INHERITED STRUCTURAL POLYMORPHISM IN HUMAN C2: EVIDENCE FOR GENETIC LINKAGE BETWEEN C2 AND Bf

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Inherited structural polymorphism in human complement proteins has been found in C3 (1, 2), properdin factor B (3), and C6 (4). Evidence has been presented that the gene for factor B (Bf) is closely linked to the major histocompatibility locus in man (5) and in the rhesus monkey (6). From linkage analysis in families, it appears that the distance between Bf and HLA-B on the sixth human chromosome is approximately 2-6 centimorgans (7-9), and the distance between these two loci in the rhesus is similar (6). Evidence for genetic linkage has also recently been obtained between C6, Bf, and HLA with an approximately distance between C6 and HLA of 15 centimorgans. Other workers have found no evidence for C6-HLA linkage (10). Other observations indicate linkage of the locus for C4 deficiency in the guinea pig with the major histocompatibility complex (MHC) in that species (11), and there may also be linkage between C4 deficiency and the MHC in man (12).

The gene for C2 deficiency in man is closely linked to the MHC (13-15), and moreover there is marked linkage disequilibrium in that, in a large number of instances, the C2 deficiency gene is in linkage with HLA-A10 B18 (13-15), HLA-Dw2 (16), and Bf S (9). The distance between the genes for C2 deficiency and for HLA-B is similar to that between the genes for HLA-B and for Bf, about 5 centimorgans (9, 17). The present studies are concerned with genetic polymorphism in C2 and possible linkage of this structural locus to Bf.

Materials and Methods

Serum Samples. Serum samples were obtained by centrifugation of whole blood allowed to clot at room temperature for about 30 min and at 4°C for 1 h. The serum was stored at -80°C and thawed before analysis. However, samples kept at 4°C for up to 1 wk or thawed and frozen up to five times gave identical patterns of normal intensity.

Isoelectric Focusing in Thin-Layer Polyacrylamide Gel. The method described by Awdeh et al. (18), modified as delineated previously (4), was used. Ampholines (LKB Instruments, Inc., Rockville, Md.) at a final concentration of 2% (six parts pH 5-8, one part pH 3.5-10) were incorporated into 5% acrylamide gels with 0.2 M taurine. Gels were polymerized with riboflavin and light. Samples were applied by moistening small rectangles of Whatman no. 1 filter paper and placing them on the gel surface near the anode.

Developing Gel. Upon completion of isoelectric focusing, the acrylamide gel was layered with

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1-mm thick 0.6% agarose in isotonic Veronal-buffered saline at pH 7.4 containing 0.1% gelatin, $1 \times 10^{-3}$ M Mg$^{2+}$ and $1.5 \times 10^{-4}$ M Ca$^{2+}$, 0.8% sheep erythrocytes sensitized with rabbit antibody (EA), and 6.7% homozygous C2-deficient serum (19). The undiluted C2-deficient serum and EA were preincubated for 70 s at 37°C and then mixed with the agarose solution in buffer at 45-50°C. After gelation, plates were placed in an incubator at 37°C. Patterns usually began to appear by 1 h and were optimal at 4 h. This method is the same as that published by Hobart and Lachmann (20).

Results

Serum from most individuals showed C2 patterns consisting of two prominent bands of lysis and two or three additional bands acidic or basic to these, as can be seen in Fig. 1. Rare sera showed basic duplication of the major bands (and perhaps of the minor bands as well), and a single sample from a Caucasian showed acidic duplication of the major and some minor bands of lysis (Fig. 1). The variant bands tended to develop more slowly than the common bands and did not achieve equal intensity by 4 h. Serum samples from five subjects homozygous for C2 deficiency gave no patterns.

The common C2 has been designated as C2 C (for common), the rare basic type as C2 B, and the rare acidic type as C2 A. The respective genes are C2 c, C2 B, and C2 A.

Table I lists the incidence of C2 variants in the various populations examined. The overall incidence of variants was 3.75%, and it is evident that they occur in all major races.

The families (LR, Th, and En) of three persons with C2 variants were studied. Family LR was reported previously in relation to C3 deficiency (21). In 11 subfamilies with one parent heterozygous for C2 A or C2 B, 24 of 51 children inherited the variant C2. Moreover, about half the heterozygotes were males, and there was male to male transmission of the C2 variant, indicating autosomal codominant inheritance. Factor B types are shown in Fig. 2 where they are informative for analysis of Bf-C2 linkage. It is seen that no instances of crossovers between C2 and Bf were encountered. The lod score for this linkage calculated from these relatively few informative matings was 3.6 at $\theta = 0.005$. This indicates the odds for linkage are nearly 4,000:1. Thus, it appears highly likely that the C2 structural locus is closely linked to Bf and, therefore, probably to the MHC and the other complement genes linked to the MHC on the sixth human chromosome.

Discussion

Isoelectric focusing in liquid medium was applied by Colten and coworkers (22) to examine possible genetic polymorphism in human C2. All human sera examined gave a single band. The failure to detect the multiple bands of C2 in all sera and the small differences in pI that characterize the genetic variants, as seen in the present study, must be ascribed to the relative insensitivity of isoelectric focusing in liquid compared with isoelectric focusing in gel.

The existence of genetic polymorphism in human C2 has been found by Hobart and Lachmann (10) who also obtained evidence for linkage between C2 and HLA in a family with four informative children and no crossing over. Their C2 2 almost certainly corresponds to C2 B of the present report.
Fig. 1. Patterns of C2 in normal human sera as obtained by isoelectric focusing in polyacrylamide gel and development of bands of lysis in an overlay agarose gel containing EA and C2-deficient human serum. Common C2 (C2 C) is shown in a homozygote, and the acidic (C2 A) and basic (C2 B) variants are shown in heterozygotes who also possess C2 C.

| TABLE I | C2 Variants Among Random Unrelated Individuals |
|---------|-----------------------------------------------|
|         | No. tested | C2 BC | C2 AC |
| Orientals | 43         | 3     | 0     |
| Caspian   | 75         | 3     | 1     |
| Negro     | 30         | 2     | 0     |
| Unspecified | 92     | 0     | 0     |

The fact that both the acidic and basic structural variants of C2 develop more slowly than C2 C and are of lesser intensity suggests the possibility that the structural mutations involved alter the hemolytic function of C2. It is also possible these structural variants are associated with diminished concentration of the gene product in serum, as occurs with structural variants of α1-antitrypsin (23).

The probable colocalization of the loci for C2 deficiency and C2 structural polymorphism on the sixth chromosome suggests that the deficiency state for C2, like that for C3 (21, 24), involves a blank allele of the structural genes. Alternatively, it might result from inheritance of a faulty regulatory gene closely situated on the chromosome to the structural gene. The proximity of the structural genes for C2 and factor B is consistent with the possibility of the origin of C2 by tandem gene duplication from Bf.

Summary

Structural variation in the second component of human complement was identified in about 4% of serum samples from random unrelated individuals of
Fig. 2. The inheritance of C2 variants in two kindred informative for linkage with Bf. Males are shown as squares, females as circles. Generation numbers are denoted by Roman numerals to the left of each family and individual identification numbers (within the larger kindred) are given at the upper right corner of each symbol. The presence of a basic variant (in the LR family) and an acidic variant (En family) is indicated by half-blackened symbols. Bf types are given below each symbol.

all the major races. Three forms of C2 have been identified by isoelectric focusing in polyacrylamide gel and development of patterns in agarose gel containing antibody-sensitized sheep red cells and C2-deficient serum: C2 C (common), C2 A (acidic), and C2 B (basic). The C2 variants were shown to be inherited as autosomal codominant traits, and suggestive evidence for close linkage between C2 and Bf was obtained.

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