Review

Developments in the scientific understanding of lupus

Stacy P Ardoin¹ and David S Pisetsky²

¹Departments of Pediatrics and Medicine, Duke University Medical Center, 2301 Erwin Road, Durham, NC 27710, USA
²Medical Research Service, Durham VA Hospital, Durham, North Carolina, 508 Fulton Street, Durham, NC 27705, USA

Corresponding author: Stacy Payne Ardoin, stacy.ardoin@duke.edu

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Abstract

Systemic lupus erythematosus is a systemic autoimmune disease characterized by the production of antinuclear antibodies (ANAs). Recent research into human and murine lupus suggests that disease susceptibility results from genetic polymorphisms regulating immune responses as well as impairing the clearance of apoptotic cells. Because the products of dead cells, including nucleic acids, have immunologic activity, this situation can promote antigen-driven ANA responses. Furthermore, immune complexes of ANAs can drive the production of proinflammatory cytokines, inducing the "interferon signature", and intensifying disease. Together, these findings point to new genetic and immunologic markers of disease as well as targets for new therapies.

Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that is characterized by the production of antibodies to nuclear molecules in association with clinical manifestations of fluctuating intensity and severity. This disease primarily affects young women and occurs with variable frequency in racial and ethnic groups. Furthermore, although SLE has a strong genetic component, its occurrence is sporadic in families and concordance is incomplete, even among identical twins. Together, these observations have suggested that the etiology of SLE has genetic and environmental components, with female sex strongly influencing pathogenesis.

Consistent with the systemic nature of SLE, the clinical manifestations of this disease are diverse, with the skin, joints, kidneys, nervous system, serosal surfaces, and blood elements prominently involved. These manifestations occur to a variable extent in the individual patient and their activity can change over time. Although lupus is classically a disease of flares, in some patients sustained remission can occur after an initial phase of activity; in other patients the disease is more sustained. The challenge in understanding SLE is therefore to explain the heterogeneity in disease course and to develop a model of pathogenesis that encompasses disparate clinical events.

During the past decade studies of the immune system in patients and animal models have provided important new insights into underlying disease mechanisms and have led to an encompassing model of pathogenesis in which antinuclear antibodies (ANAs) play a central role in promoting immune dysregulation and tissue injury. This model (Figure 1) incorporates an aberrant immune response to cell death in lupus, with immune complexes comprised of ANAs and the products of dead cells activating the innate immune system and driving inflammation and autoantibody production. This review considers new data on pathogenesis and highlights opportunities to develop new therapies.

Etiology of systemic lupus erythematosus

Genetic analysis of SLE has advanced impressively, reflecting the powerful analytic tools created by the Human Genome Project. Importantly, a combination of genome-wide scanning, family studies, and candidate gene approaches has led to identification of a series of genes that determine either susceptibility to disease or its severity (Table 1). Although it is likely that many more genes contribute to pathogenesis, the nature of genes thus far identified suggests that patients with SLE have an immune system predisposed to aberrant responsiveness. These patients may also have genetic variants that may affect the interactions among immune cells to enhance inflammation or promote vascular damage [1,2].

The study of human lupus has been complemented by a detailed analysis of the genetics of murine lupus. Through large and detailed breeding studies, investigators have dissected the gene loci that contribute to disease in mice of several strain backgrounds. These studies indicated clearly

ANA = antinuclear antibody; BlyS = B-lymphocyte stimulator; DAMP = death/damage-associated molecular pattern; HMGB1 = high mobility group protein B1; IFN = interferon; NPSLE = neuropsychiatric systemic lupus erythematosus; PAMP = pathogen-associated molecular pattern; RAGE = receptor for advanced glycation end-products; SLE = systemic lupus erythematosus; TLR = Toll-like receptor; Treg = T-regulatory (cell).
that, in inbred mice, disease is multigenic and loci can promote as well as retard disease. Furthermore, whereas a single gene locus may, for example, disturb B-cell activation, additional gene or genes must be present for a full-blown autoimmune syndrome. Another finding to emerge from this analysis concerns the linkage, in the same chromosomal location, of more than one susceptibility gene [3,4].

In addition to the role played by genetic polymorphisms in disease susceptibility, epigenetic modifications to DNA may influence risk. Such epigenetic factors include DNA methylation and post-translational modifications of histones, which can be either inherited or environmentally modified. Recent studies have indicated global hypomethylation in the T cells of patients with SLE. Furthermore, in mice drugs such as procainamide and hydralazine can promote lymphocyte hypomethylation to induce lupus [5].

Although these genetic and epigenetic factors may promote susceptibility to SLE, environmental influences probably trigger the start of autoimmunity. Among these infections, Epstein-Barr virus may promote lupus, given its extensive immune effects. Furthermore, constituent proteins of the virus resemble self-antigens and may, in genetically predisposed individuals, drive autoantibody responses by molecular mimicry [6].

Serological abnormalities in systemic lupus erythematosus

The production of antibodies to the cell nucleus (ANAs) is the serologic hallmark of SLE. Of these antibodies, anti-DNA antibodies serve as markers for diagnosis and prognosis and occur in both patients and animal models of SLE. Indeed, in animals, anti-DNA expression is the defining immunologic feature of this disease. In addition to their expression of anti-DNA, patients with SLE express other ANAs in a pattern that has been characterized as linkage. Thus, anti-DNA antibodies occur in association with antibodies to histones as well as histone-DNA complexes that comprise the nucleosome. Similarly, antibodies to Sm and RNP occur together frequently. Sm and RNP are ribonucleoprotein complexes that reside in the cell nucleus and mediate RNA processing [7,8].

Although both anti-DNA and anti-Sm are serologic criteria for classification, the expression of antibodies to nucleosomes and antibodies to RNP and Sm are independent. Whereas levels of anti-DNA vary with disease activity, anti-Sm and anti-RNP exhibit much less variation over time and have not been clearly associated with disease activity or response to therapy. The independence of these responses implies the existence of more than one pathway for autoreactivity as well as sources of autoantigen to drive autoantibody production [8]. Furthermore, in patients with SLE, autoantibody expression can predate clinical disease manifestations by many years, suggesting that for full-blown disease to develop other events must supervene to translate serologic abnormalities into active autoimmunity [9].

The generation of autoantibodies

A major question in the pathogenesis of SLE concerns the basis for autoantibody specificity. Whereas ANA production...
is common to many rheumatic diseases, the targeting of nucleic acids is a striking feature of autoimmunity in SLE. Recent research has identified possible explanations for this targeting that converge on the ability of certain self-molecules to stimulate immune responses, a concept known as danger. Stated simply, danger represents an immunologic challenge that activates the innate immune system and stimulates host defense. In the susceptible person, danger may also trigger autoimmunity.

Danger can arise from both exogenous and endogenous sources. Exogenous sources include foreign molecules known as pathogen-associated molecular patterns (PAMPs) such as endotoxin (lipopolysaccharide) and bacterial, viral, and fungal molecules. The endogenous danger molecules are called death (or damage)-associated molecular patterns (DAMPs). DAMP can arise during tissue injury or death and are self-molecules that acquire immunologic activity when they are either degraded or released from their normal intracellular location [10].

Among PAMPs and DAMPs, DNA and RNA exhibit important immunological activity. Double-stranded RNA from viruses can stimulate Toll-like receptor (TLR)3; single-stranded RNA can stimulate TLR7; and DNA from bacterial sources enriched with CpG motifs (so-called CpG DNA) can stimulate TLR9. Furthermore, although mammalian DNA itself may be immunologically inactive (because of a paucity of CpG motifs), it can nevertheless stimulate cells when it is introduced into the cytoplasm by alternative pathways such as transfection or DNA-binding proteins [11,12]. Within the context of SLE, these findings suggest that molecules inducing autoimmunity have intrinsic immunologic activity and may serve as adjuvants for their own responses as well those to molecules to which they are attached [13].

A second explanation for the targeting of nuclear molecules in SLE relates to an increase in the exposure of the immune system in lupus to ‘dangerous’ products. This increase could result from either an increase in the amount of cell death or a failure to clear the products of dead and dying cells. In the simplest conceptualization, cells can die by apoptosis or necrosis. Apoptosis is a form of programmed cell death in which macromolecules are degraded or translocated by enzyme cascades. Among these changes is the migration of nuclear antigens into surface blebs. In contrast, necrosis is an immediate or accidental form of cell death that is mediated by physical or chemical trauma that culminates in the extracellular dispersal of the contents. Importantly, many cellular and humoral systems mediate the clearance of apoptotic cells, presumably to prevent transition to secondary necrosis, which appears to be a much more proinflammatory or immunogenic state [14].

Measurement of the extent of in vivo apoptosis is difficult because of uncertainty in sampling, although it is likely that patients with SLE have increased apoptosis of peripheral blood lymphocytes. In contrast, there is strong evidence from

### Table 1

| Candidate gene | Chromosomal location | Proposed function |
|---------------|----------------------|-------------------|
| PTPN22        | 1p13                 | T cell activation |
| FCGR-2A, FCGR-2B, FCGR-3A, FCGR-3B | 1q23-25             | Fc receptors; clearance of immune complexes |
| TNF-3        | 1p36                 | TNFα expression   |
| STAT-4        | 2q32                 | T cell cytokine production and macrophage response to IFN-α |
| CTLA-4        | 2q33                 | T cell activation |
| PDCD-1        | 2q37                 | Lymphocyte differentiation |
| PXK           | 3p14                 | Unknown           |
| HLA-DR2, HLA-DR3 | 6p11-p21           | Antigen presentation |
| IRF-5         | 7q32                 | Expression of IFN-α |
| BLK-C8or13    | 8p23                 | B cell development and function |
| MBL-2         | 10q11                | Antigen presentation and immune complex clearance |
| KIAA1542      | 11p15                | Interferon alpha expression? |
| ITGAM         | 16p11                | Adherence of neutrophils and monocytes to endothelium |

BLK = B lymphocyte tyrosine kinase; CTLA = cytotoxic T-lymphocyte associated; FCGR = Fc gamma receptor; HLA = human leukocyte antigen; IFN = interferon; IRF = interferon regulatory factor; ITGAM = integrin alpha(M); MBL = mannose binding lectin; PDCD = programmed cell death; PTPN = protein tyrosine phosphatase nonreceptor; SLE = systemic lupus erythematosus; STAT = signal transducer and activator of transcription; TNF = tumor necrosis factor; TNFSF = tumor necrosis factor ligand superfamily.
both patients as well as animal models for aberrant clearance of dead cells. Genetic deficiency of C1q, for example, is highly associated with SLE. Because complement can promote removal of dead cells, a deficiency in this system can allow the accumulation of dead cells to drive the innate immune system and serve as immunogen to induce ANAs. Of note, blebs can also bind complement, with a complement deficiency allowing these structures to escape into the periphery to induce responses as well as promote immune system and vascular changes. Similar considerations pertain to the role played by other proteins such as C-reactive protein and IgM, where deficiency may lead to impaired clearance and enhanced autoreactivity [15].

Taken together, these considerations suggest that the induction of ANAs results from aberrant production or accumulation of danger molecules from dead cells, with the changes in these molecules during apoptosis enhancing immunogenicity. Furthermore, because cell death probably leads to the release of other immune mediators known as alarmins, the immune environment is replete with danger molecules that can promote immune hyperactivity and autoreactivity.

**Immunological abnormalities**

In the pathogenesis of SLE, an increase in the amount of self-antigen may not be sufficient to drive autoimmunity. Rather, intrinsic abnormalities in cells of the adaptive immune system (for example, B cells, T cells, and dendritic cells) may act synergistically to induce a mature, antigen-driven response. As shown in studies on both patients as well as animal models, SLE is associated with functional disturbances that involve the entirety of the immune system. Some of these may be genetically determined, whereas others arise secondarily in response to events such as infection. Not surprisingly, delineation of these disturbances has evolved with the development of new analytic approaches to elucidate the immune cell function and the downstream signaling pathways engaged during activation.

In peripheral blood of patients, both the B-cell and T-cell compartments exhibit functional abnormalities that could lead to the autoantibody production. Thus, among B-cell precursor populations, there is a striking shift toward autoreactivity as indicated by the binding specificity of antibody products. This shift, which could predispose to ANA generation, reflects impairment in B-cell tolerance. With a preimmune repertoire filled with autoreactive precursors, drive by autoantigen may more readily elicit a specific response [16].

Analysis of B-cell populations during disease also reveals distinctive abnormalities, including a prominent increase in plasma cells during active disease. These cells can be enumerated by flow cytometry on the basis of their expression of high levels of CD27. These changes are dynamic, however, and can respond to immunosuppressive therapy [17,18]. Although the peripheral blood has been studied in detail, few studies have characterized other B-cell compartments. Of note, an analysis of germinal centers in the tonsils of normal patients and patients with SLE revealed marked differences in the expression of an idiotypic marker that is ordinarily not expressed during tolerance induction [19]. Among influences that affect B-cell activation or differentiation, cytokines such as B-lymphocyte stimulator (BlyS) may promote these functional and phenotypic changes [20].

As shown in studies of patients as well as animal models, T cells in SLE exhibit abundant functional and phenotypic abnormalities, with the role of T-helper cells in disease suggested by the effectiveness of anti-T-cell approaches (for instance, antibodies as well as genetic knockouts) in animal models. In patients, these abnormalities can be defined by analysis of cell phenotype as well as signal transduction pathways. Thus, SLE patients exhibit evidence of increased numbers of memory T cells as well as decreases in the number or function of T-regulatory (Treg) cells. Among the cells with the highest level of CD25 expression (a marker for Treg cells) in vitro function is reduced, although this level can be restored by activation, implying that a dynamic process is at work [21,22]. Interactions of Treg cells with IFN-producing antigen-presenting cells may also impair their function [23].

An important issue concerning the role of T-helper cells for autoantibody production relates to their antigen specificity. Among antigens targeted, DNA and RNA, in their ‘naked’ form, do not appear able to bind to the T-cell receptor. Rather, in SLE, T-cell help for anti-DNA and other anti-nuclear responses may result from recognition of nucleosomes, with histone peptides serving as major autoepitopes to activate T cells and provide help for autoantibody production [24]. Because nucleosomes can arise during nuclear breakdown in apoptosis, cell death may also directly impact on T-cell autoreactivity. The induction of autoreactive T cells may be promoted during disease, because - at the molecular level - SLE T cells exhibit evidence of ‘rewiring’ and increased activation of the T-cell receptor transduction system [24,25].

**Cytokine disturbances in systemic lupus erythematosus: the role played by immune complexes**

Microarray and other molecular approaches have provided a new dimension to the analysis of immune cell function in SLE and provided dramatic evidence for cytokine disturbance. Thus, as shown by studies conducted by several investigators, peripheral blood mononuclear cells from patients with SLE exhibit patterns of gene expression consistent with in vivo stimulation by type 1 IFN. Although not all patients have this ‘interferon signature’, it nevertheless represents clear evidence of the effects of cytokines on the immune system in SLE [26-28]. The potential effects of IFN in lupus are widespread, because overproduction of this cytokine can promote expression of proinflammatory cytokines and chemokines, maturation of monocytes into dendritic cells,
activation of autoreactive B and T cells, production of autoantibodies, and loss of self-tolerance. Furthermore, IFN may adversely affect the vasculature by inducing endothelial dysfunction and depleting endothelial progenitor cells for repair. Studies conducted in animals support the critical role of IFN, because lupus mice that are deficient in type I IFN receptors have significantly reduced disease expression [29].

Although lupus nephritis has long been conceptualized as a classic immune complex disease, studies conducted in both human and murine systems have revolutionized the concept of immune complexes and have demonstrated convincingly that immune complexes can promote aberrant cytokine production, serving as potent inducers of IFN-α. Thus, as shown originally in in vitro culture systems, the blood of SLE patients contains a factor that can induce the production of IFN-α by IFN-producing cells, also called plasmacytoid dendritic cells. Original studies indicated that this factor represents immune complexes comprised of DNA and anti-DNA. Subsequent studies indicate that complexes can be assembled by mixing patient sera with the media from apoptotic cells and that antibodies to RNA binding proteins could also form immunostimulatory complexes [30,31].

The stimulation of plasmacytoid dendritic cells by immune complexes involves both TLR and non-TLR receptors, which probably respond to the nucleic acid components of the complexes. Because complexes may promote uptake into cells, the nucleic acid component may have access to other internal nucleic acid sensors, thereby eliminating the requirement for CpG motifs. In addition to the role played by pattern recognition receptors, stimulation of IFN production by complexes involves the Fc receptors as well as RAGE (receptor for advanced glycation end-products). The role played by RAGE reflects the presence in the complexes of high mobility group protein B1 (HMGB1). HMGB1, a nonhistone nuclear protein, is a prototypic alarmin that is released from apoptotic as well as necrotic cells. Because HMGB1 binds to chromatin in the cell, its presence in the complexes probably results from the release during cell death of chromatin with its attached proteins [32-34].

Consistent with a role for nucleic acids in inducing IFN via TLRs, inhibitory oligonucleotides can block the progression of SLE in animal models [35,36]. The situation with respect to effects of TLR knockouts is more complicated. Thus, in a study of disease in autoimmune MRL/Mp-Jpr/Jpr mice, although a TLR7 knockout had reduced disease severity, a TLR9 knockout had accelerated nephritis and increased mortality. Furthermore, the effects of the knockouts on various autoantibody responses differ, with TLR9 knockout mice exhibiting reduced anti-nucleosome responses and TLR7 knockout mice showing reduced anti-Sm responses. These findings indicate that the effects of activation via different TLRs may differ, with the effects on IFN also varying depending upon the TLR pathway stimulated [37].

Whatever the mechanism by which the immune complexes stimulate responses, their formation requires the availability of nuclear antigens in the extracellular milieu where antibody binding may occur. Because media from apoptotic cells can substitute for pure DNA in in vitro systems, cell death is the probable setting for the release of nuclear material for complex formation. The manner in which DNA and RNA leave the cell has not been extensively investigated, although it appears that both may be extruded from the cell during apoptosis, albeit by separate mechanisms [38]. The conditions for the conditions in which DNA and RNA exit the cell may account for the differences in the pattern of autoantibody product noted above.

**Mechanism of organ damage in systemic lupus erythematosus**

Although the immune dysregulation inherent to SLE can cause damage in nearly any organ system, the kidneys, central nervous system, and endothelium remain major sources of morbidity and mortality and have been studied intensively over the past decade.

**Kidney**

Lupus nephritis results from glomerular deposition of immunoglobulins, which in turn activate complement and promote inflammation. As in the case of cytokine production, anti-DNA antibodies play an important role in nephritis, with pathogenicity resulting from either glomerular deposition of immune complexes with nucleosomes or cross-reactive binding with proteins (possibly α-actinin) in the glomerular basement membrane. Although elevated anti-DNA levels may predict lupus nephritis, not all SLE patients with circulating anti-DNA antibodies exhibit this manifestation. These findings suggest that only certain anti-DNA antibodies are nephritogenic or that the presence of immune complexes, even when deposited in the kidney, may not be sufficient to provoke glomerular injury.

As demonstrated most clearly in studies of mice, in addition to immune complex formation, other mechanisms influence immune cell recruitment to inflamed renal tissue. Thus, mice that are deficient in the γ chain of the Fc receptor are protected from the development of nephritis, despite the presence of immune complex deposition and complement activation. T cells may also be involved in this manifestation, because, in mice, depletion of CD4+ cells and antagonism of CD28/B7, CD40/CD40 ligand, and ICAM-1/LFA (intercellular adhesion molecule-1/lymphocyte function-associated antigen) co-stimulation attenuates nephritis [39].

In renal biopsies from SLE patients with class III and IV glomerulonephritis, CD8+ T cells predominate in the inflammatory infiltrate [40]. Although renal biopsies are informative, their performance carries risk and repeat biopsies are difficult. The urine itself may provide a new source of material for assessing mechanisms of nephritis as well as clinical disease activity. Thus, urine of patients with active
The central nervous system
Neuropsychiatric SLE (NPSLE) is a clinical category that comprises a multitude of syndromes whose mechanisms probably vary significantly. At least some of these manifestations, however, may result from the direct effects of antibodies. Although a wide array of autoantibodies has been described in the serum and cerebrospinal fluid of individuals with NPSLE, studies in both human and murine lupus highlight the potential role played by antibodies to the N-methyl-D-aspartate receptors NR2a and NR2b in the cognitive dysfunction in SLE. These antibodies represent a subset of antibodies to double-stranded DNA that cross-react with the extracellular domain of NR2 receptors. These receptors occur throughout the brain and are key to learning, memory, and pathogenesis of psychosis [42].

As shown in murine models, anti-NR2 glutamate receptor antibodies can induce a noninflammatory, neurotoxic effect on neurons, particularly in the hippocampus, resulting in cognitive impairment. Importantly, disruption of the blood-brain barrier is necessary for this effect. Despite the clarity of the murine models, studies in SLE patients have yielded more mixed results, with only some showing correlations between the presence of anti-NR2 antibody and cognitive impairment. Because most of these clinical studies have assessed serum and not cerebrospinal fluid levels of the anti-NR2 antibodies, it is uncertain whether in patients a breach in the blood-brain barrier (a crucial factor in the animal models) has occurred to allow antibody penetration into the brain [43].

Among other autoantibodies, antiphospholipid antibodies promote the pathogenesis of focal ischemic disease in SLE and may also mediate more diffuse cognitive impairment [43]. More controversial in the etiology of NPSLE is the role played by anti-ribosomal P antibodies, which target three different ribosomal proteins. These antibodies were originally described in conjunction with psychosis and depression in SLE, but more recent reports have provided less clear associations [44]. Of interest, it has been demonstrated in a murine model that the intracerebral administration of human anti-ribosomal P can induce depressive behavior, with staining of antibody to various neuronal populations [45].

In addition to autoantibodies, cytokines and chemokines probably contribute to the pathogenesis of NPSLE and cognitive dysfunction. Among these mediators, interleukin-6, interleukin-8, CCL5 (C-C chemokine ligand 5, or RANTES), CX3CL1 (C-X3-C chemokine ligand 1, or fractalkine), monocyte chemotactic protein-1, and CXCL9 (C-X-C chemokine ligand 9, or MIG) are increased in the cerebrospinal fluid of patients with active NPSLE and may mediate events that promote neuronal damage or dysfunction [46,47].

Vasculature
Complications of SLE include vasculitis and atherosclerosis, reflecting the major impact of the immune system on the endothelium. In the atherosclerosis associated with SLE, traditional cardiovascular risk factors and medications do not fully account for the strikingly increased risk of atherosclerosis seen in premenopausal women with SLE. These findings suggest that features of the disease itself drive this process. Even in the absence of clinical atherosclerosis and overt disease activity, patients with SLE show evidence of impaired endothelial function [48].

Several distinct mechanisms probably promote endothelial injury on SLE. Thus, endothelial damage may result from immunologic factors that include immune complex deposition, complement activation, and direct cell-mediated cytotoxicity to the endothelium. In addition, antibodies to phospholipids, endothelial cells, and oxidized low-density lipoprotein may exert pathogenic effects. Acting together, these mechanisms may increase endothelial cell apoptosis, diminish production of endothelial-derived nitric oxide, and increase endothelial exposure of procoagulant tissue factor and phosphatidylserine. In addition, enhanced IFN levels may increase endothelial cell apoptosis and promote abnormal vasculogenesis. In the face of these insults, the endothelium of SLE patients may have limited capacity for repair, because monocyte (CD14+) and hematopoietic stem-cell derived (CD34+ and CD133+) endothelial progenitor cells, usually recruited to restore damaged endothelium, are diminished in number and function in SLE [49,50].

Conclusion
Recent discoveries concerning immune abnormalities in SLE have provided the scientific basis for more targeted treatment that may interdict key steps in the pathogenesis. Agents currently under trial or for which trials are planned based on promising results in animal models include anti-B-cell therapy (anti-CD20 and anti-CD22); CTLA-4Ig (cytotoxic T-lymphocyte associated antigen 4/immunoglobulin), which impairs T cell co-stimulation; anti-cytokine approaches directed
against BlyS, interleukin-10, tumor necrosis factor-α, and IFN-α; and TLR inhibition [51]. In addition to exploring novel therapies in SLE, recent research efforts have provided insights into the action of older agents such as hydroxychloroquine, which may be immunomodulatory because of effects on TLR9 signaling [52]. Coupled with potential new markers (for example, IFN signature and fluorescence-activated cell sorting analysis of B-cell populations), the new era of trials in SLE should refine our understanding of disease pathogenesis and hopefully provide a new generation of more effective and less toxic targeted therapies.

**Competing interests**

The authors declare that they have no competing interests.

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