Epidemiol. Infect. (1994), **113**, 275–281

Hereditary deficiency of the seventh component of complement and recurrent meningococcal infection: investigations of an Irish family using a novel haemolytic screening assay for complement activity and C7 M/N allotyping

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(*Accepted 24 April 1994*)

SUMMARY

Terminal complement component deficiency predisposes to meningococcal infection and is inherited in an autosomal co-dominant manner. An Irish family is described, in which 2 of 3 brothers had recurrent meningococcal infection. A novel screening assay was used to investigate for terminal complement deficiency and the 2 affected brothers were found to be completely deficient in the seventh component of complement (C7). Enzyme-linked immunosorbent assay for C7 revealed lower than normal levels in the remaining brother and parents. C7 M/N protein polymorphism allotyping, used to investigate the segregation of the C7 deficiency genes, showed that the apparently complement sufficient brother was heterozygous C7 deficient and a carrier of one of the deficiency genes. Complement screening should be carried out in any individual suffering recurrent meningococcal infection or infection with an uncommon meningococcal serogroup. Identification of complement deficient patients allows the implementation of strategies to prevent recurrent infection.

INTRODUCTION

The association between inherited deficiencies of terminal complement components and increased susceptibility to *Neisseria meningitidis* infection is well established [1]. The terminal complement components comprise the fifth (C5) through to the ninth (C9) complement components. Following complement activation C5 is cleaved leading to the formation of C5b. The resultant exposure

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of appropriate binding sites enables the terminal components to combine on target cell surfaces to form the potentially lytic membrane attack complex (MAC) [1]. Isolated deficiencies of all the terminal complement components have been described [1] and an Irish family with deficiency of the seventh component of complement (C7) is reported here. The structural genes for both C7 and the sixth component of complement (C6) reside close to each other on chromosome 5 [2]. There are over 80 reported cases of C7 deficiency in diverse ethnic populations [1] including one previously reported Irish case [3].

C7, like most complement proteins, is polymorphic [4]; on isoelectric focusing analysis it has one common and many rare charge variants [5]. The C7 M/N polymorphism is also a protein polymorphism and depends on the expression of an allospecific epitope [6]. The C7 variants, including C7 deficiency, are inherited in an autosomal co-dominant manner [1]. In practice, this means that C7 deficiency is found only in individuals who are believed to be homozygous deficient and the inheritance pattern of the deficiency appears to be that of an autosomal recessive condition. Heterozygote deficient individuals have reduced C7 levels, but retain sufficient complement activity to prevent overt clinical manifestation of the deficiency [1].

PATIENTS, METHODS AND RESULTS

A previously healthy 20-year-old male from the west of Ireland presented with a history of less than 12 h of headache, rash, shivering and neck stiffness. Physical examination revealed pyrexia of 39 °C, tachycardia and a blood pressure of 100/60 mmHg. The patient was drowsy, there was a purpuric rash on the trunk, meningism was present, there were no focal neurological signs and papilloedema was not present but the mild hypotension suggested the possibility of early endotoxic shock. Cerebrospinal fluid analysis showed a polymorphonuclear leucocytosis and bacterial culture yielded *N. meningitidis*, serogroup B. Blood cultures were sterile. Treatment with penicillin and chloramphenicol resulted in rapid and uncomplicated recovery.

Eleven months later the patient presented again with a history of sudden onset of headache, photophobia and sweating. Physical examination revealed pyrexia 39 °C, blood pressure 125/60 mmHg and meningism was present. There was no rash, papilloedema or focal neurological signs. Blood cultures taken subsequently grew *N. meningitidis*, serogroup Y and therapy was instituted with high dose cefotaxime. Cerebrospinal fluid culture after administration of antibiotic was sterile and again, recovery was uneventful.

Three weeks following the onset of the second episode, groups A and C polysaccharide meningococcal vaccine was administered.

The index case was the second of 3 brothers. The eldest was aged 25 years and the youngest 17 years. The eldest had a history of 2 episodes of meningococcal infection, the first at age 8 years and the second at age 12 years. Only scanty clinical details were available, but the parents gave a clear history of the first infection – he had been seriously ill. The clinical course had been complicated by pericarditis and doctors had warned that he might not survive. However, he recovered but suffered a second episode 4 years later which was less severe and recovery was complete. Meningococci were isolated from blood or cerebrospinal

fluid on both occasions, although serogrouping was not carried out. Neither parent nor the youngest brother had a history of meningococcal infection.

The recurrence of meningococcal infection in this patient and his brother prompted investigations for hereditary terminal complement component deficiency.

Complement assays

Tests for deficiency of the classical complement pathway and the alternative complement pathway were done by haemolytic assays in 1% agarose gels containing appropriate erythrocyte indicator systems [7]. Normal human serum (NHS) produced rings of haemolysis of approximately 10 mm diameter in both assays, whereas serum from the index case failed to produce haemolysis in either assay. This strongly suggested a defect in the final common lytic terminal complement pathway.

A double diffusion haemolytic assay in an agarose gel was developed in order to determine which, if any, of the terminal complement components C5, C6, C7, or C8β was absent from the index case serum. The functional indicator slab gel was 1 mm thick and contained 1% agarose in a complement fixing diluent (CFD): 3·1 mM diethyl barbituric acid, 0·9 mM sodium barbitone, 0·145 M NaCl, 0·25 mM $CaCl_2$ and 0·83 mM $MgCl_2$ (prepared diluent tablets available from Oxoid, Basingstoke, UK) and 1% sheep erythrocytes that had been sensitized with antibody and the early complement components (EAC43b) [7]. Five 3 mm holes were punched in the gel (Fig. 1), the centres of 4 peripheral holes being approximately 9 mm from the centre of the central hole. Test serum (7 μl) was inoculated into the central well and 7 μl each of human sera known to be deficient in C5, C6, C7 or C8β were inoculated into the 4 peripheral wells. The gel was incubated at 4 °C overnight and then at 37 °C for approximately 6 h. Figure 1 shows that no rings of haemolysis developed around any of the samples, but that in each instance 2 haemolytic lines developed between the C5, C6 or C8β deficient samples and the test sample. No haemolytic lines developed between the C7 deficient sample and the test sample. Haemolytic rings failed to develop because all samples were deficient in one of the terminal complement components. The development of the double lines of haemolysis between the test sample and the C5, C6 and C8β deficient samples occurred because the test sample was not deficient in any of those components and provided the C5, C6 and C8β necessary for the development of a line of haemolysis proximal to the deficient samples. The deficient samples also provided the component missing in the test sample so the lines developed proximal to the test sample. The failure of any interaction with the C7 deficient sample demonstrates that C7 is the missing component in the test sample. The results of this assay were confirmed with single diffusion C7 assay [7].

Similar assays were performed on sera from the other family members. The sera from the parents and the younger brother had normal C7 haemolytic activity, although in the single diffusion C7 assay the rings of haemolysis were smaller than that produced by control NHS. The serum from the elder brother also showed complete absence of C7 haemolytic activity. Functional assays are qualitative rather than quantitative and therefore serum C7 levels were confirmed by a specific C7 enzyme-linked immunosorbent assay (ELISA) [8].

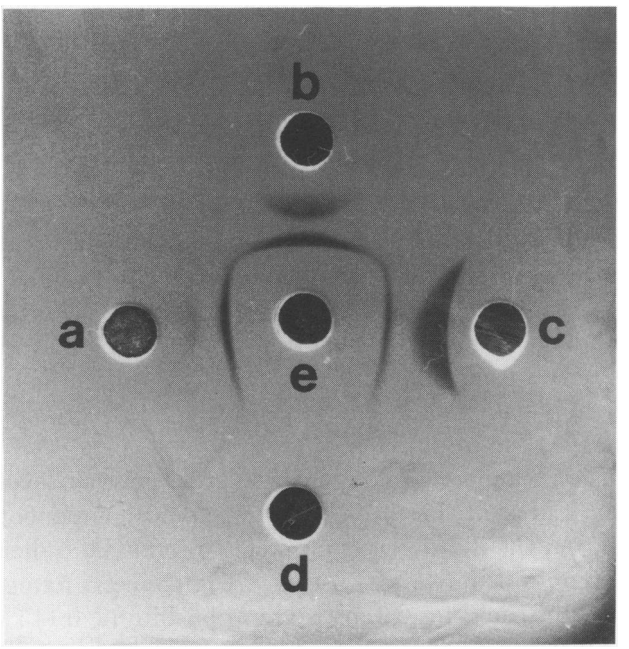

Fig. 1. The haemolytic gel contains antibody sensitized sheep red cells with the complement components C143b as described in Patients, Methods and Results. The test sample (7 μl) was inoculated into well e and 7 μl each of human C6 deficient, C5 deficient, C8β deficient and C7 deficient sera were inoculated into wells a, b, c and d respectively. Incubation was at 4 °C for 17 h and at 37 °C for 6 h. Two bands of lysis developed between the test well e and the wells a, b and c (the band nearest well a is very faint), but no lines developed between the test sample and well d. This indicates that, like the serum in well d, the test sample is C7 deficient.

The father, mother and younger brother had levels of 41, 51 and 60 μg/ml respectively. The 2 affected brothers had C7 levels less than 0·2 μg/ml (normal mean 90 ± 36 μg/ml [8]).

C7 M/N protein polymorphism allotyping

The C7 M/N protein polymorphism is based on the reactivity of an allospecific mouse monoclonal antibody that recognizes an epitope present on the C7 found in the majority of individuals [6]. This C7 is allotype C7M and C7*M has a gene frequency of 0·775 in Caucasians. The non-antibody reactive C7 is C7N and the C7*N gene frequency is 0·225. In a study similar to one described previously [9], we used C7 ELISA assays with both the allospecific monoclonal antibody WU 4-15 and a polyclonal goat anti-human C7 to determine the C7 M/N allotypes of the family members. The protein M/N allotypes in deficient individuals themselves cannot be determined because of the absence of the protein. The mother and father were obligate deficient heterozygotes and their C7 M/N phenotypes were C7M and C7N respectively, indicating genotypes C7*M/C7 deficient and C7*N/C7 deficient respectively. The C7 sufficient brother allotyped C7M indicating he had inherited the C7*M sufficient gene from his mother but failed to inherit the C7*N sufficient gene from his father. Therefore his genotype must be C7*M/C7 deficient and he is a carrier of a C7 deficiency gene.

DISCUSSION

Homozygous C7 deficient individuals have almost complete absence of detectable C7 which leads to an inability to generate the MAC [1]. Thus they lack serum haemolytic and bactericidal activity. Serum from subjects with deficiencies of other terminal components C5–C8, similarly lack haemolytic and bactericidal activity. However, the only infections to which these individuals are abnormally susceptible are neisserial infections, of which *N. meningitidis* infections are by far the most common [10], although *Neisseria gonorrhoeae* has also been reported [11]. Several other sequelae of terminal component deficiency have been postulated, but these are largely isolated cases and associations with other diseases and are unsubstantiated [1].

The strain responsible for the first episode in the index case was serogroup B, which is known to be common in the west of Ireland but the second was an uncommon serogroup Y infection [12]. Infection of a young adult as opposed to a child, with an organism of uncommon serogroup such as Y or W135, is recognized as being an indicator of disease occurring in a complement deficient subject [13]. This holds true especially in areas where meningococcal infection is non-endemic [1]. The explanation for the susceptibility to Y and W135 strains may be that these organisms are relatively resistant to opsonophagocytosis, which is the only host defence mechanism against the meningococcus in complement deficient individuals [14].

The acute episode of meningococcal infection in a terminal complement deficient patient should be treated in the same way as in a sufficient patient. The only acute management issue unique to the complement deficient patient concerns theoretical possible disadvantages in the use of plasma for endotoxic shock complicating the infection [15].

Prevention of further attacks is integral to the long-term management of these patients [16]. The Centers for Disease Control in the United States recommend the group A, C, Y and W135 polysaccharide vaccine [17], and there is a recent report of positive, although poor, antibody responses to the polysaccharide vaccine in these patients [18]. There is no group B polysaccharide vaccine available because it is a poor immunogen and there is a theoretical possibility that induced antibodies could cross-react with certain host sialogangliosides [19]. In Britain and Ireland, the commonly available vaccine is divalent and contains capsular polysaccharide from serogroups A and C only. The quadrivalent vaccine which also incorporates Y and W135 polysaccharides is available from the United States (Connaught Laboratories, Pennsylvania) or Belgium (Smith, Kline and Beecham). Therefore, the meningococcal vaccines currently available are non-protective for group B infections, which are a common cause of disease in the community [12], as well as causing disease in deficient patients [13]. Deficient patients could well benefit from stimulation of their opsonophagocytic host defence mechanisms [14] and antibodies to polysaccharide capsules are probably particularly important in this regard [18]. However, it is very difficult to predict the action of potentially complement fixing antibodies when no complement is available and, at present, there are insufficient follow-up studies to establish clearly the clinical value of meningococcal vaccines in complement deficiency. Indeed, in certain circum-

stances, meningococcal antibodies may even be detrimental to the complement deficient host [20]. Clearly, vaccination with the currently available vaccines does not preclude meningococcal infection in complement deficient patients and other preventive strategies are required.

Another approach to prevent recurrent infection is the administration of prophylactic penicillin. This has been shown to be effective in an area highly endemic for meningococcal infection [16]. The need for prophylactic antibiotics may not be as great in areas non-endemic for the disease and in patients who can be educated to recognize the early manifestations of meningococcal infection.

In this instance, in addition to education and counselling, the patients have been prescribed oral penicillin V to be kept readily available and to be taken in a dose of 1500 mg without delay should symptoms occur. It is vital that medical support is readily available to these patients who remain vulnerable to further infection. At times of potential increased risk, such as during foreign travel or local outbreaks, they receive prophylactic injections of penicillin. Also, they carry letters explaining the nature of their susceptibility.

The results of the C7 M/N allotyping allowed us to identify the C7 sufficient brother as heterozygous C7 deficient. Normal C7 levels cover a wide range and it is not possible to diagnose heterozygous C7 deficiency from the results of the C7 levels alone. Heterozygous deficiency should not compromise his complement function as heterozygous C7 deficient relatives show no increased tendency to infections [1]; however, this test does mean that we are in a position to provide the family with informed genetic counselling.

Terminal complement deficiency is an important factor increasing the risk of meningococcal infection. The nature of the deficiencies varies in different parts of the world. For instance, C6 deficiency is common in the Cape in South Africa [16] and C7 deficiency has been reported from several centres, particularly Mediterranean countries including Israel [21, 22]. There are now two documented C7 deficient families in Ireland and there may be many more such cases that are as yet undetected. Complement screening to detect underlying deficiency is important in patients who have suffered recurrent meningococcal disease and in those who are infected by uncommon meningococcal serogroups [13]. Total haemolytic complement assays can be used to detect deficiency and the screening assay presented here is a simple method for identifying which of the terminal complement components is missing in individual cases. It is important for the patients and their families that deficiencies are recognized so that appropriate measures can be taken to minimize the likelihood of further infections.

ACKNOWLEDGEMENTS

A.O. was supported by the Meningitis Trust, UK and the Health Research Board of Ireland. R.W. was supported by the Deutsche Akademische Austauschdienst, Bonn, Germany and the Arthritis and Rheumatism Council, Chesterfield, UK.

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