.\textsuperscript{97} Likewise, following the onset of nephritis, kidneys of MRL/lpr and NZB/W mice over-express IL-1\textsuperscript{98–100}, and low-dose IL-1 administration accelerated renal disease.\textsuperscript{99} Moreover, in vitro treatment
of MRL/lpr B cells with recombinant IL-1 receptor antagonist (IL-1Ra) reduced autoantibody production. In addition, IL-1 activity was also found increased in cerebrospinal fluids of patients with CNS lupus.\textsuperscript{101}

In vivo, established MRL/lpr nephritis did not respond to therapy with the IL-1 receptor antagonist\textsuperscript{102} but an alternative IL-1 targeted approach using recombinant IL-1 receptor was successful.\textsuperscript{103} Recently, in a first open trial of anakinra (IL-1Ra) in four patients with SLE and severe lupus polyarthritis, anakinra therapy appeared safe and improved arthritis in all four patients.\textsuperscript{104} This therapeutic effect ceased in two of the four patients after 6 weeks and 8 months, respectively, despite ongoing therapy, and a potential effect on lupus nephritis has not yet been investigated.

**Anti-IL-18**

IL-18 is a pro-inflammatory cytokine closely related to IL-1 and, like IL-1, activated by interleukin-1 \( \beta \) converting enzyme (ICE). Several groups found increased serum levels of IL-18 in SLE, and most saw an association with disease activity.\textsuperscript{105–109} However, in rheumatoid arthritis patients, IL-18 was found to act secondary to TNF.\textsuperscript{110} Apparently, the same is true for patients with SLE.\textsuperscript{109} The latter data suggest that the level of IL-18 is in fact associated with the TNF level rather than with disease activity itself.\textsuperscript{109}

Although no data on the expression in human lupus glomerulonephritis have been published so far, IL-18 is over-expressed in the nephritics of MRL/lpr mice.\textsuperscript{111} Moreover, MRL/lpr animals also benefited from targeting IL-18.\textsuperscript{112} So far, IL-18 blockade has not been reported in SLE patients but agents suitable for this purpose are currently being tested in other rheumatic diseases.

**Anti-IL-6**

IL-6 is another pro-inflammatory cytokine secreted predominantly by macrophages and T cells and found to be increased in SLE sera (reviewed in Refs.\textsuperscript{81,113}). In concert with type I interferons, it has been shown to activate B cells and drive plasma-cell differentiation.\textsuperscript{114} IL-6 is induced by anti-dsDNA antibodies, as well as multiple cytokines including TNF, IL-1, and interferon-\( \gamma \).\textsuperscript{69,96,115}

IL-6 is also highly expressed in SLE glomerulonephritis (reviewed in Ref. 86). Moreover, in NZB/W mice, IL-6 promotes disease, and anti-IL-6 therapy delays lupus nephritis\textsuperscript{116,117}, suggesting that IL-6 blockade might also be beneficial in SLE patients. In fact, an open label trial of IL-6 blockade reportedly is under way.\textsuperscript{113}

**Anti-IL-15**

Therapeutic agents against IL-15 are currently being tested in other autoimmune diseases. IL-15 is found increased in sera of SLE patients and associated with immune abnormalities of the disease, such as the increased percentage of CD25+ lymphocytes.\textsuperscript{107,118} However, more severe nephrotoxic serum nephritis in IL-15\( ^{-/-} \) mice\textsuperscript{119} would demand caution with regard to SLE renal disease.

**IFN-\( \alpha \)**

Recent data suggest that unabated activation of type I interferon might play a role in driving the autoimmune process in SLE. As reviewed in detail recently\textsuperscript{114,120}, critical
observations include the early finding of elevated serum levels of IFN-α in SLE patients, the more recent demonstration of a striking IFN-α signature on gene expression profiling of SLE PBMCs, and the fact that SLE serum is able to induce maturation of DCs in an immunogenic and IFN-α dependent fashion. In addition to dendritic cell activation, IFN-α has been associated with B-cell lymphopenia, germinal center differentiation, and generation of plasma cells, findings of obvious relevance to the peripheral B-cell subpopulation abnormalities characteristic of SLE. Although the precise role of IFN-α in the autoimmune process remains to be fully elucidated, the abundant evidence that IFN-α contributes to disease pathogenesis has made it an attractive therapeutic target. This concept is supported by the development of lupus-like illness in patients treated with IFN-α. Work is ongoing to define the best means of inhibiting the IFN-α pathway, and humanized antibodies are likely to be available in the near future.

**Combination therapy**

A combination of different biologic agents could potentially provide even better efficacy by modulating distinct effector mechanisms. For example, based on our understanding of how germinal center B cells and long-lived memory and plasmacytes are generated, co-stimulatory blockade with anti-CD40L or CTLA-4Ig has the potential for synergy with rituximab. Alternatively, rituximab could be complemented with newer biologic interventions that might block B-cell differentiation and survival, such as inhibition of cytokines like BAFF, IL-6, IL-10 or IFN-α. Likewise, interventions targeting B cells might also be combined with drugs blocking proinflammatory mediators, and TNF in particular.

**CONCLUSIONS**

In summary, new approaches that target both immune cells and cytokine pathways important in SLE show great promise. Several open clinical trials suggest that B-cell depletion with rituximab treatment can improve clinical manifestations of SLE, indicating that B cells are crucial not only for the development of SLE but also for continued activity of established disease. Although much smaller patient numbers were treated, the published anti-TNF experience with infliximab (and etanercept) suggests significant benefit for rapidly reducing inflammation and possible long-term effects on proteinuria, despite the transient occurrence of autoantibodies. Several other cytokines, including IL-6 and IL-18, might be targeted in the near future (Figure 2). Combination therapy with different biologic agents could potentially provide even better efficacy by synergistically targeting different arms of the immune system that are dysregulated in SLE. One more compelling argument for the continued development of biologics in SLE is the potential for induction of long-term remissions. The impact of these emerging targeted therapies on patient survival is likely to be dramatic, and studies on the immune system of patients in upcoming clinical trials should continue to provide invaluable insight into the pathogenesis of human SLE.
Practice points

General principles:
- so far, none of the biological response modifiers is approved for use in SLE
- currently, all data are based on open-label experience
  B-cell targeted therapies, e.g. rituximab:
- rituximab is the best studied B-cell depletion therapy and was well tolerated in SLE when administered with pre-medication (prednisone 40 mg or hydrocortisone 100 mg, benadryl, and tylenol)
- rituximab can be efficacious in refractory patients with prolonged remissions in some cases in uncontrolled studies
- high-dose rituximab is necessary for consistent B-cell depletion (typical lymphoma dosing or alternative administration of similar total dosages)
- serologic responses are inconsistent perhaps because of the presence of long-lived autoreactive plasma cells

Anti-cytokine therapies, e.g. infliximab:
- TNF blockade with infliximab in combination with azathioprine or methotrexate was safe in small, open-label trials and single cases
- infliximab therapy rapidly improved inflammatory lupus organ disease and apparently had long-lasting effect on lupus nephritis
- autoantibodies to nuclear material and cardiolipin increased during infliximab therapy, but this increase was transient and not associated with disease flares

Research agenda

- multi-center, randomized, controlled trials of anti-B-cell therapies, including rituximab, in SLE are needed to confirm the preliminary results of open studies
- determination of the optimal dosing of rituximab and the role of combination therapy
- multi-center, randomized, controlled trials of infliximab, and possibly other anti-TNF agents, are needed to confirm the open-label results
- open-label trials with other cytokine-directed biological response modifiers
- investigations into the frequency, consequences, and means of prevention of HACAs
- mechanistic studies of patients treated with rituximab, infliximab, and other agents, to define the basis of clinical and serologic responses and their variability

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