Genetics of the complement system

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Our knowledge of the genetics of the complement system stems from two types of information: the detection of genetic markers on complement components and the study of isolated component deficiency states in man and animals. Since there are a substantial number of complement components, the potential data yield of such studies is considerable. The Table lists the known factors involved in the complement system; whether or not they are known to show polymorphism in man and whether or not genetic deficiency states have been encountered in man or animals.

Allotype of complement components

C3. C3 is both in amount and in biological importance the major complement component. It is also the first in which the existence of population polymorphism was described (at the 1st International Symposium and Workshop; see *Vox Sanguinis*, 1973). For all these reasons, this is the allotype system that has been most extensively studied. The C3 allotypes are distinguished by their electrophoretic mobility. The two major allotypes were described as fast (gene frequency in Caucasians 0.25) and slow (gene frequency in Caucasians 0.75) and a variety of rare allotypes with different mobility also occur. The C3 allotypes do not appear to be functionally different but it has recently been claimed that C3f is better at producing rosettes with macrophages than is C3s (Arvilommi, 1974). Mendelian inheritance of the allotypes within families is readily shown.

The other allotypic systems have so far been studied less.

Factor B. Factor B shows electrophoretically detectable allotypes in a similar way to C3 (Alper *et al*, 1972). The slow variant has a gene frequency of

0.71 and the fast variant a frequency of 0.28. Occasional rare variants of factor B have also been found.

C6. Polymorphism of C6 has been investigated by both electrophoresis and by a new technique of isoelectric focusing in polyacrylamide gel slabs (Hobart *et al*, 1975). The position of the C6 bands can be detected either by immunofixation or by the use of a 'zymogram' technique in which the gel is overlaid with agarose containing red cells coated with antibody and C6-deficient rabbit serum (see below). Two common alleles for C6 have been found with frequencies of 0.63 and 0.36, and four rarer variants have also been found.

Population heterogeneity. The combination of isoelectric focusing, which has very high resolving power, and specific functional detection, promises to be a powerful tool for the investigation of complement polymorphisms. It shows complex banding patterns for all components that have so far been studied and has so far revealed population heterogeneity in C2 and factor D. It can be used as an alternative method for the detection of factor B polymorphism.

Population heterogeneity has also been reported in C4 (Rosenfeld *et al*, 1969) but it is not inherited in a clearly Mendelian pattern and its significance is still not clear. Since this technique for detecting polymorphism is highly discriminating and rapid to perform it is to be envisaged that the study of complement allotypes will rapidly expand.

Linkage. A major interest of this work is to establish whether there is linkage between the markers on different complement components and in this way to see whether information can be obtained about the evolution of complement. This interest became much more intense when it was found that Factor B showed close linkage to the

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HL-A locus in man (Allen, 1974) since this locus is intimately concerned with immunological phenomena. In general, proteins coded at or around the HL-A locus are present on cell membranes and it may be relevant that an activity resembling Factor B has been found on lymphocyte membranes (Halbwachs, McConnell, and Lachmann, 1975).

Further associations between complement and the principal transplantation locus of various species have recently come to light.

In man the 'C2-deficiency gene' (q.v.) is linked to HL-A and appears to show a particular association with the haplotype 10, W18 (Fu *et al.*, 1974). The significance of this linkage disequilibrium is obscure. However, a similar phenomenon has been reported in the guinea pig where the 'C4 deficiency gene' is associated with the B allele of the principal histocompatibility locus (Shevach, Frank, and Green, 1975). In the only known C4 deficient human family the C4 deficiency gene has been reported to be linked to the HL-A haplotype 2-T3-W10 for which the *propositus* is homozygous. In these cases it is not yet firmly established that it is the structural gene that is involved, though in the case of C2 it seems particularly likely since C2 and Factor B are homologues in the classical and alternative pathways and it is attractive to think they may be the products of duplicated genes.

In the mouse the Ss-Slp protein is coded in the H2 region and there is evidence that this may be a complement component (Demant *et al.*, 1973). It seems to be C4 (Lachmann *et al.*, 1975). C3 levels in mice are also controlled by a gene linked to H2 and at least one other not so linked (Ferrara and Nussenzweig, 1975) though the effects are modest. Since C3 can be regarded as the alternative pathway homologue of C4 this at first sight resembles the Factor B/C2 situation. However the C3 polymorphism in man is not linked to HL-A and, if it is assumed for the moment that the genetic arrangements are conserved among mammals, this means that the structural gene for at least one of the two chains making up C3 is not in the H2 region. Likewise the C5 deficiency gene in mice is not linked to H2.

The extent to which structural complement genes and/or genes regulating complement levels occur in the major histocompatibility complex will doubtless rapidly become clarified as more data are gathered.

Studies of isolated complement deficiencies

Although the first complement deficient animals were described as long ago as 1919 it is only in the

last few years, with the growing application of complement testing in clinical practice, that it has become recognized that primary genetic deficiencies of a substantial number of complement components occur. The complement factors of which deficiencies have been described are listed in the Table. It can be seen that deficiencies of all the classical complement components except C1q, C8,* and C9 have now been described in man. On the other hand no deficiencies of the alternative pathway factors have yet been found. While it is tempting to speculate that the absence of a recognized deficiency state for a particular component shows that such a deficiency would be incompatible with life, the evidence to date does not really support such a view. Many of the deficiencies listed in the Table have been described only in the last few years and until recently it was widely held that the deficiency of C3—the central and most important component of the complement system—was likely to be lethal. Now however three cases have been found. It seems more likely that it is the frequency with which components are measured that affects the likelihood of detecting deficiency states. The lack of information about the alternative pathway factors in particular is likely to be due to the fact that these components have so far been measured in a relatively small number of patients, and it remains to be seen whether with more wide spread testing deficiency states will also come to light.

In considering the clinical effects of complement deficiencies it is convenient to divide them into four groups.

Group 1. This group would include the three C3-deficient children and the unique patient with deficiency of the C3b inactivator who has a conditioned C3 deficiency due to the continuous activation to exhaustion of the C3b feedback cycle. These patients show a gross immunity deficiency towards bacterial infection that is closely similar to that seen in the antibody deficiency syndrome. It is clear that resistance to the pyococci in particular is highly dependent on complement mediated reactions going as far as C3 fixation. It seems likely that the C3 mediated adherence reactions which enhance the phagocytosis of bacteria that are particularly important in this respect.

Group 2 contains the patients with deficiency of the components required to generate the C3 convertase of the classical pathway ie, C1, C4, and C2. Of these, the deficiency that is least rare in man is

* A single case of C8 deficiency has now been reported (H. J. Müller-Eberhard, Personal Communication).

TABLE

	Genetic Markers Described	Isolated Deficiency State	
		Animals	Man (No. of distinct pedigrees reported)
<i>Components of the classical pathway</i> C1q			(Secondary only; Kohler and Müller-Eberhard, 1972)
C1r			Two (Moncada <i>et al</i> , 1972)
C1s			One (Pondman <i>et al</i> , 1968)
C4	(Population heterogeneity; Rosenfeld <i>et al</i> , 1969)	Guinea pigs (Ellman <i>et al</i> , 1970)	One (Hauptmann <i>et al</i> , 1974)
C2	(Population heterogeneity; M. J. Hobart, unpublished observations)		At least 12 (Klemperer <i>et al</i> , 1966; 1967; Cooper <i>et al</i> , 1968; Ruddy <i>et al</i> , 1970; Agnello <i>et al</i> , 1972; Day <i>et al</i> , 1973; Sussman <i>et al</i> , 1973; J. Leddy, personal communication; F. Stratton, H. Jason, J. Mowbray, and F. S. Rosen, personal communication)
C3	Yes (<i>Vox Sanguinis</i> , 1973)		Three (Alper <i>et al</i> , 1973; F. S. Rosen, personal communication)
C5		Mice (Rosenberg and Tachibana, 1962; Alper and Rosen, 1971)	One (Rosenfeld and Leddy, 1974)
C6	Yes (Hobart <i>et al</i> , 1975)	Rabbits (Rother and Rother, 1961; Lachmann, 1970) and hamsters (N. K. Day, personal communication)	Two (Leddy <i>et al</i> , 1974; H. Gewurz, personal communication)
C7			Three (Pondman <i>et al</i> , 1975; J. Boyer, personal communication; A. Pelltier, personal communication)
C8			One (H. J. Müller-Eberhard, personal communication)
C9			
<i>Factors of the alternative pathway</i> Properdin			
Factor B	Yes (Alper <i>et al</i> , 1972)		
Factor D	(Population heterogeneity; M. J. Hobart, unpublished observations)		
<i>Complement inhibitors</i> C1-inhibitors	(About 20% of deficient subjects have dysfunctional protein)		Many (NB, heterozygotes; Alper and Rosen, 1971; Beck <i>et al</i> , 1973)
C3b-inactivator (KAF)			One (Abramson <i>et al</i> , 1971; Alper <i>et al</i> , 1972)

that of C2 which is also by far the most common of the isolated component deficiencies found in man. Of the C2-deficient subjects that are now known, about half enjoy good health. However, the other half are found among patients with systemic lupus erythematosus (LE; at least four cases); Henoch-Schönlein purpura (two cases); polymyositis, and glomerulonephritis (two cases). The question has been raised whether this high incidence of what are regarded as probable immune complex diseases is a true association with C2 deficiency or whether it is an ascertainment problem due to the fact that it is mainly in patients with immune complex diseases that complement levels are measured. Although this question cannot be given a rigorous answer on available data, it does seem highly likely that the association is a real one. Two surveys of normal populations for complement deficiency have been reported. One was carried out by Hässig and his colleagues (1964) on recruits joining the Swiss Army and they found 14 cases of some type of complement deficiency in 41 000 subjects. A further study carried out by F. Stratton (personal communication) on the Manchester blood donor panel shows one patient with C2 deficiency among 10 000 blood donors. This is perhaps a more satisfactory study since the Swiss Army recruits were all young men and systemic LE is predominantly a disease of women. If these figures for the incidence of C2 deficiencies are at all representative the association in half the diseases with C2 deficiency must be accepted as real.

The two pedigrees with C1r deficiency both suffered from disease—a combination of repeated infections with renal and cutaneous disease resembling systemic LE but without the characteristic serological findings. The one patient with C1s deficiency similarly had a lupus-like syndrome and during period of observation developed anti-nuclear antibodies. The single C4-deficient patient has also clinical manifestations of disseminated cutaneous LE but has so far failed to show any of the serological stigmata of this disease. To these patients who show primary genetic deficiencies of the early acting complement components may be added the patients with C1-inhibitor deficiency who have a conditioned deficiency of C4 and C2 for the greater part of their life. Although most of these patients suffer from no disease other than hereditary angioedema it has recently become clear that both systemic LE (at least three cases—Kohler *et al*, 1974; J. Vaughan, personal communication) and glomerulonephritis (at least two cases—Pickering *et al*, 1971; D. K. Peters, personal communication) have been described among them. This again seems

to be a greatly excessive incidence. It therefore seems almost inescapable that in man the absence of the capacity to generate the classical pathway C3 convertase is associated with this group of immunological diseases. The aetiology of these diseases is not clear but there is some evidence to implicate oncornavirus in the case of systemic LE (Lerner *et al*, 1972); mycoplasma in the case of Henoch-Schönlein purpura (Sussman *et al*, 1973) and possibly a variety of viral infections in the case of glomerulonephritis. It therefore seems possible that deficiencies at this stage of the complement system may predispose to infection with organisms whose virulence is low and which give rise to diseases of allergic pathogenesis, particularly immune complex disease. Nevertheless C4-deficient guinea pigs kept in laboratory conditions appear to be quite healthy.

Group 3 includes patients deficient in C5 (two cases), C6 (two cases), and C7 (two cases). One of the C5-deficient cases has systemic LE while the other is healthy. The two C6-deficient patients seem to have no diseases relevant to their complement deficiency and this is probably also true of the two C7-deficient cases. In a similar way, the C5-deficient mice and the C6-deficient rabbits kept under laboratory conditions appear to be quite healthy. Although it remains to be seen whether an increased incidence of systemic LE exists among patients with deficiencies of C5, C6, and C7 it would seem at the present time that deficiencies of this stage of the complement sequence are relatively well tolerated. In fact in the C6-deficient rabbit it is possible to discern a potential advantage in their deficiency as these animals are relatively resistant to endotoxin shock (Brown and Lachmann, 1973) which produces death in the rabbit as a result of complement activation and disseminated intravascular coagulation resulting from it.

Group 4 included the patients with hereditary angioedema associated with deficiency of the C1-inhibitor. This deficiency is unique among the complement deficiencies in that it is the heterozygote that develops the disease which is therefore transmitted as an autosomal dominant. The C1-inhibitor or α_2 neuraminoglycoprotein is an important inhibitor not only of C1 but of a number of other plasma esterases including plasmin, kallikrein, and factors X1a and X11a of the coagulation system. However, it is the complement system that appears to be important in the pathogenesis of the angioedema since the C2-deficient subject will not give a wheal and flare on injection of C1. In the absence of the inhibitor the unrestrained action of C1 or C4

and C2 in the fluid phase produces a 'kinin-like' fragment which seems to be the main mediator of the disease (Donaldson *et al*, 1970). The characteristic attacks of the disease appear to be brought about by the exhaustion of the small amount of inhibitor available at an extravascular site, probably as a result of the activation of any of the enzymes with which it reacts. This is followed by the (pseudo) autocatalytic activation of C1 and the destruction of C4 and C2. Attacks of the disease have been successfully treated by giving fresh plasma containing the missing inhibitor (Pickering *et al*, 1969) and prevented by the long-term administration of epsilon amino-caproic acid or its analogues which prevent the activation of the whole range of enzymes with which C1-inhibitor reacts and thus exert an 'inhibitor sparing' effect (Hadjijannaki and Lachmann, 1971). Of the now quite numerous pedigrees with hereditary angioedema, about 80% have no cross-reacting protein and about 20% have a dysfunctional protein which while antigenically normal is without enzyme inhibitory activity.

Conclusions

The complement system, unlike the coagulation system, was largely characterized by *in-vitro* techniques which did not make use of genetically deficient plasmas. The existence of the genetically deficient subjects therefore has served largely to increase our knowledge of the *in-vivo* role of complement. At the present time its clearest role is in the resistance to infection; obviously in the case of C3 deficiency and bacterial infection and possibly more subtly in the case of deficiency of the early active complement components and low virulence organisms. There is so far no evidence that genetic complement deficiency interferes with antibody formation or with the generation of tolerance as has been suggested in the past (Azar *et al*, 1968; Dukor and Hartmann, 1973).

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