Supplement Review

Complement and systemic lupus erythematosus

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Chapter summary

Complement is implicated in the pathogenesis of systemic lupus erythematosus (SLE) in several ways and may act as both friend and foe. Homozygous deficiency of any of the proteins of the classical pathway is causally associated with susceptibility to the development of SLE, especially deficiency of the earliest proteins of the activation pathway. However, complement is also implicated in the effector inflammatory phase of the autoimmune response that characterizes the disease. Complement proteins are deposited in inflamed tissues and, in experimental models, inhibition of C5 ameliorates disease in a murine model. As a further twist to the associations between the complement system and SLE, autoantibodies to some complement proteins, especially to C1q, develop as part of the autoantibody response. The presence of anti-C1q autoantibodies is associated with severe illness, including glomerulonephritis. In this chapter the role of the complement system in SLE is reviewed and hypotheses are advanced to explain the complex relationships between complement and lupus.

Keywords: C1q, complement, glomerulonephritis, lupus, SLE

Introduction

The major discoveries that led to the modern era of work on systemic lupus erythematosus (SLE) were those of Hargraves *et al.* in 1947 of the lupus erythematosus (or 'LE') cell phenomenon [1], followed by the discovery of autoreactivity to nuclei [2,3] and to nucleoprotein [4]. Contemporaneous with these findings came the discovery that complement levels were abnormal in patients with SLE [5,6] and the discovery a few years later that complement was deposited in inflammatory lesions in tissues [7]. These findings led to a model for the pathogenesis of SLE in which autoantibodies formed immune complexes with their autoantigens, the resulting immune complexes activated complement, and the products of complement activation caused tissue injury and disease.

Subsequent findings have shown that the associations of complement with SLE are much more complex and it is now clear that complement may be friend as well as foe. The first finding was of a rare subgroup of patients with SLE with inherited homozygous deficiencies of certain complement proteins, particularly proteins of the early part of the classical pathway of complement activation. Subsequently it was discovered that up to a third of patients with SLE had high levels of autoantibodies to some complement proteins, especially to C1q, the very first protein in the classical pathway of complement.

These data allowed three deductions to be made about the association of complement with SLE. The first is that SLE is associated with complement activation, which may

A glossary of specialist terms used in this chapter appears at the end of the text section.

cause tissue injury. The second is that hereditary complement deficiency may cause SLE. The third is that the disease processes in SLE cause the development of autoantibodies to certain complement proteins. At first sight these statements appear to be mutually contradictory and difficult to reconcile one with the other.

In this chapter I will illustrate the evidence in support of these three deductions and develop some hypotheses that may explain these complex abnormalities of the complement system found in association with SLE.

The first section of this review will first describe the clinical associations of complement abnormalities with SLE, followed by the mechanisms of these associations. Finally a hypothesis will be proposed to explain the associations and consider the therapeutic implications.

Complement and SLE: the clinical observations

Complement is activated in SLE

Complement activation is easy to demonstrate in the plasma and tissues of patients with SLE. Indeed measures of complement activation are part of the standard repertoire of laboratory tests to which most patients with SLE are subjected on a regular basis. In this section I will describe the evidence for complement activation in plasma, on cells and in tissues. I will also discuss abnormalities that have been discovered in an important complement receptor, complement receptor type 1 (CR1), expressed on erythrocytes and cells of the immune system. I will review the evidence that measurements of complement activation correlate with disease activity, and the value of such measurements in the assessment and management of patients with SLE.

Complement activation in plasma

Assays of complement levels in serum are one of the standard assays used to assist the clinical management of patients with SLE [8]. The majority of laboratories measure antigenic concentrations of C3 and C4. A smaller number of laboratories also routinely provide a functional measurement of the activity of the whole complement pathway from classical pathway activation through to formation of the membrane attack complex, such as the CH50 (complement haemolysis 50%) test.

The dominant pathway for complement activation in SLE is the classical pathway, triggered by the interaction of C1q with immune complexes. Classical pathway complement protein levels are reduced in association with active disease, especially C1, C4 and C2 levels. Levels of C3 are typically at the lower end of the normal range and only occasionally severely depressed. Levels of C3 are maintained because of the regulatory mechanisms that control classical pathway complement activation *in vivo*, especially the activity of C4 binding protein that inhibits classical pathway activation [9]. When C3 levels are reduced, this is usually associated with reduced levels of factor B, indicating amplification of C3 turnover *in vivo* by the amplification loop of the alternative pathway [10,11].

Complement levels, however, provide a rather poor surrogate of clinical disease activity [12–14] for four reasons. Firstly, there is wide variation in normal complement protein levels between different individuals, partly due to genetic polymorphisms and partly for unknown reasons. The second reason is that protein levels are controlled by the balance of protein synthesis and catabolism, and the increase in catabolism of complement proteins caused by immune complexes in SLE is balanced by a variable response in complement protein synthetic rates between individuals [15–17]. As an additional factor that affects protein synthetic rates, many complement proteins are acute phase reactants, including C3 and C4. Such proteins display an increase in synthetic rate in response to inflammatory stimuli, which may compensate for the hypercatabolism of complement secondary to complement activation [18]. Thirdly, measurement of complement levels in serum may not reflect accurately what is occurring in tissues. This can be illustrated by diseases such as myasthenia gravis in which complement activation occurs at the motor neurone end plate [19,20], or membranous nephritis in which complement activation occurs at the subendothelial surface of the glomerular basement membrane [21,22]. Experimental models show that, in these diseases, complement activation is a key part of the inflammatory injury, but complement activation is virtually undetectable in peripheral blood. By contrast, when complement activation occurs in peripheral blood, for example in autoimmune haemolytic anaemia, then serum levels of C4 and C3 may be severely reduced. The fourth reason why complement levels are a poor surrogate of disease activity is that autoantibodies to complement proteins, especially to C1q, may be associated with profound activation of the complement pathway *in vivo*, and the level of complement activation may be associated with the levels of these autoantibodies rather than by disease per se. Autoantibodies to C1q are reviewed later in this chapter.

In patients with established disease, regular measurement of complement activity is a helpful guide to disease activity [23], though only as one of a series of assessments, of which the most important relate to clinical symptoms and signs of disease. In an attempt to improve the value of complement measurements in the assessment of disease activity, investigators have measured the levels of serum markers of complement activation, such as anaphylatoxin levels [24], C1r-C1s-C1 inhibitor complexes [18], C4d levels [25], iC3b or C3dg levels [26,27] and membrane attack complex levels [28,29]. Although there is evidence that measurement of these complement activation products correlates

somewhat better with disease activity than measurement of total C4 and C3 levels [30–32], none of these assays has been widely adopted in clinical practice. There are two reasons for this: each of these complement activation products is highly unstable *in vivo* and therefore difficult to measure with any useful reliability outside a research setting; and the plethora of different causes of complement activation in lupus, which were discussed earlier.

In spite of all of these caveats it is worthwhile to measure complement activity in patients with SLE. At the onset of disease, complement measurements may have diagnostic value. Evidence of complement activation is a marker of the family of diseases in which immune-complex-mediated pathology is prominent. Although a rare finding, it is also important to consider whether a patient might have an inherited complement deficiency underlying the disease. Assay of C4 and C3 levels alone may not alert the practitioner to the presence of complement deficiency. Indeed in the case of C1 deficiency, C4 and C3 levels may be high because of reduced consumption of classical pathway proteins [33]. For this reason, a functional assay of the pathway, such as a CH50, should be considered in all patients with suspected SLE.

The second diagnostic value of complement measurement in SLE is that evidence of marked complement activation should alert the clinician to one of three possibilities. The first is the presence of anti-C1q antibodies, which I will discuss below. The second is the presence of haemolytic anaemia; a Coombs' test should be performed in patients in whom this possibility is suspected. The third is the presence of active disease with the presence of glomerulonephritis. Finally, regular measurements of complement may disclose changes in complement levels that, when considered with the clinical findings and the results of other serological assays, may point towards changes in disease activity. There is a series of studies (reviewed in [34]) that show that changes in complement, anti-double-stranded (ds) DNA and anti-C1q antibody levels may predict the onset of disease flares [23,35–39].

Complement activation on cells and in tissues

Many tissues from patients with SLE show deposits of antibodies and complement proteins [7,40]. The demonstration of these in biopsies can be of diagnostic value, for example as part of the characterization of the histology of renal biopsies, or at the dermo-epidermal junction in the skin.

There is usually a correlation between the presence of antibody deposits and the presence of complement proteins. However, there is no simple correlation between the presence of antibody or complement deposits and the presence of tissue inflammation. For example, biopsies of clinically normal skin typically show deposits of antibody and complement at the basement membrane at the dermo-epidermal junction, and this has some diagnostic value for SLE, known as the 'lupus band' test. Attempts have been made to identify markers of complement activation in tissues that correlate better with the presence of inflammation. One such marker is the presence of the membrane attack complex, which is more prominent in inflamed tissues compared with clinically normal tissues from patients with SLE [41–44]. Complement may also be detected on the cellular elements in blood and this is especially prominent in patients with autoimmune haemolytic anaemia or thrombocytopenia as part of the spectrum of disease.

Red cells and CR1

Erythrocytes in primates bear a complement receptor known as complement receptor type 1, usually abbreviated as CR1 (reviewed by [45]). Alternative names for this protein include the immune adherence receptor and CD35. The receptor has specificity for C3b, C4b and iC3b, in order of binding strength (C3b highest). On erythrocytes CR1 is present at low copy number (between 70 and 700 molecules per cell) but is organized in clusters that allow high avidity binding. Erythrocyte CR1 levels in patients with SLE are reduced, and before discussing the explanation and possible significance of this finding, a brief preamble will be given describing the biology of this interesting molecule.

Complement receptor type 1 has several biological activities. It acts as a binding and transport molecule for particles in the circulation bearing C3b clusters, for example parasites and bacteria. Red cells are thought to act as particulate carriers of pathogens bearing C3b and C4b in the circulation, transporting them to the fixed mononuclear phagocytic system in the liver, spleen and bone marrow.

Complement also has a biochemical activity as a cofactor to the serine esterase factor I in the cleavage of C3b to inactive products. Factor I cleaves C3 sequentially to iC3b, then to C3dg plus C3c. Factor I may use one of several cofactors for the first cleavage reaction, including the plasma protein, factor H, and the cell membrane proteins CR1 or membrane cofactor protein (also known as CD46). However, CR1 is the sole known cofactor for the second cleavage reaction by factor I of iC3b to C3dg and C3c.

Complement receptor type 1 is also present on other cell types, including B cells (on which ligation of complement receptors modulates the threshold for B cell activation), neutrophils and other phagocytic cells on which CR1 contributes to the recognition, uptake and destruction of particles such as pathogens or immune complexes carrying C3 and C4 split products. Finally CR1 is present on antigen-presenting cells and reservoirs of antigen such as follicular

dendritic cells that act as a long-lived 'sump' of foreign antigens, preserving immunological memory.

It had been known since the 1930s that there was variation between normal humans in the expression of CR1 levels on erythrocytes [46], and a study in the 1960s provided evidence that this variation was inherited [47]. These studies were lost in the pre-Medline mists but interest in CR1 in SLE was aroused when it was discovered that erythrocyte CR1 levels were reduced on the erythrocytes of patients with SLE [48] and evidence emerged that inherited factors might contribute to this reduction [49,50]. However, there is now a large body of evidence (reviewed in [51]) that shows that CR1 levels on erythrocytes from patients with SLE are reduced as a consequence of disease activity [52]. In addition, although there is a genetic polymorphism that determines CR1 levels on normal erythrocytes [53], the distribution of the allele of CR1 associated with reduced CR1 expression shows no increase in frequency amongst patients with SLE [54,55].

Most data now point to low levels of CR1 in SLE being caused by the removal of the receptor from the cell. This is associated with evidence of complement activation in the vicinity of red cells with low CR1 numbers (i.e. a correlation has been found between raised levels of C3dg deposited on erythrocytes and reduced CR1 numbers [56]). Erythrocytes that were transfused into patients with SLE rapidly lost CR1 expression from the cell surface [57]. Similarly, when the formation of immune complexes *in vivo* was monitored in humans, clearance of immune complexes was accompanied by deposition of C4 and C3 on erythrocytes and loss of CR1 from these cells [58]. Proteolysis of the receptor, which is highly susceptible to cleavage by trypsin-like enzymes [59], is the most likely mechanism for loss of the receptor from erythrocytes [60,61]. Studies in nonhuman primates have shown that when erythrocytes are infused bearing immune complexes ligated to CR1 there is concerted removal of immune complexes from erythrocytes together with a reduction in CR1 levels [62–64]. This reaction, including the loss of CR1, has been modeled *in vitro* by studying the transfer of immune complexes from erythrocytes to macrophages [65]. Indeed, loss of erythrocyte CR1 may be a physiological release mechanism that facilitates the transfer of opsonised immune complexes and pathogens from red cells to tissue macrophages.

CR1 levels are also low on certain other cell types in SLE, most notably on glomerular epithelial cells [66,67]. Soluble CR1 was detected in the urine of patients with SLE on microvesicles that were derived from kidney tissue, most probably from glomerular podocytes [68]. Both enhanced microvesiculation and proteolytic cleavage are candidates for the mechanism of removal of CR1 from podocytes in SLE [69,70].

Autoantibodies to complement proteins in patients with SLE

A series of autoantibodies have been described that bind with high affinity to complement proteins. The majority of these are directed to 'neoepitopes'. These are defined as epitopes that are not expressed in the native protein and are only exposed in protein that has been modified by a change in structure. Such a change in structure to reveal a neoepitope may follow proteolytic cleavage, a conformational change following activation or following binding of the protein to another protein. Amongst the earliest autoantibodies to complement proteins to be identified were immunoconglutinins and these have been found in the sera of patients with SLE [71,72]. These autoantibodies bind to an activation product of C3 known as iC3b and are something of an esoteric curiosity since, although quite common, they have never been shown to play any direct role in disease.

In contrast, subsequently identified autoantibodies to complement proteins are more malign. They include C3 nephritic factor, associated with partial lipodystrophy, and a form of mesangiocapillary glomerulonephritis with electron dense deposits in glomerular basement membranes. A small number of patients with C3 nephritic factor and SLE have been identified [73]. Another important, albeit rare, autoantibody to a complement protein is anti-C1 inhibitor autoantibody [74,75], occurring particularly in some patients with lymphoma [76–78].

In SLE, the most important autoantibody to a complement protein is anti-C1q, which is found in approximately a third of patients with SLE. In the next few paragraphs I will review the nature and significance of anti-C1q autoantibodies. Before doing so I should note that other autoantibodies to complement proteins that have been described in a small number of patients with SLE are against CR1 [79,80] and against the C4b2a C3 classical pathway convertase enzyme [81]. These antibodies are similar to immunoconglutinins in being of doubtful pathological significance.

Autoantibodies to C1q in hypocomplementaemic urticarial vasculitis

The major clinical features of the clinical syndrome hypocomplementaemic urticarial vasculitis (HUVS) are well encompassed by its cumbersome name. This syndrome is associated with evidence of intense activation of the classical pathway, with very low levels of C1q, C4 and C2, and moderately reduced levels of C3, in serum samples from patients. This syndrome is defined serologically by the presence of very high titres of autoantibodies to C1q [82], which were identified originally as C1q precipitins [83]. These autoantibodies react with a neoepitope in the collagenous region of C1q that is not exposed in the C1 complex, comprising one molecule of C1q combined with two C1r and two C1s molecules. The epitope

is only exposed when the C1 complex is activated with the removal of the C1r and C1s molecules in combination with C1 inhibitor.

In addition to chronic urticaria, with its accompanying cutaneous leukocytoclastic vasculitis, other clinical features of the disease can include angioedema, glomerulonephritis, neuropathy and airways obstruction [84]. No other autoantibodies have been identified in HUVS but some of the features of this syndrome may be found in patients with 'full-blown' SLE who also have anti-C1q autoantibodies. The relationship between HUVS and SLE is analogous to that between the primary antiphospholipid syndrome and SLE. In the case of the latter association, patients with the primary antiphospholipid syndrome have a clinical syndrome of recurrent thromboses and abortions in the presence of autoantibodies to cardiolipin and to β2-glycoprotein-I. Approximately a third of patients with SLE also have these autoantibodies as part of their autoantibody spectrum and may also have some of the clinical features of the antiphospholipid syndrome.

Anti-C1q autoantibodies in SLE

During the 1970s and 1980s there was a great deal of interest in trying to measure pathogenic immune complexes in those diseases that were thought to be mediated by immune complexes (reviewed in [85]). Very many assays were devised, including two based on the capacity of immune complexes to activate complement by the binding of C1q. These assays were the fluid phase and solid phase C1q-binding assays. The fluid phase assay was conducted by adding radiolabelled C1q to serum; some of this bound to immune complexes and the bound material could be measured as a coprecipitate with the immune complexes by addition of polyethylene glycol [86,87]. The solid phase C1q-binding assay was conducted by incubating serum samples in microtitre plates coated with C1q [88]. Immune complexes were thought to bind to the C1q and could be detected using labelled antiglobulins that bound to the antibodies within the immune complexes. Positive results for 'immune complexes' using both of these assays were found in patients with SLE and correlated with the presence of active disease, the presence of hypocomplementemia and elevated titres of anti-DNA antibodies [86,87,89,90]. There was a poor correlation, however, between results obtained using the fluid phase and solid phase C1q-binding assays [91]. It was subsequently discovered that the explanation for this discrepancy was that the solid phase C1q-binding assay is mainly a measure of autoantibodies to C1q rather than of immune complexes. This was discovered in the following way.

Agnello and colleagues [83] had observed that C1q precipitins could be characterized in a small number of patients with SLE and some of these sedimented at 7S, the sedimentation constant of monomeric IgG. These observations were confirmed in two further studies of the size of 'immune complexes' in the sera of patients with SLE [92,93]. These results raised the possibility that the C1q binding activity was attributable to an autoantibody rather than to circulating immune complexes that could bind C1q. This was found to be the case [94–96] and it was shown that the majority of IgG binding to C1q in solid phase assays was attributable to autoantibodies reacting with a neoepitope in the collagenous portion of C1q [97].

The assay has now been adapted to provide a specific measurement of anti-C1q autoantibodies. The adaptation to give this specificity is simply the addition of sodium chloride (1M) during the incubation step of serum with the C1q-coated plates. This high ionic strength prevents the binding of immune complexes to C1q but is insufficient to prevent high affinity binding of antibody to antigen [98].

The ready availability of simple quantitative assays for autoantibodies to C1q has allowed many studies of the prevalence and clinical association of C1q autoantibodies in patients with SLE (reviewed in [99]). The main conclusions of these are as follows. Approximately a third of patients with SLE have elevated levels of anti-C1q antibodies and these patients often have severe disease [100]. Several studies have shown an association between the presence of anti-C1q autoantibodies and glomerulonephritis [39,101–105]. There is usually evidence of intense activation of the classical pathway of complement, with very low C1q and C4 levels. A small number of patients with high titres of anti-C1q autoantibodies have been described with angioedema, resembling that of hereditary angioedema. These patients have low levels of C1 inhibitor and of classical pathway complement proteins [106]. Levels of C3, which tend to be well maintained in patients with SLE, probably because of effective regulation of classical pathway activation by C4 binding protein, may be substantially below the normal range in patients with anti-C1q autoantibodies [107]. Autoantibodies to C1q have been described in mice with lupus-like disease as well as in humans [108,109].

The association between the presence of anti-C1q autoantibodies and classical pathway, both in HUVS and SLE, leads to the obvious question, which is whether the C1q autoantibodies cause or amplify the classical pathway activation. There is not a certain answer to this. Simply adding anti-C1q antibodies to normal serum does not cause complement activation [110], which may reflect the fact that these autoantibodies are to a neoepitope of C1q not expressed in the intact C1q complex. A more likely explanation is that anti-C1q antibodies fix to C1q that is bound to immune complexes on cells and within tissues and that this causes the amplification of the complement activation by immune complexes. There is some

experimental evidence in support of this hypothesis [111]. An alternative theory is that anti-C1q antibodies arise in response to intense activation of the classical pathway with large-scale exposure of an autoantigenic neoepitope in C1q, and that the anti-C1q antibodies are a consequence and not a cause of the complement activation. As I will discuss below, the correct answer may be a combination of these hypotheses.

Complement deficiency is associated with the development of SLE

Homozygous hereditary deficiency of each of the early proteins of the classical pathway of complement activation is very strongly associated with the development of SLE [112]. Indeed such deficiencies are the strongest disease susceptibility genes for the development of this disease that have been characterised in humans. The association shows a hierarchy of prevalence and disease severity according to the position of the protein in the activation pathway. The most prevalent and most severe disease is associated with deficiency of the proteins of the C1 complex and with total C4 deficiency. More than 75% of all individuals with deficiency of one of these proteins have SLE, which is commonly severe. By contrast, C2 deficiency is associated with a much lower prevalence of disease, estimated at approximately 10%. Deficiency of C3 is only very uncommonly associated with the development of SLE, but because of the rarity of homozygous C3 deficiency, only limited data exist [113].

These clinical data suggest that there is an activity of the early part of the classical pathway of complement that is protective in normal individuals against the development of SLE. Deficiency of this activity predisposes very strongly to the development of disease, as shown by the observation that almost every human, so far identified, who lacks C1q has developed SLE.

It is always a concern that a reported disease association is due to an ascertainment artefact rather than due to a true causal association. In the case of complement and SLE, it could be argued that because complement is frequently measured in patients with SLE, it is not surprising that complement deficiency turns up in these patients.

There is a group of arguments that militate against this artefactual explanation for the association. Complement deficiency has been sought in large normal populations, but no classical pathway deficiencies have been identified. The clearest example is the enormous study of Japanese blood donors, amongst whom no hereditary classical pathway deficiency states were found [114,115]. There is a reported Japanese patient, however, with C1q deficiency and SLE [116]. Another argument is that the observed intrafamilial disease concordance for SLE in combination with complement deficiency is extremely high. In the case of C1q deficiency it is more than 90%, a figure that far exceeds the observed concordance of SLE between siblings in monozygotic twin pairs (24%) [117]. The third argument is that disease associated with hereditary complement deficiency tends to be of early onset and the male to female ratio approximates to unity, unlike the high female preponderance amongst the majority of SLE patients without complement deficiency.

Each of these observations illustrates the strength of the disease association and provides evidence that the association between complement deficiency and SLE is not likely to be explained by ascertainment artefact. A further argument for a causal link is the finding that acquired complement deficiency states also predispose to the development of SLE. There is an increased prevalence of SLE amongst patients with hereditary angioedema who, because of inherited partial deficiency of C1 inhibitor, fail to regulate classical pathway complement activation [118–120] and amongst patients with C3 nephritic factor [73].

This evidence leaves no doubt that homozygous classical pathway protein deficiencies are causally associated with the development of SLE. There is less certainty in the case of partial inherited complement deficiencies. There has been particular interest in the associations between partial deficiencies of C4 and SLE. In humans, C4 is encoded by two tandemly duplicated genes within the major histocompatibility complex (MHC), encoding respectively isotypes known as C4A and C4B [121]. In common with other MHC genes, each of the C4 genes is highly polymorphic and, included in the polymorphisms, are null alleles from which no protein is produced. These null alleles are very common and approximately 6% of most populations have homozygous deficiency of either C4A or C4B. It should be noted that total C4 deficiency is exceptionally rare because haplotypes encoding null alleles at both the C4A and C4B loci are very rare. The functions of the two C4 isotypes differ slightly. The internal thioester bond of C4A shows preferential binding to amino groups with the formation of covalent amide bonds between C4A and proteins. Preferential binding to hydroxyl groups is shown by C4B, typically in carbohydrates, forming ester linkages [122].

There are disease susceptibility genes for SLE in the MHC (reviewed in [123,124]). Following the discovery of the two isotypes of C4 and the existence of null alleles at both loci there have been many studies to attempt to determine whether null alleles of C4 might play a role in determining disease susceptibility. Initial studies showed an increased prevalence of C4A null alleles in Caucasoid patients with SLE [125,126]. However, null alleles for C4A are found commonly in a particular MHC haplotype, HLA-A1, B8, C4AQ0, C4B1, DR3, and it is exceptionally difficult to determine which is the real susceptibility gene or genes

for SLE and which are the 'passengers'. The literature abounds with case-control and family studies either confirming or refuting associations between C4 null alleles and SLE. A definitive answer is likely to emerge in the next few years as large scale genotyping methods are applied to the very large family collections of patients with SLE that are being formed around the world.

What is the explanation for these clinical findings?

The role of complement in the pathogenesis of inflammatory lesions in SLE

The traditional view of the pathogenesis of SLE is that immune complexes containing autoantigens and autoantibodies activate complement, and that this causes inflammatory injury to tissues. Although this model is biologically plausible, it cannot account for all of the clinical observations that link the complement system and SLE. In particular, the observation that complement deficiency causes lupus is hard to reconcile with the concept that complement activation products are the major cause of inflammatory injury in the disease.

The development of gene-targeted strains of mice that lack individual protein constituents of inflammatory pathways has led to a plethora of apparently inconsistent findings on mechanisms of immune-complex-mediated inflammation. There is a large array of mice lacking complement proteins and Fc receptors, and an equally large array of studies of these mice. In this section the mechanisms of complement activation in SLE will be reviewed briefly and the pathogenesis of inflammation caused by immune complexes will be considered.

How is complement activated in SLE?

The cause of complement activation in SLE is the formation of immune complexes, which in turn activate complement, predominantly by means of the classical pathway. Complement activation is normally measured in clinical practice by estimation of antigenic levels of both C3 and C4, and measured functionally using the CH50 assay. In the majority of patients with moderate or active SLE, reduced levels of C4 are detected, with the level of C3 varying between normal to slightly reduced. The CH50 is typically below normal.

Up to 25% of patients with SLE, however, may have much more dramatic reductions in C4 levels, typically associated with significantly subnormal C3 levels and CH50. Three subsets of patients make up the majority of these individuals. There are those subjects who have evidence of autoimmunity to red blood cells. This may manifest as overt autoimmune haemolytic anaemia, but it may be more subtle and be detectable only by means of direct antiglobulin test (Coombs' test) positivity for IgG, C3 and C4 deposition on red cells. Some of these patients also have antiphospholipid (also known as anticardiolipin) autoantibodies. A study in a murine model of the antiphospholipid syndrome has recently shown that antiphospholipid antibodies cause foetal resorption by means of complement activation in the decidua in the pregnant uterus [127]. The complement activation at this site was shown to cause inflammatory injury, which appeared to be responsible for the foetal death and resorption.

Another subset comprises patients with very systemic disease, typically associated with high levels of anti-dsDNA autoantibodies and, sometimes, with the presence of type III cryoglobulins [128] in serum containing polyclonal IgG and C3.

The third group of patients comprises those individuals who have anti-C1q autoantibodies. These subjects often have the most severe evidence of classical pathway complement activation, with profoundly reduced C4 levels and moderate to substantial reductions in C3 levels. It is thought that anti-C1q autoantibodies are the cause and not the consequence of the complement activation measured in serum, and possible mechanisms were reviewed above.

Mechanisms of immune-complex-mediated injury

Until very recently it was believed that tissue injury in SLE was caused by the formation of immune complexes that caused complement activation, which in turn caused inflammatory injury. Tissue injury was thought to be mediated by the direct effects of activation of the triggered enzyme cascades of complement, coagulation and kinin pathways, coupled with an influx of inflammatory cells, including polymorphonuclear leukocytes and monocytes. The activities of complement in the mediation of inflammation include the ligation of complement receptors for the opsonic components of complement subcomponents C3b and C4b, the effects of the anaphylatoxins C5a and C3a, and the effects of insertion of sublethal amounts of the membrane attack complex into cell membranes.

The development of mice with null mutations in selected proteins of inflammatory pathways has enabled the precise dissection of their role in both host defence and the causation of inflammatory injury. There are many experimental models of injury caused by immune complexes and the use of these in gene-targeted mice has led to a reappraisal of the role of complement and Fc receptors in inflammatory responses to immune complexes. The major conclusions from these experiments are as follows. Firstly, it is clear that all immune complexes are not equal in the manner in which they cause tissue injury. The site of immune complex formation [129], the species [130] and strain [131] of animal, and the nature of the antigen [132], as well as the antibody, may affect the inflammatory response. Secondly, in many models it has been found that the ligation of Fc receptors by immune complexes is

the dominant cause of tissue injury, and complement plays no important role in the induction of tissue injury [133]. Indeed, in experimental models of complement deficiency, it has been shown that immune-complex-mediated glomerulonephritis can develop spontaneously in the presence of genetic deficiency of the complement activation pathways [134,135]. In other experimental models, however, complement activation, and in particular C5a production, have been found to be essential for the full expression of tissue injury [136]; inhibition of C5a activity is therapeutic [137]. Thirdly, it has been found that complement may provide some degree of protection against inflammatory injury induced by immune complexes in some models of experimental glomerulonephritis [138]. We will consider possible mechanisms for this in a subsequent section of this chapter. Finally, it is clear that in mice and humans, inflammatory pathways that operate downstream from the activation of complement, and the ligation of Fc and complement receptors play crucial roles in the expression of inflammation that is mediated by immune complexes [139].

Complement deficiency and SLE

The associations of complement deficiency with SLE have been reviewed in the Introduction. Here we shall consider three hypotheses that have been advanced to explain the mechanism of the association. The first two of these are closely related, proposing mechanisms that could operate in tandem. These hypotheses are that complement prevents the development of SLE through a role in the processing and clearance from the body of immune complexes, and dying and dead cells. In the absence of these activities, autoantigens may be presented to the immune system in the context of inflammatory injury and this may drive the development of autoimmunity. The third hypothesis invokes a role for complement in the development of self-tolerance to the autoantigens of SLE, and proposes that B cells with specificity for lupus autoantigens are not effectively silenced or eliminated in the absence of complement. Each of these hypotheses will be explored briefly in this section.

Complement and immune complex processing

Michael Heidelberger in the 1940s demonstrated a role for complement in the modification of immune complex lattices [140]. Since then there have been many studies that showed that complement promotes the inactivation and clearance of immune complexes by two main mechanisms. One of these is the reduction in the size of immune complex lattices (reviewed in [141,142]). This is achieved by the interaction of C1q with immune complexes, which interferes with Fc–Fc interactions that stabilize immune complexes, and by the covalent binding of C4b and C3b to antigens within the immune complexes. This binding interferes with the binding of antigen to antibody by effectively reducing the valency of antigen for antibody. As well as reducing the size of immune complexes, complement provides additional ligands within the immune complex, promoting the clearance of immune complexes by complement as well as Fc receptors. As discussed in earlier in this chapter, immune complexes that have bound C4b and C3b can bind to CR1 on erythrocytes in the circulation that promote the clearance of immune complexes to the fixed mononuclear phagocytic system.

These observations led to the hypothesis that complement deficiency may promote the development of SLE by impairment of the normal mechanisms for clearance and processing of immune complexes. These could cause inflammatory injury in tissues, resulting in the release of autoantigens in an inflammatory context, promoting the development of an autoimmune response [143]. There is abundant evidence that immune complex processing in SLE is abnormal and related to abnormal complement function [144–146]. Both the discovery that complement deficiency is compatible with the normal spontaneous development of glomerulonephritis in murine models of SLE [134,135] and the surprising finding that induced glomerulonephritis may be exacerbated in the presence of complement deficiency [147] are compatible with this proposed role for complement in protection against immune-complex-mediated injury.

This hypothesis is complementary to and was the precursor of the 'waste disposal' hypothesis, developed in the next section, which advances the hypothesis that complement provides protection against the development of SLE by impairment of the physiological waste disposal of autoantigens released by dying and dead cells.

Complement and the clearance of apoptotic cells

A central question about the aetiology of SLE is to understand how an autoimmune response develops to autoantigens that are found ubiquitously in cells of the body. There is abundant evidence that the established autoantibody response in SLE is driven by the actual autoantigens (as opposed to being part of a polyclonal antibody response or driven by cross-reacting antigens). What is the source of these autoantigens? Cell death is an obvious potential source of autoantigens that are otherwise hidden from immune receptors in the heart of living cells. However, cell death is a highly regulated process and the normal mechanisms of apoptosis ensure that dying cells are cleared without the induction of tissue inflammatory responses [148]. One possible source is dead or dying cells from sites of inflammation and tissue injury. A possible connection between apoptotic cells and the autoantibody response of SLE was established by Rosen and his colleagues, who showed that a number of lupus autoantigens were located at the surface of apoptotic bodies and on apoptotic blebs [149].

These observations have been followed by a series of studies of the mechanisms of apoptotic cell clearance in SLE. It was shown that macrophages derived from peripheral blood of patients with SLE showed defective uptake of apoptotic cells [150]. The idea that complement might play a role in the clearance of apoptotic cells came from the observation that C1q bound to apoptotic keratinocytes [151]. An excess of apoptotic cells was observed in kidneys from C1q-deficient mice [152]. It was later shown that elicited peritoneal macrophages from C1q-deficient mice showed defective clearance of injected apoptotic thymocytes and the human lymphocyte cell lines known as Jurkat cells [153]. Similarly, monocyte-derived macrophages from a small number of C1q-deficient humans showed defective clearance of apoptotic cells, a defect that could be reversed by the addition of purified C1q. It was also found that the clearance of apoptotic cells could be mediated by bound C3 [154], although there may be an additional role for earlier proteins of the classical pathway in mediating and/or augmenting apoptotic cell clearance. This work has led to the hypothesis that complement plays a role in the prevention of autoimmunity through a role in the disposal of dying and dead cells. Absence of this activity, possibly occurring in the context of an inflammatory environment, may promote the development of an autoantigen-driven autoimmune response [155,156]. It has also been shown that C4-deficient mice are prone to the development of lupus-like autoimmunity [157,158], which provides further evidence that complement-deficient mice are a useful model to study the association between complement deficiency and SLE, first described in humans.

The steps from defective clearance of apoptotic cells to the development of an autoimmune response remain unknown. At least in mice, there is evidence that other genes contribute to the autoimmune phenotype in the context of C1q deficiency, which is associated with lupus in mice on a hybrid (129 strain × C57/BL6 strain) background. It also causes disease acceleration in the lupus prone MRL.mp mouse. However, no autoimmunity is seen in C1q-deficient mice on 129 and C57/BL6 inbred strains [152,159]. These data show the necessity for the presence of other disease-modifying genes that enable the potential autoimmune consequences of C1q deficiency to be expressed in mice. In humans, the strength of the contribution of C1q deficiency to disease appears higher than in mice, as the majority of humans with C1q deficiency express some manifestations of SLE [112]. However, only approximately a third of patients develop glomerulonephritis, again indicating that C1q deficiency is not sufficient for the development of glomerular inflammation. Lupus in humans and mice is subject to the influence of multiple genetic and, probably also environmental, factors.

Complement and the induction of tolerance

An alternative hypothesis to explain the link between complement deficiency and SLE is that complement plays a role in the induction of tolerance to autoantigens [160]. Deficiency of this activity disturbs the normal tolerance mechanisms of lymphocytes leading to the induction of SLE. This hypothesis is difficult to reconcile with the observations that the complement system plays a physiological role in the augmentation of antibody responses. Pepys showed many years ago that depletion of complement C3 was associated with reduced primary and secondary antibody responses to T-cell-dependent antigens [161], a finding that has subsequently been reproduced in complement genetically-deficient mice and guinea pigs [162,163]. The corollary of these observations was the engineering of complement as an adjuvant by the covalent attachment of oligomers of C3d to an experimental antigen [164].

Using a transgenic model to study tolerance to hen egg lysozyme, expressed as an autoantigen, it was found that tolerance was disturbed in C4-deficient, but not C3-deficient, mice [165]. In similar experiments with C1q-deficient mice, however, tolerance induction to hen egg lysozyme as an autoantigen was normal [166]. This model of tolerance induction has important limitations as a model for exploring SLE, as in this disease, the majority of the autoantigens are cell-associated, as will be reviewed in the next section. Experimental studies are underway exploring the role of complement deficiency in tolerance induction in experimental animals expressing transgenic lupus autoantibodies, which may provide a clearer test of the hypothesis that complement is involved in the induction of tolerance.

A unifying hypothesis that links C1q deficiency, C1q autoantibodies and SLE

A prominent feature of the autoantibody response in SLE is that it is directed not against isolated proteins but typically against whole complexes of proteins and nucleic acids [167–169]. Autoantibodies are found in clusters reactive against the different protein, nucleic acid and phospholipid components of these complexes. For example anti-dsDNA autoantibodies are usually associated with antihistone autoantibodies and antibodies reacting with conformational determinants of chromatin. The autoantigens in this case are thought to be nucleosomes. Anti-Sm and antiribonucleoprotein specificities are directed against different proteins in the spliceosome complex. Anti-Ro and anti-La are directed against the major protein component of a small cytoplasmic ribonucleoprotein complex. These clinical findings point to actual autoantigenic particles in lupus being these large complexes and there is evidence that B cells play a key role as antigen-presenting cells in lupus [170–172]. A B cell bearing an antigen receptor for a component of one of

these complexes can internalize the complex and then act as antigen-presenting cell for other peptides within the complex. In this way it is likely that the autoantibody response in SLE is diversified and amplified.

How do these data explain how on the one hand C1q deficiency causes SLE and on the other that SLE causes an autoantibody response to C1q? The hypothesis has been reviewed above that C1q and other complement proteins prevent lupus by binding to apoptotic and necrotic cells and promoting their clearance. The absence of C1q promotes autoimmunity by allowing these autoantigens to drive an autoimmune response.

This hypothesis implies that C1q binds to the autoantigens of SLE. In doing so, it may itself become part of the autoantigenic complexes that characterize the disease. Although it is unusual for plasma proteins to be targets for the autoimmune response in SLE, there is an important and informative analogy. Approximately a third of patients with SLE develop antiphospholipid autoantibodies. These are directed against negatively charged phospholipids, especially phosphatidylserine, which normally reside on the inner lamella of cell membranes. However, they are translocated to the outer lamella of apoptotic cells, which reinforces the possible role of these cells as the source of the autoantigens that drive SLE [173,174]. Antiphospholipid autoantibodies in SLE are typically associated with an autoantibody response to the plasma proteins β2-glycoprotein-I and annexin V, that binds to negatively-charged phospholipids [175,176].

Thus, the observation that C1q is itself an autoantigen in many patients with SLE reinforces the hypothesis that C1q and the complement system may prevent disease by binding to and promoting the clearance of autoantigens.

Complement and the treatment of disease

It is not straightforward to treat SLE by the manipulation of the complement system. On the one hand complement deficiency, as we have seen, is a powerful cause of SLE and this provides arguments for increasing complement activity by repletion of classical pathway proteins, at least in the case of the rare hereditary deficiencies. On the other hand, there is evidence that complement, especially the anaphylatoxins and the membrane attack complex, may be a cause of tissue injury and this raises the possibility of the treatment of SLE by therapeutic inhibition of the complement system. Both approaches to therapy have been attempted in different contexts in SLE and will be reviewed in the next few paragraphs.

Complement is a potent cause of tissue injury in ischaemia-reperfusion injury and therefore is a therapeutic target in patients with myocardial infarction and stroke. Because these are highly prevalent and disabling diseases there have been many studies that have validated the potential importance of complement inhibition as a treatment for them. There are several reviews that illustrate the range of diseases that are under investigation and the many approaches to treatment by inhibition of the complement system [177–182].

Anti-C5 therapy

There is increasing evidence that the key mediators of injury induced by the complement system are the anaphylatoxins, especially C5a, and the membrane attack complex. An anti-C5 monoclonal antibody reduced the expression of glomerulonephritis and increased the lifespan of NZB/W F1 hybrid mice [183]. Similar anti-C5 antibodies have also been found to be therapeutically effective in other disease models where complement is thought to play an important role, such as ischaemia-reperfusion injury in the heart [184] and hyperacute xenograft rejection [185]. There have been preliminary studies of this approach to treatment in humans undergoing coronary artery bypass surgery on cardiopulmonary bypass, which is known to be a potent means of activating the complement system [186].

These studies, however, have to be balanced against the findings that lupus nephritis may develop in humans lacking classical pathway proteins and in mice which are either unable to activate C3 [134] or genetically deficient in this protein [135]. Trials have been proposed of anti-C5 therapy in patients with SLE and nephritis.

Complement repletion therapy

Given that deficiency of classical pathway complement proteins is a potent cause for the development of SLE, one obvious approach to therapy is replacement of the missing protein. However, there are potential pitfalls to this approach. Purified or engineered complement proteins are not available for treatment purposes. Therefore, whole plasma preparations have to be used, which entail all of the complications of plasma treatment, including hypersensitivity reactions and the potential for transmitted viral infections. Another pitfall is the possibility that replacement of a missing complement protein in a patient with months or years of accumulated immune complexes in tissues may be followed by complement activation in tissues causing inflammatory injury. A further issue is that, following exposure to a protein that is genetically deficient and therefore 'foreign', antibodies may develop, preventing treatment by replenishment.

There are several anecdotal reports of the treatment of complement-deficient patients with plasma; benefit has been reported in two patients with C2 deficiency [187,188]. There was no significant clinical response in a patient with C1q deficiency who had received briefly fresh-frozen plasma, but antibodies developed to C1q [189].

Treatment with plasma of two dogs with hereditary C3 deficiency and mesangiocapillary glomerulonephritis was not successful and led to a worsening of disease [190].

Many tissues synthesize complement proteins. The bone marrow is the major source of C1q [191] and this raises the possibility that C1q replenishment in patients with C1q deficiency and severe SLE might be achieved by bone marrow transplantation.

C1q absorption

Another approach that is undergoing experimental evaluation in small numbers of patients with SLE is immunoabsorption by perfusion of plasma over columns coupled with C1q [192,193]. These columns can remove anti-C1q autoantibodies from serum and may also be capable of extracting immune complexes from the circulation. This is an interesting approach, particularly in the light of the evidence discussed above that anti-C1q antibodies may play a pathogenic role in disease. It is too early to know whether this approach will have any important role to play in the management of SLE.

Glossary of terms

C = complement component; CH50 = complement haemolysis 50% test; CR = complement receptor; HUVS = hypocomplementaemic urticarial vasculitis.

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