

REVIEW

Pathogenesis of systemic lupus erythematosus (SLE)

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Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with clinical features that include glomerulonephritis, rashes, serositis, haemolytic anaemia, thrombocytopenia, and central nervous system involvement [1]. The characteristic immunological abnormality is the presence of high affinity, somatically mutated IgG autoantibodies to ubiquitous intracellular, cell-surface and extracellular autoantigens. The spectrum of intracellular autoantigens includes the proteins and DNA that make up chromatin, proteins of the spliceosome complex and the Ro/La small cytoplasmic ribonucleoprotein particle [2]. Autoantibodies to cell-surface antigens include anti-phospholipid antibodies, associated with thrombotic complications of disease, and autoantibodies to red cells, white cells and platelets resulting in cytopenias [3]. Autoantibodies to the plasma protein C1q, the first component of the classical pathway of complement [4] are strongly associated with the development of glomerulonephritis [5].

In addition to human SLE, a very similar disease develops spontaneously in mice and several important transgenic models of SLE have been developed. Our knowledge of the pathogenesis of SLE comes from intensive study of both human disease and its murine counterpart. This review is divided into three sections. In the first section - Genes and environment - we discuss the evidence that proves the role of both genetic and environmental factors in the pathogenesis of lupus. In the second section - The autoimmune response in lupus - the evidence implicating both B and T cells in pathogenesis, the mechanisms by which immunological tolerance may be broken, and the origin of the autoantigens involved in the

autoimmune response, are reviewed. The last section - Immune complex-mediated inflammation in lupus - is concerned with immune-mediated inflammatory processes that result from the autoimmune response. In this section we consider how immune complexes may fail to be cleared from the circulation, deposit in the glomerulus, and cause inflammation.

Genes and environment

Human lupus has an important genetic component as illustrated by a 24% concordance of disease between monozygotic twins, compared with 2% between dizygotic twins [6]. However, the observation that the second twin does not develop lupus in three-quarters of monozygotic twin pairs also implies an important role for environmental or stochastic factors. The genetic susceptibility to lupus has recently been reviewed [7]. Here we will describe the linkage studies that have mapped the genetic loci (containing multiple genes) that influence the development and expression of disease in humans and mice. We will then discuss specific genes that have been implicated in the pathogenesis of lupus.

Genetic loci linked to lupus

There are a number of strains of mice in which lupus reproducibly develops in all the members of colonies, held worldwide, in standard animal houses. These strains include the NZB/W F1 hybrid, MRL/lpr and BXSB. By setting up informative crosses between these strains and non-autoimmune partners it has been possible to map more than 20 loci associated with different aspects of the autoimmune phenotype. Genes present in the major histocompatibility complex (MHC) have been implicated in disease pathogenesis in all three of the major lupus-prone strains; NZB/W, MRL/lpr and BXSB [7]. Several loci on

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chromosome 1 have also been identified in each of these mouse strains and also in humans. However, as yet it is not known what is the identity of the specific genes mapped by these studies.

The genes that cause increased susceptibility to SLE appear to fall into three functional categories. The first is genes that control autoimmune responses to particular sets of autoantigens; these may encode proteins that facilitate the clearance of these autoantigens within the body or bind to autoantigens, masking them from the immune system. The second category is genes that control the thresholds of T and B lymphocytes for activation. The third is genes that influence the expression of inflammatory disease in particular organs. We will consider examples of each of these.

Genetic studies of spontaneous murine lupus have shown also that multiple loci interact to cause the development of disease. For example, C57BL/6 mice whose genome contained one locus (*Sle1*) from an NZB/W-derived strain developed antichromatin antibodies without glomerulonephritis. C57BL/6 mice whose genome contained another locus (*Sle3*) developed polyclonal B and T cell activation and a low incidence of glomerulonephritis. However, mice congenic for both loci developed anti-dsDNA antibodies, splenomegaly and a high incidence of glomerulonephritis [8].

Specific genes associated with lupus

The genetic basis for SLE is not known in the vast majority of cases. However, in a tiny minority of humans with SLE and in some strains of mice, abnormalities in single genes play a powerful role in determining disease susceptibility. In humans, certain inherited deficiencies of proteins of the classical pathway of complement are almost uniformly associated with the development of SLE. Although these cases of disease are extremely rare, they are also extremely important as an understanding of the mechanism of this association may provide clues to the aetiology of SLE.

There is a hierarchy of susceptibility to the development of SLE, and the severity of disease according to the position of the deficient complement protein in the activation sequence of the classical pathway. The most severe and highly prevalent lupus is associated with a deficiency of C1q, C1r and C1s, or C4. The frequency of lupus in C1q, C1r/C1s, and C4 deficiency is approximately 92, 57 and 75%, respectively. In the case of C2 deficiency, the frequency of lupus has probably been overestimated due to ascertainment artefact. There is likely to be a bias towards publishing C2-deficient subjects with SLE and not the occurrence of healthy C2 deficient individuals. Calculations based on the frequency of C2 deficiency amongst cohorts of patients with lupus, and the estimated

frequency of C2 deficiency in the general population, suggest that around 10% of C2-deficient patients develop lupus [9]. In the case of C3 deficiency, pyogenic infections are the major clinical feature, and lupus is uncommon, being recorded in only 3 out of 23 cases. These data imply that there is an activity of the early part of the classical pathway of complement that is protective in the majority of people against the development of SLE. We will consider hypotheses for this protective mechanism below.

Amongst mice, the MRL strain is spontaneously prone to the development of lupus. However, the susceptibility of these mice to lupus is dramatically enhanced by the presence of the *lpr* gene. This was shown to encode a mutated *fas* gene, whose product is a cell-surface receptor regulating the development of apoptosis in lymphocytes and some other cell types [10]. In the absence of normal *fas* function, huge numbers of lymphocytes accumulate, causing gross lymphadenopathy and splenomegaly, and the development of accelerated lupus with fatal glomerulonephritis. However, even in the presence of this gross defect regulating lymphocyte apoptosis, other genes play a vital role in determining the presence or absence of disease. When the *lpr* gene was crossed on to the C57BL/6 strain of mice, lymphadenopathy and some autoantibodies developed, but no significant inflammatory change of lupus such as glomerulonephritis [11]. After the identification that the *lpr* gene encoded a mutant *fas* protein, it was discovered that the *gld* gene, associated with a very similar lupus phenotype to *lpr*, encoded a mutant *fas*-ligand [12]. Examples of both *fas* and *fas*-ligand gene mutations were subsequently discovered in humans. Interestingly, although both are associated with some autoimmune abnormalities, the major disease association in humans is with a syndrome known by the acronym, ALPS, standing for autoimmune lymphoproliferative syndrome, rather than with typical SLE [13,14].

Many genetic association studies have been performed amongst populations of humans with SLE. As in mice, there is strong evidence for the participation of the products of genes of the major histocompatibility complex in causing disease susceptibility. Certain polymorphic products of MHC class II genes, influencing the selection of peptides to be presented to T cells, are strong candidates for modifying disease susceptibility. However, there is also evidence that other genes encoded in the region of the MHC may be involved, including, in humans, the complement genes encoding the two isotypes of C4, C4A and C4B, and the C2 gene, and the genes for the cytokines, TNF-alpha and lymphotoxin-alpha [9].

There are several genes on chromosome 1 that have been associated with the development of SLE and/or lupus glomerulonephritis, known as candidate disease

susceptibility genes. These include polymorphic variants of the Fc receptors, Fc gamma RIIA and Fc gamma RIII [15,16]. However, many genetic association studies in SLE have been performed using relatively small populations of cases and controls and these are liable to give false-positive or -negative results, discussed in [17].

Environmental factors

A rate of concordance of 24% between monozygotic twins was cited above as evidence for a genetic component to the pathogenesis of lupus. However, this figure also suggests an important role for environmental influences or for stochastic effects. The very low prevalence of SLE in rural black populations in West Africa compared with a prevalence of as high as 1 : 250 female African Americans suggests a role for important environmental influences in protection against the development of lupus in Africa. It has been speculated that the high incidence of infectious disease, especially malaria, in Africa may protect against the development of SLE [18].

The best characterized environmental agent for the development of lupus in humans is the intake of certain drugs, such as hydralazine and procainamide. However the phenotype of drug-induced lupus is rather different from the idiopathic disease, with a low prevalence of glomerulonephritis and predominance of antihistone rather than anti-dsDNA autoantibodies [19].

In mice, polyclonal B cell activators such as pristane [20] and endotoxin [21], may accelerate or induce SLE. These are discussed in the second section. The mechanisms by which drugs may cause lupus are also considered in the next section. Dietary factors have also shown to play a role in animals; cynomolgus macaques that were fed alfalfa sprouts developed a lupus-like illness. The amino acid L-canavanine appeared to be the key constituent of alfalfa in inducing this effect [22,23].

Viral infections have been implicated in the pathogenesis of several autoimmune diseases including diabetes and lupus, and an association was reported between Epstein-Barr virus (EBV) infection and lupus in young adults and children [24]. There is some evidence for the involvement of a transmissible agent in canine lupus; cell-free spleen extracts from dogs with lupus resulted in the generation of anti-nuclear antibodies when given to newborn puppies [25]. This led to the suggestion that human lupus might be a zoonosis transmitted from dog to man. However despite suggestions of immunological abnormalities in dogs belonging to humans with lupus [26,27], this was not confirmed in other large, well-controlled studies [28,29,30].

In summary, despite the evidence that the environment influences susceptibility to lupus in humans, no environmental agent responsible for lupus in humans has been

identified, with the exception of the rare cases of drug-induced disease.

The autoimmune response in lupus

Introduction

In the first section we discussed the evidence that both genes and environment interact to cause lupus, without examining the mechanisms responsible for the development of lupus. In this section we focus on the mechanisms by which the autoimmune response characteristic of lupus arises. Early studies demonstrated that a tendency to produce autoantibodies and develop glomerulonephritis could be transferred from NZB mice to normal mice by bone marrow cells [31]. These experiments did not clearly address the cell types involved, and it seems likely that both myeloid and lymphoid cells were transferred. More recent studies have provided information on the role of specific lymphocyte types in pathogenesis. Here we will review the evidence that both B and T lymphocytes play a role in lupus, and then explore the ways in which mechanisms that normally maintain tolerance may be broken in lupus. These include; reduced thresholds for B and/or T cell activation, and the presence of excessive amounts of autoantigen.

The role of B cells

Pathogenic autoantibodies produced by B cells are an important cause of tissue damage in SLE. That B cells were required for murine lupus was shown by abrogation of disease in MRL/lpr mice that were genetically manipulated to be deficient in B cells [32]. Evidence for the pathogenicity of autoantibodies, in particular anti-DNA antibodies, comes from the observation that tolerization to DNA abrogates disease in NZB/W mice [33]. Other sections below describe further evidence that supports a role for antibody and B cells in the pathogenesis of lupus. These include; manipulations affecting B and T cell interactions that modify disease, evidence supporting a role for polyclonal B cell activation in disease expression, and a discussion of the pathogenicity of autoantibodies

A requirement for T cells

Two important observations in humans suggest a role for T cells in the pathogenesis of lupus. Firstly, the association of SLE with MHC class II (reviewed in [7] and discussed above under genetic factors), and, secondly, the autoantibodies found in lupus show features associated with T cell dependent responses including isotype-switching and somatic mutation [34]. Several different experimental approaches have been used to more directly demonstrate

this T cell requirement in animal models. Antibodies against CD4 abrogated disease in both MRL/lpr and NZB/W mice, demonstrating a role of T helper cells in both models [35]. More recently, confirmatory evidence of a role for T helper cells has come from gene deletion studies. For example, MRL/lpr mice deficient in MHC class II had a decreased susceptibility to disease compared to unmanipulated MRL/lpr mice [36]. Manipulations to inhibit B and T cell interaction with CTLA4-Ig or anti-CD40 ligand have also reduced the expression of disease in NZB/W mice [37,38,39]. These data suggest that T helper cells are important in the provision of help in order to generate the autoantibodies characteristic of lupus.

If T cells are required for autoantibody production, then there must be a peptide epitope to be presented to the T cell receptor. In the case of anti-DNA antibodies this may be derived from the histone components of the nucleosomes, or from some other DNA-binding protein such as the anti-DNA antibody itself. Nucleosome-specific T cells have been isolated from patients with lupus and from lupus-prone mice, and recent studies have suggested that tolerization with nucleosomal peptides can halt the progression of established disease in NZB/SWR mice [40,41,42,43,44]. Peptides derived from anti-DNA antibody have been shown to stimulate T cells and accelerate disease in NZB/W mice, suggesting that at least some helper T cell epitopes may originate in DNA-binding antibodies [45,46]. In addition, the induction of tolerance to similar peptides can prevent the development of disease [47]. These data suggest that nucleosomes and/or anti-DNA antibody may provide T cell epitopes for the T cell-dependent antibody response seen in lupus.

Abnormal thresholds for lymphocyte activation

There is extremely strong evidence that the mature autoantibody response in SLE is driven by antigen. However, there is also evidence that B cells in mice and humans show some degree of polyclonal activation and it is likely that this reflects abnormalities in the threshold for activation of lymphocytes that may play a role in disease susceptibility. Thus, the earliest immunological abnormality that can be identified in young NZB/W mice is polyclonal B cell activation [48].

The idea that polyclonal B cell defects contribute to the pathogenesis of lupus is also suggested by the observation that mutant mice with defects of B cell negative regulation develop a lupus-like illness, although glomerulonephritis has not been reported in all of these mice. These include mice deficient in Lyn [49], CD22 [50], SHP-1 [51] and Fc gamma RIIB [52]. The effect on B cell activation was additive for heterozygous deficiencies of Lyn, CD22 and SHP, suggesting multiple genetic factors may affect the

threshold for B cell signalling, and interact to predispose to autoantibody production [53].

Environmental polyclonal activators have also been shown in animal models to augment or accelerate disease. Pristane administered to normal mice resulted in anti-nuclear antibodies [20], although anti-dsDNA and anti-nucleosome antibodies were not found, and anti-ssDNA and antihistone antibodies were IgM only. Endotoxin is another polyclonal B cell activator, and has been shown to accelerate disease in various strains of long-lived lupus mice [21]. However, the effects of endotoxin are wide ranging, and it is difficult to be sure that B cell activation was solely responsible for these findings.

In addition to polyclonal B cell activation, general hyperactivity of T cells may also result in lupus. Two lines of evidence that support this suggestion will be discussed. Firstly, lupus develops in gene-targeted mice with T cell hyperactivity. Mice deficient in the membrane-bound protein Pten have been shown to have abnormal T cell reactivity and to develop a lupus-like illness [54]. A similar situation is seen in mice deficient in the cell cycle inhibitor p21 [55]. Mice deficient in the protein PD-1 also develop a lupus-like illness [56]. This protein inhibits both B and T cells, and it is not clear if the important abnormality is a hyper-reactivity of B cells, T cells or both. Secondly, alloreactive T cells may cause lupus when parental T cells are injected into F1 offspring of mixed genetic background. Autoantibody production is thought to occur by stimulation of 'host' B cells by 'grafted' alloreactive T cells [57,58,59].

These data support the idea that one of the factors that may predispose to the development of SLE is reduced thresholds for activation of B and T lymphocytes. These 'hypersensitive' lymphocytes, when driven specifically by autoantigens, may generate the autoantibody responses of SLE.

The source of autoantigens

As we have discussed, there is much evidence that it is the autoantigens themselves that drive the autoimmune response in SLE. What is the source of these autoantigens, which are ubiquitous and abundant components of all healthy tissues in the body?

The link between complement deficiency and the development of lupus led to the development of a hypothesis relating to the clearance of autoantigens from the sites of tissue injury. It has been known since the 1940s that complement has an important role in the clearance and removal of immune complexes from tissues by the mononuclear phagocytic system. The hypothesis was developed that, in the presence of complement deficiency, the clearance of immune complexes from sites of tissue injury may be defective, leading to enhanced local

inflammatory damage, with the release of autoantigens that could drive the autoimmune response [60].

Growth, development and repair all depend on the ability to remodel tissues. In the case of growth and development, this happens without the development of overt inflammation. In the case of repair, an initial injury is followed by a phase of resolution in which inflammatory cells are removed. Cell death is a key feature of each of these processes and this normally occurs by the mechanism of apoptosis, or programmed cell death. Apoptotic cells are removed from tissues by uptake by phagocytosis by other cells by means of a variety of specific receptors [61].

A breakthrough in our recognition of the possible source of the autoantigens of SLE came with the observation that many of the most characteristic autoantigens of SLE were presented in surface blebs of apoptotic cells [62]. This led to the hypothesis that it might be apoptotic cells and bodies that are the source of the autoantigens that drive the autoimmune response in SLE.

This hypothesis led in turn to the hypothesis that failure of the normal mechanisms of physiological disposal of apoptotic cells, or of the clearance of autoantigens released from dead or dying cells, might predispose to the development of SLE. This led to an extension of the hypothesis linking complement deficiency and lupus, i.e. that complement may play a role not only in the clearance of immune complexes but also in the binding and clearance of apoptotic cells [63]. Failure of each of these 'waste disposal' activities of the complement system may contribute to the autoantigen drive in SLE in the context of complement deficiency.

Several studies have now confirmed a role for the complement system *in vitro* and *in vivo* in the binding and clearance of apoptotic cells [64,65]. In particular, C1q-deficient mice showed a defect in the clearance of apoptotic cells injected *in vivo* [66].

Observations in two other lines of mice that are genetically deficient in proteins that may be important in the clearance pathways of tissue debris have supported this hypothesis, reviewed in [67]. Some strains of mice deficient in serum amyloid P component, SAP, which binds to chromatin [68], or deficient in DNase 1, which cleaves extracellular chromatin [69], develop lupus. In addition, mice deficient in the expression of secreted IgM develop a lupus-like illness [70,71]. One role for IgM could be to enhance the clearance of cellular debris.

Immune complex-mediated inflammation in lupus

In the previous section we discussed how autoimmunity may arise in lupus. This section considers how this autoimmune response leads to tissue inflammation. We have considered above the evidence that B cells are

required for disease. Here we will discuss how antibody localizes to glomeruli and the direct evidence that this may lead to inflammation.

Pathogenicity of autoantibodies

Anti-DNA and anti-nucleosome antibodies are thought to be a major cause of tissue damage in SLE and many studies have addressed how these may cause glomerulonephritis. Anti-DNA antibodies have been eluted from kidneys of both humans [72] and mice [73] with lupus. However, anti-DNA antibodies are neither necessary nor sufficient for disease and certain strains of mice as well as humans have high titres of anti-DNA antibodies without evidence of nephritis. In addition, nephritis may be present in the absence of circulating anti-DNA antibodies. The mechanisms by which autoantibodies bind to the glomerulus of patients with lupus is debated. The glomerular basement membrane is negatively charged and it has been suggested that positively charged antibodies with an alkaline pI may preferentially locate there [74]. However, other studies have not confirmed this [75].

Patients with lupus have a high incidence of DNA [72, 76] and histones [77] present in their glomeruli compared to controls. These observations of the presence of nuclear material in lupus kidneys are consistent with several different mechanisms of immune complex localization in glomeruli and there is evidence from rodent perfusion studies to support each of these. These include complexes of antibody and DNA-binding to histones planted in glomeruli [78], complexes of antibody and intact nucleosomes binding to glomeruli [79,80], and binding of free antibody to planted nuclear material (DNA or nucleosomes) [81].

We have described how an antibody may localize in glomeruli. What is the evidence that autoantibodies cause glomerulonephritis? Much of the evidence discussed above suggesting a role for B cells indirectly shows a pathogenic role for autoantibodies. Strong evidence implicating antibodies in disease pathogenesis comes from a study in which NZB/W mice were made deficient for Fc γ receptors I and III. These mice did not develop glomerulonephritis despite deposition of immune complexes [82]. In parallel with these findings are data showing that complement activation by immune complexes in experimental models is not necessary for the development of glomerulonephritis [83].

However, a note of caution is necessary in weighing the balance between the roles of ligation of Fc receptors and the activation of complement in the induction of tissue injury by immune complexes. Significant production of anaphylatoxins and the membrane attack complex may play a proinflammatory role in lupus. In this context it is

noteworthy that treatment of lupus-prone mice with antibodies to C5 was found to ameliorate disease [84].

Conclusions

We have analysed the pathogenesis of lupus by considering firstly the role of genes and environment, secondly how an autoimmune response arises, and thirdly how this autoimmune response causes inflammation. This complex and fascinating disease is caused by the interaction of multiple genetic and environmental factors. Deficiency of classical pathway complement components are strongly associated with the development of lupus and this is likely to be due to a failure to clear apoptotic cells and/or immune complexes. Other genes that predispose to features of lupus have been found in mice, and may soon be identified in humans. Identification of these may open new avenues of treatment.

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