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*F1, and two rarer alleles, BF*F1 and BF*S1 (22). The structural locus for BF is closely

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

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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 desialylated 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 Na₂EDTA. For the detection of C4 half-null haplotypes, desialylated plasma samples were subjected to crossed immunoelectrophoresis as described previously (14). C4 structural variants were detected by electrophoresis of desialylated plasma in agarose gel (ICN Nutritional Biochemicals, Cleveland, Ohio) and immunofixation with goat anti-human C4 antisera (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,0, 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,DR2,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>
<thead>
<tr>
<th>Patient</th>
<th>BF</th>
<th>HLA</th>
<th>C4 Haplotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td>S.S.</td>
<td>S/S</td>
<td>A3,B18,DR2/A10,B14</td>
<td>A4,B2/A4,B2</td>
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<tr>
<td>M.C.</td>
<td>S/S</td>
<td>A10,B18/A10,B18</td>
<td>A4,B2/A4,B2</td>
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<tr>
<td>A.O.</td>
<td>S/S</td>
<td>A2,B18,DR2/A26(10),B12</td>
<td>A4,B2/A4,B2</td>
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<tr>
<td>E.M.</td>
<td>S/S</td>
<td>A2,B18,DR2/A26(10),B12</td>
<td>A4,B2/A4,B2</td>
<td></td>
</tr>
<tr>
<td>G.R.</td>
<td>S/S</td>
<td>A25(10),B18,DR3,GLO2/A2,B18,DR2,GLO 1</td>
<td>A4,B2/A4,B2</td>
<td>(28)</td>
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<tr>
<td>L.M.</td>
<td>S/S</td>
<td>A10,B18/DW2/A10,B18</td>
<td>A4,B2/A4,B2</td>
<td>(1, 26)</td>
</tr>
<tr>
<td>A.B.</td>
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<tr>
<td>B.S.</td>
<td>S/S</td>
<td>A10,B18,DW2/A10,B18</td>
<td>A4,B2/A4,B2</td>
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<td>L.Sh.</td>
<td>S/S</td>
<td>A2,B18,DR2/A26(10),B12</td>
<td>A4,B2/A4,B2</td>
<td>(1, 26)</td>
</tr>
<tr>
<td>L.So.</td>
<td>S/S</td>
<td>A25(10),B18,DR3,GLO2/A2,B18,DR2,GLO 1</td>
<td>A4,B2/A4,B2</td>
<td></td>
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<tr>
<td>K.R.T.</td>
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<td>A4,B2/A4,B2</td>
<td></td>
</tr>
<tr>
<td>A.F.</td>
<td>S/S</td>
<td>A25(10),B18,DR3,GLO2/A2,B18,DR2,GLO 1</td>
<td>A4,B2/A4,B2</td>
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* 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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REFERENCES


