Chapter 2
The Immunopathogenesis and Immunopathology of Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by autoantibody production in association with systemic inflammatory manifestations that vary in pattern and severity. This disease occurs primarily in young women, with a peak incidence during the childbearing years suggesting an important influence of sex on disease pathogenesis. Like most autoimmune diseases, lupus results from the interplay of genetic and environmental factors which together promote immune hyperactivity. While the precise environmental triggers for lupus are unknown, these factors likely induce changes in both the innate and adaptive arms of immune system as well as initiate or potentiate the production autoantibodies to components of the cell nucleus. These autoantibodies (antinuclear antibodies or ANAs) are directed to proteins, nucleic acids, and protein–nucleic acid complexes and represent the serological hallmark of SLE.

While the pathogenesis of SLE has elements in common with other autoimmune diseases, studies on human lupus as well as mouse models of this disease have established a strong conceptual and experimental framework to understand the generation of ANAs as well as the mechanisms for tissue inflammation and damage. Key to this framework is the recognition that the nuclear macromolecules that are the target of ANA reactivity have potent immunological activities that can promote both underlying immune disturbances and tissue injury. Indeed, the immunological activities of these target antigens may distinguish SLE from other autoimmune diseases (whose target antigens lack intrinsic immune activity) and account for its characteristic clinical features as well as the response to therapy.

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For lupus, like other autoimmune diseases, current models of pathogenesis posit that disease arises in a genetically susceptible individual in whom a propensity to autoreactivity is triggered by an environment agent. This event in turn initiates a cascade of B and T cell disturbances that lead to autoantibody production. For SLE, the consequences of autoantibody production are amplified by the formation of immune complexes that have pro-inflammatory properties that reflect the intrinsic immune activity of their component antigens. Thus, understanding the immunopathogenesis of SLE requires consideration of the determinants of susceptibility, the nature of the underlying immune disturbances, the properties of autoantibodies, and the role of immune complexes in pathology.

Determinants of Disease Susceptibility

One of the most notable features of SLE is its striking occurrence in women. Thus, depending on the study, SLE is 5–10 times more common in females compared to males and, furthermore, has a peak incidence during their childbearing years. Since the incidence of disease is more similar between males and females before puberty and after menopause in women, these findings suggest an important role for sex hormones in creating a susceptibility to autoreactivity. This susceptibility extends to many autoimmune diseases, suggesting a general influence of female sex on immune regulation.

While the female preponderance of lupus has long been recognized, little is known about the precise effects of hormones on the immune system. The susceptibility of women to autoimmunity, however, has been linked to aspects of the immune system regulation related to pregnancy. Pregnancy represents an extraordinary immune challenge since a woman bearing a child must accept what is in essence a foreign tissue graft for 9 months. No doubt there are many modifications in the immune system necessary to accommodate the fetus that bears a set of histocompatibility antigens from the father. While these modifications would be expected to lead to immune suppression during pregnancy, they may actually modulate immune cell function to increase, rather than decrease, immune responsiveness.

Estrogen and progesterone have diverse effects on various cells of the innate and adaptive immune systems. In terms of B cells, these actions can affect fundamental events in the establishment of tolerance, skewing the antibody repertoire to increase ANA generation. Hormones can also affect T cells and macrophage function, for example, although hormonal effects may not be the only influence conferred by sex. Thus, differences in the expression of genes on X and Y chromosomes as well as the number of X chromosomes may also determine the tendency to autoreactivity. Since manipulation of sex hormones, including androgens, can modify disease in murine models, direct effects of these mediators appear important.

As shown in both family studies and population-based genome-wide association studies (GWAS), genetic factors have a powerful influence on disease susceptibility. Thus, SLE shows a high concordance among identical twins and occurs at increased

frequencies among first-degree relatives; furthermore, in extended family pedigrees, SLE may occur along with other autoimmune diseases such as rheumatoid arthritis or thyroiditis. These findings suggest an inheritable tendency toward autoreactivity that may be shaped by either other inherited factors or environmental exposures to induce a specific pattern of disease.

Building upon studies of family as well as candidate gene approaches, GWAS analyses have now identified over 30 different loci that contribute to disease susceptibility in lupus. These genes include HLA-DRB1, IRF5, STAT4, BLK, and ITGAM among many other. While identifying loci with susceptibility alleles, in general, these studies diseases have not yet defined the actual gene involved or the functional changes conferred by a polymorphism. Even for genes whose link to disease seems compelling, the relationship to pathogenesis is nevertheless speculative and will require extensive deep sequencing as well as functional studies to clarify the effect on immune system.

From these studies, certain conclusions about the genetics of lupus are possible. Thus, lupus appears to be a multigenic disease, with the contribution of each locus to susceptibility limited. Whether the risk to disease is additive or synergistic is not known although abnormalities affecting different arms of the immune systems seem necessary to create a tendency to autoreactivity. Significant heterogeneity in the genetic substrate in disease is therefore likely especially if disease susceptibility involves a threshold effect in which a certain number of susceptibility alleles need to be present. This situation suggests that screening for genetic risk factors will be difficult especially if disease is also influenced by private variants that are not detected by current GWAS technique.

The genetic analysis of lupus suggests possible mechanisms by which inherited factors influence pathogenesis. Thus, gene variants thus far identified appear to influence elements of the immune system related to both the induction of immune responses and inflammation. With respect to B and T cells, the effects of these variants could occur at key steps in immune activation affecting the establishment of central or peripheral tolerance as well as the triggering of cellular responses during disease. Other polymorphisms could influence the function of neutrophils and their role in inflammation. Finally, as suggested by studies on complement genes, variation in copy numbers could affect the overall capacity to clear foreign or self-antigen that could trigger immune responses.

Studies on genetics of lupus in mouse models in general preceded the work on human disease. Murine studies have been of two major types. The first involves detailed dissection of the genetics of an inbred lupus strain such as the (NZB/NZW) hybrid or the NZM2410 inbred strain. The second type is the development and characterization of a knockout or transgenic strain with a lupus phenotype. In general, these genetic models have indicated that a multitude of disturbances of B and/or T cell function can lead to lupus although other genes in the strain background can influence the severity of disease. These studies have also demonstrated a role for genes encoding complement and other proteins (e.g., SAA) involved in the clearance of microorganisms or cellular debris in creating a predisposition to autoimmunity.

The studies on the lupus models have been informative in showing that lupus is multigenic, with the clinical outcome reflecting a summation of positive and negative influences. These genes can regulate the function of B and T cells. While a single locus can lead to serological disturbance, the development of a full-blown lupus syndrome (i.e., nephritis) requires the presence of more than one locus. Interestingly, among loci identified, a gene for interferon response is important in the NZB/NZW hybrid, while, in the BXSB mouse, duplication in the gene encoding TLR7, the toll receptor that recognizes single-stranded RNA, is important for the development of a Y-linked lupus illness. Both interferon and toll-like receptor (TLR) systems appear important in human lupus, suggesting similarity in pathogenetic mechanisms among species.

Serological Disturbances in Lupus

ANA production is a defining immunological disturbance in both human and murine disease. While patients with lupus produce over 100 different autoantibodies, certain specificities have diagnostic and prognostic significance and are associated with particular disease manifestations. Regarding ANA, two specificities represent criteria in the classification of patients with SLE: anti-DNA and anti-Sm. DNA is deoxyribonucleic acid, a polymeric macromolecule that encodes genetic information. Sm (or Smith antigen) is a complex of uridine-rich RNA molecules in association with a series of proteins to which the antibodies are directed. Sm and the related molecular complex called RNP play important roles in RNA processing. While antibodies to RNP occur commonly in SLE, often in association with anti-Sm, they have a wider distribution among other rheumatological disorders and do not have status as classification criteria.

While DNA displays a potentially enormous array of antigenic determinants corresponding to base sequence, antibodies in SLE patient sera predominantly bind conformational determinants on the DNA backbone, either double-stranded (ds) or single-stranded (ss) structure. These backbone structures are highly conserved and occur widely on DNA independent of species origin or basic composition. While, by definition, anti-DNA antibodies are autoantibodies, they are not specific for human (self) DNA since they also react with foreign DNA. These antibodies display high affinity for DNA, likely reflecting the interaction of each Fab binding site with a determinant along an extended polymeric structure. This type of interaction is termed monogamous or bivalent. Anti-dsDNA and anti-ssDNA antibodies commonly coexist in patient sera because many antibodies can bind both ss- and dsDNA. Nevertheless, anti-dsDNA directed to the B conformation of the DNA helix occurs almost exclusively in SLE; anti-ssDNA can occur in other clinical settings.

While anti-DNA can be analyzed by using biochemically and purified DNA, these antibodies are part of a spectrum of specificities that bind to chromatin, the form of DNA in the cell nucleus. In chromatin, DNA is wrapped around a core of histones to form nucleosomes which are the essential building block for chromatin

Table 2.1 Properties of anti-DNA autoantibodies

Subset of antibodies to nucleosomes
Bind conserved sites on single- and double-stranded DNA backbone
Monogamous or bivalent interaction
Can form pathogenic immune complexes
Levels can vary with disease activity

structure and are likely the source of antigenically active DNA. Consistent with a role of DNA as a component or epitope of a larger antigenic structure, anti-DNA antibodies appear commonly in association with antibodies to nucleosomes and histones. These responses are all closely linked, allowing anti-DNA to be studied as a representative of a large antibody family.

The expression of anti-DNA occurs in approximately 30–70 % of patients at some time during the course of their disease but shows marked variability in levels. Anti-DNA levels can rise and fall in association with disease activity, in particular, glomerulonephritis. These fluctuations, which underlie anti-DNA as a measure of disease activity, are a distinctive feature of this response that is not observed commonly with other autoantibody responses. These fluctuations also indicate sensitivity of antibody expression to agents such as glucocorticoids or cyclophosphamide, which, when used to treat flares, can reduce and even eliminate anti-DNA production. Table 2.1 summarizes properties of anti-DNA antibodies.

In contrast to anti-DNA, anti-Sm antibodies bind to proteins even though the Sm antigen has both protein and RNA components. Unlike levels of anti-DNA, levels of anti-Sm (and anti-RNP) tend to be stable during the course of disease, limiting delineation of an association with disease manifestations. Furthermore, in the USA, anti-Sm antibodies have a higher expression in African-Americans than European-Americans; while linked to anti-RNP, anti-Sm is expressed independently of anti-DNA. These features suggest that anti-DNA and anti-Sm antibodies result from different underlying immune disturbances that culminate ultimately in their distribution among B cell subsets, including long-lived plasma cells.

Other autoantibodies found in SLE have diverse antigenic targets and potential roles in disease. Thus, antibodies to the Ro and La antigens bind RNA–protein complexes and occur in SLE as well as Sjogren's syndrome and rheumatoid arthritis; in the context of SLE, anti-Ro and anti-La antibodies are associated with the neonatal lupus syndrome including congenital heart block, as well as subacute cutaneous lupus. Like anti-Sm and anti-RNP, anti-Ro and anti-La are linked.

Another important group of autoantibodies has been identified as lupus anticoagulants and includes antibodies to phospholipids and the lipid-binding protein β2-glycoprotein 1. These antibodies occur prominently in patients with the antiphospholipid syndrome (APS) which is characterized by venous and arterial thrombosis, pregnancy loss, and thrombocytopenia; in this syndrome, antibodies may promote clotting disturbances by their in vivo interaction with clotting factors or endothelial cells. Finally, among antibodies with a potential role in pathogenesis, antibodies to the NMDA receptor, which can cross-react with DNA, have been

Table 2.2 Clinical associations of SLE autoantibodies

Anti-DNA and nephritis
Anti-Ro and neonatal lupus and subacute cutaneous LE and photosensitivity
Anti-ribosomal P and CNS lupus
Anti-RNP and Raynaud's
Antibodies to phospholipids and clotting
?Anti-NMDA antibodies and cognitive dysfunction

implicated in CNS on the basis of studies in mouse models. While the data in the murine models indicate that such antibodies can induce behavioral and cognitive disturbances, the complexity of neuropsychological manifestations of patients has limited determination of the role of these antibodies in human lupus. Table 2.2 summarizes clinical association of selected lupus autoantibodies.

The Induction of ANA

The most striking feature of SLE autoantibody production relates to the targeting of nucleic acids or nucleic acid-containing complexes, which are highly conserved molecules key to essential cell functions such as replication, transcription, and translation. Furthermore, as a group, the nuclear antigens targeted in SLE display features which may be relevant to ANA induction: (1) these molecules can have intrinsic immunological activity; (2) the intracellular location of these molecules is dynamic and can vary depending on the state of the cell, including extracellular translocation; and (3) as conserved molecules, nuclear antigens bear determinants that can displayed by foreign versions of the same molecule or a foreign molecule that is structurally and functionally similar. As such, induction of ANA responses by the mechanism of molecular mimicry may be particularly likely. Of note, intrinsic immune disturbances in patients may predispose to molecular mimicry and the generation of cross-reactive antibodies with autoantibody activity.

The expression of ANA can be divided to three stages: antibody induction, antibody maintenance, and antibody reduction of elimination. Importantly, in current models for pathogenesis, the antigens involved in the first two stages may not be the same. Indeed, there is evidence that bacterial and viral antigens can induce ANA production while self molecules may mediate antibody maintenance. The stage of antibody elimination and reduction usually occurs in response to immunosuppressive therapy; since agents used for this purpose have broad immunomodulatory (and even cytotoxic activity), antibody elimination may be nonspecific and not directly involve a role of antigen in stimulating a regulatory response.

As shown in both patients and animal models, the production of antibodies to RNA-binding proteins (RBPs) (e.g., Sm, RNP, Ro, and La) may differ mechanistically from the production of antibodies to DNA. Thus, antibodies to RBPs may temporally precede those to DNA in humans and may evolve from cross-reactions to foreign responses such as proteins in the Epstein–Barr virus, which can bear

sequences similar to those in the RBP. Furthermore, immunization of normal mice with such EBV antigens can lead to generation of autoantibodies as well as the spreading of the response to other self-antigens, including DNA. In contrast, in spontaneous autoimmune mice, the expression of anti-RBP antibodies is much less common than anti-DNA expression, which is essentially universal in murine lupus.

The induction of antibodies to DNA, while a feature of spontaneous autoimmune disease, is much more difficult to replicate by immunization of normal animals. Even if dsDNA is coupled to an immunogenic protein and presented in an adjuvant, immunization of normal mice leads to very limited antibody production. This finding initially suggested that DNA is immunologically weak or inert and that anti-DNA production arises by a mechanism other than direct DNA immunization. Such an alternative mechanism could be either polyclonal activation or molecular mimicry.

While usual models for molecular mimicry for anti-DNA production implicate foreign proteins as the inducing molecule, foreign DNA can serve that function. As shown in studies in vitro and in vivo, DNA is not uniform in its immunological properties, with viral and foreign DNA demonstrating potent immunostimulatory properties. This stimulation arises from interaction of DNA with toll-like receptor 9 (TLR9) as well as other non-TLR internal nucleic acid sensors. These sensors are likely important elements of the innate immune system and serve as pattern recognition receptors (PRR) that respond to foreign molecules termed PAMPs for pathogen-associated molecular patterns.

For bacterial DNA, key determinants of stimulation of TLR9 result from short DNA sequences that center on unmethylated cytosine-guanosine dinucleotide repeats (CpG motifs). These motifs occur much more commonly in bacterial than mammalian DNA because of differences in the pattern of base methylation and a phenomenon known as CpG suppression. Because of CpG suppression, the CpG dinucleotide occurs much less commonly in mammalian DNA than would be predicted on the basis of the base sequence. The combination of base methylation and CpG suppression leads to a PAMP structure that can stimulate TLR9. Stimulation of non-TLR sensors may reflect the intracellular localization of DNA as opposed to a unique structural element, with access to these sensors occurring during infection with viruses or bacteria.

Even though anti-DNA antibodies are considered essentially unique to lupus, anti-DNA production also occurs in normal individuals, with levels approaching those found in patients with lupus. The antibodies in normal individuals, however, differ markedly from those in SLE with respect to specificity and immunochemical properties. Thus, antibodies in normal individuals bind with high selectivity to DNA from particular bacterial species but do not cross-react with either mammalian DNA or other bacterial DNA. These antibodies predominantly bear the IgG2 isotype and κ light chain, whereas anti-DNA antibodies in patients with lupus are predominantly IgG1 and IgG3 and have a more equivalent expression of κ and λ. Table 2.3 describes properties of anti-DNA antibodies in normal human subjects.

The development of antibodies to foreign DNA occurs with DNA from certain bacteria or viruses and does not occur with DNA from *E. coli*, for example; the high

Table 2.3 Properties of anti-DNA in normal subjects

Bind to non-conserved sites on bacterial and DNA
High affinity
IgG2 predominance
κ light chain restriction
Inducible in normal mice by immunization

specificity of these antibodies for DNA from particular species likely accounts for the previous failure to detect this type of anti-DNA antibodies in many studies. The rules for the antigenicity of bacterial and viral DNA are not known although the type of exposure (e.g., infection vs. colonization or location such as skin vs. lung) may influence antibody production. Whatever the basis of this response, the generation of antibodies to foreign DNA may occur because bacterial and viral DNA is immunostimulatory and has sequential determinants not present in mammalian DNA.

In the context of lupus, the immunogenicity of bacterial or viral DNA provides a mechanism for the induction of anti-DNA antibodies that has analogy to the induction of anti-RBP antibodies. Thus, in patients with lupus, bacterial DNA, rather than inducing antibodies to non-conserved sequential determinants, may stimulate a cross-reactive response to conserved backbone determinants shared by bacterial and mammalian DNA; viral DNA may play a similar role. These cross-reactive antibodies would have autoreactivity even if the inducing antigen was foreign nucleic acid. Furthermore, deficient expression in lupus of the normal IgG2 response to bacterial DNA may promote the cross-reactive response because of more prolonged exposure to bacterial DNA and persistent immune stimulation.

Studies in mice support this possibility and demonstrate differences in the immune recognition of DNA in normal and autoimmune animals which likely have a counterpart in patients. Whereas immunization of normal and autoimmune mice with mammalian DNA (as protein complexes in complete Freund's adjuvant) fails to induce an anti-DNA response, immunization with bacterial DNA induces a significant anti-DNA production. In normal mice, the induced antibodies are specific for bacterial DNA, while in autoimmune NZB/NZW mice, the induced antibodies cross-react with both bacterial and mammalian DNA. These findings suggest that an autoimmune background may determine the pattern of antibody recognition of DNA, leading to antibodies to conformational rather than sequential determinants. Since recognition of conformational determinants can confer autoreactivity, an alteration in binding specificity may be key to development of SLE.

An interesting aspect of the immunization model concerns the differences in the response to bacterial and mammalian DNA, in particular, in the autoimmune NZB/NZW mice. These mice are destined to produce anti-DNA spontaneously as they age and can produce anti-DNA in response to immunization with bacterial DNA. Nevertheless, they do not produce anti-DNA in response to immunization with mammalian DNA. These findings suggest that self DNA is poorly immunogenic but that response can occur once tolerance is broken by immunization with a foreign molecule. Coupled with studies on response to protein antigens, these

observations highlight the potential role of infection in initiating lupus. While exposure to bacterial and viral antigens may stimulate autoantibody production, self-antigen may underlie ongoing antibody production and affinity maturation.

Cellular Immune Disturbances

Studies on both murine and human SLE demonstrate phenotypic and functional abnormalities of B cells, T cells, macrophages, dendritic cells, and neutrophils among elements of the immune system. While these abnormalities may reflect primary disturbances which are genetically determined, others may be secondary to other events in lupus, in particular, cytokine production. Among these cytokines, type 1 interferon appears to have an important role in pathogenesis. As shown in studies of patients with lupus, peripheral blood cells display patterns of gene expression consistent with stimulation by type 1 interferon. This pattern, which can be demonstrated by either microarray analysis or PCR analysis of selected genes, is termed the interferon signature. An increase in interferon can also be demonstrated by the ability of patient serum to stimulate transcription of interferon responsive genes in cell lines.

The interferon signature may be important in pathogenesis since interferon can induce autoimmunity in patients treated for other diseases. Furthermore, genetic studies point to an etiological role of genes involved in the response to interferon. Interferon has multiple actions that can impact on immune cell function. In addition, interferon can also modulate endothelial cells, an important target tissue in lupus, and lead to vasculopathy. While the interferon signature is present in only some patients, its association with antibodies to RBPs and DNA suggests a mechanism by which these antibodies may promote immunological disturbance in SLE.

In a scenario in which anti-DNA and anti-RBP antibodies drive pathogenesis, a self-reinforcing cycle of autoantibody production leads to the formation of immune complexes which in turn drive interferon production to potentiate inflammation and antibody production. As shown in in vitro systems, antibodies to RBPs and DNA antigen can form immune complexes which stimulate interferon production by plasmacytoid dendritic cells (pDCs). This stimulation involves TLR and non-TLR sensors and occurs most likely because autoantibodies allow internalization of DNA and RNA to access the internal nucleic acid receptors; depending on intracellular location, DNA can become immunoactive and may not need CpG motifs to induce stimulation. Since nucleic acids originating from cells may be attached to nuclear proteins such as the high mobility group box 1 protein (HMGB1), the RAGE receptor (receptor for advanced glycation end products), which binds this protein, may also contribute to immune stimulation; HMGB1 also has immune activity and is a prototype for an alarmin or not of DAMP [a death (or damage)-associated molecular pattern] by analogy with a PAMP (pathogen-associated molecular pattern).

Evidence for the role of stimulatory complexes containing DNA and RNA comes from studies on the inhibitory effects of antagonists of TLR9 and TLR7, receptors

for DNA, and single-stranded RNA, respectively, in both in vitro systems as well as lupus mice. Importantly, these mechanisms derive from the intrinsic immunostimulatory activity of DNA and RNA although the presence of these molecules in immune complexes is critical for the stimulation of the pDCs.

In addition to aberrant cytokine production, the pathogenesis of SLE involves intrinsic immune cell abnormalities that may alter, for example, thresholds for activation and signaling. The end result is aberrant tolerance among B and T cells, either peripherally or centrally. These abnormalities perturb the checkpoints for normal deletion or inactivation and can skew the respective repertories to predispose to autoreactivity. In the B cell compartment, these repertoire disturbances are characterized by an increased number of autoantibody precursors that show broad reactivity among autoantigens including DNA. With an increase in autoantibody precursors in the pre-immune repertoire, stimulation by DNA or a cross-reactive antigen can induce anti-DNA in a way not possible with the ordinary pre-immune repertoire.

Once an autoantibody response is initiated among such precursors, affinity maturation occurs, with related members of clones showing the hallmark of antigen-driven selection in turns of somatic mutations. For anti-DNA antibodies, these mutations can lead to the presence of arginine and other positively charged amino acids to promote the binding of negatively charged DNA. While affinity maturation may occur in lupus, the outcome may differ from that of a conventional antibody response because of the distortion in the pre-immune repertoire, the adjuvant activity of the antigen, the presence of the antigen in a complex with other molecules (e.g., histones), and promiscuous T cell help. These processes could lead to the expression of antibodies that prefer conformational epitopes and display polyreactivity.

Consistent with ongoing immune stimulation, patients with lupus show an increase in the number of plasma cells in the peripheral blood. The level of these cells, which may result from germinal center activity, is dynamic and can correlate with disease activity. The role of plasma cells in autoantibody production is also important in terms of disease pathogenesis since these can be very long-lived cells and difficult to eliminate using current therapy including anti-B cell reagent such as anti-CD20. The presence of cytokines such as B cell activating factor (BAFF) can contribute to the activity of these cells.

The Role of Self-Antigen

For DNA and other nuclear autoantigens to induce responses or form immune complexes, they must exist outside of cells in an immunologically relevant form. The translocation process appears to occur most commonly during cell death, either physiologic or pathologic. During apoptosis, a regulated process called programmed cell death, nuclear molecules undergo extensive cleavage and translocation, appearing on the surface as well as in small subcellular structures called blebs. Furthermore,

as apoptosis proceeds, the cell itself can undergo compaction and fragmentation, leading to production of apoptotic bodies. These bodies contain DNA and other nuclear molecules; microparticles which may correspond to blebs also form and detach to cells. While circulating nuclear molecules can be both free and particulate, most of the RNA appears to be in particles. The processes of RNA and DNA release from cells may be distinct, perhaps accounting for the difference in expression of antibodies to RBP and DNA.

Reflecting cell death during ordinary turnover, the blood of even normal subjects has significant amounts of circulating DNA. These levels can increase during a wide variety of conditions, including lupus, that are characterized by inflammation or cell death although these processes can commonly coexist. Thus, levels of circulating DNA can rise because of either an increased cell death or impaired clearance of dead and dying cells. The body has extensive systems for removal of these cells which includes cellular elements such as macrophages as well as humoral elements such as complement, C-reactive protein, and IgM. With impairment of these systems, dead cells may persist and can spill or leak their contents.

A role of impaired clearance in disease pathogenesis can explain the association of SLE with genetic abnormalities in elements of the clearance system, whether cellular or humoral immune. As such, an increase in apoptosis and a decrease in clearance of dead cells in patients may represent fundamental abnormalities that boost the supply of self-antigen to drive autoantigen-specific response or form immune complexes. The consequences of this increase may be particularly important because of the immunostimulatory activity of nuclear molecules as well as subcellular structures such as HMGB1 and microparticles.

Mechanisms of Tissue Inflammation and Damage

Given the array of cellular and humoral disturbances in patients with lupus, tissue inflammation is most likely multifactorial and diverse, differing among organ systems that are commonly affected. For many manifestations, the mechanisms of inflammation are unknown although in general autoantibodies have been implicated as important effectors. These antibodies can cause functional disturbances (e.g., clotting system for antibodies to phospholipid antigens), induce cytotoxicity or promote elimination (e.g., anti-red blood cell antibodies), or form immune complexes with tissue deposition. Abnormal levels of cytokines such as interferon or TNF may either directly induce inflammation or increase inflammation resulting from these other mechanisms. Interferon, for example, can perturb endothelial cell function and promote vasculopathy.

Of organs involved in SLE, the kidney has been studied most intensively to elucidate immunopathogenesis. Compelling data indicate that glomerulonephritis results from the deposition of immune complexes that are comprised of DNA and anti-DNA. This evidence for this mechanism includes the observations as summarized in Table 2.4. While evidence that lupus is an immune complex disease is

Table 2.4 Mechanisms of SLE renal disease

Association with anti-DNA immune complexes
High anti-DNA and low complement with activity
"Full house" immune deposition (IgG, IgA, IgM, and C3)
Immune complexes in sub-epithelial and sub-endothelial location
Impact of antibody and antigen charge on glomerular localization

strong, many aspects of this process remain unknown. Thus, it is not clear whether immune complexes form systemically in the circulation or locally in the kidney. Furthermore, if the complex formation is local, it may occur in a two-step process in which DNA first binds to the glomerular basement membrane to form a nidus to bind anti-DNA. Alternatively, the process may involve the local generation of nucleosomal antigen for assembly of an immune complex within the confines of the kidney. For some anti-DNA antibodies, direct binding to a glomerular antigen may occur by cross-reactivity.

For immune complexes, whether formed in the circulation or in the kidney, the properties of both antibody and antigen can influence renal deposition; DNA is a negatively charged molecule although, when present in nucleosomes, it can display regions of positive and negative charge; charged antigens may have a predilection for glomerular binding. Similarly, anti-DNA antibodies are charged and also may contribute to glomerular interaction. In the kidney, the immune complexes can activate complement, promoting inflammation that can ultimately lead to scarring and fibrosis.

The operation of these mechanism can be assessed by a variety of serological and immunopathological assays. Thus, active nephritis can be associated with high levels of anti-DNA and decreased complement; cytokines can also appear in the urine, indicative of renal inflammation. By histopathology, the immune complexes in the kidney can be detected by immunofluorescent microscopy which shows the presence of IgG, IgM, IgA, and complement in a pattern called "full house." By electron microscopy, the complexes appear as electron-dense material that can localize to either sub-epithelial or sub-endothelial sites. The site of localization can affect the pattern of nephritis and the occurrence of nephritic and nephrotic disease.

Figure 2.1 presents an overall picture of lupus pathogenesis, indicating the role of immune complexes in both cytokine disturbance and nephritis. Although immune complexes may contribute to both facets of pathogenesis, the anti-DNA complexes that drive cytokine production may differ from those that stimulate nephritis, for example. Complexes of other specificities may also contribute to these and other manifestations. While the term "nephritogenic" denotes anti-DNA antibodies that can cause nephritis, a term for antibodies that drive cytokines has not yet been developed. Furthermore, assays to distinguish those specificities with various pathogenic properties (i.e., nephritis and cytokine production) would be very valuable but are not yet available.

Lupus is associated with a dramatically increased frequency of atherosclerosis, an important source of morbidity and mortality. This manifestation is likely multifactorial

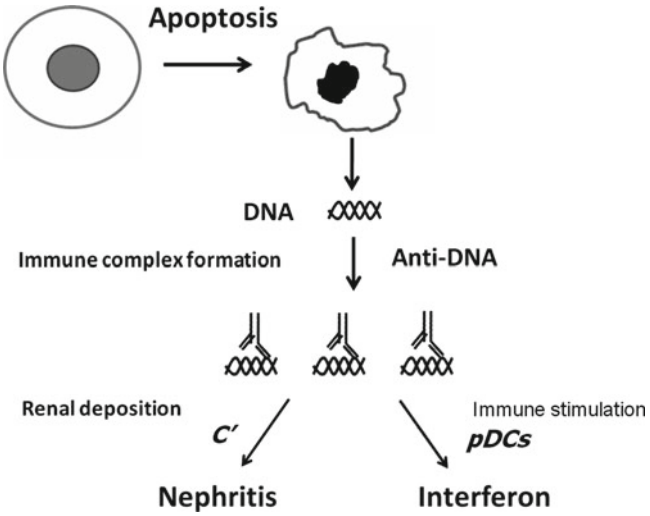

Fig. 2.1 The role of immune complexes in lupus pathogenesis. As illustrated in this figure, the pathogenesis of SLE is critically dependent on the formation of immune complexes whose antigenic components can derive from dead and dying cells. The levels of these components may rise because of impairment of the cellular and humoral immune systems involved in the clearance of dead and dying cells. In the presence of extracellular nuclear antigens, immune complexes can form with antibodies to DNA and other nuclear molecules; this figure depicts the DNA system. These complexes can in turn deposit in the kidney to incite nephritis or stimulate pDCs to produce cytokines such as type 1 interferon. Interferon has broad immunological activities that can impact on other cells to intensify immune disturbances. This schema indicates steps where genetic disturbances can promote susceptibility and, correspondingly, steps where treatment can ameliorate disease

and results from inflammation, vasculopathy, and thrombosis that characterize lupus. Some of these effects may relate to cytokines while others may relate to autoantibodies (e.g., antiphospholipid antibodies) that promote thrombosis in coronary or cerebral arteries. The recognition of this manifestation emphasizes the importance in disease management of agents to reduce cholesterol, lower blood pressure, and prevent clotting (i.e., anticoagulants) as part of the overall therapeutic approach.

Conclusion

SLE is a systemic autoimmune disease that results from interaction of genetic and environmental factors which together promote autoantibody production directed prominently to nuclear macromolecules. Since the nuclear molecules can have intrinsic immunological activity, the resulting immune complexes can cause potent stimulation of the immune system including the production of type 1 interferon.

In addition, these complexes can deposit in the kidney to incite inflammation and damage. The formation of the immune complexes requires an available source of nuclear material. In SLE, this material may arise because of an increase in the amount of cell death as well as impaired clearance dead and dying cells. Thus, pathogenesis of SLE entails important abnormalities that affect both autoantibody and autoantigen production.

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