Review

Developments in the clinical understanding of lupus

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Abstract

Advances in genetics and new understanding of the molecular pathways that mediate innate and adaptive immune system activation, along with renewed focus on the role of the complement system as a mediator of inflammation, have stimulated elaboration of a scheme that might explain key mechanisms in the pathogenesis of systemic lupus erythematosus. Clinical observations identifying important comorbidities in patients with lupus have been a recent focus of research linking immune mechanisms with clinical manifestations of disease. While these advances have identified rational and promising targets for therapy, so far the therapeutic trials of new biologic agents have not met their potential. Nonetheless, progress in understanding the underlying immunopathogenesis of lupus and its impact on clinical disease has accelerated the pace of clinical research to improve the outcomes of patients with systemic lupus erythematosus.

Introduction

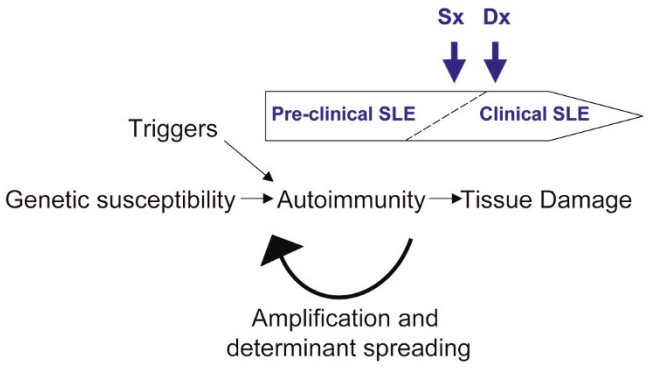
Systemic lupus erythematosus (SLE) is often considered the prototype systemic autoimmune disease, as virtually all components of the immune system contribute to the characteristic autoimmunity and tissue pathology. The utility of lupus research extends beyond defining lupus-specific mechanisms, as the disease can serve as a model system for consideration of immune system responses to microbial infection and control of hematologic malignancies. Especially in recent years, as new concepts have evolved to explain mechanisms that link the nucleic acid targets of lupus autoantibodies to immune system activation and inflammation, the intellectual rewards of research on this most complex medical syndrome have grown. Yet this is a disease with high impact on patients, particularly women in the reproductive years. The satisfaction derived from new understanding of disease mechanisms will only be fully realized when those insights are translated into new therapies. Despite some frustrations in efforts to develop new lupus drugs, clinical care of lupus patients continues to improve, and the scope of clinical research in search of new lupus therapies has significantly expanded to include both traditional and new biologic agents.

The etiopathogenesis of lupus comprises genetic contributions, environmental triggers, and stochastic events, as demonstrated in murine models in the late 1980s [1]. These factors play out at the level of the immune system, with multiple genetic hits and an undefined complement of exogenous or endogenous triggers required for initiation of autoimmunity. When the genetic load is sufficient, immune triggers are available and chance favors effective immune system activation, the disease process can move forward [2] (Figure 1). A concept that has been developed in recent years considers the kinetics of the disease, with lupus autoantibodies present in serum of lupus patients up to 5 years prior to the development of clinical manifestations of disease [3]. It is notable that autoimmunity, when considered in a population of lupus patients, develops in a stereotypical manner, with anti-Ro and anti-La antibodies, common to several systemic autoimmune diseases, developing early in the pre-clinical stage of disease, while anti-Sm and anti-RNP antibodies, those that are more specific for SLE, developing very close to the time that disease becomes clinically apparent.

It is now recognized that autoantibodies and their associated nucleic acids can play an amplifying role in immune system activation, most likely through stimulation of innate immune pathways. Insights into the genetic variations that are associated with lupus, along with this new awareness of how autoimmunity, immune dysfunction, and tissue damage develop over time, are providing a more complete picture of disease risk, the steps in pathogenesis, and most significantly, new therapeutic targets.

ACR = American College of Rheumatology; BLyS = B lymphocyte stimulator; dsDNA = double-stranded DNA; FDA = Food and Drug Administration; GWAS = genome-wide association study; HMGB1 = high mobility group box 1; ICOS = inducible costimulator; IFN = interferon; IL = interleukin; MMF = mycophenolate mofetil; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; TLR = Toll-like receptor; TNF = tumor necrosis factor.

Figure 1



Stages of lupus pathogenesis. Genetic factors and environmental triggers, whether exogenous or endogenous, along with stochastic events, act on the immune system to initiate autoimmunity. Autoantibodies and their antigens, cytokines and chemokines amplify immune system activation and generate tissue damage. Autoantibody production occurs years prior to the development of clinical signs and symptoms of systemic lupus erythematosus (SLE). Organ damage has likely occurred by the time lupus is diagnosed. Sx, symptoms; Dx, diagnosis.

New concepts in lupus pathogenesis

Genetics

Two types of genetic variants associated with a diagnosis of SLE, common single nucleotide variants and rare genetic mutations, are stimulating studies of functional alterations in molecular pathways important in lupus pathogenesis. A third type of genetic variant, copy number variation, has been observed in a murine model of lupus, the BXSB mouse, where a duplication of a region of the X chromosome containing the Toll-like receptor (TLR) 7 gene (*TLR7*) is associated with increased type I IFN production, macrophage activation, autoantibody production and poor survival [4-6].

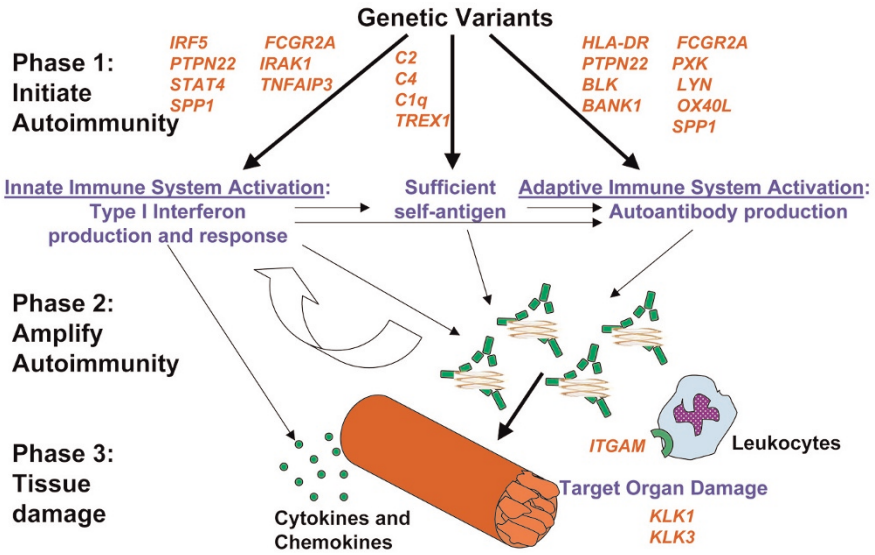
Establishment of large collections of DNA samples from lupus patients and controls, along with advances in technology that have made large scale studies of genetic variants more affordable, have led to successful genome-wide association studies (GWAS) supported by government agencies, foundations, industry and academic centers [7-10]. Data from these studies have confirmed several candidate genes previously associated with lupus, identified some new lupus-associated genes and gene loci, and identified variants in a gene (*ITGAM*) whose protein product had been studied in SLE but was not previously known to have a genetic association with lupus [11]. An earlier publication in this series, 'Developments in the Scientific Understanding of Lupus', has listed some of the genes showing a statistical association with a diagnosis of lupus in GWAS [12]. Several, including *PTPN22*, *IRF5*, *STAT4*, *FCGRIIA*, and of course the HLA region, have been previously described prior to the publication of the GWAS data. Some recently identified lupus-associated genetic variants, including *BLK*, *PXK*, and *BANK1*, may modify lymphocyte signaling and provide new insights into molecular pathways relevant to lupus pathogenesis. The protein product of *ITGAM*, also identified as a lupus-associated gene and known as CD11b, Mac1 and complement receptor 3, had not been previously linked to lupus at the genetic level but its expression was known to be increased on neutrophils of active lupus patients and it can mediate adhesion to endothelial cells [11]. In recent months additional lupus associated genes have been described, including *LYN*, a src-tyrosine kinase, *IRAK1*, an IL-1 receptor associated kinase, *TNFAIP3*, which encodes A20, and *OX40L*, a costimulatory molecule [13-16]. *KLK1* and *KLK3*, encoding kallikreins, have been associated with altered protection from anti-glomerular basement membrane disease and lupus nephritis [17].

What is striking about most of these lupus-associated genes is that their function is most likely associated with activation or regulation of the immune response. Based on identification of these genes and their known functions, we can hypothesize a role for activation of the innate immune response through TLRs (*IRF5*, *FCGRIIA*, *TNFAIP3)*, response to cytokines (*STAT4*, *IRAK1*), or lymphocyte activation and regulation (*PTPN22*, *PLK*, *BANK1*, *LYN*, *OX40L*, *SPP1*) [18-22] (Figure 2). In addition, some of these genetic variants might contribute to directing the immune response to target organs and contribute to tissue inflammation and damage (*ITGAM*).

In addition to the GWAS, which identify common genetic variants, old observations of the high risk of SLE in rare patients with C2, C4 and C1q deficiencies have now been supplemented with data from several groups identifying lupus in patients with mutations in a DNase encoded by *TREX1* [23]. Rare mutations in that gene are associated with a lupus-like syndrome characterized by anti-DNA antibodies, high levels of IFN-alpha and neurologic disease and have led to studies of lupus cohorts and detection of occasional *TREX1* mutations. It appears that altered structure or function of the *TREX1*-encoded DNase results in inefficient clearance of intracellular DNA rich in endogenous genomic repeat element sequences and induction of type I IFN [24].

To some extent, data from genetic studies are confirming what we have known - that the immune response underlies lupus pathogenesis [7]. But those studies are also providing some surprises, such as the *TREX1* observation, that will lead to research on previously unsuspected pathways. Clinical insights from genetic data are just beginning to emerge. For example, recent data identify variations in *LYN* that confer protection from hematologic manifestations in a lupus subgroup defined by the presence of certain autoantibodies [13], and the association of IFN-alpha and neurologic manifestations in patients with *TREX1* mutations may lead to greater understanding of the molecular basis of neurologic involvement in patients with SLE. Analysis of the function of

Figure 2



Genetic determinants of lupus pathogenesis. Genome-wide association studies are confirming previous data identifying genetic variants that are statistically associated with systemic lupus erythematosus and are finding new lupus-associated genes. Most lupus-associated genes represent common variants, but several (C2, C4, C1q and *TREX1*) are characterized by rare mutations. We suggest that lupus-associated genes contribute to one or more essential mechanisms that must be implemented to generate lupus susceptibility. Some genetic variants will facilitate innate immune system activation, particularly type I IFN production; other genetic variants will result in increased availability of self-antigen; and other genetic variants will alter the threshold for activation or regulation of cells of the adaptive immune response, resulting in production of autoantibodies. Additional genetic variants might promote inflammation and damage to target organs or fail to protect those organs from proinflammatory mediators. The lupus-associated genetic variants prepare the immune system and target organs to be responsive to exogenous or endogenous triggers. Lupus-associated genes are shown in red.

the lupus-associated genetic variants should provide important insights into pathogenic mechanisms that can be applied to development of highly targeted therapeutics.

Apoptotic cells

Apoptotic cells remain attractive candidates as a source of self-antigens that may initiate and direct the autoimmune response. Longstanding observations have documented the concentration of lupus autoantigens in apoptotic cell blebs [25], and *in vitro* studies have demonstrated stimulation of autoreactive T cells by dendritic cells that have processed autologous apoptotic cell components [26]. Some lupus patients demonstrate increased spontaneous apoptosis or impaired clearance of apoptotic peripheral blood cells [27,28]. Recent data have supported the hypothesis that components of the classical complement pathway are required for phagocytic clearance of apoptotic cells, providing a possible explanation for the high frequency of SLE among the rare individuals with genetic deficiencies of those components, particularly C1q [29]. In addition to C1q, similar molecules with collagen-like structural features, including mannose-binding lectin and ficolin 3, can contribute to uptake of late apoptotic cells by macrophages [30]. The mechanisms that might account for induction of immune dysregulation and autoimmunity by apoptotic cell components are of great interest. Recent data support a role for high mobility group box 1 (HMGB1)-nucleosome complexes derived from apoptotic cells in the induction of pro-inflammatory mediators, dendritic cell maturation, and anti-double-stranded DNA (anti-dsDNA) autoantibodies [31,32].

Innate immune response

Among the autoimmune and rheumatic diseases, studies of SLE have arguably provided the strongest evidence for an essential role of TLRs and the innate immune response in disease pathogenesis [33-35]. The immunomodulatory effects of the HMGB1-nucleosome complexes are apparently mediated by interactions with TLR2 [32]. In addition, several lupus genes encode proteins that mediate or regulate TLR signals and are associated with increased plasma IFN-alpha among patients with particular autoantibodies. Those antibodies could potentially deliver stimulatory nucleic acids to TLR7 or TLR9 in their intracellular compartments [36-40]. Activation of the IFN pathway has been associated with the presence of autoantibodies specific for RNA-associated proteins, and the current literature supports RNA-mediated activation of TLR as an important mechanism contributing to production of IFN-alpha and other proinflammatory cytokines [41]. Activation of the IFN pathway is associated with renal disease and many measures of disease activity [42-45]. Ongoing studies are evaluating the temporal relationship between expression of IFN-inducible genes in peripheral

blood mononuclear cells of SLE patients and disease flares, as measured by conventional tools such as the British Isles Lupus Assessment Group (BILAG) index or the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). In some patients, increases in IFN-inducible gene expression precede flares in disease activity by several months, suggesting that the increased IFN activity might contribute to increased immune system activity and tissue damage. In view of the wide effects of type I IFN on immune system function, including induction of macrophage differentiation toward a dendritic cell phenotype, increased immunoglobulin class switching and generalized priming of the immune system for increased responsiveness to subsequent stimuli, IFN-alpha represents a rational therapeutic target [35,46].

Adaptive immune response

Activated T and B cells are features of SLE, and many of the genetic variants that are being studied in association with SLE are likely to contribute to immune activation and clinical disease by altering the threshold for lymphocyte activation or modifying the capacity of inhibitors of signaling pathways to appropriately function. Analysis of cell surface molecules on lupus cells has led to descriptions of the phenotype of lymphocytes from patients with increased disease activity. Broad polyclonal activation of T cells is detected by increased or prolonged expression of CD40 ligand, and circulating B cells with a memory cell phenotype are increased in patients [47,48]. The soluble TNF family member B lymphocyte stimulator (BLyS) is increased in serum of many lupus patients and promotes B cell survival and differentiation [49], and interactions between co-stimulatory ligands and receptors on T and B cells, including CD80 and CD86 with CD28, inducible costimulator (ICOS) ligand with ICOS, and CD40 ligand with CD40, contribute to B cell differentiation to antibody producing plasma cells [48]. The autoantibodies produced as a result of these T and B cell interactions may directly contribute to inflammation and tissue damage in target organs but also amplify immune system activation and autoimmunity through their delivery of stimulatory nucleic acids to TLRs, as described above. The contribution of T and B cells in lupus pathogenesis is not restricted to their role in inducing autoantibodies, but likely also includes their production of cytokines and chemokines that shape the immune response and promote tissue damage. The anecdotal reports of excellent therapeutic responses in some patients treated with co-stimulatory molecule blockade or anti-B cells agents, in spite of persistent autoantibody titers, suggest that those additional mechanisms of lymphocyte function are likely contributing to clinical disease [50].

Target organ damage

Effector functions of the immune system, particularly those induced by Fc receptor ligation and complement activation, contribute to tissue damage through complex mechanisms that include induction of reactive oxygen intermediates, recruitment of inflammatory cells, induction of proinflammatory mediators such as TNF, and modulation of the clotting cascade. In fact the complement system, for many years only assessed as a measure of immune complex-mediated activation, is increasingly recognized to play a prominent role in many lupus-associated inflammatory states, including some that do not involve a major role for immune complexes. Antiphospholipid antibodies binding to membranes of the placenta can contribute to complement activation, placental inflammation and fetal loss in a murine system [51,52]. The presence of complement and complement regulatory proteins in association with high density lipoprotein particles suggests that one function of those particles might be to deliver complement regulators to the vasculature where chronic inflammation can take place, possibly modulating atherosclerotic mechanisms [53].

Autoantibody mediated tissue damage has been proposed as a possible mechanism that contributes to central nervous system manifestations of SLE, particularly cognitive dysfunction [54]. Antibodies that react with both DNA and glutamate receptors on neurons are proposed to mediate excitotoxic neuronal cell death. In addition to autoantibodies or immune complexes, cytokines might contribute to central nervous system dysfunction and clinical symptoms. As noted above, high levels of IFN-alpha have been associated with central nervous system disease in patients with TREX1 mutations [23]. In addition, administering recombinant IFN-alpha to patients with hepatitis C infection can lead to depression and cognitive dysfunction, perhaps similar to those manifestations in SLE. In recent studies, immune complexes present in cerebrospinal fluid were shown to provide potent induction of type I IFN in target cells [55]. TNF is another cytokine that is likely to contribute to inflammation and tissue damage. Small studies using TNF antagonist therapy in patients with arthritis or nephritis suggest some efficacy of that approach, although controlled studies are needed [56]. Together, these observations suggest that cytokines, particularly IFN-alpha, may contribute to target organ damage.

While antibodies, immune complexes, cytokines, and products generated by Fc receptor ligation and complement activation likely represent important mediators of tissue damage in SLE, the cells that produce some of those products deserve further study. The properties of macrophages, dendritic cells and lymphocytes that infiltrate the kidney and other target organs might suggest cell surface molecules or components of signaling pathways that could be therapeutically targeted to relieve some of the damage mediated by those cells [57,58]. The strong association of a polymorphism in the *ITGAM* gene raises the possibility that leukocytes expressing the lupus-associated *ITGAM* variant might demonstrate a propensity to adhere more avidly to the local renal vasculature. In addition to augmented inflammatory mechanisms, target organ damage, particularly in the kidney, might be amplified by impaired protective mechanisms.

Recent data demonstrating an association of variants of *KLK1* and *KLK3* with lupus nephritis suggest a possible defect in the protective function of kallikreins in some lupus patients [17].

A summary of current concepts of lupus pathogenesis would include an important role for genetic variants that prime both innate and adaptive immune systems for increased responsiveness to cell activation, increased production of and response to IFN-alpha, increased capacity to generate autoantibodies, and perhaps an increased targeting of inflammatory cells - or decreased protection from the products of those cells - to target organs. As additional genetic data are collected and analyzed, we will gain a better understanding of how lupus susceptibility genes interact and the level of risk conferred by each additional variant. Recent data suggest that the risk of each disease-associated single nucleotide polymorphism in IRF5 and STAT4 confers additive risk of disease [59]. While the manner in which environmental triggers interact with genetic risk remains to be understood [60], we have already gained substantial insights into the major pathways used by the immune system to initiate and amplify immune system activation and inflammation. The new information regarding candidate protective mechanisms in target organs should stimulate new attention to the response of tissue to the insults delivered by the immune system and might suggest very novel and as yet unexplored approaches to organ protection or repair.

Recent focus on comorbidties

The characteristic clinical features of SLE, including those included in the American College of Rheumatology (ACR) classification criteria, tend to be the focus of patient management and therapy. But the past 10 years have witnessed increased attention to comorbidities that have substantial impact on patient outcomes and quality of life. These comorbidities, beyond their impact on patients and their medical management, have provided opportunities for novel research observations with impact beyond SLE. Three comorbidities that are associated with, but not exclusive to, SLE will be briefly discussed: accelerated atherosclerosis, antiphospholipid syndrome, and fetal loss.

Accelerated atherosclerosis

With the description of increased occurrence of myocardial infarction by Urowitz in 1976 [61] and the ready availability of tools, such as carotid ultrasound, to detect preclinical atherosclerotic lesions, the rheumatology community is now well aware of the additional risk of accelerated atherosclerosis conferred by lupus beyond that attributable to traditional cardiovascular risk factors [61-64]. Studies from Manzi and colleagues [63], Roman and colleagues [64], and others have documented the high prevalence of premature atherosclerosis in SLE patients compared to control populations without lupus, with Roman and colleagues' study demonstrating carotid plaque in 37% of SLE patients compared to 15% of age, race, gender and hypertension-matched control subjects. In follow-up studies 28% of those SLE patients developed new or more extensive plaque over approximately 3 years, with plaque progression associated with increased homocysteine levels [65]. In addition to plaque, radial applanation tonometry was used to show that SLE patients also demonstrate increased vascular stiffness that was associated with duration of disease, cholesterol, and serum IL-6 and C-reactive protein levels [66].

In addition to the data pointing to pro-inflammatory cytokines and homocysteine as possible mediators in the development of cardiovascular disease, data from several groups have linked IFN-alpha to decreased availability of endothelial precursor cells and impaired endothelial function [67,68]. Even when SLE patients and controls have a similar degree of atherosclerotic plaque, the SLE patients show increased endothelial dysfunction, as measured by flow-mediated dilatation [69]. In that study endothelial dysfunction was associated with disease activity. A role for type I IFN in the premature atherosclerosis of lupus patients is an attractive concept in light of the growing literature implicating this cytokine in many aspects of altered immune function in SLE. But investigation of mechanisms that provide a functional link between homocysteine and arterial stiffness might be another fruitful research direction. At this time, it is advisable to be vigilant in addressing traditional cardiovascular risk factors in management of lupus patients. Additional translational and clinical studies will be needed to better define the mechanisms that account for the added risk experienced by lupus patients beyond that in the general population.

Catastrophic antiphospholipid syndrome

The facilitated communication and collaboration presented by the internet has been utilized by rheumatologists to gain new knowledge about a significant cause of morbidity and mortality among lupus patients: the catastrophic antiphospholipid syndrome [70]. A website was established by the European Forum on Antiphospholipid Antibodies that provides a site for collection and analysis of clinical data on those patients, whether associated with a diagnosis of SLE or not [71-73]. This severe but rare clinical syndrome, seen in perhaps 1% of patients with antiphospholipid syndrome, is associated with SLE in approximately half the cases [74,75]. The clinical manifestations can appear suddenly, often precipitated by an infection or tissue trauma such as surgery. Occlusion of small or large vessels with thrombi can result in renal disease, cerebrovascular thrombosis, gastrointestinal or pancreas involvement, acute respiratory distress syndrome, severe thrombocytopenia, peripheral gangrene, and other manifestations. An analysis of 280 patients enrolled in the registry documented a mortality rate of 44% [75]. Treatment with anticoagulation, steroids, and plasma exchange or intravenous gamma globulin resulted in the best survival (63%). Ongoing studies are investigating anti-B cell therapy in this dramatic syndrome. While the mechanisms by which a

precipitating event and antiphospholipid antibodies might induce the multisystem failure seen in these patients are not understood, the system established by this investigator group provides novel opportunities to share observations, compare results, and organize patient data to gain improved knowledge of a clinical syndrome with very high mortality.

Fetal loss

Antiphospholipid antibodies have also been implicated in pregnancy complications in lupus patients, including fetal loss. Data from studies of the effect of those antibodies in murine models established a contribution of complement activation to the placental inflammation, TNF production, neutrophil accumulation and fetal death that mimics the events that sometimes occur in lupus patients with antiphospholipid antibodies [51,52]. Those antibodies are rapidly adsorbed onto the membranes of placental trophoblast cells and trigger activation of the complement system. One of the interesting observations from these studies that impacts our understanding of current therapeutic approaches, while not substantially changing them, is that heparin, commonly used to prevent fetal loss in patients with previous losses, may be beneficial by virtue of its inhibition of the complement system rather than its anticoagulant effects [76].

Nephritis in systemic lupus erythematosus

Nephritis remains the most significant major organ system manifestation of SLE and continues to be a therapeutic challenge. In 2004 a revision of the pathologic classification of lupus nephritis sponsored by the International Society of Nephrology and the Renal Pathology Society was published, and in 2009 a beautifully illustrated discussion of this classification was presented [77]. The revised classification devotes special attention to qualitative as well as quantitative morphologic data and distinguishes segmental (involving less than half of a glomerular tuft) from global disease. The classification also notes the presence of tubulointerstitial components and vascular lesions. Tubulointerstitial inflammation often accompanies proliferative glomerulonephritis, with T cells, plasma cells and macrophages prominent in the infiltrate [57,58]. Focal tubulitis can be present in active disease, and tubular atrophy and interstitial fibrosis characterize chronic renal disease, contributing to impaired renal function. The degree of tubular atrophy and interstitial fibrosis can be useful in predicting time to dialysis in lupus nephritis. A morphometric measure of chronic renal damage, based on image analysis and an index of chronic damage as a proportion of cortical area, was developed and was a strong indicator of risk of progression to renal failure [78]. The poor prognosis associated with renal damage was also shown in data from the LUMINA study, describing lupus patients of African-American, Hispanic or Caucasian ethnicity [79]. The renal domain of the Systemic Lupus International Collaborating Clinics (SLICC) damage index was independently associated with a shorter time to death when poverty was excluded from a multivariate analysis.

Vascular lesions are another important component of lupus nephritis that deserve more investigation. In addition to immune complex-mediated vasculopathy, thrombotic microangiopathy and occasionally necrotizing vasculitis of intrarenal arterioles and small arteries can occur [77]. Endothelial damage may be a common mechanism when vascular damage is present, although diverse mediators can be responsible for that damage, including antiphospholipid antibodies. As renal thrombotic microangiopathy can occur even in the absence of glomerular immune complexes and can be associated with hypertension and renal fibrosis, its mechanisms deserve further study. A recent report implicates activation of the classical complement pathway in this setting, with a strong relationship between glomerular deposition of C4d and presence of microthrombi [80].

Old treatments for systemic lupus erythematosus

The advances in basic science related to the TLR family have stimulated new concepts of lupus pathogenesis. They have also provided a possible mechanistic basis for the broad and generally effective use of antimalarial therapy in SLE. Choroquine and hydroxychloroquine are weak bases and gain access to late endosomal vesicles where they can raise the pH. *In vitro* studies have documented the capacity of these agents to inhibit induction of type I IFN and other proinflammatory mediators by lupus immune complexes. While additional mechanisms relevant to lupus pathogenesis may also come into play, the effect on TLR signaling provides considerable rationale for use of hydroxychloroquine to control disease activity and perhaps inhibit the amplification of immune system activation mediated by type I IFN.

A randomized placebo-controlled study of the withdrawal of hydroxychloroquine treatment in clinically stable SLE patients was published in 1991 by the Canadian Hydroxychloroquine Study Group and showed a 2.5-fold increase in flare rate and a shorter time to flare in those patients who received placebo for 24 weeks [81]. After more than 3 years of follow-up, those who had been randomized to continue hydroxychloroquine had a relative risk of hospitalization for major flare of 0.58 compared to those who received placebo [82]. A subsequent controlled trial of chloroquine supported its utility in reducing steroid requirements and avoiding disease flare [83]. These studies initiated a shift from the previous practice of using hydroxychloroquine and related agents predominantly for management of skin and joint symptoms toward a broader and more consistent use in many lupus patients [84].

A recent review has summarized the available literature addressing the impact of hydroxychloroquine on lupus activity and its comorbidities [85]. While severe lupus requires addition of more active therapeutic agents, the current recommendation is for use of this drug throughout the course of disease.

Development of new therapies for systemic lupus erythematosus

Aspirin, hydroxychloroquine and prednisone remain the only US Food and Drug Administration (FDA)-approved drugs for SLE, and in spite of the improved outcomes associated with wider use of hydroxychloroquine, there is an urgent need for improved therapies for active SLE, its significant organ involvement and its comorbidities. One approach that has been taken to identify more effective therapies is to extend the use of drugs first studied for other diseases to treatment of SLE. This approach is being used for both immunosuppressive agents as well as biologic therapies. Particularly with the biologic therapies, the growing knowledge of lupus pathogenesis is stimulating studies of therapeutic approaches that appear rational and likely to target important mechanisms of autoimmunity and inflammation. Unfortunately, this latter approach has only recently begun to demonstrate efficacy in randomized clinical trials of biologic agents. In contrast to the success that has been met in rheumatoid arthritis (RA), where TNF antagonists, CTLA4-Ig and anti-CD20 therapies are significantly better than the placebo comparators in clinical trials, leading to FDA approvals, only one controlled clinical trial in SLE has met its primary outcome measure. Nonetheless, introduction of mycophenolate mofetil (MMF) has increased therapeutic options for lupus nephritis and off-label use of available biologics has proved successful in select cases, with case studies and anecdotal reports supportive of their use. Definition of the clinical manifestations that are most responsive to biologic agents is needed. Perhaps future clinical trials that focus on defined clinical subgroups rather than 'all comers' will result in more positive results.

Mycophenolate mofetil

The application of MMF, a drug approved for use in prophylaxis of organ rejection, to treatment of lupus nephritis has provided a new alternative to cyclophosphamide for this severe manifestation of SLE [86]. Ginzler and colleagues [87] initiated a 24-week randomized, open-label, non-inferiority trial comparing oral mycophenolate mofetil (1 g per day, increased to 3 g per day) with monthly intravenous cyclophosphamide and reported that more patients receiving MMF than those receiving cyclophosphamide achieved complete remission, and a comparable number of patients in the two groups achieved partial remission. There were fewer infectious complications in the MMF group. The results from an international randomized, controlled trial comparing MMF to intravenous cyclophosphamide for induction therapy in 370 patients with lupus nephritis were recently published [88]. The primary outcome - decrease in urine protein/creatinine ratio and stabilization or improvement in serum creatinine - was similar between the two groups. Adverse events were also similar between the two groups, although there were more deaths in the MMF group. While it was hoped that MMF might prove superior to cyclophosphamide, demonstration of equivalence provides additional support for this approach as an appropriate therapeutic option for lupus nephritis.

Biologic therapies

As described above, it is recognized that T and B lymphocytes collaborate to generate lupus autoantibodies. Interruption of interaction between these cell types or selective inhibition of their activation or survival represents a promising therapeutic strategy.

The soluble inhibitor of interaction between CD28 on T cells and CD80/86 on antigen presenting cells, CTLA4-Ig (abatacept), improves joint pain and swelling in RA. However, the controlled trials of abatacept in SLE have not yet met their defined endpoints. In data presented at the ACR Annual Scientific Meeting in 2008, SLE patients selected for active polyarthritis, serositis or discoid lesions received 10 mg/kg abatacept or placebo over 1 year, along with 30 mg/day prednisone that was tapered after the first month. The outcomes for the abatacept and control subjects were comparable, as measured by new flares. In spite of these negative data, some hint of possible efficacy was suggested by improved quality of life related to physical health and less fatigue in the abatacept group. Inhibition of T cell activation remains a rational therapeutic approach. Future studies of abatacept, along with tests of biologics targeting CD40 ligand or the ICOS-ICOS ligand pathway, will provide additional data related to T cell function in SLE.

B cells, the precursors of autoantibody-producing plasma cells, are currently the most popular candidate therapeutic target for clinical investigation in SLE. In addition to their role in differentiating to antibody-producing cells, B cells can potentially contribute to SLE pathophysiology through their capacity to focus relevant antigens for presentation to T cells, by production of cytokines, through their role in organizing the anatomy of the germinal centers and other sites of productive immune responses, and perhaps other functions. Recent studies have defined a B cell phenotype that is associated with lupus disease activity [47].

B cell depletion is an approach borrowed from the lymphoma field, and anti-CD20 monoclonal antibody (rituximab) is increasingly used for treatment of lupus patients refractory to more traditional therapies [50,89-92]. As CD20 is expressed on mature B cells but not on plasma cells, it is not surprising that rituximab therapy does not deplete serum immunoglobulin or autoantibodies, even in the context of effective peripheral B cell depletion. Studies of B cell depletion in target organs are limited in SLE, but in RA, several studies have shown extensive variability in depletion of B cells in the RA synovial membrane, perhaps a correlate of clinical response. Case studies and anecdotal reports of rituximab therapy in patients with active SLE have supported use of this agent in clinical practice [50], but randomized, placebo-controlled clinical trials of rituximab in generalized non-renal

lupus, and more recently in lupus nephritis, have not met their primary or secondary outcome measures. Results of the phase II/III study of rituximab compared to placebo over one year in patients with moderate to severe active lupus in 257 subjects on stable immunosuppressive therapy were presented at the 2008 ACR meeting. Neither primary nor secondary endpoints were achieved. Active debate in the clinical research community has included the possibility that prednisone, administered early in the trial and then tapered, might have blunted differences in the responses of the rituximab and placebo groups. It must also be acknowledged that targeting the B cell, or the B cell depletion approach, might not have the anticipated impact on the relevant pathogenic mechanisms in the lupus patients studied. Future studies might focus on defined clinical subgroups reported to benefit from anti-B cell therapy in anecdotal reports, such as those characterized by cytopenias. Review of protocol design as well as careful comparison of data from responders and non-responders will help to guide future trials.

Additional approaches to targeting of B cells in SLE may provide support for the value of moving forward with a range of B cell therapies. While abetimus (LJP394), a putative B cell tolerogen, reduced anti-dsDNA antibody levels but did not reduce time to lupus flare, other B cell targeted therapies may be more promising [93]. Non-depleting anti-B cell monoclonal antibodies and inhibitors of BLyS and a proliferation-inducing ligand (APRIL) pathway are being tested and will provide informative data. BLyS and APRIL provide survival and differentiation signals to B cells [94]. TACI-Ig (atacicept), a soluble receptor that is predicted to block both of these factors, may reduce serum IgG levels, as may anti-BLyS monoclonal antibody (belimumab). Results from a 52-week double-blind placebo-controlled trial of belimumab in 449 SLE patients showed sustained improvement in disease activity through 3 years of therapy in seropositive patients (antinuclear antibody (ANA) test >1:80 or anti-dsDNA >301 units), representing 72% of the original cohort, but not in the total enrolled patient group. With the use of a new composite outcome measure, a phase III trial of belimumab has recently been reported to have met its primary endpoint. Clinical studies continue to evaluate these agents, along with a monoclonal antibody reactive with the IL-6 receptor, in SLE [95]. Together, these studies and linked evaluation of immune mechanisms affected by those interventions should allow a fair assessment of the value of B cell targeted therapies in SLE as well as new insights into the underlying disease mechanisms.

With the recognition of the possibly central role of innate immune system activation and nucleic acid-triggered TLRs in the pathogenesis of SLE, increasing interest in inhibiting that pathway has moved toward clinical trials of new biologic agents. Several distinct anti-IFN-alpha monoclonal antibodies are being tested in early phase clinical trials, with some indication of blockade of IFN-inducible gene expression. Additional approaches that are rational yet may encounter challenges with delivery, stability or specificity include oligonucleotide inhibitors of TLRs or inhibitors of downstream signaling pathways.

Conclusion

Paradigm-changing advances in basic immunology have led to significant progress in characterizing key pathogenic mechanisms in SLE. New focus on the activation of the innate immune response by nucleic acid-containing immune complexes that signal production of IFN-alpha and other pro-inflammatory mediators through TLRs has enriched our understanding of initiation and amplification of autoimmunity and inflammation. Lupus-associated genetic variants support the important contributions of altered regulation of T and B cell activation, along with the TLR pathways. The role of complement activation in target organ damage has gained renewed attention. All of these mechanisms are being applied to improved understanding of the diverse clinical manifestations of lupus disease. Clinical observations of co-morbidities associated with lupus are stimulating more comprehensive management of lupus patients as well as research studies to determine lupus-related mechanisms involved in premature atherosclerosis, catastrophic anti-phospholipid syndrome, and fetal loss. Each of these developments has contributed to accelerated efforts in drug development for lupus patients. While more consistent use of hydroxychloroquine and addition of MMF to the armamentarium of therapeutic options for lupus patients have improved patient management, the lupus community still awaits the pay-off that should follow from the insights into mechanisms and the development of biologic therapies.

Competing interests

MKC has served as a consultant for the following companies related to development of lupus therapeutics or diagnostics: Biogen-IDEC, Bristol-Myers Squibb, Genentech, Idera Pharmaceuticals, MedImmune, Merck Serono, Novo-Nordisk, Roche, Teva, XDx.

10 years AR&T

The Scientific Basis of Rheumatology: A Decade of Progress

This article is part of a special collection of reviews, *The Scientific Basis of Rheumatology: A Decade of Progress*, published to mark *Arthritis Research & Therapy*'s 10th anniversary.

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