Complement is an important effector pathway of innate immunity. In this review the role of complement in the pathogenesis of systemic lupus erythematosus (SLE) is described. There are important associations between both inherited and acquired complement component deficiencies and SLE. These have focused attention on identifying the relevant physiological role of the classical pathway of complement activation that appears to protect against the development of SLE. There is also unequivocal evidence that major histocompatibility complex (MHC) genes play an important role in determining both disease susceptibility and phenotype in SLE and its subsets. Accordingly, the role of complement genes located in the class III region (C4A, C4B and C2) in the genetic susceptibility to SLE is reviewed. The utility of the measurement of complement levels in monitoring disease activity and the clinical significance of hypocomplementaemia are discussed. Homozygous C1q deficiency is the strongest genetic susceptibility factor for SLE that has been identified in humans, but, paradoxically, SLE causes C1q consumption and is commonly associated with anti-C1q antibodies. The role of complement in the pathogenesis of lupus, with particular emphasis on the role of C1q, is discussed in order to explain this paradox.

Homozygous classical pathway component deficiency and SLE

Homozygous hereditary deficiency of each of the classical pathway components (C1q, C1r, C1s, C4, and C2) is associated with an increased susceptibility to SLE [1]. Both the severity of disease and the strength of this association is greatest for homozygous C1q deficiency followed in turn by homozygous C4 and C2 deficiency. Thirty-eight of the 41 patients with homozygous C1q deficiency reported to date have developed a clinical syndrome closely similar to SLE (recently reviewed in [2]). In the affected patients rash occurred in 36, glomerulonephritis in 16, and cerebral disease in seven. Antinuclear antibodies (ANA) were reported in 24 and antibodies to extractable nuclear antigens in 15 of these patients, but notably, the incidence of anti-double-stranded DNA antibodies was low, positive in only five of the 24 patients tested.

Hereditary deficiency of C1s and C1r is rarer than that of C1q deficiency and, in the majority of cases, deficiencies of both components coincide [3–5]. In such cases C1r levels are usually absent with levels of C1s below 50% of normal. As in homozygous C1q deficiency, SLE occurs in the majority of these individuals (seven of the 11 reported cases). Selective C1s deficiency, i.e. with normal C1r levels, has been reported in association with SLE [6]. In homozygous C4 deficiency, the prevalence of SLE is approximately 75% and the lupus illness is of moderate severity [7].

Homozygous C2 deficiency is the commonest inherited classical pathway complement deficiency with an approximate prevalence in Western European Caucasoid populations of 1:20 000. In contrast to homozygous C1q deficiency, the majority of affected individuals are probably healthy. SLE may occur in up to 33%, although this figure may be an overestimate due to ascertainment artefact, and the severity of the illness is comparable to that seen in SLE patients without homozygous complement deficiency. In contrast to the classical pathway components, homozygous C3 deficiency predisposes to recurrent pyogenic infections and membranoproliferative glomerulonephritis (MPGN), but SLE is rare [8].

What do the clinical observations in individuals with homozygous classical pathway component deficiency teach us about the pathogenesis of SLE? They suggest that there is a physiological function of the classical pathway of complement activation that protects against the development of SLE. Furthermore, the hierarchy of susceptibility and the severity of lupus, according to the missing classical pathway protein (C1q > C4 >> C2), suggest that the early part of the classical pathway plays a key protective role against the disease. Since one of the major roles of the classical pathway is that of host defence against infectious disease, one hypothesis is that classical pathway component deficiency predisposes to SLE because of impaired resistance to an infectious trigger.

There is little evidence in favour of this hypothesis. Classical pathway complement deficiencies are not associated with an overt increase in susceptibility to viral or fungal infections. Although complement deficiency is strongly associated with the development of pyogenic
bacterial infections, such infectious agents are not strong candidates for the induction of SLE. Other immunodeficiencies associated with increased susceptibility to pyogenic bacterial infections are not associated strongly with the development of SLE.

Complement plays a key role in the clearance and processing of immune complexes and cellular debris and the evidence will be summarized that it is the role of the classical pathway that protects against the development of SLE.

C4 and C2 null alleles and SLE

In view of the link between homozygous complement deficiency and SLE, it was hypothesized that partial deficiencies of C4 or C2 might also increase disease susceptibility. C4 is the product of two genes, C4A and C4B, and the expressed C4A and C4B proteins have slightly different functional activities. There is good evidence that C4A null alleles are important in SLE. An increased prevalence of C4A null alleles in patients with SLE has been found in many different populations, independent of other MHC associations. These data are reviewed below.

C4 null alleles

C4 is encoded by two tandemly duplicated genes, C4A and C4B, within the class III MHC region. Human C4A and C4B are highly polymorphic with variants including non-expressed or null alleles for which no protein product is identifiable (expressed as C4AQ*0 and C4BQ*0, i.e. quantity zero). A single C4 null allele may be seen in up to 30% of healthy Caucasoid subjects with approximately 4% having homozygous C4A deficiency and 1% homozygous C4B deficiency. Different allotypes of C4A and C4B can be detected by differences in electrophoretic mobility [9], but phenotyping is difficult in patients with active disease in whom C4 levels are usually very low because of complement activation and consumption in vivo. Following the molecular cloning of the C4 genes [10], molecular typing mechanisms have enabled more accurate determination of C4 heterozygous and homozygous deficiency states.

There is a strong association between C4AQ*0 null alleles and SLE in Caucasoid patients [11–25]. In some studies, the association shows a gene ‘dose-dependent’ effect: relative risk estimates for SLE in the case of homozygous C4AQ*0 deficiency have been between 9.7 [25] and 16.86 [14], whilst estimates in heterozygous deficiency range from 2.3 [25] to 4.87 [22]. However, the major source of C4AQ*0 alleles is the commonest Caucasoid MHC haplotype: HLA-A1, -B8, CW-, -DR3, C4AQ*0, C4*B1, C2*C, B*IS. In view of this, it is extremely difficult to identify which is the relevant disease-susceptibility gene within the MHC, since the particular allotypic variants of the many genes within the MHC are usually inherited en bloc, in what is termed an MHC haplotype.

There are two ways around this problem. The first is to try to identify lupus patients bearing relatively unusual haplotypes which are the products of recombination at some point in human history. However, individuals bearing these recombined haplotypes are uncommon. In one study, the C4AQ*0 null allele frequency was determined in Caucasoid SLE patients who were HLA-DR3 negative. Eighteen of 30 (60%) lupus patients had extended haplotypes containing a C4 null allele compared with 22 of 60 (37%) healthy Caucasoid controls ($P < 0.05$) [26].

The second approach is to analyse the HLA associations of SLE in ethnic groups in which other HLA haplotypes are present, to test the hypothesis that the association with C4AQ*0 is present in different populations. A number of studies showed significant associations between C4AQ*0 deficiency and SLE without any consistent association with HLA-DR3 [14, 15, 27–31]. For example, in African-American patients the presence of a C4AQ*0 allele confers a relative risk of 4.5 despite no association with HLA-DR3 [29]. Furthermore, in Japanese populations where the prevalence of HLA-DR3 is extremely low, an association with C4AQ*0 was demonstrated [30].

However, there are also a significant number of studies which have not shown a significant association between SLE and the presence of C4AQ*0 alleles. In a study of French-Canadian and non-French-Canadian lupus patients, a significant association with C4AQ*0 could be demonstrated only in the non-French-Canadian group [32]. Additionally, a Spanish [33], Greek [24] and a recent Caucasoid study [34] failed to detect a significant association between lupus and the presence of C4AQ*0 null alleles. It remains uncertain what is the relevant disease-susceptibility gene or genes within the MHC.

Other candidate genes within the MHC include those encoding (i) class II gene products, controlling the repertoire of peptide presentation to T cell receptors; (ii) components of the antigen processing machinery, e.g. HLA-DM, -DO and proteins of the TAP (transporters associated with antigen processing) complex; (iii) the cytokines, tumour necrosis factor-alpha and lymphotoxin and (iv) the many other proteins encoded in the MHC. Recent work has demonstrated that B cells and resting T cells from healthy HLA-B8, -DR3 positive individuals express markedly reduced levels of Fas (CD95/APO-1) compared with cells from HLA-B8, -DR3 negative matched controls [35]. This is of direct relevance to SLE where, as discussed below, inefficient clearance of apoptotic cells has been implicated in the disease pathogenesis.

Evidence for an association between C4BQ*0 alleles and SLE is much less strong than for C4AQ*0 alleles. Indeed the majority of studies have found no increase in the C4BQ*0 allele frequency in Caucasoid [12, 14, 15, 21, 26, 34], Asian [15, 27, 31] and African-American populations [14, 28, 34].

It is therefore necessary to explain why a partial deficiency of C4A and not C4B might predispose to SLE. The C4A isotype shows preferential binding to amino groups, forming amide bonds, and binds
Complement abnormalities and SLE

particularly to proteins, for example in immune complexes [36], whilst C4B is haemolytically more active than C4A, reflecting increased ester binding of C4B to carbohydrates on erythrocytes. It could be hypothesized that deficiency of C4A results in impaired processing of immune complexes or apoptotic cells which, as discussed below, may be important in the pathogenesis of SLE.

C2 null alleles

Although one early study did detect an association between heterozygous C2 deficiency and SLE [37], later studies have not confirmed this [12, 18, 38, 39]. The C2 null allele (C2Q*0) occurs, in the majority of cases, in association with the haplotype HLA-A25, B18, CW-, DR2, C4A*4, C4B*2, Bf*S [40–42]. The molecular basis of C2 deficiency associated with this extended haplotype consists of a 28 base pair genomic deletion that causes skipping of exon 6 during RNA splicing, resulting in the generation of a premature stop codon [43]. The frequency of this extended haplotype was not raised in a study of 248 European lupus patients [38] and none of the HLA-B18 positive lupus patients in another Caucasoid study possessed the compleotype, C4A*4, C4B*2, Bf*S [12]. Furthermore, the frequency of the 28 base pair deletion associated with type I C2 deficiency was not significantly elevated in a study of 86 Swedish lupus patients [39]. Therefore, it appears unlikely that partial C2 deficiency is a disease-susceptibility factor for the development of SLE.

Acquired deficiencies of complement proteins and SLE

SLE has also been associated with acquired complement deficiency states, particularly in patients with reduced levels of C2 and C4 caused by the effects of inherited C1 inhibitor deficiency. The presence of the autoantibody, C3 nephritic factor, which causes C3 consumption, has also been associated with the presence of SLE. These conditions are discussed briefly below.

C1 inhibitor deficiency

C1 inhibitor deficiency may be hereditary (hereditary angioedema) or acquired. Hereditary C1 inhibitor deficiency is transmitted as an autosomal dominant trait and heterozygous individuals experience recurrent subcutaneous and submucosal swelling (recently reviewed in [44]). In normal circumstances, this inhibitor, a serine proteinase inhibitor (serpin), binds and inactivates enzymatically active C1r and C1s. Deficiency results in uncontrolled fluid phase classical pathway activation and consequently reduced levels of both C4 and C2. Following the observation that homozygous C4 and C2 deficiency predisposes to the development of SLE, it might be predicted that individuals with C1 inhibitor deficiency may have an increased likelihood of developing lupus and this does appear to be the case [45–52]. The incidence of SLE in individuals with hereditary C1 inhibitor deficiency has been estimated at approximately 2% [46].

C3 nephritic factor

C3 nephritic factor is an IgG autoantibody directed against a neo-epitope on the fluid phase and cell-bound alternative pathway C3 convertase (C3bBb and C3bBb, respectively) [53]. The binding of C3 nephritic factor to the C3 convertase renders it resistant to inactivation by factor H. The consequent uncontrolled alternative pathway activation results in secondary C3 deficiency. C3 nephritic factor is most commonly associated with partial lipodystrophy and MPGN type II. However, associations between C3 nephritic factor and SLE have been reported [54–58]. In these reports the onset of SLE was between 2 and 24 yr after the onset of partial lipodystrophy or nephritis. The significance of this association is uncertain. It may be that common factors cause both the development of the C3 nephritic factor and SLE. However, it is rare to see C3 nephritic factor as part of the spectrum of SLE autoantibodies. Alternatively, prolonged C3 deficiency caused by the C3 nephritic factor, may predispose to the development of SLE. However, as discussed above, SLE is rare in homozygous C3 deficiency. Furthermore, homozygous factor H deficiency, which also results in severe C3 deficiency, has only been associated with SLE in one individual who was also found to have homozygous C2 deficiency [59]. Similarly, homozygous factor I deficiency, again resulting in secondary C3 deficiency, is predominantly associated with recurrent pyogenic infections but not SLE [8].

Complement and disease activity

The measurement of complement antigenic levels and functional activity in serum is commonly used as a marker of disease activity in SLE. During periods of active disease, serum complement activity is usually reduced. Typically, levels of the classical pathway components (C1q, C2, C4) are low and, especially in patients with severe disease, may be accompanied by a reduction in C3 levels. However, in cohorts of patients with SLE, levels of individual complement components only approximately correlate with disease activity and levels of C4 may remain low when the patient is well [60]. This may partially be explained by the fact that the level of any serum protein is related to the rate of synthesis as well as catabolism. Determinants of protein synthetic rate are not well understood and studies have shown that hypercatabolism of C3 in disease may be associated with increased or decreased synthetic rates [61–63]. This limits the value of the simple measurement of protein concentration in serum.

The activation or breakdown products of complement proteins have been assayed in an attempt to circumvent this problem. Levels of a variety of such products have been reported to correlate with disease activity more strongly than simple measurements of total C3 and C4 levels. Examples of assays for complement activation products in SLE include C3a [64], iC3b [65], C3d [66] and C4d [67]. Furthermore, in pregnancy, where increased C3 synthesis occurs, a reduction in the ratio
of total haemolytic activity (CH50) to the alternative pathway activation product Ba, has been shown to be the most reliable complement assay in distinguishing active lupus from pre-eclampsia [68]. However, complement activation products are highly unstable and usually have a very short half-life in plasma. At present, all complement assays are at best a very crude surrogate marker of disease activity in SLE.

It should also be appreciated that other factors, independent of disease activity, may affect complement levels in lupus patients. The most important are a series of autoantibodies to complement proteins which include: anti-C1q autoantibodies [69], anti-C1 inhibitor antibodies [70, 71], classical pathway nephritic factor (an autoantibody to the classical pathway C3 convertase, C4b2a) [72] and C3 nephritic factor [53].

It has been suggested that the measurement of complement activity may identify patients at risk of developing lupus nephritis. An early study demonstrated that normal C3 levels were rarely detected in the presence of active nephritis [60]. More recently, the presence of anti-C1q antibodies in SLE has been found to correlate strongly with the presence of proliferative nephritis with a positive predictive value of 50–58% and a negative predictive value of 96.5–100% [73, 74]. Anti-C1q antibodies are IgG autoantibodies that are directed against neo-epitopes on the collagen-like region of the C1q molecule [69]. These antibodies, found in 34–59% of lupus patients [75], show a significant inverse correlation with levels of C1q, C3 and C4 [73, 76]. Increases in anti-C1q antibody titres have been shown to precede renal involvement in SLE [76] and, in contrast to increases in anti-DNA antibody titres, appear specifically to increase prior to renal relapse [77]. Furthermore, these antibodies have been recovered from renal biopsies of lupus patients with proliferative nephritis in higher concentrations than that of serum [78], supporting the hypothesis that they play a direct role in the pathogenesis of the nephritis.

**Infection and hypocomplementaemia in SLE**

There is one extremely important clinical association of hypocomplementaemia in SLE. Patients with chronic hypocomplementaemia are at particular risk of developing serious infection with encapsulated organisms such as *Streptococcus pneumoniae* and *Neisseria meningitidis*. These patients can be considered to be ‘functionally asplenic’ because the hypocomplementaemia, in addition to causing defective opsonization and local phagocytosis, also results in reduced splenic clearance of these organisms [79]. There is a strong case that such patients, analogous to standard post-splenectomy prophylaxis, receive prophylactic penicillin therapy and be considered for both pneumococcal and meningococcal vaccination [80].

The role of complement in the pathogenesis of SLE

One of the important physiological functions of the classical pathway of complement activation is the clearance of circulating immune complexes. The importance of this in the pathogenesis of SLE and the role of complement in immune complex-mediated tissue inflammation are discussed. Recent data suggesting a role for C1q in the clearance of apoptotic cells and possible involvement of the classical pathway in maintaining B cell tolerance are summarized.

**Role of complement in immune complex processing and immune complex-mediated tissue inflammation**

The role of complement in the processing of immune complexes has been reviewed in detail recently and will only be summarized here [81]. The classical pathway of complement activation inhibits the formation of precipitating immune complexes in plasma [82] and alternative pathway activation is able to solubilize immune complexes that have formed or are deposited in tissues. In vivo clearance studies, following intravenous injection of complement-fixing immune complexes, have demonstrated that circulating immune complexes bind to CR1 (CD35, immune adherence receptor, C3b/C4b receptor) present on the surface of erythrocytes [83]. The complement ligands for CR1 include C3b, C4b and iC3b, and recent data suggest that C1q may also bind to CR1 [84]. C3b covalently incorporated into immune complex lattices interferes with the interaction between antibody and antigen, limiting the formation of large complexes. In addition, incorporation of C3b in circulating immune complexes, via its interaction with erythrocyte CR1, allows immune complexes to be transported to the fixed cells of the mononuclear phagocytic system, predominantly in the liver and spleen. Therefore, chronic hypocomplementaemia would be expected to be associated with defective clearance of circulating complement-fixing immune complexes. This has been tested experimentally. Two studies found that hypocomplementaemic patients showed reduced splenic but enhanced hepatic uptake of injected radio-labelled circulating immune complexes [85, 86]. Despite enhanced hepatic uptake, immune complexes were retained in the liver inefficiently and slowly released back into the circulation. A striking demonstration of the role of complement in the splenic uptake of immune complexes was shown in a C2-deficient individual in whom splenic uptake, initially absent, was restored following administration of fresh frozen plasma [86]. The observation that hypocomplementaemia is associated with defective clearance of immune complexes has led to the hypothesis that complement deficiency, as a consequence of this defective processing, results in increased tissue deposition of immune complexes. Subsequent inflammation could then result in exposure of autoantigens, driving an autoantibody response and the development of autoimmunity.

However, the presence of immune complexes in tissues with associated complement activation does not always result in tissue inflammation. It has been established that immune complexes, C1q and C3 may be present in tissues despite the absence of clinical and histological inflammation. This is clearly illustrated in the skin of
patients with SLE where dermal–epidermal deposits of immunoglobulins, C1q and C3 may be found in normal as well as inflamed lesional skin, the so-called ‘lupus band’ test [87]. Recently, comparative studies in complement-deficient and Fc receptor-deficient mice, have highlighted the important role of Fc receptors (cellular receptors for IgG) in the development of immune complex-mediated inflammation [88]. For example, Fc-γ chain-deficient NZB/NZW mice are protected from developing severe nephritis despite the presence of immune complexes in the kidney [89]. Furthermore, mice deficient in the inhibitory Fc receptor, Fc-γRII, develop severe immune complex alveolitis using doses of immune complexes that do not elicit inflammation in wild-type controls [90]. In a murine model of the reverse passive cutaneous Arthus reaction, C3–/– and C4–/– mice developed inflammatory responses comparable with those seen in wild-type control mice, whereas the response of Fc-γ chain-deficient mice was significantly reduced [91]. This would suggest that inflammation in this model is independent of complement activation. Moreover, it has recently been shown that the spontaneous glomerulonephritis observed in C1qa–/– mice is independent of C3 activation [92].

However, in some experimental models there are clear data showing a role for complement in the induction of inflammatory injury. For example, mice deficient in the C5a anaphylatoxin receptor show marked reduction in inflammation in both peritoneal and cutaneous reverse passive Arthus reactions [93]. Moreover, both complement activation and activation of Fc-γRI on peritoneal macrophages were required to initiate inflammation in a murine model of immune complex peritonitis [94].

These findings, showing that the mechanism of induction of inflammation by immune complexes may be complement-independent or complement-dependent, should not be interpreted as contradictory. They serve to illustrate the complexity of mechanisms of inflammation in disease mediated by immune complexes. The size, composition and location of immune complexes may each modify whether and how inflammation ensues.

**C1q- and receptor-mediated clearance of apoptotic cells**

There is present much interest in the hypothesis that a major source of the autoantigens driving the immune response in SLE is apoptotic cells. This has recently been reviewed in [95]. In the context of this hypothesis, inherited defects in the clearance mechanisms of apoptotic cells would be strong candidates as disease-susceptibility genes for SLE. The role of apoptosis in SLE has been discussed in an earlier article in this series [96].

There is a small body of recent evidence that suggests that the complement system, particularly C1q, may be involved in the clearance of apoptotic cells. It has been demonstrated that, in vitro, human keratinocytes bind C1q in the absence of antibody when rendered apoptotic by UVB exposure [97]. Apoptotic keratinocytes exhibit surface blebs which have been shown to contain many lupus autoantigens (e.g. nucleosomal DNA, Ro, La, Sm and small nuclear ribonucleoproteins) and have phosphatidylserine present on their surface [98, 99]. In addition to being concentrated in the surface blebs, these autoantigens may also be altered by apoptotic-specific proteases allowing the exposure of potentially immunogenic cryptic epitopes [100–102]. Furthermore, normal mice injected intravenously with syngeneic apoptotic thymocytes, have been shown to develop ANA, anti-single-stranded DNA and anti-cardiolipin antibodies [103].

It may also be that proteins that bind to apoptotic cells may become part of complexes of autoantigens. This could explain the finding that a number of proteins, such as β2-glycoprotein I and annexin V, that bind to phosphatidylserine, a negatively charged phospholipid exposed on the outer lamella of apoptotic cell membranes, elicit an autoantibody response in many patients with SLE. Another such binding protein to apoptotic cells may be C1q, which could explain the high prevalence of autoantibodies to C1q in SLE, although it is not yet known to what moiety on apoptotic cells C1q binds.

Further evidence supporting a role for C1q in the clearance mechanisms of apoptotic cells follows the observation that C1qa–/– mice, on a mixed genetic background (129/Ola × C57BL/6), develop proliferative glomerulonephritis characterized by the presence of large numbers of glomerular apoptotic bodies [104]. The finding of excess glomerular apoptotic bodies in non-diseased C1qa–/– kidneys compared with non-diseased genetically matched controls, suggested that this was the primary defect. The C1qa–/– male mice on a mixed genetic background also developed significantly higher titres of ANA at the age of 8 months compared with controls (54 vs 33%).

Although many aspects of this hypothesis remain untested, it could provide a unifying explanation for the paradoxical finding that homozygous C1q deficiency causes SLE whilst many patients with SLE have anti-C1q antibodies. According to this hypothesis, in both situations the interference with the putative physiological role of C1q receptor-mediated clearance of apoptotic cells would result in autoimmunity.

**Complement and B cell tolerance in SLE**

Complement plays an accessory role in the efficient production of antibodies to T-cell-dependent antigens by reducing the threshold for B cell activation. Complement deficiency would therefore be predicted to impair antibody responses to T-cell-dependent antigens. Mice genetically deficient in C1q [105], C3 [106], C4 [106] and complement receptors CD35 and CD21 (C2–/– mice) [107] demonstrate impaired antibody production to T-cell-dependent antigens. In addition, humans with homozygous C3 deficiency show mild impairment of antibody responses. The two principle mechanisms involve the covalent attachment of C3b in immune complexes which allows localization of antigen to the germinal centres of lymph nodes where it interacts with CR1 (CD35) on follicular dendritic cells. Secondly,
ligation of CR2 (CD21) on B cells by iC3b or C3dg lowers the threshold for B cell activation following binding of antigen to the B cell receptor. It has also been shown using a transgenic model to explore mechanisms of B cell tolerance that CR1, CR2 and C4, but not C3, are involved in the negative selection of autoreactive B cells [108, 109]. However, the significance of this in relation to autoimmunity is uncertain and further work is needed.

Conclusion

The links between complement and SLE are complicated and fascinating. In the case of C1q: (i) C1q deficiency causes SLE, (ii) SLE causes activation and consumption of C1q, (iii) autoantibodies to C1q are commonly found in patients with SLE! After many years of study, hypotheses are developing which may begin to explain these apparent paradoxical findings. It is becoming clear that complement, rather than being the villain in SLE as a mediator of inflammatory injury, may in fact play a DD. Deletion of C4A genes in patients with systemic lupus erythematosus: importance of null alleles of C4A and C4B in determining disease susceptibility. Br Med J 1983;286:425–8.

Acknowledgements

The authors gratefully acknowledge support for their work from the Arthritis Research Campaign and from the Wellcome Trust.

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