Complement–Human Histocompatibility Antigen Haplotypes in C2 Deficiency

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ABSTRACT C4 allotyping 13 homozygous C2-deficient individuals demonstrated 23 of 25 haplotypes to be of the relatively rare type *C4A*4 B*2*. This is of the same magnitude as the association of *C2*Q0* with *HLA-DW2/DR2*.

INTRODUCTION

Deficiency of the second component of complement (*C2*Q0*) has been extensively studied and reported and has a gene frequency near 1% (K. Stratton. Personal communication.). *C2*Q0* is known to be *HLA*-linked (1–4) and to be a silent allele at the *C2* structural locus (5). The structural locus for *C2* is also known to be linked to the *HLA* region (6–10). The structural polymorphism of the fourth component of human complement (C4) has been shown to be caused by two closely linked loci, associated with Rodgers and Chido positivity (11–13). Deficiency of one or the other of these proteins is relatively common, whereas the haplotype deficient for both is very uncommon. By desialation before electrophoresis and subsequent crossed immunoelectrophoresis, it is possible to detect individuals heterozygous for these partial deficiencies (14). Using desialated plasma, prolonged agarose gel electrophoresis, and immunofixation, six common structural variants at the Rodgers locus and two common variants at the Chido locus have been defined (15). The nomenclature used by us to describe the genetic variants of C4 is the same as that proposed earlier (15). The alleles of the acidic, Rodgers-positive, [Rg(a+)], *C4* alleles are designed *C4A*1-6* and those for the basic, Chido-positive, [Ch(a+)] alleles *C4B*1,2*. There is a null allele at each locus designated *C4A*Q0* and *C4B*Q0*. The linkage of a *C4* structural locus to *HLA* was first inferred from the study of two C4-deficient families (16–18). The model of O'Neill et al. (11, 12) also postulates linkage to *HLA* of the two closely linked *C4* loci. The red cell antigens Rodgers and Chido carried on C4 have been independently shown to be HLA linked (19–21). The structural polymorphism of *C4* has also been shown to be linked to *C2* and *BF* (15), and no crossovers have been observed among these loci. Properdin factor B (BF)[1] of the alternative pathway of complement activation also exhibits a common polymorphism with two common, *BF*F* and *BF*S*, and two rarer alleles, *BF*F1* and *BF*S1* (22). The structural locus for *BF* is closely

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[1] *Abbreviation used in this paper*: BF, properdin factor B.

linked to *HLA* in man (23). No crossover has yet been observed between *C2*Q0* and *BF* (24) or *C2* and *BF* (10). Finally, the loci for *C2*, *BF*, *C4A*, and *C4B* are thought to be close to *HLA-D* and *HLA-DR* with no observed recombination between them (25).

The present report concerns the haplotypes of *HLA*, *BF*, and *C4* alleles associated with *C2*Q0* in 13 homozygous C2-deficient individuals.

METHODS

The 13 subjects in this study are all homozygous C2-deficient individuals. They were unrelated except that K.R.T. is the daughter of G.R. Blood was collected into EDTA, and plasma was separated, stored at −80°C, and thawed just before analysis. For BF typing, samples were subjected to agarose gel electrophoresis and immunofixation with goat antiserum to human factor B (22). Plasma samples were desialated by incubation with neuraminidase from *Clostridium perfringens* (Type VI, Sigma Chemical Co., St. Louis, Mo.) at a concentration of 10 mU enzyme/μl of plasma for 15 h at 4°C while dialyzed against 0.1 M phosphate buffer, pH 6.8 containing 0.005 M Na_2EDTA. For the detection of C4 half-null haplotypes, desialated plasma samples were subjected to crossed immunoelectrophoresis as described previously (14). C4 structural variants were detected by electrophoresis of desialated plasma in agarose gel (ICN Nutritional Biochemicals, Cleveland, Ohio) and immunofixation with goat anti-human C4 antiserum (Atlantic Antibodies, Scarborough, Me.) as described previously (15). Homozygous C2 deficiency was determined by previously published methods (4, 24, 26–28).

RESULTS

Of the 13 homozygous C2-deficient individuals tested for C4, 11 were apparent homozygotes at both C4 loci and had a C4 type of C4A 4, C4B 2 and were presumably of genotype *C4A*4,C4B*2/C4A*4,C4B*2*. The two exceptions were a man and his daughter. The man was homozygous deficient at the *C4B* locus, heterozygous at the *C4A* locus, had a C4 type of C4A 3,4, C4B Q0, and was therefore of the genotype *C4A*4,C4B*Q0/C4A*3, C4B*Q0*. His daughter had inherited *C4A*4 C4B*2* in linkage with *C2*Q0* from her mother and *C4A*4, C4B*Q0* from her father. The C4,BF, and HLA types of these deficient subjects are given in Table I. *C2*Q0* has a gene frequency of about 1%, and *C4A*4,B*2* has a haplotype frequency of about 6.5%, whereas *HLA-DW2* has a frequency of about 8%. From Table I, it is seen that 23 of 25 independent *C2*Q0* haplotypes were *C4A*4,C4B*2*, and 24 of 25 were *C4A*4*.

DISCUSSION

All cases for which information on C2 deficiency have been available are Caucasians. In contrast, the majority of patients with complement deficiencies of late-acting components have been Negroes. It has been calculated from a review of the literature (29) that C2 deficiency (*C2*Q0*) is in linkage disequilibrium with *HLA-B18* on approximately 56% of chromosomes but with *HLA-DW2* on about 94% of chromosomes. The linkage disequilibrium reported here between *C2*Q0* and *C4A*4,C4B*2* is of the same order of magnitude as that between *HLA-DW2* and *C2*Q0* suggesting that the loci for the complement components *C4A*, *C4B*, *BF*, and *C2* are closer to *HLA-D* and *DR* than to *HLA-B*. If one assumes that the mutation giving rise to C2 deficiency occurred only once on a chromosome that was *HLA-A10,B18,DW2,DRW2,C4A*4,C4B*2,BF*S*, then the current and previous findings suggest that the *C2* locus is very close to *BF* and the two C4 loci, *C4A* and *C4B*. The haplotype *C4A*4,B*Q0* has been observed only this once among all individuals studied to the present (more than 300 normal haplotypes). This makes it most likely that this haplotype arose either by deletion from *C4A*4,C4B*2* or by a crossover between

TABLE I
Chromosome Six Markers In C2-deficient Individuals

Patient	BF	HLA	C4 Haplotype	Reference
A.W.	S/S	A10,B18/A1,B8	A4,B2/A4,B2	(27)
S.S.	S/S	A3,B18,DR2/A10,B14	A4,B2/A4,B2	
M.C.	S/S	—	A4,B2/A4,B2	
A.O.	S/S	—	A4,B2/A4,B2	
E.M.	S/S	A2,B18,DR2/A26(10),B12	A4,B2/A4,B2	
G.R.	S/S	A10,B18/A10,B18	A4,BQ0/A3,BQ0*	
L.M.	S/S	A25(10),B18,DR3,GLO2/A2,B18,DR2,GLO 1	A4,B2/A4,B2	(28)
A.B.	S/S	—	A4,B2/A4,B2	
B.S.	S/S	—	A4,B2/A4,B2	
L.Sh.	S/S	A10,B18,DW2/A10,B18,DW2	A4,B2/A4,B2	(1, 26)
L.So.	S/S	—	A4,B2/A4,B2	
K.R.T.	S/S	—	A4,B2/A4,BQ0*	
A.F.	S/S	A25,B18,DW2/A25,B18,DW2	A4,B2/A4,B2	

* Related individuals G.R. and K.R.T.

*C4A*4* and *C4B*2* with a *C4B*Q0* being substituted. This suggests that *C4A* is closer to *C2* than is *C4B*. Similarly, the presumed substitution in the past of *HLA-DR3* for *HLA-DR2* in individual L.M. is consistent with the possibility that the complement genes are located between *HLA-B* and *HLA-DR*.

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