Studies of a Hypomorphic Variant of Human C3

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ABSTRACT A hypomorphic electrophoretic variant of C3 with the mobility of C3 F was found in the serum of a healthy man, his mother, and one of his two sons. Serum C3 concentrations were normal in these subjects as were hemolytic complement levels. Metabolic studies with radiolabeled purified C3 FF and C3 SS in the propositus suggested, but did not prove, that the variant C3 F gene was hyposynthetic. The designation C3 f was therefore proposed for this allele.

INTRODUCTION

Extensive genetic polymorphism of the third component of complement, C3, has been found in man (1–4). Among Caucasians, two alleles, $C3^S$ with a gene frequency of approximately 0.77 and $C3^F$ with a frequency of 0.22, control the synthesis of this protein in most individuals. Gene products are recognized by their relative electrophoretic mobilities in prolonged agarose-gel electrophoresis (3) or high voltage starch-gel electrophoresis (4). In heterozygotes, the concentration of the two allelic gene products is approximately the same. In one individual of apparent type C3 FS, the concentration of C3 F was found to be distinctly less than that of C3 S, and studies of this subject and his family form the basis of this report.

METHODS

Serum. All sera were frozen promptly at −80°C and thawed immediately before analysis.

Determination of C3 concentration. Concentration of C3 in serum was determined by both an electroimmunochemical (5) and a nephelometric technique (6) using monospecific rabbit or goat antisera to human C3 prepared by the method of Nilsson and Müller-Eberhard (7).

Electrophoretic methods. Prolonged electrophoresis of whole serum in agarose gel (8) was performed as described previously (3), and the gels were either stained with Amido Black for C3 typing or were subjected to immunofixation (9, 10) with anti-C3, or were used for antigen-antibody crossed electrophoresis (11).

Metabolic studies with radioiodine-labeled C3. C3 was purified from the plasma of one subject who was C3 FF and one who was C3 SS. C3 FF was labeled with ^{125}I and C3 SS with ^{131}I by the iodine monochloride technique (12). A mixture of these two labeled preparations was administered to each subject. The collection and processing of samples and methods of calculation have been described previously (13).

Subjects. The propositus, the living members of his family (Boe), and the normal subjects were all in excellent health.

RESULTS

The C3 in the serum of the propositus showed two bands on prolonged agarose-gel electrophoresis which corresponded to C3 FS in mobility. The concentration of C3 F was, however, considerably lower than that of C3 S (Fig. 1). Identical C3 patterns were observed when his serum was examined by immunofixation after prolonged agarose-gel electrophoresis. Over 100 samples of other C3 FS sera examined in our laboratory have shown bands of approximately equal density. Patterns similar to that of the propositus were observed, however, when the sera of his mother and one of his two sons were studied. By elution of stained electrophoretic patterns, it was estimated that 40% of the total serum C3 had the mobility of C3 F, whereas 60% had the mobility of C3 S. A similar, but less accurate, estimate was obtained by antigen-antibody crossed electrophoresis, as shown in Fig. 2. In C3 FS patterns from six individuals, the average F : S ratio was 52 : 48. Serum C3 concentrations of all the available family members are given in Table I and were normal. It, therefore, seems reasonable that the gene product with the mobility of C3 F was reduced in concentration. Hence, the designation C3 f is proposed for this allele. As is evident from Table I, hemolytic complement was normal in all the family members.

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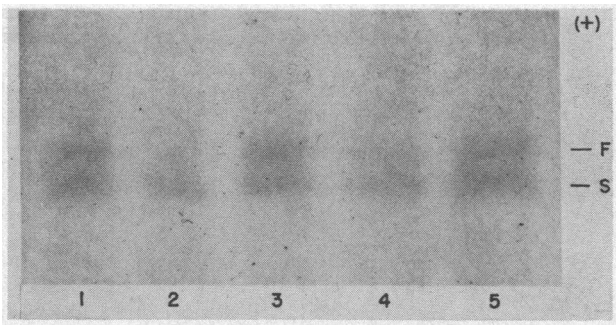

FIGURE 1 Prolonged agarose-gel electrophoresis showing the C3 area. Samples 1, 3, and 5 were C3 FS serum, whereas samples 2 and 4 were from the Boe family propositus and contained C3 fS. The anode was at the top.

The results of the metabolic studies with labeled purified C3 FF and C3 SS in the C3 fS propositus, a C3 FF subject, and a C3 SS subject are given in Table II. The two C3 preparations gave closely similar fractional catabolic rates and extravascular : plasma pool ratios in all subjects, including the C3 fS individual. Synthesis rates for C3 FF were low in all subjects but particularly so in the C3 fS propositus. The serum concentration of C3 in the C3 FF subject was 90 mg/100 ml (below the normal range of 96.9–203.7, as determined in our laboratory). The extravascular : plasma pool ratios for C3 FF were low in all subjects.

DISCUSSION

The present results provide evidence for a C3 allele with a gene product of the same electrophoretic mobility as C3 F but occurring in lower concentration in serum than that of the other C3 allele. The inheritance of this trait, as is true of the other C3 alleles, appears to be autosomal (since there is male to male transmission and there are male heterozygotes) and codominant. It is only possible to detect this form of C3 with certainty in the heterozygote and if an analogous gene, C3 s, occurs, it will be much more difficult to identify because of the higher frequency of C3 S compared with other alleles in all populations studied to date.

TABLE I

C3 Concentration, C3 Type, and Hemolytic Complement in Sera of Members of the Boe Family

Subject	C3 concentration	C3 type	CH_{50}
	mg/100 ml		*U/ml*
Propositus	132	fS	32
Mother	146	fS	
Wife	157	SS	48
Son 1	135	fS	34
Son 2	144	SS	47
Normal range	97–204		32–45

TABLE II

Metabolism of C3 FF-^{125}I and C3 SS-^{131}I in Subjects of Different C3 Types

Recipient C3 type	C3 preparation	Catabolic rate	Allelic synthesis rate*	Extravascular pool / Plasma pool
		% plasma pool/hr	*mg/kg per hr*	
fS	SS	1.65	0.61	0.52
	FF	1.42	0.35	0.29
SS	SS	1.76	0.47	0.56
	FF	1.53	(0.41)	0.41
FF	SS	1.80	(0.43)	0.58
	FF	1.57	0.38	0.37
11 Subjects of unknown type (Mean ±2SD)	—	1.26–3.36	(0.43–0.95)	0.49–1.31

* In C3 homozygous subjects (C3 SS or C3 FF), it was assumed that each gene produced C3 at the same rate. Thus, allelic synthesis rates are one-half those of total C3. The rates in parentheses are "theoretical" since C3 SS subjects produce no C3 FF or vice versa.

Since the serum concentration of C3 f in this family was at least one-half the concentration of C3 S, the total concentration in affected persons was normal, owing to the large range of normal C3 concentration. For similar reasons, the normal hemolytic complement levels were not surprising.

Genes analogous to C3 f are known in other systems. Among the genes controlling the synthesis of haptoglobin, one is associated with a low relative concentration of Hp 2 compared with Hp 1 in heterozygotes and

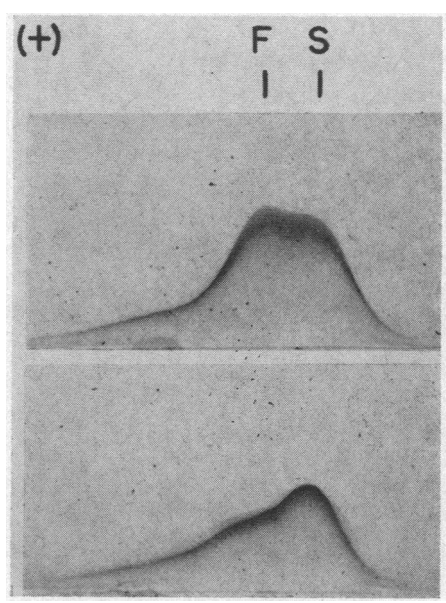

FIGURE 2 Antigen-antibody crossed electrophoresis showing C3 FS (top) and C3 fS (bottom). The positions of C3 F and C3 S are indicated. The anode for the first separation was at the left and for the second separation was at the top.

produces the pattern of Hp 2-1 M (14, 15). Indeed, there appears to be a spectrum of such Hp alleles (16). Similarly, among the genes controlling α_1-antitrypsin synthesis, Pi^P, Pi^S, Pi^W (17) and, most notably, Pi^Z (18, 19), are associated with low concentration gene products.

The metabolic studies with purified labeled C3 FF and C3 SS suggest, but do not prove, that $C3^f$ is hyposynthetic in comparison with $C3^S$. Since it is possible that C3 F and C3 f differ structurally, it would have been more definitive had the metabolism of C3 f been studied. Unfortunately, it is not yet possible to isolate C3 f from the serum of C3 fS persons and C3 ff has not yet been found. The present studies, therefore, do not rule out the possibility that C3 f is unstable, either at the site of synthesis or in the plasma.

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