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REVIEW

Cytokine disturbances in systemic lupus erythematosus

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Abstract

The pathogenesis of systemic lupus erythematosus (SLE) is complex, and the resulting disease manifestations are heterogeneous. Cytokine dysregulation is pervasive, and their protein and gene expression profiles may serve as markers of disease activity and severity. Importantly, biologic agents that target specific cytokines may represent novel therapies for SLE. Four cytokines (IL-6, TNFα, IFNα, and BLyS) are being evaluated as therapeutic targets in SLE. The present review will examine the roles of each of these cytokines in murine and human SLE, and will summarize results from clinical trials of agents that target these cytokines.

Introduction

Cytokines collectively play key roles in the regulation of systemic inflammation, local tissue damage, and immunomodulation. Not surprisingly, cytokines often play direct roles in disease pathogenesis, including that of systemic lupus erythematosus (SLE). To the chagrin of both investigator and practitioner, the effects of cytokines are pleiotropic and include both synergistic and antagonistic effects on other cytokines – thereby introducing tremendous complexity that has often led to diametrically opposing conclusions in different studies. In the present review, we focus on four cytokines that have received great attention either as candidate biomarkers for disease activity and/or as candidate targets of novel biologic agents.

Interleukin-6

General biology of IL-6

IL-6 is a pleiotropic 26 kDa protein produced by a panoply of cell types, and affects the function of an equally broad spectrum of cell types. Of greatest relevance to SLE is the ability of IL-6 to promote activation and/or differentiation of cells central to the development of systemic autoimmunity and the attendant pathologic inflammatory responses, including T cells, B cells, macrophages, and neutrophils [1].

IL-6 signal transduction occurs via coordinated interactions between the 80 kDa IL-6 binding chain (IL-6 receptor (IL-6R), IL-6R α chain, CD126) and the 130 kDa signal transducing chain (gp130, IL-6R β chain, CD130). IL-6 can bind to membrane IL-6R, thereby inducing homodimerization of gp130 and leading to activation of gp130-associated JAK1 and tyrosine phosphorylation of gp130. Alternatively (and not mutually exclusively), IL-6R can be enzymatically cleaved from the cell surface, thereby producing soluble IL-6R. Soluble IL-6R can then bind circulating IL-6 and form IL-6/IL-6R complexes that act on cells expressing gp130 [1].

IL-6 in murine systemic lupus erythematosus

Studies in multiple murine SLE models point to an essential role for the IL-6 pathway in SLE. Genetic deficiency of IL-6 in MRL/lpr mice delays disease development, reduces $CD4^+$ and $CD8^+$ T-cell numbers, decreases glomerular IgG and C3 deposition, downregulates renal parenchymal vascular cell adhesion molecule-1 (VCAM-1) expression, and diminishes kidney macrophage infiltration [2]. This last effect may be especially important, in as much as a close association between activated renal macrophages and disease has been documented in (NZB x NZW)F1 (BWF) mice [3].

Additional important observations have been made with BWF mice. Exogenous IL-6 increases IgG anti-DNA autoantibody production by B cells isolated from clinically affected BWF mice, whereas neutralization of IL-6 via either addition of an anti-IL-6 mAb or macrophage depletion decreases production of such autoantibodies [4-6]. Building on these *ex vivo* findings, administration of human IL-6 to 6-month-old female BWF mice promoted accelerated membranoproliferative glomerulonephritis associated with marked upregulation of mesangial MHC class II antigens and glomerular intercellular adhesion molecule-1 (ICAM-1) expression.

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Treatment with cyclosporin inhibited the development of glomerulonephritis, distinguishing the effects of IL-6 on systemic inflammation from its direct effect on renal mesangial cells [7]. More impressive were the significant decreases in mortality, progression of proteinuria, and anti-dsDNA antibody levels in BWF mice chronically treated from 3 months of age with anti-IL-6 mAb or anti-IL6 receptor antibody [8,9].

IL-6 is also associated with SLE in other murine SLE models. In pristine-induced SLE, renal disease was milder, and high levels of IgG anti-single-strand DNA, anti-dsDNA, and anti-chromatin antibodies were absent in IL-6-deficient mice [10]. In $JunB^{\Delta ep}$ mice, development of an SLE-like phenotype (including skin lesions and immune complex glomerulonephritis) was linked to increased epidermal IL-6 secretion that arose from the specific loss of epidermal JunB. Intercrosses with IL-6-deficient mice abrogated the SLE phenotype [11].

IL-6 in human systemic lupus erythematosus

As in murine SLE, serum levels of IL-6 are elevated in human SLE and have correlated with disease activity or anti-dsDNA levels in some studies [12-14]. The increased frequency of IL-6-producing peripheral blood mononuclear cells correlates with disease severity/activity and treatment response [15,16]. Of note, SLE B cells express IL-6R spontaneously and produce great amounts of IL-6 [17,18]. Moreover, autoreactive T-cell clones from SLE patients also produce large amounts of IL-6, and thereby promote B-cell activation and autoantibody production [19]. Indeed, the copious spontaneous production of immunoglobulin by SLE B cells can be enhanced by exogenous IL-6 and diminished by neutralizing antibodies to IL-6 [13].

Aside from its systemic effects, IL-6 is closely linked with specific disease manifestations. Elevated cerebral spinal fluid levels of IL-6 are found in SLE patients with psychosis and may afford an effective measure of its diagnosis [20]. In SLE nephritis patients, urinary levels of IL-6 are elevated, correlate with titers of anti-dsDNA antibodies, and decrease following treatment [14,21]. Local expression of IL-6 was documented in kidney tissue of SLE nephritis patients [22], arising at least in part from mesangial cells [23].

Therapeutic blockade of IL-6 in systemic lupus erythematosus

Given the considerable evidence pointing to a contributory role for IL-6 in SLE pathogenesis, the *a priori* likelihood that therapeutic targeting of this pathway in SLE will be efficacious is high. Tocilizumab, a mouse–human chimeric anti-human anti-IL-6R mAb, is already US Food and Drug Administration approved for the treatment of patients with rheumatoid arthritis. Tocilizumab effectively inhibits IL-6 signal transduction by its ability to bind both mIL-6R (preventing IL-6 binding to cell-surface mIL-6R/gp130 complex) and sIL-6R (preventing formation of IL-6/sIL-6R complex, which could bind to cell-surface gp130).

Although initial results suggest that tocilizumab can control SLE disease activity, an increased risk of infections may limit its ultimate clinical utility. In an open-label phase I dosage-escalation study (Table 1), 16 patients with mild to moderate disease activity (presence of chronic glomerulonephritis or extrarenal SLE Disease Activity Index – SELENA modification score of 3 to 10) received one of three doses of tocilizumab (2, 4, or 8 mg/kg) every other week for 12 weeks [24]. Disease activity showed improvement, with a decrease of ≥4 points in activity scores for eight of 15 evaluable patients. Additionally, arthritis improved in all seven patients who had arthritis at baseline, and completely resolved in four of them. There were also significant decreases in the frequency of circulating plasma cells and in levels of anti-dsDNA antibodies. Tocilizumab also led to a similar decline in levels of C3 and the complement activation products, iC3b and C5b-9 (terminal activation complex). Tocilizumab treatment led to dose-related decreases in the absolute neutrophil count, however, with two patients experiencing severe neutropenia (absolute neutrophil count of 500 to 1,000/μl). The decline in neutrophil counts probably had clinical consequences – 16 infections developed in 11 patients, 10 of whom required systemic antibiotics or antivirals. Additional studies are clearly needed before tocilizumab or other IL-6 antagonists can be considered viable therapeutics in human SLE.

Tumor necrosis factor alpha

General biology of TNFα

TNFα is expressed as a 26 kDa transmembrane protein that can be cleaved by TNFα-converting enzyme to release a 17 kDa soluble protein [25]. Like IL-6, TNFα is produced by a variety of cells, including activated macrophages and monocytes as well as B cells, T cells, keratinocytes, renal cells, and fibroblasts.

Depending upon the conditions, TNFα can trigger either proinflammatory or anti-inflammatory pathways by engaging one or both of two distinct transmembrane receptors: the type I, p55 tumor necrosis factor receptor (TNFR1), and the type II, p75 TNFR (TNFR2) [26]. TNFR1 may trigger apoptotic and anti-inflammatory signals through Fas-associated death domain (FADD) and activation of the caspase cascade. Conversely, it may also trigger anti-apoptotic and proinflammatory responses through recruitment of TNF receptor-associated factor 2 (TRAF-2), which activates NF-κB, c-Jun NH2-terminal kinase (JNK), and mitogen-activated protein (MAP) kinase. Binding of TNFα to TNFR2 also leads to

Table 1. Cytokine-targeting agents and their clinical trials in SLE

Cytokine target	Therapeutic agent	Stage of clinical trial	References
IL-6	Tocilizumab	Phase I	[24]
TNFα	Infliximab, etanercept	Phase I; phase II and III trials terminated prematurely	[53-55]
IFNα	Sifalimumab (MEDI-545), rontalizumab (rhuMab)	Phase I; phase II ongoing	[102,103]
BLyS	Belimumab	Phase III completed	[115-121]
	A-623	Phase I; phase II suspended/restarted	[123]
	LY2127399	Phase III planned	[125]
BLyS and APRIL	Atacicept	Phase II/III initiated	[122]

recruitment of TRAF2 and promotes proinflammatory responses. The TNF/TNFR system can thus serve dually both as a potent proinflammatory mediator and as a key immune modulator as a function of the immunological setting [25,26].

TNFα in murine systemic lupus erythematosus

Several studies point to a pathogenic role for TNFα in murine SLE. TNFα expression is increased in the sera and kidneys of MRL/lpr mice and correlates with disease activity [27,28]. Indeed, TNF antagonism/inhibition in such mice has clinical benefit. Furthermore, in C3H.SW mice with induced SLE, treatment with anti-TNFα mAb results in decreased development of anti-DNA antibodies, proteinuria, and glomerular immune-complex deposition [29,30].

In sharp contrast, the administration of recombinant TNFα or TNFα-inducing agents to BWF mice is clearly beneficial [31-33]. Moreover, TNFα deficiency in other SLE mice has resulted in disease induction/exacerbation. Autoimmunity and fatal SLE nephritis develop in NZB mice when crossed with TNFα-deficient mice [34]. Similarly, TNFα deficiency in mice bearing a mixed B6/129 genetic background (H-$2^{b/b}$) develop IgG_{2b} and IgG_3 anti-DNA antibodies, glomerular IgG deposition, and alterations in glomerular structure resembling SLE nephritis [35].

These often conflicting effects of TNFα in different murine SLE models underscore this cytokine's dual proinflammatory and immunoregulatory roles. These conflicting effects are also disease stage specific and may reflect the cell type expressing the receptors as well as differential expression of the receptors themselves by a given cell type. Although the underlying molecular and cellular mechanisms remain largely enigmatic, at least part of TNF's functional duality might segregate at the level of the two TNFα receptors [35,36]. Indeed, in anti-GBM antibody-induced glomerulonephritis, TNFR2 deficiency is protective, whereas TNFR1-deficient mice develop proteinuria and renal pathology similar to those in wild-type controls [37]. In addition, TNFR1 deficiency greatly accelerates lymphoadenopathy, autoantibody production, and mortality in C57BL/6-lpr mice [38]. In NZM2328 mice singly deficient in one TNFR, however, signaling through the other receptor can compensate and permit persistence of the wild-type clinical and pathological phenotypes. Deletion of both receptors, with complete abrogation of TNFα signaling, results in significantly accelerated disease [39].

TNFα in human systemic lupus erythematosus

As in animal models, the evidence has been conflicting regarding the role of TNFα in human SLE. Some studies have shown that serum TNFα levels are elevated in SLE patients and correlate with disease activity [40,41]. Other studies, however, have shown otherwise. One study demonstrated that elevated TNFα plasma levels do not correlate with SLE disease activity, and another study showed that TNFα levels were actually higher in patients with inactive disease, suggesting a protective role for TNFα in SLE [42,43]. Differences in patient characteristics, assays for TNFα, and study designs probably each contribute to the somewhat inchoate picture that emerges, highlighting the pleiotropic and oftentimes unpredictable nature of TNFα.

In addition to systemic production of TNFα, local production of TNFα may contribute to SLE. Studies of local production of TNFα in the kidneys have documented upregulated TNFα protein and gene expression in renal biopsy tissue from 52% of SLE nephritis patients [22]. Local upregulated TNFα protein and gene expression within the resident glomerular, tubular, and mesangial cells has also been demonstrated [23].

The effects of systemically produced TNFα may be dissociated from the effects of locally produced TNFα. For example, DR3-positive or DR4-positive SLE patients, who genetically are high TNFα producers in response to appropriate induction, are not predisposed to nephritis. In contrast, DR2 and DQw1-positive SLE patients, who genetically are low TNFα producers following induction, have an increased incidence of SLE nephritis [44]. As another example, the TNFα adaptor proteins TNF receptor type 1-associated DEATH domain (TRADD), FADD, receptor interacting protein-1 (RIP-1), and

TRAF-2 are downregulated in SLE peripheral blood mononuclear cells, and their decreased expression correlates with disease activity [45]. The downregulation of TRADD and FADD (which can recruit the caspase cascade) may thus promote an anti-apoptotic effect that leads to survival of autoreactive cells. Of note, TRADD, RIP-1, and TRAF-2 (which can activate the NF-κB pathway) are upregulated, rather than downregulated, in renal tissue from SLE nephritis patients, suggesting that TNFα may promote immunoregulatory functions systemically but may mediate inflammation locally [46].

Therapeutic blockade of TNFα in systemic lupus erythematosus

Use of TNFα antagonists is well established in the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, chronic inflammatory bowel disease, and ankylosing spondylitis [47]. Consistent with the competing proinflammatory and anti-inflammatory effects of TNFα, use of TNFα antagonists in multiple sclerosis patients has led to immune activation and disease exacerbation [48]. Moreover, TNFα antagonists have triggered the development of autoantibodies, neuroinflammatory disease, or SLE-like features in some patients with rheumatoid arthritis or chronic inflammatory bowel disease [49-51]. Indeed, depending on the disease, two-thirds of previously antinuclear antibody (ANA)-negative patients became ANA-positive after initiation of TNFα-antagonist therapy. A recent report described six rheumatoid arthritis patients who developed active SLE associated with major organ involvement and life-threatening manifestations and required additional treatment beyond cessation of the TNFα-antagonist regimen [52].

Nevertheless, TNFα-antagonist therapy has been successful in some SLE patients. Six patients with moderately active SLE (three with nephritis, two with arthritis refractory to other therapies, and one with both joint and renal involvement) were given four infusions of 300 mg doses of infliximab, in combination with immunosuppression with azathioprine or methotrexate [53]. Patients with joint involvement experienced remission of arthritis, which relapsed 8 to 11 weeks after the last infliximab infusion. In the four patients with nephritis, proteinuria decreased by >60% within 8 weeks. Of note, anti-dsDNA and anti-cardiolipin antibodies increased in four patients. A number of long-term studies also revealed modest clinical benefit, but there were several adverse events, including deep-vein thrombosis, infections, central nervous system lymphoma, and Legionella pneumonia [54,55].

The need to evaluate safety and efficacy of TNFα blockade in SLE prompted two large randomized phase II and phase III trials with etanercept and infliximab (Table 1), but both studies (NCT00447265 and NCT00368264) were terminated prematurely (although this may have had more to do with corporate business matters than with medical matters). As with neutralization of IL-6, additional studies with TNFα antagonists will be needed before use of such agents becomes a routinely viable option in human SLE.

Type I interferons

General biology of type I interferon

Type I interferon represent a large family of cytokines that includes many IFNα subtypes, a single IFNβ, and multiple IFNω subtypes that exert their expansive biological properties through engagement of a common heterodimeric receptor (type I interferon receptor (IFNAR)) composed of IFNAR1 and IFNAR2 subunits. These broad effects include activating dendritic cells; promoting proliferation, survival, and differentiation of monocytes into antigen-presenting cells and B cells into plasma cells; stimulating the Th1 pathway and preventing apoptosis of activated cytotoxic T cells; and suppressing regulatory T cells, enhancing natural killer cell activity, and modulating each of these cells' respective cytokine production and signaling responses.

Within the type I interferon family, the IFNα subtypes are arguably the biologically most important in general, and this certainly is the case for SLE. The major producers of IFNα are plasmacytoid dendritic cells, although virtually any cell type can elaborate interferon [56]. IFNα is produced both in response to exogenous stimuli, such as bacterial and viral pathogens, and to endogenous stimuli, such as self-nucleic acids and nucleic acid-containing immune complexes, via Toll-like receptor (TLR)-dependent and TLR-independent pathways. Among the TLR-dependent pathways, engagement of TLR7 and TLR8 by single-strand RNA-containing complexes and engagement of TLR9 by dsDNA-containing complexes may be especially relevant to SLE pathogenesis [56].

Type I interferon in murine lupus

Numerous studies have collectively demonstrated the complexity of the type I interferon/IFNAR axis in the pathogenesis of murine SLE. In several strains of mice with SLE-related autoimmune phenotypes, type I interferon/IFNAR signaling promotes autoantibody production and development of renal disease. Indeed, genetic deletion of the *Ifnar1* gene prevents the development of severe clinical and pathological disease in SLE-prone NZB and NZM2328 mice [57,58]. Similarly, upregulation or administration of IFNα to BWF mice, (NZWxBXSB)F1 mice, B6.Sle123 mice, or NZM2328 mice markedly accelerates nephritis and death [59-61], and in BWF mice is associated with unabated expansion

of short-lived plasma cells [62]. Nevertheless, type I interferon/IFNAR signaling is not always deleterious. IFNAR-deficient MRL/lpr mice develop more severe disease than do their IFNAR-sufficient counterparts [63], an observation that may relate to the ability of IFNβ to ameliorate disease manifestations in MRL/lpr mice. Importantly, these counterintuitive effects of type I interferon are not limited to MRL/lpr mice, in that blockade of type I interferon in B57BL/6 or B6.Sle2 mice led to increased, rather than decreased, levels of IgG autoantibodies [64].

In addition to studies that directly manipulated type I interferon and/or IFNAR expression, studies focused on TLR (especially TLR7 and TLR9) have also pointed to a vital role for IFNα in murine SLE. Interestingly, TLR7/9 doubly-deficient MRL/lpr mice display significantly reduced ANA titers, proteinuria, and kidney disease [65]. In agreement with these results, treatment of BWF mice with a dual inhibitor of TLR7 and TLR9 demonstrated significant reductions in interferon produced by plasmacytoid dendritic cells; decreased proteinuria; reduced glomerulonephritis, serum autoantibody levels, and end-organ damage; and increased survival [66]. Indeed, administration of bacterial or viral TLR ligands to SLE-prone mice leads to increased IFNα production and disease exacerbation, whereas disease is not exacerbated in similarly treated IFNAR-deficient SLE-prone mice [67].

Type I interferon and human systemic lupus erythematosus

Serum type I interferon activity is increased in SLE patients and their first-degree relatives, and ages of peak type I interferon are associated with ages of peak SLE incidence [68,69]. Type I interferon levels have correlated with disease activity in a number of cross-sectional studies, although longitudinal correlations could not be established in other studies [70-72]. The use of elevated serum levels of type I interferon-regulated chemokines as biomarkers for disease activity was suggested in early studies [73] and was later replicated and validated by associations between elevated transcript levels of these chemokines with disease activity and organ damage [74,75]. By relying on expression of type I interferon-inducible genes rather than on circulating type I interferon protein levels (which are often barely detectable or not detectable), most SLE patients have been demonstrated to unequivocally express type I interferon-inducible genes in peripheral blood mononuclear cells. This so-called interferon signature has been convincingly correlated with active disease, renal manifestations, and increased damage index [70,76,77]. Furthermore, the interferon gene signature has been found in glomerular and synovial tissue, suggesting local organ involvement of type I interferon [78,79]. Indeed, plasmacytoid dendritic cells – the main producers of IFNα – accumulate in the glomeruli of SLE nephritis patients and can also be found in cutaneous lesions where they promote continuous IFNα release [80,81].

In terms of potential interferon contribution to lymphocyte autoreactivity in SLE, interferon not only promotes B-cell activation, antibody production, and class switching, but can also decrease B-cell selectivity for CpG-rich DNA, thereby activating TLR9, and can also allow stimulation of B cells by non-CpG DNA [82,83]. Moreover, interferon can potentially promote survival of autoreactive B cells, since it can prevent B-cell apoptosis and enhance proliferation even in the absence of mitogenic stimuli [84].

T-cell function is also modulated by interferon in SLE. Ample literature has demonstrated dysfunction of regulatory T cells in SLE, with SLE regulatory T cells being inefficient suppressors of inflammation and T-cell proliferation [85,86]. Part of this dysfunction appears to be due to interferon production by antigen-presenting cells [87].

The *ex vivo* and *in vitro* findings that suggest a role for type I interferon in SLE pathogenesis are supported by the development of SLE or SLE-like syndromes (malar rash, oral ulcers, photosensitivity, renal involvement, and anti-Sm and anti-dsDNA antibodies) following administration of IFNα [88,89]. Occasionally, IFNα-induced SLE has led to life-threatening multiorgan involvement [90].

Recent investigations into the genetics of SLE have also revealed a strong connection between the type I interferon pathway and SLE. The gene coding for the transcription factor IRF5 was the first gene involved in IFNα production to be associated with SLE susceptibility [91]. Further evidence of genetic association between SLE and IRF5 and for interaction between IRF5 and TYK2, a Janus kinase involved in cytokine signaling that binds to IFNAR, has also been demonstrated [92]. Moreover, IRF5 risk loci are also associated with high serum type I interferon activity and the development of autoantibodies to dsDNA and RNA-binding proteins. [93]. Subsequently, the related IRF7 has also been associated with SLE [94]. Furthermore, the STAT4 gene, which codes for a protein that interacts with the cytoplasmic portion of IFNAR, is strongly associated with SLE. STAT4 risk loci increase sensitivity to IFNα, are associated with a more severe phenotype that includes anti-dsDNA antibodies and renal involvement, and interact with IRF5 susceptibility loci [95-97]. Another SLE susceptibility gene codes for IRAK1, which is involved in TLR-triggered signal transduction [98].

IFNα, independent of its effects on the immune system, may be especially important in promoting atherosclerotic disease in SLE. The dysfunction of circulating endothelial progenitor cells and myelomonocytic circulating

angiogenic cells in SLE appears to be mediated by IFNα, in as much as neutralization of IFNα restores a normal endothelial progenitor cell/circulating angiogenic cell phenotype [99]. Indeed, SLE patients with robust interferon signatures have decreased endothelial function [100], and interferon signatures correlate with carotid intima media thickness [101].

Therapeutic blockade of type I interferon in systemic lupus erythematosus

The fact that type I interferon exacerbates SLE in some mouse strains but ameliorates SLE in others [59-61,63] raises the possibility that markedly divergent clinical responses to type I interferon antagonists might emerge among individual SLE patients. Experience to date with antagonists against type I interferon in SLE is very limited (Table 1). Treatment of SLE patients (n = 62) in a phase I trial with anti-IFNα mAb sifalimumab (MEDI-545) in single escalating intravenous doses of 0.3, 1.0, 3.0, 10.0, or 30.0 mg/kg led to dose-dependent reversal of the interferon signature in both the blood and skin, downregulation of several cytokines, and reduced disease activity without any increase in serious infections [102]. Results from a phase I trial with the anti-IFNα mAb rontalizumab (rhuMab IFN-α) in SLE patients (n = 32) replicated this dose-dependent reduction in the interferon signature [103]. Two phase II trials are currently ongoing to evaluate the effects of anti-IFNα in SLE (NCT01031836 and NCT00657189), but as yet it will be some time before the utility of type I interferon antagonists in SLE is known.

B-lymphocyte stimulator

General biology of BLyS

BLyS (also known as BAFF) is a cytokine that is essential for survival of most B cells beyond the transitional 1 stage [104]. BLyS binds to three receptors: BCMA, TACI, and BR3 (also known as BAFFR). Two of these receptors (BCMA and TACI), but not the third (BR3), also bind APRIL, a cytokine closely related to BLyS that shares some, but not all, of its biological properties. Whereas survival of plasma cells, which express TACI and BCMA, is supported by either BLyS or APRIL, the survival of pre-plasmablast mature B cells, which express much BR3 but little TACI and essentially no BCMA, is supported only by BLyS. Of note, memory B cells appear to be independent of both BLyS and APRIL.

In addition to affecting B-cell survival/function, BLyS can affect other cell types that express BLyS receptors. Specifically, BR3 is expressed on T cells, although its role in T-cell signaling/stimulation is controversial [104]. Moreover, dendritic cells also express BLyS receptors, and BLyS-stimulated dendritic cells upregulate co-stimulatory molecules and produce inflammatory cytokines and chemokines such as IL-1, IL-6, CCL2, and CCL5.

BLyS in murine systemic lupus erythematosus

There is an irrefutable link between BLyS and murine SLE. Constitutive overexpression of BLyS in BLyS-transgenic nonautoimmune-prone mice leads to SLE-like features, including elevated levels of multiple autoantibodies (including anti-dsDNA), circulating immune complexes, and glomerular immunoglobulin deposition [105]. Moreover, BLyS overexpression accelerates the development of SLE-like features in mice that bear an autoimmune diathesis but otherwise do not develop overt SLE [106]. On the other hand, SLE-prone NZM2328 mice genetically deficient in BLyS are largely spared from overt disease (severe proteinuria and premature death), although the lifelong absence of BLyS does not protect them from ultimately developing serological autoimmunity and renal pathology [107]. Most importantly, treatment of BWF mice, (NZMxBXSB)F1 mice, MRL/lpr mice, or NZM2410 mice with either TACI-Ig (which neutralizes both BLyS and APRIL) or BR3-Ig (which selectively neutralizes BLyS) is effective at preventing clinical disease and ameliorating renal injury [108]. Intriguingly, IFNα-driven exaggerated disease in several SLE-prone mouse strains is associated with increases in serum BLyS levels [59-61], and IFNα-driven exaggerated disease is completely blocked in BLyS-deficient NZM2328 mice [109], indicating that BLyS is a vital contributor to the IFNα-driven pathogenic pathway in SLE.

BLyS in human systemic lupus erythematosus

Not only is BLyS associated with murine SLE, but is also associated with human SLE. Circulating BLyS levels are elevated in as many as 50% of SLE patients [110], and disease activity correlates with blood leukocyte expression of BLyS mRNA [111]. Aberrant expression of BLyS on multiple immune cells has been observed in SLE. Although BLyS is largely expressed in myeloid lineage cells, activated B cells can also express both BLyS and APRIL. In SLE patients, B cells and plasma cells express high levels of BLyS and APRIL mRNA, which correlate with disease activity and levels of anti-dsDNA antibodies [112].

In addition to the biologic studies summarized above, investigations into genetic susceptibility loci in SLE have revealed associations between polymorphisms in BLyS and APRIL genes with human SLE [113,114]. In light of the results from murine SLE models, from *ex vivo* studies of human SLE leukocytes, from BLyS serum analyses, and from the genetics of SLE, BLyS has emerged as a highly attractive cytokine target in SLE.

Therapeutic blockade of BLyS in human systemic lupus erythematosus

The greatest experience to date with BLyS antagonists (Table 1) has accrued with belimumab, a fully human

$IgG_{1\lambda}$ mAb that binds and neutralizes soluble BLyS [115]. Belimumab was shown to be safe in a randomized, double-blind, placebo-controlled phase I trial of SLE, in which the prevalence of adverse events was no different between belimumab-treated and placebo-treated patients [116]. Of note, only modest reductions in peripheral blood B cells were observed among belimumab-treated patients. No clinical efficacy was demonstrated in this phase I trial, but the small number of patients (n = 70) and the very brief treatment schedules (single infusion or two infusions 3 weeks apart) and follow-up period (12 weeks after final infusion) precluded demonstration of clinical benefit.

Disappointingly, the subsequent phase II trial (n = 449) failed to meet its co-primary endpoints [117]. Extensive *post hoc* analysis, however, led to a novel composite index of clinical response (SLE responder index) [118] and demonstrated significantly increased clinical response among belimumab-treated patients at 52 weeks among the patients who were seropositive (ANA titer ≥1:80 and/or positive for anti-dsDNA antibodies) at entry. Using this novel SLE responder index, two separate large randomized, double-blind, placebo-controlled phase III trials (n = 865 and n = 819, respectively) of belimumab in seropositive SLE patients each met their primary endpoints (increased percentage of responders at 52 weeks) [119,120]. Importantly, analysis of the combined 1,864 SLE patients in both trials pointed to reductions in disease activity and prevention of worsening across vital internal organ systems [121]. Although questions remain regarding the durability of the clinical response, a US Food and Drug Administration advisory panel in November 2010 recommended approval for belimumab in the treatment of SLE, and final approval by the US Food and Drug Administration was given on 9 March 2011.

Concern has been raised regarding the ostensibly lower clinical efficacy of belimumab in human SLE in comparison with the ostensibly more robust clinical efficacy of BLyS antagonists in murine SLE. This difference in clinical response between human and murine SLE may be more apparent than real. First, the clinical response in the phase III human trials was based on a composite of several instruments each rooted in multiple organ systems [118]. In contrast, clinical response in the murine trials was simply the absence of pre-moribund proteinuria and death. (Belimumab in all likelihood will prove to be very effective in preventing pre-moribund proteinuria and death; however, it will take many years of follow-up to formally prove this point.) Second, human SLE patients received standard-of-care therapy in addition to belimumab in the human clinical trials, whereas murine SLE patients in the murine clinical trials received no treatment other than a BLyS antagonist. (Human SLE patients treated with belimumab alone would undoubtedly do much better than untreated patients, but such a human trial would be entirely unethical.)

In addition to belimumab, several other BLyS antagonists are undergoing clinical evaluation in SLE. The one furthest advanced in clinical evaluation is atacicept, a fusion protein between one of the BLyS receptors (TACI) and the Fc portion of IgG. Favorable safety and tolerability were demonstrated in a randomized, double-blind, placebo-controlled phase I trial (n = 49) [122]. Dose-dependent reductions in peripheral blood B cells and in circulating immunoglobulin levels were noted, but clinical efficacy could not be demonstrated due to the limited treatment and limited follow-up period. Of concern, an increased risk of severe infections was observed in a subsequent trial involving patients with SLE nephritis who were concurrently taking mycophenolate mofetil and corticosteroids (NCT00573157). As a consequence, this trial was prematurely terminated. Nevertheless, a separate phase II/III trial of atacicept in SLE has recently been initiated (NCT00624338). Whether atacicept achieves clinical success from efficacy and safety standpoints remains to be determined.

A third BLyS antagonist being tested in clinical trials is A-623 (previously known as AMG 623), a fusion between the Fc portion of IgG and a peptide sequence selected for its ability to bind with high affinity to BLyS. In a double-blind, placebo-controlled phase I trial, SLE patients received a single dose (n = 54) or four weekly doses (n = 63) of escalating doses of AMG 623 or matching placebo [123]. A dose-independent decrease in naive and total peripheral blood B cells was accompanied by an increase in memory B cells – an observation that has now also been made in patients treated with atacicept or belimumab [122,124]. Clinical responses were not reported, so the relevance of the disparate changes among B-cell subsets to clinical parameters remains unknown. A phase II trial of A-623 in SLE had been initiated but was suspended due to 'structural failure identified in some product vials', but the trial was recently resumed (NCT01162681).

A fourth BLyS antagonist in clinical development for SLE is LY2127399, a mAb that binds both soluble and membrane BLyS [125]. Two phase III trials in SLE are planned (NCT01205438 and NCT01196091), but neither has yet started recruiting patients. It remains to be determined whether neutralization of soluble plus membrane BLyS (as with LY2127399) will have greater therapeutic efficacy than neutralization of soluble BLyS alone (as with belimumab).

Concluding remarks

Cytokine-targeted therapy may prove effective in the treatment of SLE and offer less toxic options

in comparison with 'conventional' therapies such as glucocorticoids or cytotoxics. In addition to the four cytokines reviewed above, there are many more cytokines – including IFNγ, IL-1, and IL-17 – that have been implicated in SLE. As has become painfully evident, cytokine biology is extremely complex – and increasingly so in a complex disease such as SLE. Continued investigations of cytokine pathways *in vivo* in animal models, *ex vivo* in human SLE tissues, and in the genetics of SLE will reveal the roles of additional cytokines in disease pathogenesis and should offer additional novel targets for treatment.

Autoimmune Basis of Rheumatic Diseases

This article is part of a series on *Systemic lupus erythematosus*, edited by David Pisetsky, which can be found online at http://arthritis-research.com/series/lupus

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Abbreviations

ANA, antinuclear antibody; APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor of the TNF family; BCMA, B-cell maturation antigen; BR3, BLyS receptor 3; BLyS, B-lymphocyte stimulator; BWF, (NZB x NZW)F1; dsDNA, double-strand DNA; FADD, Fas-associated death domain; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IFNAR, type I interferon receptor; IL, interleukin; IL-6R, IL-6 receptor; mAb, monoclonal antibody; MHC, major histocompatibility complex; NF, nuclear factor; RIP-1, receptor interacting protein-1; SLE, systemic lupus erythematosus; TACI, transmembrane activator and calcium modulator and cyclophilin ligand interactor; Th, T-helper type; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRAF, TNF receptor-associated factor; TRADD, TNF receptor type 1-associated DEATH domain; VCAM-1, vascular cell adhesion molecule-1.

Competing interests

WS has conducted clinical trials in systemic lupus erythematosus with belimumab (sponsored by Human Genome Sciences). NJ has no competing interests.

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