Inherited Deficiency of the Third Component of Human Complement (C'3)

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ABSTRACT A kindred has been investigated in which seven individuals were found to have half-normal serum concentrations of the third component of complement (C'3). This partial deficiency was transmitted as an autosomal dominant trait. Affected individuals were entirely healthy. Hemolytic complement titers were slightly reduced but immune adherence titers and reagent titrations of the classical complement components were normal.

Examination for C'3 allotypes revealed that all affected individuals had patterns resembling those of homozygotes. Analysis of the inheritance of C'3 structural genes disclosed that the most likely mechanism for partial C'3 deficiency in this family was nonexpression of one allele.

INTRODUCTION

Hereditary deficiencies of human plasma proteins have been known for many years, having first been detected in the proteins involved in blood coagulation. For the most part, the deficiency states thus far characterized were initially detected by finding very low biological activity in some functional assay in the plasma or serum of affected individuals. It is now clear that such low activity may be associated either with a deficiency of the protein bearing this activity or with normal concentrations of presumably aberrant protein. This has been well illustrated in hereditary angioneurotic edema where all affected individuals lack demonstrable C'1 esterase inhibitor activity (1, 2). In most affected kindred, individuals with the disease have very low serum concentrations of the inhibitor by immunochemical assay, whereas in other families affected individuals have normal concentrations in the serum of a functionless protein, antigenically indistinguishable from active C'1 esterase inhibitor (3).

Hereditary deficiency states of one or another of the complement components have been documented in experimental animals and in man. There are inbred strains of rabbits with C'6 deficiency (4, 5), of mice with C'5 deficiency (6, 7), and a strain of guinea pigs deficient in one of the proteins comprising the classical third component of complement (8, 9). C'2 deficiency has now been well characterized in at least two families (10–12).

It is the purpose of this report to present observations in a family with some members whose C'3 concentration is about one-half normal. Because of the recent demonstration of inherited structural polymorphism in human C'3 (13), it has been possible to study the genetic basis of this defect.

METHODS

Serum. All sera were analyzed immediately after separation by centrifugation from whole clotted blood or were frozen promptly at −80°C and thawed immediately before analysis.

Determination of C'3 concentration. Monospecific rabbit antisera prepared against human C'3 purified in this laboratory by the method of Nilsson and Müller-Eberhard (14) were used to determine the concentration of C'3 in fresh serum by both an electroimmunochemical (15) and a nephelometric (16) technique. All determinations were done in duplicate by each method. The absolute concentration of C'3 in a reference serum was established as described elsewhere (17).

Genetic typing of red cell and serum protein groups. The red cells of family members were tested for the presence or absence of the following antigens using appropriate specific antisera: A, A_1, B, H; Rh 1, 2, 3, 4, 5, and 8; P_1; K 1, 3, and 4; Le^a and Le^b; M, N, S, and s; Vw, M^g, Lu^a, Fy^a, Jk^a, and Wr^a. The sera of these individuals were also typed for haptoglobin and transferrin by starch gel electrophoresis

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(18, 19) and for Gm 1, 2, 3, and 5, and for Inv 1 using appropriate reagents. The results of these typings were compatible with the family relationships as given.

Hemolytic complement activity. Hemolytic complement activity was measured as $C'H_{50}$ U/ml by a modification (10) of Mayer's method (20).

Immune adherence activity. Immune adherence titers were determined by a minor modification of the method of Nishioka (21) as described elsewhere (13), using antibody-sensitized sheep red cells and human red cells.

Titration of serum complement components. Titrations of the four classical complement components with human reagents were carried out as described previously (10), except that purified C'3 was added to the R4 (22).

C'3 genetic typing. The C'3 of all family members was typed by prolonged agarose electrophoresis (13).

Antigen-antibody crossed electrophoresis. C'3 in the fresh sera of affected individuals was examined by the antigen-antibody crossed electrophoresis technique of Laurell (23) and Laurell and Lundh (24), using rabbit antisera to purified C'3.

Clinical details of individuals with decreased C'3 concentrations. The propositus (III-3) came to our attention by chance. During hospitalization for investigation for "failure to thrive" a serum sample was dispatched for a determination of BEI (butanol extractable iodine), since his father (II-1) had known hyperthyroidism. The requested test was misinterpreted as β_{1C} (C'3). When the initial C'3 concentration was found to be low, two additional analyses over a 4 month period were done with consistently low results. At this point, investigation of the family of the propositus was begun. Other than the low C'3 concentration in serum, no abnormalities were found during the hospitalization of the propositus. There was no history of undue susceptibility to infection, nor were there any allergies. The peripheral blood karyotype, determined in the Cytogenetics Laboratory of the Children's Hospital Medical Center, was normal. All other family members appeared to be in good health with the exception of the father of the propositus, as stated above.

RESULTS

The mean concentration of C'3 in serum from 60 apparently healthy adults chosen at random was 150.3 mg/100 ml with a normal range of 96.9–203.7 (mean ±2 SD). 7 of 16 available members of the Hub family had serum C'3 concentrations that were below this range, whereas 9 had normal C'3 concentrations. The mean C'3 concentration of the 7 family members with subnormal C'3 concentrations was 69.3 mg/100 ml, whereas that of the other family members was 142.6. Thus, the affected persons had C'3 concentrations in serum that were approximately half of normal, and approximately half of that of the remaining family members. The *P* value for the significance of the difference between the mean C'3 concentration of affected persons and either the control population or the unaffected family members was < 0.001.

Table I presents the serum C'3 concentration of all family members as well as the results of titrations of hemolytic complement activity. As can be seen, hemolytic complement was slightly reduced in some affected family members. Immune adherence activity, individual classical complement components as judged by reagent titrations, and bactericidal activity (11) were normal.

TABLE I

C'3 Concentration, C'3 Type, and Hemolytic Complement in Sera of Members of the Hub Family

Subject	C'3 concentration	C'3 type	$C'H_{50}$
	mg/100 ml		*U/ml*
I-3	74	F-	45.3
I-4	148	FS	
II-1	207	FS	54.5
II-2	62	S-	33.3
II-3	110	FS	
II-4	52	S-	28.5
II-5	141	SS	38.1
III-1	138	FS	
III-2	119	FS	
III-3	73	F-	29.6
III-4	81	S-	36.5
III-5	133	SS	31.5
III-6	142	SS	
III-7	77	S-	24.2
III-8	60	S-	
III-9	143	FS	
Control	149	FS	45.0

It was of interest to determine the form in which C'3 was present in the sera of affected persons, since the antiserum used for the immunochemical estimations reacted with conversion products such as $beta_{1A}$-globulin and $C'3_i$ as well as with native C'3. No significant conversion products were found when the sera of individuals with one half of the normal concentration of C'3 were examined in antigen-antibody crossed electrophoresis. The expected minor C'3 peaks were detected and there were no other minor peaks seen.

The genealogy of the Hub family is depicted in Fig. 1 where affected individuals are indicated by half-blackened symbols. It can be seen that the trait for half of the normal level is inherited in autosomal dominant fashion and that the pedigree is consistent with affected persons being heterozygous for complete C'3 deficiency, a postulated total or almost total lack of C'3.

The results of C'3 typing are also included in Fig. 1 and a typing electrophoretic pattern is shown in Fig. 2. In the latter figure, it is particularly clear that C'3 concentrations are about half of normal in the two affected sera.

Examination of the pedigree in Fig. 1 sheds light on the genetic mechanism of C'3 deficiency in this family. All affected persons showed only single-banded patterns. There are two possible mechanisms whereby C'3 may

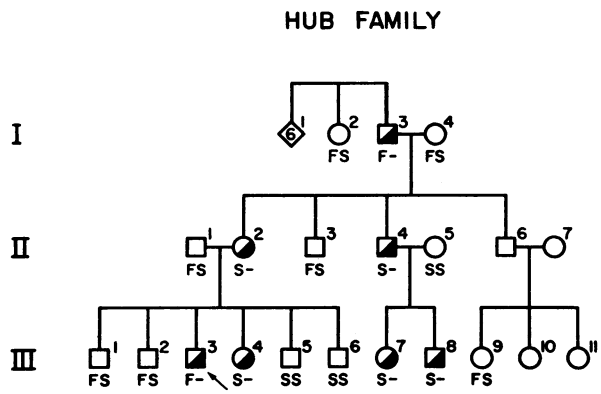

FIGURE 1 The genealogy of the Hub family with partial deficiency of C'3. The Roman numerals at the left of the pedigree refer to generations, the Arabic numerals at the upper right of each circle (female) or square (male) are designations of individuals within each generation, and the letters below them are C'3 types. Individuals with half the normal serum C'3 types. Individuals with half the normal serum C'3 serum concentration are indicated by half-blackened symbols. Where no C'3 types are given, those individuals were not tested. A diamond with an Arabic numeral within it indicates the number of untested siblings.

be half of the normal concentration in these individuals. Either they may have two genes each synthesizing protein at half of the normal rate or they may have one gene synthesizing at a normal rate and the allelic gene producing no protein. If the latter is the case, all affected individuals should be "hemizygous" or, in terms of the typing procedure, apparent homozygotes. In addition, the affected children of a heterozygote (FS) and an affected apparent homozygote can be apparent homozygotes of either type (F or S). The family members II-2, II-4, and III-3 are products of such matings. Since the blood group typings of the kindred are compatible with the relationships as stated, including C'3 types in unaffected members, it is reasonable to assume that their C'3 types provide evidence for an allele that produces no detectable C'3. Thus, I-3 must be of genotype F-, not FF, since an FF individual cannot have an SS child (or two such children, in this case, II-2 and II-4). By extension of this concept, all affected persons in this family are probably of C'3 types F- or S-.

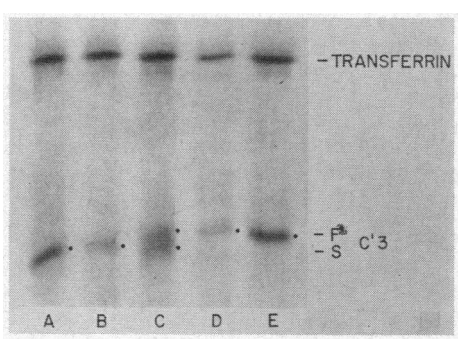

FIGURE 2 Prolonged agarose electrophoresis patterns showing the C'3 area. The sera examined were from (A) an individual with C'3 SS; (B) individual II-2 of the Hub family; (C) an individual with C'3 FS; (D) the propositus (III-3) of the Hub family; and (E) an individual with C'3 FF.

DISCUSSION

The observations in this report indicate that genetically controlled deficiency of C'3 occurs in man as an autosomal trait. In the family described, only the heterozygous state has been found. It seems highly probable that individuals homozygous for this trait exist and that they should have little or no C'3 in their plasma. The homozygous condition should be detectable at birth, since the C'3 in cord serum is of fetal origin with no detectable maternal contribution (25).

Although definite proof is lacking, it seems most probable that defective synthesis of C'3 by a mutant gene is responsible for the partial deficiency state. There is some precedence for allelic exclusion, at least at the cellular level, in higher organisms in the mechanism proposed in the Lyon hypothesis (26). The latter postulates that the genes on only one of the two X chromosomes of normal women are expressed, the genes of the other X chromosome being inactivated. Studies of somatic cells from women heterozygous at the glucose-6-phosphate dehydrogenase locus provide convincing support for the Lyon hypothesis (27). Individual cells from such women produce only one or the other form of the enzyme, but not both. Complete genomic exclusion has been reported to occur after conjugation in the protozoa, tetrahymena pyriformis (28), accompanied by characteristic karyotypic alterations. The karyotype obtained from peripheral blood cells from our propositus showed no abnormalities.

Although the genetic mechanism for C'2 deficiency in humans has not been elucidated, the inheritance patterns are similar to those of C'3 deficiency, in that partial and complete deficiency are transmitted as autosomal dominant and recessive traits, respectively, with heterozygotes having somewhat less than one-half of the normal concentration of C'2 by functional assay (10, 11) and immunochemical estimation (12, 29). By contrast, the deficiency of C'1 esterase inhibitor activity in patients with hereditary angioneurotic edema, whether associated with quantitatively or qualitatively deficient protein (1–3), is transmitted as an autosomal dominant trait.

The heterozygous and homozygous deficiency states for alpha$_1$ antitrypsin are inherited in autosomal codominant fashion (30) and a genetic mechanism similar to that described for C'3 deficiency is suggested by the study of a family with a structural variant and the

heterozygous deficiency state (31), although the authors do not draw this conclusion. Anhaptoglobinemia as a genetically determined disorder appears to be inherited as a variant of Hp 2-1M (32).

Whether the postulated synthetic defect is the result of a mutation in a structural or regulatory gene is, of course, unknown. It is conceivable that an aberrant gene product might be so unstable that it precipitates or is destroyed at the cellular site of synthesis in the liver (33) and never enters the plasma. Alternatively, a mutation in a structural gene might interfere with translation and polypeptide chain assembly, as has been postulated in thalassemia (34).

Affected family members in this report differ in many respects from the individual we described (35) with low serum C'3 concentration secondary to continuous increased conversion of C'3 to C'3$_i$ and the rapid catabolism of the inactivated protein. The synthesis of C'3 was entirely normal in that patient, as determined by metabolic studies with labeled C'3. His total immunochemical C'3 serum concentration was about 25–30 mg/100 ml, but of this amount only about 8 or 9 mg/100 ml was native C'3, since the major portion was C'3$_i$. By contrast, no C'3$_i$ was detectable by antigen-antibody crossed electrophoresis in the sera of the present family members. More importantly, the individual reported previously had markedly impaired complement-mediated functions, such as C'H$_{50}$, chemotaxis for leukocytes, serum Gram-negative bactericidal activity, and opsonization. Individuals partially deficient in C'3 in the present family show no serum abnormalities of complement function except for mildly reduced hemolytic complement and about half-normal opsonization as measured by Nitroblue tetrazolium (35, 36) reduction. The results of the latter test, as applied to serum from individuals with partial C'3 deficiency will be reported in detail elsewhere.[1] It appears that C'3 is the limiting complement component in this assay. Finally, in contrast with the patient with increased catabolism of C'3, thought to be associated with the congenital absence of a protein required for the in vivo stability of C'3, individuals with partial, genetic C'3 deficiency are not subject to increased susceptibility to infection, nor do they have any detectable clinical abnormality.

Since individuals with partial deficiency of C'3 have approximately 50% of the normal concentration of this protein, the synthesis of C'3 is probably independent of the plasma concentration. This conclusion is in accord with the findings obtained from metabolic studies with isotopically labeled C'3 (17).

[1] Johnston, R. B., Jr., M. R. Klemperer, C. A. Alper, and F. S. Rosen. The enhancement of bacterial phagocytosis by serum factors: the role of complement components and two serum cofactors. In preparation.

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