Am J Hum Genet 31:35–41, 1979

Mapping of the Structural Gene for the Second Component of Complement with Respect to the Human Major Histocompatibility Complex

DONALD RAUM,[1] DAVID GLASS,[2] CHARLES B. CARPENTER,[3]
PETER H. SCHUR,[2] AND CHESTER A. ALPER[1]

SUMMARY

Families have been HLA typed, and allotypes of the second component of complement and properdin factor B determined. The lod score for the *C2* structural gene and *HLA-B* from the study of 11 families and 55 informative meioses was 14.39 at maximum likelihood estimate of the recombination fraction of .02. This is related to other estimates of the distance between these two genes. The relative kinetic activities of the C2 allotypes were studied and no differences were demonstrated. No crossovers between *Bf* and *C2* were observed.

INTRODUCTION

The human major histocompatibility genes (*HLA-A, B, C, D*) collectively referred to as the major histocompatibility complex (MHC), are closely linked on chromosome 6 [1] to the structural genes for properdin factor B (*Bf*) [2], and the second component of complement (*C2*) [3]. In addition, the MHC is closely linked with deficiency of the second component of complement in man (*C2d*) [4, 5]. It is also known that the MHC of rhesus monkey (*RhLA*) is closely linked to the properdin factor B locus (*RhBf*) [6]; and the MHC of guinea pig to deficiency of the fourth component of complement [7]. It has been shown that the *H-2* linked Ss protein controlling mouse complement [8] is the murine equivalent of the fourth component of human complement. Conflicting evidence has been presented concerning the order of human genes of the MHC and *Bf*. Studies by Lamm et al. [9] and Hauptmann et al. [10] suggest that *Bf* is between *HLA-B* and *HLA-D*. We have presented data which suggest that the order of genes is *HLA-A*,

Received July 22, 1978; revised September 22, 1978.

This work was supported in part by U.S. Public Health Service grants AI 14157, AI 13626, AM 16392, AI 15033, and AM 11414, grant 6-183 from The National Foundation–March of Dimes, The Hood Foundation, The Charles E. Merrill Trust, Clinical Research Center grant FR 128, and Histocompatibility Research Fund of the Peter Bent Brigham Hospital.

[1] Center for Blood Research, Boston, Massachusetts 02115.

[2] Robert B. Brigham Hospital, Boston, Massachusetts 02120.

[3] Howard Hughes Medical Institute, Peter Bent Brigham Hospital, Boston, Massachusetts 02115.

B, D, Bf [11]. We observed no crossovers between loci for *Bf* and *C2d*, suggesting that the locus for *C2d* is likewise located outside of the MHC. Here we present further data suggesting that the structural gene locus for *C2* is located outside the MHC.

MATERIALS AND METHODS

Population Studied

Families studied were those utilized in investigations of acquired abnormalities of the complement system in patients with rheumatic diseases [12], of the relationship of inherited C2 deficiency to HLA types [13, 11], and to rheumatic diseases [14], and of the association between HLA and necrotizing venulitis [15]. In addition, 100 parents of children with juvenile rheumatoid arthritis were surveyed for C2 allotypes. Parents and children were studied in cases in which at least one parent was heterozygous for C2.

C2 Allotypes

Allotypes of the second component of complement were determined by the method previously described [3]. Serum is subjected to isoelectric focusing in polyacrylamide gel and developed with an overlay gel containing sheep erythrocytes sensitized with antibody and C2-deficient human serum (or dilute normal human serum). There are two common alleles at the *C2* locus, *C* (for common), and *B* (for basic gene products). The gene frequency in a Caucasian population is $C = .975, B = .025$ [3]. A rare allele observed only twice is called *A1*.

Properdin Factor B Allotype, HLA and Red Blood Cell Typing

Properdin factor B allotypes were determined as previously described [16]. Typings for red cell antigen markers (A_1, A, H, B, D, C, E, c, e, C, f, V, G, P, K, k, Kp^a, Kp^b, Js^a, Le^a, Le^b, M, N, S, s, V^w, He, M^g, Lu^a, Lu^b, Jk^a, Wr^a, Vel, Yt^a, I, Xg^a, and Fy^a) and serum protein allotypes (Gm, Inv, Hp, C3, Tf, Gc, Or, Cp, and Pi) were performed to assure that stated family relationships were correct. HLA typing was done by the microdroplet lymphocyte cytotoxicity test [17].

RESULTS

Figure 1 presents lytic patterns of human C2. From left is serum with the common pattern C, then serum from a heterozygous parent BC with a set of bands shifted

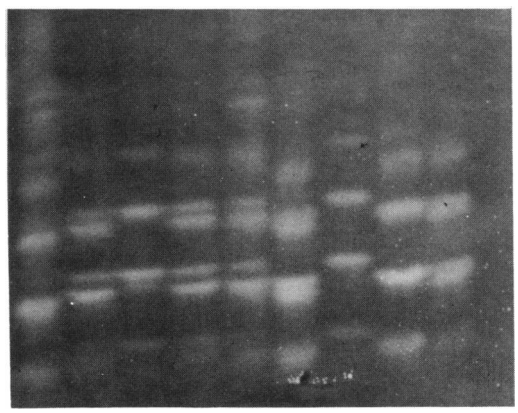

FIG. 1.—Lytic patterns of human C2

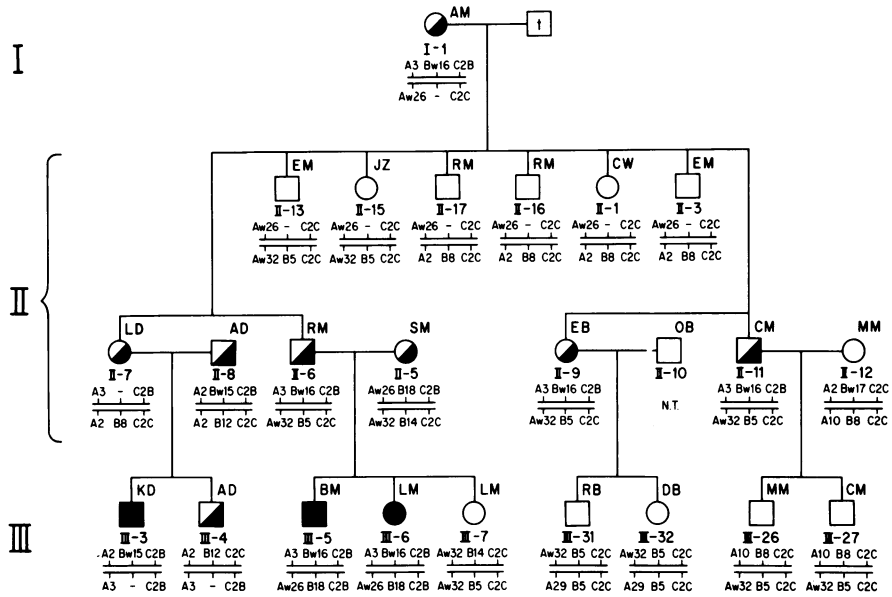

FIG. 2.—Pedigree M demonstrating the inheritance of *C2* alleles with regard to *HLA-A* and *-B* types. *C2BC* individuals are half black and *C2B* individuals are completely black.

towards the cathode, serum from a homozygous B offspring, and serum from the other heterozygous BC parent, an equal mixture of serum of homozygous B and C individuals, serum of an acidic variant heterozygote (A1C), serum of a homozygous B, of a homozygous C and of another A1C individual.

Figure 2 demonstrates the inheritance of *C2* alleles. C2 heterozygote A.M. has passed the *B* allele to four of 10 children. Coincidentally, two of her children (L.D. and R.M.) have married C2 heterozygous BC individuals and have produced offspring with phenotypes B, BC, and C.

Figure 3 shows a family in which a crossover of *C2* has been observed with respect to the MHC. The genotypes of the parents of A.T. have been inferred from two other children. In this figure, the haplotype *A2,B7* is assumed to be in coupling with *C2B*. Glyoxylase I typing was uninformative, and the family was unavailable for D- typing.

The current lod score of the *C2* structural locus to *HLA* was obtained by study of 11 families with 18 informative matings and 55 meioses. We observed one recombination, with a recombination fraction of .02 and a maximum lod score at $\theta = .02$ of 14.39. The lod score of the properdin factor B (*Bf*) locus to *C2* structural locus from study of seven families with 11 informative matings and 28 meioses and no recombinations gives a lod score at $\theta = .001$ of 8.409.

In this study, families originally studied for inherited deficiency of C2 [11] were allotyped. Of 28 matings, all C2-deficient heterozygotes and their mates were C.

To ascertain whether structural variants have the same enzymatic activities, the ability of sera from individuals of each of the cell types was compared for the rate at

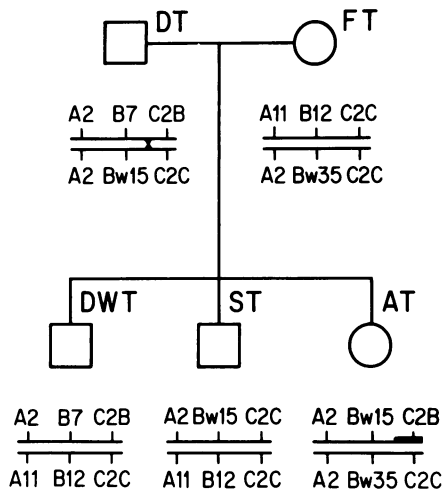

FIG. 3.—Pedigree T demonstrating a crossover of *C2* with respect to *HLA-A* and *B* loci.

which they caused lysis of sensitized red cells. Tables 1 and 2 present the results of kinetic studies of the allotypic C2 proteins. There are no differences in the functional means or the rates at which they mediate hemolysis (T_{max}'s) for CC, BC, or BB individuals even though the B bands develop more slowly on plates.

A significant association is seen between the HLA allele *Bw15* and *C2B*. In our data, from individuals pooled from all the material studied (more than 300 unrelated families), six of 16 independently observed *B* alleles were genotypically associated with *Bw15*, for which $\Delta = .33$ [18]. Two of the 16 independently observed *B* alleles were genotypically associated with *B18*, for which $\Delta = .08$. Three of these six *Bw15*'s were also associated with *A2*, while a total of five of 16 were associated with *HLA-A2* for which $\Delta = .29$. The frequency of *Bw15* in this population is .11, of *B18* is .15, and of *A2* is .56. In the case of *C2A1*, the two independent alleles were associated with different *HLA* haplotypes, *A2,Bw17* and *A3,Bw21*.

A linkage disequilibrium [18] is seen between *Bf* and *C2* alleles. All C2B's whose *Bf* genotype could be ascertained were *BfS*. In the case of *C2A1*, both independently occurring alleles were associated with BfF. No crossover has been observed between *Bf* and *C2*.

TABLE 1

T_{max} OF C2 ALLOTYPIC VARIANTS

No. Samples	Average T_{max} Minutes	Average of C2CC Controls Minutes	Allotype
6	5.83	5.00	BB
7	4.43	4.29	CC
2	4.50	4.00	BC

TABLE 2

FUNCTIONAL ACTIVITY OF C2 VARIANTS

No. Samples	Mean Functional Activity	Allotype
14	21,966	BC
4	18,008	BB
10	21,180	CC
5	16,753	A1C

DISCUSSION

The results demonstrate inheritance of C2B as a Mendelian autosomal codominant trait and product of a variant allele at the *C2* locus. In kinetic studies there is no functional difference between C2C and C2B allele products. The association of *C2B* with *HLA-Bw15* and *BfS* suggests it is a recent mutation, and the association of *C2A1* with *BfF* suggests that *A1* is a separate mutation.

These data extend previous studies [3, 19] on the genetic polymorphism and distribution of alleles of *C2*. Another probable variant, C2A2, has been found which is presumably inherited, but the single individual has no parents or offspring to test. These studies confirm the linkage disequilibrium noted between *C2B* and *HLA-Bw15* by Meo et al. [20, 21]. In these papers, $C2^2$ is equivalent to C2B and $C2^1$ to C2C.

We have observed no crossover between *Bf* and *C2* suggesting that these loci are intimately situated. The observed recombination fraction of .02 between *C2* and *HLA-B* is in accord with that observed in this population between *C2d* and *HLA-B* (.03) and *Bf* and *HLA-B* (.04) [11]. In this study, errors of ascertainment of C2 phenotype as C, BC, or A1C are less likely than ascertainment of C2 phenotype as C2 heterozygous deficient. In the first case, phenotype is determined by observation of an allotype, while in the second, it depends on several observations which fall outside a normal range. Therefore, estimates of distance by use of C2 allotypes should be more accurate. In a separate study, *C2d* has been shown to be a mutation at the *C2* structural locus [22]. Friend et al. [23] have also presented evidence that *C2d* is located outside the MHC. Published recombination fractions for *Bf* and *HLA-B* include Olaisen et al. [24] 2.1% and Albert et al. [25] 1.66% and Raum et al. [11] 4.2%. Only one group, Arnason et al. [26] reported a substantially lower fraction. Taken together, these data tend to support the argument that the *Bf* locus is outside *HLA-D* in that estimates of the map distance between *HLA-B* and *HLA-D* are lower than those for *Bf* and *HLA-B* which range from 0.4 to 1.8 centimorgans [27]. The MHC is on the short arm of chromosome 6 [28, 29], and the suggested order of genes is pter - *HLA-A, B, D, (Bf, 2C), GLOI, PGM3* . . . centromere.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent assistance of Vicki Mattson Cairns and Jean Pruss.

REFERENCES

1. LAMM LU, FRIEDRICH U, PETERSEN GB, JORGENSEN J, NIELSEN J, THERKELSEN AJ,

KISSMEYER-NIELSEN F: Assignment of the major histocompatibility complex to chromosome 6 in a family with a pericentric inversion. *Hum Hered* 24:273–284, 1974
2. ALLEN FH JR: Linkage of HL-A and GBG. *Vox Sang* 27:382–384, 1974
3. ALPER CA: Inherited structural polymorphism in human C2: evidence for genetic linkage between *C2* and *Bf*. *J Exp Med* 144:1111–1115, 1976
4. FU SM, KUNKEL HG, BRUSMAN HP, ALLEN FH JR, FOTINO M: Evidence for linkage between HL-A histocompatibility genes and those involved in the synthesis of the second component of complement. *J Exp Med* 140:1108–1111, 1974
5. DAY NK, L'ESPERANCE P, GOOD RA, MICHAEL AF, HANSEN JA, DUPONT B, JERSILD C: Hereditary C2 deficiency: genetic studies and association with the HL-A system. *J Exp Med* 141:1464–1469, 1975
6. ZIEGLER JB, ALPER CA, BALNER H: Properdin factor B and histocompatibility loci linked in the rhesus monkey. *Nature* 254:609–611, 1975
7. KRÖNKE M, GECZY AF, HADDING U, BITTER-SUERMANN D: Linkage of C4 and C4 deficiency to Bf and GPLA. *Immunogenetics* 5:461–463, 1977
8. DÉMANT P, CAPKOVÁ J, HINZOVÁ E, VORÁCOVÁ B: The role of the histocompatibility-2-linked *Ss-Slp* region in the control of mouse complement. *Proc Natl Acad Sci USA* 70:863–864, 1973
9. LAMM LU, JØRGENSEN F, KISSMEYER-NIELSEN F: Bf maps between HLA-A and D loci. *Tissue Antigens* 7:122–124, 1976
10. HAUPTMANN G, SASPORTES M, TONGIO MM, MAYER S, DAUSSET J: The localization of the Bf locus within the MHS region on chromosome no. 6. *Tissue Antigens* 7:52–54, 1976
11. RAUM D, GLASS D, CARPENTER CB, ALPER CA, SCHUR PH: The chromosomal order of genes controlling the major histocompatibility complex, properdin factor B, and deficiency of the second component of complement. *J Clin Invest* 58:1240–1248, 1976
12. SCHUR PH: Complement in lupus, in *Clinics in Rheumatic Diseases, Systemic Lupus Erythematosus,* Vol 1, edited by ROTHFIELD NS, London, Saunders, 1975, pp 519–543
13. GIBSON DJ, GLASS D, CARPENTER CB, SCHUR PH: Hereditary C2 deficiency: diagnosis and HLA gene complex associations. *J Immunol* 116:1065–1070, 1976
14. GLASS D, RAUM D, GIBSON D, STILLMAN JS, SCHUR PH: Inherited deficiency of the second component of complement. *J Clin Invest* 58:853–861, 1976
15. GLASS D, SOTER NA, GIBSON D, CARPENTER CB, SCHUR PH: An association between HLA and cutaneous necrotizing venulitis. *Arthritis Rheum* 19:945–949, 1976
16. ALPER CA, BOENISCH T, WATSON L: Genetic polymorphism in human glycine-rich beta-glycoprotein. *J Exp Med* 135:68–80, 1972
17. MITTAL KK, MICKEY MR, SINGAL DP, TERASAKI PI: Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation (Baltimore)* 6:913–927, 1968
18. CAVALLI-SFORZA LL, BODMER WF: *The Genetics of Human Population.* San Francisco, W. H. Freeman, 1971
19. HOBART MJ, LACHMANN PJ: Allotypes of complement components in man. *Transplant Rev* 32:26–42, 1976
20. MEO T, ATKINSON J, BERNOCO M, BERNOCO D, CEPPELLINI R: Mapping of the HLA locus controlling C2 structural variants and linkage disequilibrium between alleles $C2^2$ and Bw15. *Eur J Immunol* 6:916–919, 1976
21. MEO T, ATKINSON JP, BERNOCO M, BERNOCO D, CEPPELLINI R: Structural heterogeneity of C2 complement protein and its genetic variants in man: a new polymorphism of the HLA region. *Proc Natl Acad Sci USA* 74:1672–1675, 1977
22. PARISER KM, RAUM D, BERKMAN EM, ALPER CA, AGNELLO V: Evidence for a silent or null gene in hereditary C2 deficiency. *J Immunol.* In press, 1978
23. FRIEND PS, HANDWERGER BS, KIM Y, MICHAEL AF, YUNIS EJ: C2 deficiency in man. Genetic relationship to a mixed lymphocyte reaction determinant (7a*). *Immunogenetics* 2:569–576, 1975
24. OLAISEN B, TEISBERG P, GEDDE-DAHL T JR, THORSBY E: The *Bf* locus in the HLA region

of chromosome 6: linkage and association studies. *Hum Genet* 30:291–296, 1975
25. ALBERT ED, RITTNER CH, GROSSE-WILDE H, NETZEL B, SCHOLZ S: Recombination frequency and linkage disequilibrium between HL-A and Bf, in *Histocompatibility Testing*, edited by KISSMEYER-NIELSEN F, Copenhagen, Munksgaard, 1975, pp 941–943
26. ARNASON A, LARSEN B, MARSHALL WH, EDWARDS JH, MACKINTOSH P, OLAISEN B, TEISBERG P: Very close linkage between HLA-B and Bf inferred from allelic association. *Nature* 268:527–528, 1977
27. LAMM LU, KRISTENSEN T, KISSMEYER-NIELSEN F, JØRGENSEN F: On the HLA-B, –D map distance. *Tissue Antigens* 10:394–398, 1977
28. OTT J, LINDER D, MCCAW BK, LOVRIEN EW, HECHT F: Estimating distances from the centromere by means of benign ovarian teratomas in man. *Ann Hum Genet* 40:191–196, 1976
29. FRANCKE U, PELLEGRINO MA: Assignment of the major histocompatibility complex to a region of the short arm of human chromosome 6. *Proc Natl Acad Sci USA* 74:1147–1151, 1977

FIFTH INTERNATIONAL WORKSHOP IN HUMAN GENE MAPPING (sponsored by the National Foundation–March of Dimes) July 9–13, 1979, at the University of Edinburgh, UK.

This meeting will be limited to a total of 150 participants. Most contributions will be in the form of posters, and invited speakers will contribute to two plenary sessions. Those wishing to attend should apply, indicating the contribution that they propose to make, to Professor H. J. Evans, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK. CLOSING DATE FOR APPLICATIONS—APRIL 1, 1979.

The first 1979 meeting of the Clinical Genetics Society will take place at Queen's College, Oxford, on April 9th and 10th. It is likely that we shall have a full program of proffered papers. It is anticipated that we shall have some opportunity for sight-seeing and a concert in one of the colleges during the course of the meeting.

I shall be pleased to supply any members of the American Society of Human Genetics who might like to attend either of these meetings with further particulars, booking forms, and the like. Please contact K. M. Laurence, Hon. Secretary, Clinical Genetics Society, Dept. of Child Health, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, England.