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A Novel Polymorphism of Human Complement Component C3 Detected by Means of a Monoclonal Antibody

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Abstract. A mouse monoclonal antibody, HAV 4-1, obtained after immunization of a BALB/c mouse with purified C3F, detected a novel genetic polymorphism of human complement component C3 in a simple immunoblotting system. The frequency of *HAV 4-1*-positive genes was 20.1%. Reactivity of HAV 4-1 was closely related to C3F, but certain individuals with the *C3F* allele did not react with HAV 4-1. Conversely, certain C3S homozygous individuals did react with HAV 4-1. The polymorphism detected by this monoclonal antibody is therefore different from the previously described polymorphism based on charge differences.

Introduction

The complement system is an important part of an organism's defence system against pathogenic microorganisms. The third component of complement is the key component of this system. Activation of either the classical complement pathway (Reid and Porter 1981) or the alternative complement pathway (Müller-Eberhard and Schreiber 1980) leads to the formation of a C3 convertase. This causes the formation of the opsonin C3b and the anaphylatoxin and inflammation-inducing fragment C3a from C3. The activation of C3 is also the first step required for the formation of the cytolytic membrane attack complex (Podack and Tschopp 1984). A genetic polymorphism of human C3 was first demonstrated by Wieme and Demeulenaere (1967) and Alper and Propp (1967) by means of prolonged electrophoresis of human serum proteins. In this system, two common allelic forms, C3S and C3F, were demonstrated. In addition, several rare C3 variants were defined by their electrophoretic mobility. Today, more than 20 alleles of C3 are known (Rittner and Rittner 1974). We have defined a novel type of genetic polymorphism of human C3 based on the reactivity of a mouse monoclonal antibody, HAV 4-1, and have demonstrated that this polymorphism is distinct from the previously described C3S/C3F polymorphism, although a close relation exists between the HAV 4-1-reactive C3 type and C3F.

Materials and Methods

Plasma samples. Blood samples from healthy individuals were from the Blood Bank, Statens Seruminstitut, Copenhagen, Denmark. Blood was brought to a concentration of 10 m*M* with ethylenediaminetetraacetate, and plasma was obtained after centrifugation at 2000 rpm for 15 min. Plasma samples were kept at −80 °C.

Electrophoresis. Ten milliliters of 1% agarose (HSA, Litex, Denmark) in electrophoresis buffer (Tris-barbital, pH 8.6) was poured onto a 10 × 10 cm glass plate. When the gel was solid, 3 µl samples of human plasma diluted 1 : 10 in 1% glycine were applied using a sample application foil (catalog no. 1850-902, LKB instruments, Stockholm, Sweden). Electrophoresis was run for 2 h at 20 V/cm.

Immunoblotting. Immunoblotting was carried out as described by Koch and co-workers (1985). In brief, a 10 × 10 cm piece of nitrocellulose paper (BA 85, Schleicher and Schuell, Dassel, Federal Republic of Germany) was placed on top of the gel, and pressure was sustained for 10 min. Remaining active sites on the nitrocellulose paper were blocked by incubation with 0.2% Tween 80. The nitrocellulose paper was incubated overnight with the monoclonal antibody and then with peroxidase-conjugated rabbit antimouse immunoglobulin (Ig) (Dako code no. P260, Dakopatt, Copenhagen, Denmark). Staining was performed with 3,5,3′,5′ tetramethylbenzidine (catalog no. 8622, Merck, Darmstadt, Federal Republic of Germany) and H_2O_2.

Purification of human C3S and C3F. C3S and C3F were purified from plasma pools obtained from human C3S and C3F homozygous donors. Purification was performed according to the method of Hammer and co-workers (1981) with minor modifications.

Monoclonal antibodies. Mouse monoclonal antibodies to human C3 were produced after cell fusion (Köhler and Milstein 1975) with spleens from BALB/c mice immunized with purified C3S or C3F. Culture supernatants were tested in a direct enzyme-linked immunosorbent assay on microtiter plates coated with either C3S or C3F.

Results

Mouse monoclonal antibodies were produced against human C3 by standard methods using purified C3S or C3F as immunogens. From the screening of the culture supernatants from a cell fusion where C3F had been used as antigen, we detected one monoclonal antibody, HAV 4-1, which by a simple immunoblotting method could be shown to react only with the C3F band and not with the C3S band after electrophoresis of human plasma (Fig. 1). The reactivity of HAV 4-1 could not only be demonstrated after electrophoretic separation, but also by a simple dot-blot technique (not shown). In both assays a different, pan-reactive human C3-specific monoclonal antibody (HAV 3-5) obtained from the same cell fusion was used as a control.

Screening of a large panel of individual human plasma samples (203 individuals were tested), where electrophoretic C3S/C3F typings performed with the HAV 3-5 monoclonal antibody were compared to the C3 typing with HAV 4-1, revealed that certain C3S individuals were indeed reactive with HAV 4-1. Conversely, certain individuals with the *C3F* allele did not react with HAV 4-1. Figure 2 illustrates such patterns of reactivity. From the data presented in Table 1, it could be estimated that the frequency with which *C3S* alleles were positive with HAV 4-1 was 1.9%, and that the frequency with which *C3F* alleles were HAV 4-1-negative was 11.3%. The overall frequency of *HAV 4-1*-positive genes was 20.1% and that of *HAV 4-1*-negative genes was 79.9%.

Two families with "unexpected" reactivity were analyzed (Fig. 3), and it was demonstrated that reactivity to C3S (family A) and lack of reactivity to C3F (family B) in these two families segregated as a genetic trait.

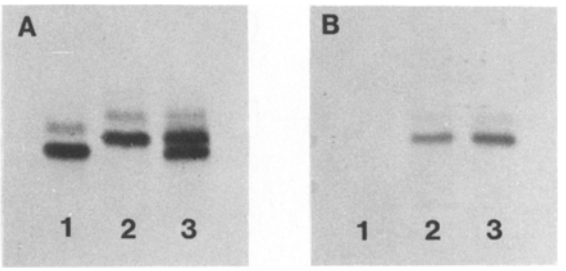

Fig. 1A and B. Prolonged electrophoresis of human plasma in agarose gel followed by immunoblotting onto nitrocellulose. **A** Incubation with monoclonal antibody HAV 3-5 and peroxidase-conjugated rabbit antimouse Ig. Staining was performed with 3,5,3′,5′ tetramethylbenzidine and H_2O_2. **B** Same treatment as in Figure 1A, except that the first incubation was with monoclonal antibody HAV 4-1. In Figure 1A and B, lane 1 is a C3S homozygous individual, lane 2 a C3F homozygous individual, and lane 3 a C3S/F heterozygous individual. In Figure 1B, only the F bands are stained

Table 1. Analysis of 203 healthy individuals for the occurrence of C3S and/or C3F bands

F/S genotype (n = 203)	HAV 4-1$^+$	HAV 4-1$^-$
S/S 126	6	120
F/S 69	61	8
F/F 8	8	0

The gene frequency of C3S was 79.1% and the gene frequency of C3F was 20.9%. Typing with HAV 4-1 revealed six individuals among the *S/S* homozygotes who reacted with this monoclonal antibody, and eight *F/S* heterozygous individuals had F bands which did not react with this monoclonal antibody. A general maximum likelihood computer program (Larsen 1982) was used to estimate the gene frequencies. The frequency of *HAV4-1*-negative genes was 79.9%, and that of *HAV4-1*-positive genes was 20.1%. The frequency with which *F* alleles were negative with HAV 4-1 was 11.3%, and the frequency with which *S* alleles were reactive with HAV 4-1 was 1.9%.

Discussion

We have demonstrated a novel type of genetic polymorphism of human C3, which is not based on charge differences but is based on the reactivity of a mouse monoclonal antibody with the C3 molecule. At present we do not know which of the described C3 polypeptide chains (α or β) or cleavage products carries the determinant.

Because of the close relation of the HAV 4-1-reactive molecular forms to the *C3F* genotype, it seems most probable that the polymorphism at the genetic level is related to the gene product of one single *C3* locus, an assumption which is also in accordance with

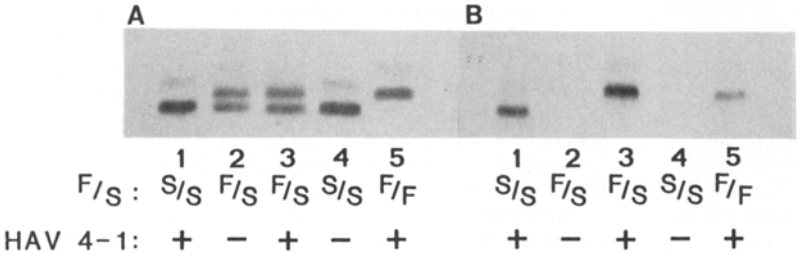

Fig. 2. Prolonged electrophoresis of human plasma samples, essentially as described in the legend to Figure 1, but including plasma samples from individuals with the less common positive reactivity of a C3S band to HAV 4-1 and the lack of reactivity of an F band to HAV 4-1

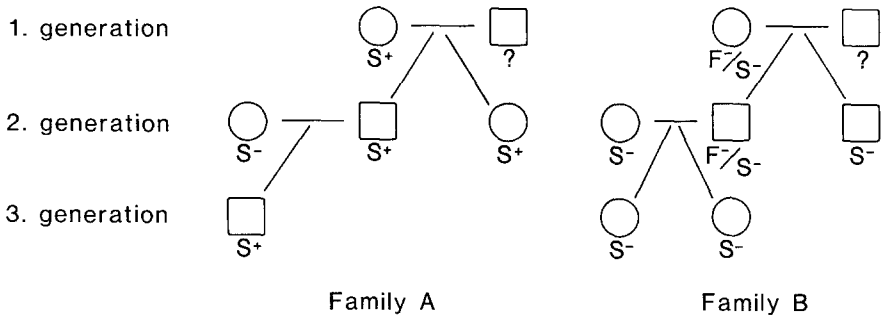

Fig. 3. The F/S phenotypes and reactivity to HAV 4-1 (+/−) of two families. Family A has an S form which is reactive with HAV 4-1 and segregates in three generations. Family B has an F form which does not react with HAV 4-1 and segregates in two generations. ○, female; □, male

the recent demonstration that the human genome carries only one *C3* gene (Whitehead et al. 1982) located on chromosome 19.

Recently, genetic polymorphism of human C3 has also been demonstrated at the DNA level as a restriction fragment length polymorphism (Davies et al. 1983). It should be mentioned that in mice a genetic polymorphism of C3 has been described which is defined by the reactivity of mouse polyclonal alloantibodies (Natsuume-Sakai et al. 1979).

The existence of multiple molecular forms of a certain protein molecule implies the possibility of differences in functional activities of the various allelic forms (Markert 1968). With respect to the immune system, of which the complement is part, there has thus been great interest in the existence of linkage between certain major histocompatibility complex alleles and certain diseases. In the case of human C3, a preponderance of C3F has been demonstrated in patients with hypercholesterolemia (Berg and Heiberg 1978), rheumatoid arthritis (Brönnestam 1973), ischemic heart disease in connection with essential hypertension (Kristensen and Bruun-Petersen 1979), and in old people with arteriosclerosis (Sørensen and Dissing 1972). A hypomorphic variant of C3F has also been described which is associated with the occurrence of an immune complex type of glomerulonephritis and arthritis (McLean et al. 1978). Finally, it has been reported that C3F has a higher capacity than C3S for binding to receptors on mononuclear cells (Avrilommi 1974).

Our finding of an allotypic difference independent of charge differences, but still closely related to the C3F form raises the question of whether the functional differences between C3F and C3S described previously might be due to differences in this new allotypic system.

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