Pathogenesis of human systemic lupus erythematosus: recent advances

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Systemic lupus erythematosus (SLE) is an autoimmune disease with manifestations derived from the involvement of multiple organs including the kidneys, joints, nervous system and hematopoietic organs. Immune system aberrations, as well as heritable, hormonal and environmental factors interplay in the expression of organ damage. Recent contributions from different fields have developed our understanding of SLE and reshaped current pathogenic models. Here, we review recent findings that deal with (i) genes associated with disease expression; (ii) immune cell molecular abnormalities that lead to autoimmune pathology; (iii) the role of hormones and sex chromosomes in the development of disease; and (iv) environmental and epigenetic factors thought to contribute to the expression of SLE. Finally, we highlight molecular defects intimately associated with the disease process of SLE that might represent ideal therapeutic targets and disease biomarkers.

Introduction

Significant progress has been made in the past decade since the pathogenesis of systemic lupus erythematosus (SLE) (Box 1) was last reviewed in this journal [1]. New loci and genes have been associated with the expression of SLE. Extensive studies have shed light on the aberrant biochemistry that governs T cell function and cytokine production in SLE [1]. Exploitation of these abnormalities has been proposed for development of therapeutic targets and biomarkers. Although studies have classically focused on the dysregulation of adaptive immunity in SLE, elements of the innate immune system have been identified as major contributors of disease pathogenesis. Furthermore, recent studies highlight the significance of epigenetic alterations in the aberrant expression or function of immune factors. Yet, despite these significant advances, the scarcity of novel therapies continues.

In this review, we discuss molecular and cellular aberrations of the immune system in patients with SLE. We discuss these alterations according to the main cell involved. However, one of the important messages that this article intends to communicate is that the immune system is broadly compromised in patients with SLE and that deregulation of single elements lead to altered behavior of the whole system.

Genes and genetics in SLE

Genetic predisposition influences the development of SLE in major ways [2]. Although in rare cases this is caused by deficiency of a single gene (e.g. C1q) [1,2], it commonly results from the combined effects of a large number of genes. Each allele contributes only mildly (odds ratio ~1.5) and the accumulation of several genes is presumed necessary to significantly increase the risk of SLE. The combinations of risk alleles that lead to predisposition and the mechanisms through which they contribute to autoimmunity are poorly understood. In fact, most single nucleotide polymorphisms (SNPs) associated with SLE fall within non-coding DNA regions and represent markers of co-segregated alleles. Notwithstanding, most of them are associated with genes presumed to be involved in the immune response.

During the past few years, genome-wide analyses have substantially increased the number of candidate genes associated with SLE [3–6] (Figure 1). Their function is variable. Some, such as IRF5 [7], STAT4 [8], osteopontin [9], IRAK1 [10], TREX1 [11] and TLR8 [12], are involved in nucleic acid sensing and interferon (IFN) production, whereas others are involved in T cell (PTPN22 [13], TNFSF4 [14], PDCD1) or B cell (BANK1, BLK [5], LYN [15]) signaling pathways (e.g. PTPN22 regulates lymphocyte activation [13]). BCL6 (Figure 1) is the lineage-specific transcription factor of follicular helper T cells (Tfh), a T cell subset that provides help to B cells in germinal centers [16]. Interestingly, IRF5 and STAT4 additively increase the risk of SLE [8]. Some genes have been associated with several autoimmune diseases (e.g. STAT4 with rheumatoid arthritis; PTPN22 with rheumatoid arthritis and diabetes), yet others appear to specifically increase the risk of SLE.

A recent large-scale replication study confirmed some of the above-mentioned associations and identified TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for SLE [17]. Although promising, the loci identified so far can account only for ~15% of the heritability of SLE [18]. Thus, although the identification of candidate genes and alleles represents an important step in our understanding of the pathogenesis, the relative importance of each gene in the
overall disease process and its particular contribution to phenotype and severity remain to be defined. The pathways affected by the genes associated with SLE and their relationship with T and B cell functional aberrations described in this review will need to be addressed in future studies.

T Lymphocytes and cytokines in lupus

In SLE, T cells provide excessive help to B cells and mount inflammatory responses while failing to produce sufficient interleukin-2 (IL-2). Biochemical and gene expression defects have been identified that account for their aberrant function [19].

Cellular activation is altered in T cells from SLE patients. Engagement of the T cell receptor (TCR) CD3 leads to an early and enhanced signaling response manifested by increased intracytoplasmic calcium flux and cytosolic protein tyrosine phosphorylation. The CD3 complex in SLE T cells is rewired whereby the CD3ζ chain is replaced by the FcRγ common chain [19] (Figure 2). When FcRγ is present, the signal relies on the spleen tyrosine kinase (Syk) and not on the canonical ζ-associated protein ZAP-70 [20]. Multiple molecular mechanisms have been described in SLE T cells that contribute to the diminished expression of CD3ζ. These include decreased transcription, abnormal mRNA splicing, decreased mRNA stability and increased caspase 3-mediated protein degradation [21].

Another factor contributing to the enhanced T cell activation is the aggregation of lipid rafts on the cell surface [22]. These high-cholesterol membrane zones rich in signaling molecules polarize upon cell activation. Evidence for the in vivo relevance of these findings was provided by a study that showed that administration of an agent that enhances lipid raft clustering accelerates disease onset in a murine model of lupus (MR1/lpr), whereas injection of a drug that disrupts lipid raft clustering has the opposite effect [23]. Additional signaling abnormalities noted in T cells from SLE patients are listed in Table 1.

Transcription factors and gene expression

The abnormal gene transcription profile observed in lupus T cells is complex. This altered pattern produces a characteristic phenotype that in some aspects resembles that of activated T cells, and in others shares characteristics with anergic cells.

NFAT and AP1

Nuclear factor of activated T cells (NFAT) is a transcription factor regulated by calcium influx. Upregulation of NFAT in SLE T cells can account for the increased expression of the co-stimulatory molecule CD40L. However, it fails to upregulate IL-2, because, in certain gene promoters it requires the concurrent binding of AP1, and AP1 is decreased in SLE T cells [24].
Active Elf-1 binds to the promoter of the CD3 gene, it represses its activity. The decreased levels of DNA-binding Elf-1 found in SLE T cells could thus explain decreased expression of CD3ζ and increased expression of FcRγ [28]. It appears that PP2A is upstream of Elf-1 and indirectly determines the expression of CD3ζ and FcRγ [29].

**CD4 helper T cells and cytokine production**

After exposure to antigen, naïve CD4 T cells develop into effector subsets defined by the expression of distinct transcription factors that induce a particular phenotype and cytokine production profile. SLE patients have aberrant cytokine production and therefore their effector capacities are compromised.

**IL-2**

The production of IL-2, a cytokine centrally involved in the process of T cell activation and proliferation, is defective in T cells from patients with SLE [19]. This could account for the known decreased cytotoxic activity, defective regulatory T cell (Treg) function and decreased activation-induced cell death in SLE patients.

**IL-17**

IL-17A and IL-17F are mainly produced by activated T cells and play important roles in the immune response against certain bacteria and fungi [30]. IL-17-producing cells have been implicated in the pathogenesis of several autoimmune diseases including multiple sclerosis and SLE [30,31]. Sera of patients with lupus contain abnormally high levels of IL-17 [32] and a high fraction of CD4⁺ and CD4⁺CD8⁻ (double-negative) T cells in these patients produce IL-17 [33]. Furthermore, IL-17-producing T cells have been found within kidney infiltrates of patients with lupus nephritis [33]. Increased production of IL-17 in patients with SLE correlates with disease activity [32,34]. Release of IL-17 amplifies the inflammatory response by recruiting effector cells to target organs. Furthermore, IL-17 contributes to the formation of germinal centers and, acting in concert with a B cell-activating factor, increases the survival and proliferation of B cells and their transformation into antibody-secreting cells [32]. Support for the role of IL-17 in the pathogenesis of SLE has been provided by studies in lupus-prone mice [35], and mice deficient in lupus autoantigen Ro52 [36] and IRF-4-binding protein (IBP) [37,38] which develop SLE.

Differentiation of T cells into the T_{H17} subset is induced when priming occurs in the presence of TGF-β and certain inflammatory cytokines such as IL-1β, IL-6 or IL-21 [30]. Interestingly, the differentiation of naïve cells into this proinflammatory subset has been proposed to occur in a reciprocal manner with the development of T_{reg} cells, and the presence of an inflammatory signal seems to be the factor that determines whether proinflammatory or suppressive cells are generated [30]. Increased levels of the T_{H17}-determining cytokines IL-6 [39], which might be linked to the increased expression of STAT3 [40], and IL-21 [41] are present in patients with SLE.

**T follicular helper cells**

T_{FH} comprise a recently described CD4 helper subset. Like T_{H17} cells, their differentiation is induced by IL-6 and...
IL-21, but depends on the inducible co-stimulatory molecule ICOS and the absence of TGFβ [16]. TFH cells localize in the B cell zones of lymph nodes and produce IL-21 and express CD40L. Their main function is to provide B cells with signals for immunoglobulin production, isotype switching and somatic hypermutation. In murine lupus, IL-21 and TFH cells are necessary elements for disease development [42,43] and treatment with IL-21R.Fc reduces disease progression [44]. Deficiency of ICOS protects MRL/lpr mice from lupus. This effect depends on the absence of a particular CD4 T cell type that promotes immunoglobulin production in extrafollicular compartments. This cell type, observed in autoimmune-prone mice, is analogous to TFH cells [42].

**Regulatory T cells**

Most reports are in agreement that CD4+FoxP3+ regulatory T cells (Treg) are low and functionally abnormal in patients with SLE [45]. However, the magnitude of the contribution of these defects to SLE course and pathology is still unknown. Moreover, other unconventional regulatory cell subsets have recently been reported to be associated with SLE clinical remission, namely CD4+NKG2D+ [46] and CD8+FoxP3+ cells [47].

**CD8 T cells and cytotoxic responses**

Several reports point to deficient CD8 cytotoxic capacity [48]. SLE CD8 cells cannot suppress self B cells infected with Epstein–Barr virus (EBV) [49], and perforin-deficient mice have accelerated humoral autoimmunity and lupus-like disease in the MRL background [50]. Furthermore, under certain conditions, CD8 T cells give rise to double-negative T cells [51]. The differentiation of functional subsets of CD8 T cells and its contribution to SLE pathogenesis must be clarified in future work.

**Double-negative T cells**

T lymphocytes that lack the CD4 and CD8 co-receptors are called double-negative (DN) T cells. These cells comprise a scarce population in normal individuals (<5% of T lymphocytes), but are significantly expanded in patients with SLE and induce anti-DNA antibody production by autoreactive B cells. Recent work has shown that they also secrete other cytokines such as IL-1β and IL-17, and are found within cellular infiltrates in kidney biopsies of patients with lupus nephritis [33]. DN cells represent a fraction of CD8+ T cells that, upon activation, acquires a distinct gene expression profile that induces the loss of CD8 expression and the capacity to produce proinflammatory cytokines [51].

**Adhesion molecules**

The expression of the adhesion molecule CD44 is abnormally increased on T cells from patients with SLE [22]. Also, SLE T cells migrate at increased rates in response to the chemokine CXCL12; this is most likely because they express more CXCR4 receptors, which causes their increased capacity to migrate into inflamed organs [22,52]. The expression of two of the many CD44 variants, CD44v3 and v6, is increased on T cells from lupus patients. This correlates with disease activity, presence of renal disease and antibodies against double-stranded DNA [53]. The importance of these findings is further supported by the fact that T cells that infiltrate kidneys from SLE patients express CD44v3 and CD44v6 [54].

**B lymphocytes**

Similar to T lymphocytes, B cells are commonly affected in patients with SLE. B cell lymphopenia and overactivity are among the most striking abnormalities encountered in SLE. B lymphocytes in SLE produce an array of autoantibodies against soluble and cellular constituents but most commonly against intranuclear antigens.

Analysis of B cell subpopulations in patients with juvenile-onset SLE revealed that autoreactive B cells in SLE arise early in B cell ontogeny and that the usual tolerance checkpoints imposed during B cell development are violated [55]. Despite this overactive state, immunization of SLE patients with tetanus toxoid results in decreased amounts of specific antibody production [56].

**B cell subpopulations**

In patients with active SLE, there is a marked reduction in the numbers of naive (CD19+CD27- ) B cells and enhanced numbers of CD27highCD38+CD19dimIgloowCD20+CD138+ plasma cells in the periphery [57]. Additionally, increased numbers of CD27high plasma cells correlate with increased disease activity, suggesting that this could be a valuable marker of disease activity [58].

**B cell signaling**

Stimulation of circulating B cells from patients with SLE through their surface IgM or IgD B cell receptor (BCR) produces significantly higher fluxes of intracytoplasmic calcium and cytosolic protein tyrosine phosphorylation [59]. Complement receptor 2 (CR2) expression is decreased in SLE B cells, yet its engagement leads to increased BCR-mediated responses. Conversely, expression of the inhibitory receptor FcγRIIB on memory B cells and plasma cells is decreased in patients with SLE [60] and its ligation provides limited inhibition, suggesting that defects in co-stimulatory molecule function might account for increased BCR signaling [61]. A SNP in the promoter of CR2 with functional repercussions might regulate its expression and function in SLE [62]. Likewise, a SNP in the human FCGR2B promoter (particularly found in European–American patients with SLE) results in decreased transcription thus providing a molecular explanation for the dysregulation of FcγRIIB in some patients with SLE [63]. Protein tyrosine kinase Lyn has been reported to be decreased in the cytoplasm of B cells from two-thirds of patients with SLE [64]. Lyn is crucial for the function of several other signaling inhibitory molecules of B cells such as the surface receptors CD22 and FcγRIIB, suggesting that decreased Lyn could contribute to lupus B cell overactivity [65]. Studies performed in mice suggest that the presence of a risk-conferring allele of the Ly108 gene (a member of the SLAM family) can facilitate autoimmunity by lowering the intensity of B cell signaling. This effect is explained by its consequences on B cell ontogeny. Autoreactive B cells can transmit through tolerance checkpoints without being deleted [66].
**Dendritic cells in SLE**

*Phenotypic characteristics of dendritic cells in SLE*

Although earlier reports described normal and/or decreased numbers and function of SLE dendritic cells (DCs) (compared with cells from normal individuals) [67], recent studies have reported an overstimulated phenotype and function of SLE monocytes and DCs [68]. These discrepancies probably relate to the fact that most studies use monocyte-derived DCs whose phenotype varies according to the environment in which they differentiate. Factors present in SLE sera, including IFN-α, CD40L, free nucleosomes and autoantibody-DNA complexes cause differentiation and activation of normal DCs [69–71] and stimulate their production of cytokines, including IFN-α.

**DCs and apoptotic cell clearance in SLE**

Under physiological conditions, the presence of apoptotic cells is interpreted by the immune system as an anti-inflammatory signal. Thus, when DCs pick up apoptotic cell fragments, autoantigens are presented in a manner that leads to the inactivation of possible autoreactive T cells [72]. Whereas ingested necrotic cells are able to induce DC maturation, apoptotic cells fail to activate DCs under normal circumstances [73]. Disturbances in apoptosis and/or clearance of apoptotic cells can play an important role in the pathogenesis of SLE [74]. Moreover, SLE macrophages have an impaired capacity for taking up apoptotic cell material *in vitro* [75]. It has been shown that the interaction with iC3b-opsonized apoptotic cells results in tolerizing DCs (with low expression of CD86 and MHC class II) [76] as well as DC interaction with thrombospondin-1 produced by apoptotic cells [77]. By contrast, high mobility group box protein 1 (HMGB1), which binds and stabilizes the structure of nucleosomes inside the nucleus, acts as a proinflammatory mediator when released from dying cells [78]. In SLE patients who are known to have a defect in apoptotic cell clearance, non-phagocytosed apoptotic cells undergo secondary necrosis [75]. HMGB1–nucleosome complexes are released from secondary necrotic cells and can be found in the blood of SLE patients [79] and induce cytokine expression in macrophages and maturation of DCs [80] (Figure 3).

**DCs and nucleic acid sensors in SLE**

Recent findings suggest that exogenous, pathogen-associated nucleic acids can exacerbate SLE pathology through stimulation of Toll-like receptors (TLRs) [81]. Moreover, the discrimination by TLRs between pathogen and self nucleic acids is not perfect and endogenous nucleic acids can also activate TLRs [82]. The apoptotic cells and necrotic debris can function as an endogenous source of nucleic acids. Bone marrow-derived myeloid DCs are activated by anti-nucleosome antibodies bound to chromatin [83] or by U1 small nuclear ribonucleoprotein (U1snRNP) immune complexes bound with anti-Sm antibodies [84]. However, these and other results indicate that DCs have an additional, TLR-independent, mechanism of nucleic acid sensing in the cytosol that triggers the secretion of IFN-β and IL-1β [85]. One of the new candidate molecules is the DNA-dependent activator of IFN-regulatory factors (DAI or DLM-1/ZBP1), which is able to trigger a strong type I IFN response upon activation [86]. Another candidate is the interferon-inducible HIN-200 family member Absent in Melanoma 2 (AIM2). This molecule binds directly to cytoplasmic DNA and triggers the assembly of an AIM2 inflammasome, resulting in caspase-1 activation and the maturation of IL-1β [87]. Recent reports have claimed an association between this gene family and SLE [88–90]. However, so far there is no information about the exact role of DAI and AIM2 in SLE and DCs.

**DCs and complement system deficiencies in SLE**

Complement factors have been shown to mediate efficient clearance of apoptotic cells by means of macrophages [91,92] and the generation of tolerizing DCs [76]. C1q is a recognition protein of the classical pathway of the complement system. Immature DCs produce high amounts of C1q and are known to induce tolerogenic responses [93]. DC activation leads to maturation associated with a reduced capacity to produce C1q and the induction of immunogenic responses [94]. It appears that this could represent another mechanism that links C1q deficiency to the development of SLE. Complement has both beneficial and deleterious roles in the pathogenesis of SLE [95]. The complex role of C1q and other complement proteins in the pathogenesis of SLE is explained by their role in the clearance of apoptotic cells as opsonins (‘waste disposal’ hypothesis). The differentiation of DCs in the presence of C1q gives rise to CD11c+, DC-SIGN+ cells with high phagocytic capacity, impaired ability to stimulate alloreactive T cells and reduced production of IFN-γ [96]. By contrast, immobilized C1q is able to activate DCs and induce DC maturation, cytokine production, expression of co-stimulatory molecules and T cell stimulatory capacity [97]. Thus, C1q plays a protective role against the development and amplification of autoimmune responses by its participation in the clearance of apoptotic cells and immune complexes and perhaps by regulating DC activation.
**DCs and the interferon signature in SLE**

The expression levels of IFN-inducible genes are upregulated in SLE patients compared with normal controls or patients with other rheumatic diseases [98]. Plasmacytoid DCs secrete large amounts of type I IFN upon viral infection owing to the activation of TLR7 and TLR9 [99], and probably represent the main source of IFN-α in SLE patients. Plasmacytoid DC numbers are reduced in SLE peripheral blood, perhaps owing to their accelerated migration to inflammatory sites [69]. Indeed, plasmacytoid DCs massively infiltrate lupus skin and renal lesions [100].

DCs play a fundamental role in the development of immune responses. However, their broad capacity to detect immune complexes and nucleic acids provide them with the power to amplify the autoimmune response in patients with SLE.

**Environmental contributions to the expression of SLE**

The contribution of the environment to the expression of SLE is unquestionable, as exemplified by the fact that clinical concordance of SLE in identical twins is limited to less than half of the pairs. Epigenetic changes such as DNA methylation have also been attributed to environmental factors associated with SLE. Exposure to ultraviolet light is a known risk factor in clinical disease, and various environmental toxins, including smoking, have been implicated in epidemiological studies [101].

Viral infections, including parvovirus B19 and cytomegalovirus (CMV), are common in patients with SLE [102]. Notably, much discussion has centered around the concept that a viral infection could trigger SLE [103]. The high prevalence of EBV in the adult population makes it difficult to draw any definitive conclusions about causality, but convincing evidence that EBV precedes SLE development was presented in a study in which serum samples of EBV-seropositive children with lupus-prone mice [108]. Although hormones influence SLE development in mice, it has recently been shown that the sex chromosomes themselves influence the expression of SLE. In gonadectomized female and male mice, that have been genetically manipulated to express XX, XO (female), XY or XXY (male), the presence of two X chromosomes increases the severity of SLE disease [109]. Although it is clear that hormones can influence autoimmune development in murine models, the use of oral contraceptives does not influence SLE disease flares [110].

**Drugs and epigenetic regulation of gene expression**

DNA accessibility and thus gene expression is regulated by DNA methylation and histone modifications (acetylation and methylation). SLE patients exhibit DNA hypomethylation in CD4 T cells. This defect has been shown to underlie the overexpression of several genes. Although it occurs spontaneously in SLE patients, some commonly used drugs, such as hydralazine and procainamide, inhibit DNA methylation and can induce lupus in healthy individuals [114]. DNA demethylation causes increased CD4 T cell cytokine production and IgG hyperproduction by B cells that overexpress the co-stimulatory molecules CD70 and CD40L. Furthermore, hypomethylated T cells respond to antigen-presenting cells with empty MHC class II pockets, and overexpression of LFA-1 on these T cells can lead to killing of antigen-free macrophages [115].

The mechanisms involved in DNA hypomethylation are not clearly defined as conflicting studies have reported a decrease or no change in the transcription levels of various DNA methyltransferases [116,117], and one study demonstrated increased expression of the catalytic subunit of PP2A in the presence of a methylation inhibitor [118]. Defective activity of PKCθ, also reported in SLE T cells, has been associated with hampered DNA methyltransferase 1 activity through a pathway that depends on the faulty activity of mitogen-activated protein (MAP) kinases [119]. Elevated levels of IL-6, a proinflammatory cytokine produced in excess by mononuclear cells from lupus patients, have been reported to abrogate DNA methylation in B cells [120].

Abnormal histone acetylation has also been proposed to alter gene expression in SLE T cells. Some of the effects of CREM, particularly its effect on the IL2 promoter, depend on its capacity to recruit histone deacetylase 1 (HDAC1) [121]. PP2A also regulates the activity of HDAC, and some of its effects are known to be mediated through histone acetylation [122]. Treatment of SLE T cells with trichostatin A, a HDAC inhibitor, diminished the expression of CD40L and the production of IL-10, suggesting that histone acetylation plays a role in the overexpression of these molecules in lupus [123].
Tissue injury in SLE

The role of immune complexes in the expression of tissue injury in SLE cannot be overstated. Immune complexes are cleared through FcR and complement receptors, and the fact that a strong genetic association has been noted between polymorphisms in the FcR genes [124] and tissue injury in SLE cannot be overestimated. Immune complexes are best represented by complement activation, as was the case with the identification of genes encoding kallikreins, but these discoveries should be followed by functional studies testing the contribution of these genes to disease. Accordingly, it is hoped that this line of investigation, that is the search for local factors that contribute, control or even instigate autoimmune tissue injury, might lead to the elucidation of completely different therapeutic approaches.

Prospects for the development of novel therapeutics

Patients with SLE are currently treated with non-specific immunosuppressive drugs. Although morbidity and mortality have been improved significantly over the past few decades, treatment-related morbidity remains a significant problem [131,132]. Advances in our understanding of the autoimmune-mediated organ damage has led to the introduction of successful biologics in the treatment of rheumatoid arthritis, spondyloarthropathies and Crohn’s disease, but the development of a new disease-specific therapeutic agent for SLE is elusive. The clinical heterogeneity of the disease, the lack of reliable biomarkers and the failure to design proper clinical trials have frequently been responsible for the failure. In Table 2, we have listed clinical trials which have either been concluded or are still in progress.

BLyS is a cytokine that is involved in the survival of immature B cells particularly at the transition stage, a major checkpoint for elimination of autoreactive B cells. Blockade of BLyS [133] with an anti-BLyS antibody (belimumab) has gained attention. In the largest clinical trial ever done in SLE patients (865 patients), high dose anti-BLyS was found to be superior to placebo (57.6% vs. 43.6% patients reached the primary endpoint at 52 weeks) [134]. Although moderate, this effect is significant enough to make anti-BLyS a good therapeutic option for patients with mild to moderate SLE.

Table 2. Biologics in the treatment of SLE

<table>
<thead>
<tr>
<th>Target</th>
<th>Treatment</th>
<th>Mode of action</th>
<th>Clinical trials</th>
<th>References</th>
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<tbody>
<tr>
<td>BLYS APRIL</td>
<td>Belimumab (anti-BLYS antibody) TACI-Ig</td>
<td>Blocks BLYS (and/or APRIL) effect on B cells</td>
<td>Efficacious in animal models. Positive large trial, although with moderate effects in patients with SLE (phase III).</td>
<td><a href="http://clinicaltrials.gov/ct2/results?term=lupus">http://clinicaltrials.gov/ct2/results?term=lupus</a></td>
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<tr>
<td>Interleukin-6 receptor (IL-6R)</td>
<td>Monoclonal antibody</td>
<td>Blocks IL-6 effect on lymphocytes, such as immunoglobulin production</td>
<td>Successful in mice. Phase I in patients underway.</td>
<td></td>
</tr>
<tr>
<td>Interferon (IFN)</td>
<td>ruthMABINF alpha</td>
<td>Blocks IFN effects</td>
<td>Successful anti-IFN-γ treatment in murine lupus. Phase I of anti-IFN-α in patients with SLE.</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>Monoclonal antibody</td>
<td>Blocks membrane attack complex (MAC) formation</td>
<td>Successful in mice. Effective and safe in patients with PNH.</td>
<td></td>
</tr>
<tr>
<td>CD20</td>
<td>Chimeric antibody</td>
<td>B cell depletion</td>
<td>Phase II/III trial in patients with SLE is ongoing.</td>
<td></td>
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<tr>
<td>CD22</td>
<td>Humanized antibody</td>
<td>Modulation of B cell signaling</td>
<td>Safe in phase I in SLE patients. Phase II is ongoing.</td>
<td></td>
</tr>
<tr>
<td>CD40L</td>
<td>Monoclonal antibody</td>
<td>Blocks T–B cell crosstalk</td>
<td>Effective in mice and patients with SLE. Significant side-effects led to discontinuation of the trials.</td>
<td></td>
</tr>
<tr>
<td>dsDNA B cell receptor</td>
<td>Abetimus</td>
<td>Blocks the production of anti-dsDNA antibodies</td>
<td>Some effects on quality of life; better effect in patients with high levels of anti-dsDNA antibodies.</td>
<td></td>
</tr>
<tr>
<td>CD28/CD80/86</td>
<td>CTLA-4.Ig</td>
<td>Prevents stimulation of T cells</td>
<td>Proven effectiveness in mice (with and without cyclophosphamide). Phase II/III in SLE patients.</td>
<td></td>
</tr>
<tr>
<td>ICOS-B7RP-1</td>
<td>Antibody against B7RP-1</td>
<td>Prevents T–B cell interaction</td>
<td>Effective in animals. Phase I trial in SLE patients.</td>
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Abbreviations: PNH, paroxysmal nocturnal hemoglobinuria; BLYS, B lymphocyte stimulator; APRIL, a proliferation inducing ligand; TACI, Transmembrane activator and calcium modulator and cyclophilin ligand interactor; CD40L, CD40 ligand; ICOS, Inducible T cell costimulator; B7RP-1, B7-related protein-1; CTLA4-Ig, Cytotoxic T lymphocyte antigen-4-immunoglobulin.
IL-6 promotes antibody production in humans and mice with lupus [135] and is present in the urine of patients with lupus nephritis [136]. An anti-IL-6 receptor monoclonal antibody was tested in phase I clinical trials in patients with SLE (Table 2). This treatment led to a decrease in acute phase reactants but at the same time caused neutropenia in two patients. Given the role of IFN in the development of murine lupus and the established upregulation of IFN-α-inducible genes, the use of anti-IFN-α antibodies to treat SLE is currently under consideration. Phase I trials showed safety and a phase II trial is underway. Complement activation is profoundly increased in SLE patients and inhibition of C5 with an antibody, which proved efficacious in the treatment of patients with paroxysmal nocturnal hemoglobinemia, should be considered.

B cell depletion in the treatment of autoimmune and rheumatic diseases showed clinical efficacy. A chimeric anti-human CD20 antibody (rituximab) showed initial promise in small studies and case series in SLE [137], but a trial of rituximab with mycophenolate mofetil in SLE nephritis failed to reach its primary endpoint of remission at 52 weeks of treatment. Another phase II/III trial of rituximab in moderate to severe non-renal SLE is currently underway. CD22 is a molecule that is expressed on the surface of mature B cells but not plasma cells or memory B cells. A humanized anti-CD22 antibody (epratuzumab) is currently undergoing a phase II trial in patients with moderately active SLE. Given the initial failure of anti-CD20 treatment in lupus nephritis, the exact role, if any, of B cell depletion in SLE is unclear. Successful B cell depletion treatment might not come to fruition in SLE until we understand which subset of B cells (autoactive vs. non-autoactive) repopulate the empty B cell space, and whether the production of B cell–tropic cytokines is altered following depletion. For example, increased production of BlyS following B cell depletion could negate the expected clinical benefit.

Attempts to reestablish B tolerance in patients with SLE have sufficient rationale. LJP-394 (abetimus sodium) is an artificial compound made of four deoxynucleotide-like molecules bound together, and thus resembles dsDNA (double-stranded DNA). It can theoretically bind to auto-reactive B cell receptors that recognize dsDNA. In clinical trials of SLE patients, abetimus led to a decrease in dsDNA antibody levels. However, clinical trials in humans have not been successful aside from improving quality of life measures [138].

Considerations to block the cognate interaction between T and B cells have led to the use of a fusion molecule of CTLA4 with immunoglobulin (Abatacept). Abatacept with background treatment with mycophenolate mofetil or cyclophosphamide is under investigation in a phase II/III clinical trial in SLE patients with nephritis. The ICOS interaction with its ligand B7-related peptide-1 presents another co-stimulatory pair, and blockade of this cognate interaction with a human antibody is currently in a phase I clinical trial in SLE patients. Finally, the co-stimulatory pair CD40–CD40 ligand has been demonstrated to be important in the production of autoantibodies. Clinical trials have been conducted with two different types of anti-CD40L (BG9588 and IDEC-1), and although some clinical efficacy was noted (only by BG9588) both trials were suspended because of unexpected thromboembolic events [139].

Failure to develop new drugs has not quenched effort and enthusiasm. The gained experience has highlighted the absolute need for biomarkers, the design of better trials and consideration of the heterogeneous nature of the disease, and should meet success in the near future.

### Concluding remarks and future directions

The main question in the field of human SLE is why there has been no new drug for SLE in the past five decades. Is this because of a lack of proper biologics, poorly designed trials, lack of proper biomarkers, or the clinical and pathogenic heterogeneity of the disease? (Box 2).

There is no doubt that the heterogeneity of the disease and lack of adequate tools to diagnose and follow disease progress present major impediments. Serum levels of autoantibodies, complement levels and the clinical tools available for characterizing the symptoms certainly have some value but they have proved inadequate in capturing the spectrum of the disease. Several laboratories have identified molecular abnormalities that appear to contribute to the molecular pathogenesis of the disease and could prove useful in defining subsets of patients with SLE.

Similarly, the available tools have proven not sufficiently powerful to provide biomarkers of disease activity. Suitable disease biomarkers are needed to conduct clinical trials. It is proposed that a good biomarker should be easily evaluated and should somehow contribute to the expression of disease pathology. Here, we have discussed several potential biomarkers, including the expression of CD44. This molecule, along with phosphorylated ezrin, radixin and moesin (pERM), was shown to be expressed by T cells in the peripheral blood and kidneys of patients with lupus nephritis [22]. Assuming that CD44+ pERM+ cells appear first in the peripheral blood prior to entering the kidney, and, assuming that, after entering the kidney, they contribute to tissue damage, their detection in the peripheral blood might represent a proper biomarker.

Emerging evidence at this point suggests that local factors could contribute, in parallel with the immune abnormalities, to the expression of end-organ damage in patients with SLE. Therefore, we need to better understand the

### Box 2. Issues that remain to be addressed

1. Identify the gene alleles that predispose to SLE and define the biologic mechanisms that underlie such predisposing effects.
2. Identify the functional relationships between predisposing genes that produce autoimmune proclivity.
3. Determine whether the biochemical defects of the SLE T cell represent a generalized T cell phenomenon or the signature of a pathogenic subset.
4. Determine the relationship between the abnormal transcription factor pattern and the skewed effector cell differentiation (e.g. abnormal Treg function, increased double-negative T cell generation).
5. Understand better the underlying biology that accounts for the failure of biologics, particularly the B cell-depleting ones, to produce therapeutic efficacy.
6. Identify the organ-specific factors that determine susceptibility or resistance to immune-mediated tissue injury.
mechanisms whereby these local factors contribute to disease pathology. It is expected that understanding of the immune and non-immune mechanisms involved in the damage of specific organs will allow us to treat patients more effectively.

The available biologies (e.g. anti-CD20 antibodies) accomplish what is expected of them with certain limitations. If anti-CD20 antibodies destroy B cells through antibody-mediated cell cytotoxicity, then patients with FeR isoforms that do not bind the Fe portion of the antibody with sufficient avidity should display limited B cell depletion. If the biologic is an antibody that destroys its target by activating complement, then SLE patients with decreased levels of complement will respond poorly. Here, we have discussed several molecular abnormalities expressed by SLE immune cells. It is expected that correction of these abnormalities will result in normalization of immune cell function and abrogate their ability to contribute to organ damage.

References

17. Gateva, V. et al. (2009) A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. Nat. Genet. 41, 1228–1233
immunosuppressive and inversely correlated with disease activity in progression.

Rheum.

effector phenotype.

Rheum.

lupus-prone mice.

J. Immunol.

pathogenesis of lupus nephritis.

Science

103, 3589–3580

Tian, J. et al. (2007) Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMBG1 and RAGE.


8, 497–500


J. Exp. Med.

Nature

Nature

Nature

J. Exp. Med.

Trends in Molecular Medicine Vol.16 No.2

Herber, D. et al. (2007) IL-21 has a pathogenic role in a lupus-prone mouse model and its blockade with IL-21R:Fc reduces disease progression.

J. Immunol.

45


Autoimmun. Rev.

4


J. Exp. Med.

47


J. Immunol.

48


Arthritis Rheum.

Puliaeva, I. et al. (2009) Therapeutic potential of CD8+ cytotoxic T lymphocytes in SLE.

Autoimmun. Rev.

5


J. Immunol.

51

Crispin, J.C. and Tsokos, G.C. (2009) Human TCR-ab+CD4+CD8- T cells can derive from CD8+ T cells and display an inflammatory effector phenotype.

J. Immunol.

52

Estess, P. et al. (1998) Functional activation of lymphocyte CD44 in peripheral blood is a marker of autoimmune disease activity.


53


(in press)


54


J. Exp. Med.

55


Arthritis Rheum.

56


Hum. Genet.

57

Crispin, S.N. et al. (1996) B cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early signal transduction events.


58


J. Immunol.

59

MacKay, M. et al. (2006) Selective dysregulation of the FcyRIIB receptor on memory B cells in SLE.

J. Exp. Med.

60

Boackle, S.A. et al. (2001) Cr1, a candidate gene in the murine Sle1c locus, encodes a dysfunctional protein.

Immunity

61


62


Immunity

63

Kumar, K.R. et al. (2006) Regulation of B cell tolerance by the lupus susceptibility gene Ly108.

Science

64


SLE patients.

Int. Immunol.

65


J. Immunol.

66


Science

67

Decker, P. et al. (2005) Nucleosome, the main autoantigen in systemic lupus erythematosus, induces direct dendritic cell activation via a MyD88-independent pathway: consequences on inflammation.

J. Immunol.

68


69


70


J. Immunol.

71


Arthritis Rheum.

72

Verbovetski, I. et al. (2002) Opsosization of apoptotic cells by autologous i3C3 facilitates clearance by immature dendritic cells, down-regulates DR and CD86, and up-regulates CC chemokine receptor 7.

J. Exp. Med.

73

Krispin, A. et al. (2006) Apoptotic cell thrombospespondin-1 and heparin-binding domain lead to dendritic-cell phagocytic and tolerizing states.

Blood

74

Tian, J. et al. (2007) Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMBG1 and RAGE.

Nat. Immunol.

75


76

Urbanovicuicite, V. et al. (2008) Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE.

J. Exp. Med.

77


J. Am. Soc. Nephrol.

78


Springer Semin.

79


J. Exp. Med.

80


Blood

81


Nature

82

Takaoka, A. et al. (2007) DAI (DLN-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response.

Nature

83


84

Roberts, T.L. et al. (2009) HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA.

Science

85


86


87

Botto, M. et al. (1998) Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies.

Nat. Genet.

88


J. Exp. Med.

89

Reis, E.S. et al. (2007) Complement components, regulators and receptors are produced by human monocyte-derived dendritic cells.

Immunobiology

90


Blood

91

56
promoter determines CREB binding and activity. J. Immunol. 182, 1500–1508


120 Garaud, S. et al. (2009) IL-6 modulates CD5 expression in B cells from patients with lupus by regulating DNA methylation. J. Immunol. 182, 5623–5632


136 Tesi, C.Y. et al. (2000) Increased excretions of beta2-microglobulin, IL-6, and IL-8 and decreased excretion of Tamm–Horsfall glycoprotein in urine of patients with active lupus nephritis. Nephron 85, 207–214


