Brief Definitive Report

HEREDITARY C2 DEFICIENCY: GENETIC STUDIES AND ASSOCIATION WITH THE HL-A SYSTEM*

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Isolated hereditary deficiency of C2 in man has been shown to be transmitted as an autosomal recessive trait (1–5). Recently, Fu et al. demonstrated that hereditary C2 deficiency segregated within a single family in close linkage to a particular HL-A haplotype, HL-A10,W18 (6). Genetic studies by Allen (7) have shown that the genetic polymorphism of factor B (C3 proactivator, glycine-rich β-glycoprotein), a component of the alternate pathway, is controlled by a gene located within the major histocompatibility region (MHR).

The genetic control of hemolytic complement (C) in mice is also governed by genes within the *H-2* system (8). These genes map very closely to the Ss-Slp region and there is evidence that the protein, Ss, is in fact a component of the C system in these animals (9,10). Ferreira and Nussenzweig recently showed that serum C3 levels in mice are determined by gene(s) linked with the *H-2* complex (11). These findings indicate that the MHR in mice as well as in man is involved in the genetic control of the C system. In the present study, we have investigated another larger pedigree of paternal members of a C2-deficient patient. This patient has been previously described to have systemic lupus erythematosus (12).

Materials and Methods

Buffers used for C assays; EDTA, gelatin veronal buffer, and glucose gelatin veronal buffer (ggvb) with and without metals (Mg^{++} and Ca^{++}) have been described by Mayer (13). Intermediate cells $EAC1^{sp},4^{hu}$ were prepared according to the method of Borsos and Rapp (14). $T_{max} < 5$ min.

Method of Titration of C2. Sera for C2 titration were diluted in $ggvb^{++}$ in 0.5-ml vol. 0.5 ml $EAC1^{sp},4^{hu}$ cells ($T_{max} < 5$ min) at a concentration of 1×10^8/ml were added to each tube. The tubes were incubated at 30°C for T_{max} time. 0.5 ml of 0.01 M EDTA was added to each tube, followed by 1 ml of guinea pig C 1/25 dilution in 0.04 M EDTA solution. The tubes were incubated at 37°C for 90 min, diluted with 5 ml of normal saline to stop the reaction, centrifuged, supernatant fluids read at 412 μm, and CH50 obtained as described previously (13).

HL-A Typing. A standard one-step lymphocytotoxicity test described by Kissmeyer-Nielsen (15) was used. Mono- and duospecific typing sera determining all W. H. O. defined HL-A specificities and most Workshop specificities were applied. The results of HL-A typing and the haplotyping of the

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family were carried out independently and without prior knowledge of the actual C2 levels in the serum of these individuals.

Results

94 family members of the paternal side of the C2-deficient patient were accounted for (Fig. 1). The individuals are numbered consecutively in the figure. The hemolytic C2 levels of 64 members and the HL-A typing of 58 members are

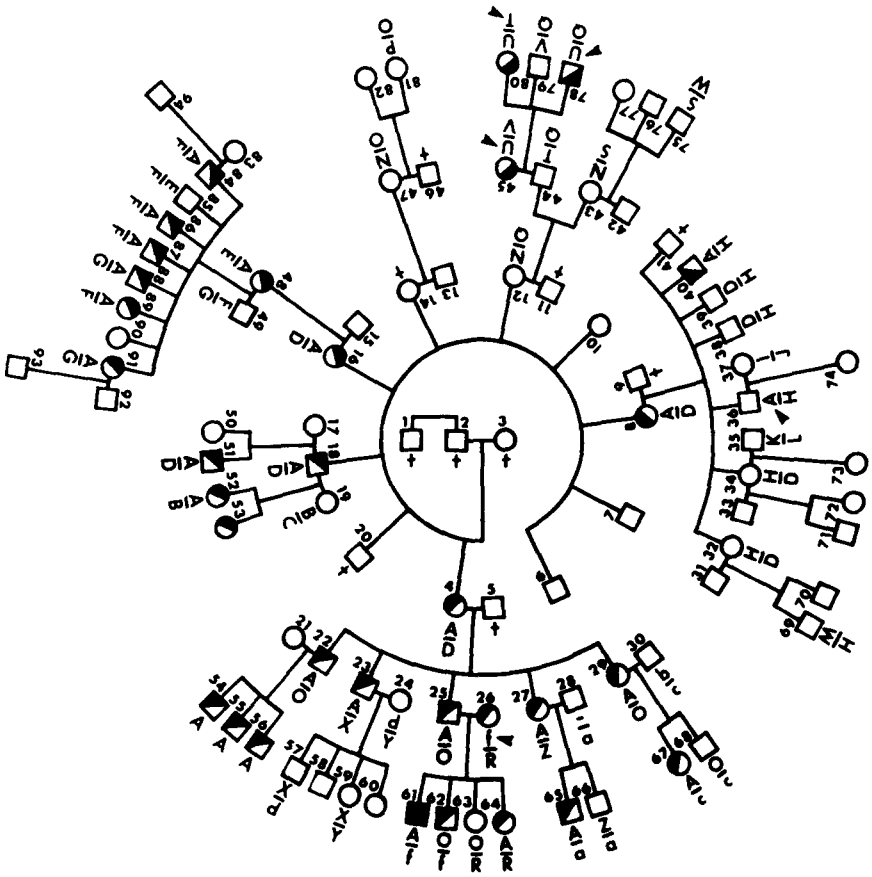

FIG. 1. The pedigree of the paternal members of the C2-deficient SLE patient. The shaded areas represent the hemolytic C2 values. These levels were 2 SD below the normal mean. (Normal mean of 40 healthy adults, 1,350 CH50 U/ml ±500.) The HL-A typing is represented below each individual.

represented in Tables I, II, III, and IV. The legend for the HL-A typing is presented in Fig. 2. In Table I, with the exception of one individual (no. 36), all the others (27 individuals) had low hemolytic C2 levels associated with an infrequent HL-A haplotype 2,4A2*. This was generally consistent, irrespective of whether the determinations were carried out once or on three different samples. Individual no. 36 with HL-A2,4A2* haplotype had elevated C2 levels at two different occasions (average, 2,488 CH50 U/ml). Another unusual finding was that an unrelated female individual (no. 45) married to a family member (no. 44)

TABLE I
*Low Hemolytic C2 Levels of Individuals Carrying HL-A2,4A2** Haplotype

No. of individual‡	C2 levels§	HL-A‖	No. of individual‡	C2 levels§	HL-A‖
4¶	635↓↓	A/D	53	158↓↓	A/D
8¶	626↓↓	A/D	54	265↓↓	A
16¶	626↓↓	A/D	55	470↓↓	A
18	184↓↓	A/D	56	490↓↓	A
22	830↓↓	A/O	61	<5↓↓	A/f
23¶	781↓↓	A/X	64	730↓↓	A/R
25	360↓↓	A/O	65	655↓↓	A/a
27	617↓↓	A/Z	67¶	228↓↓	A/c
29¶	450↓↓	A/O	84¶	430↓↓	A/F
36¶	2,488↑↑	A/H	86	130↓↓	A/F
40	164↓↓	A/H	87	250↓↓	A/F
48¶	587↓↓	A/E	88¶	428↓↓	A/G
51	790↓↓	A/D	89¶	452↓↓	A/F
52	215↓↓	A/B	91¶	404↓↓	A/G

‡See Fig. 1.
§N, normal, 1,350 ± 500 CH50 U/ml; (↓), below 1 SD and (↑), above 1 SD: 850–1,850 CH50 U/ml; (↓↓), below 2 SD: (↑↑), above 2 SD, 350–2,350 CH50 U/ml. These symbols and this legend are the same for Tables II, III, and IV.
‖See Fig. 2.
¶Mean of determinations on three separate samples.

TABLE II
Low Hemolytic C2 Levels of Individuals Carrying HL-A2,W18 Haplotype

No. of individual*	C2 levels‡	HL-A§
45‖	250↓↓	U/V
78‖	503↓↓	U/Q
80‖	150↓↓	U/T

*See Fig. 1.
‡See footnote (§) of Table I.
§See Fig. 2.
‖Mean of determinations on three separate samples.

TABLE III
Low Hemolytic C2 Levels of Individuals Carrying HL-A10,W18 Haplotype

No. of individual*	C2 levels‡	HL-A§
26	270↓↓	f/R‖
61	<5↓↓	A/f‖
62	340↓↓	O/f‖

*See Fig. 1.
‡See footnote (§) of Table I.
§See Fig. 2.
‖HL-AW25,W18 is the same as HL-A10,W18.

carried the haplotype 2,W18 which was also associated with low levels of C2 (Table II). This haplotype was transmitted to two of her three children (nos. 78 and 80). C2 levels were low in all three individuals (250, 503, and 150 CH50 U/ml, respectively).

Most important of all were the findings that in the parents and siblings of the homozygous C2-deficient patient, a third haplotype associated with low C2 values was introduced, HL-A10,W18 (Table III). This haplotype, 10,W18 was found in the mother (no. 26) of the homozygous C2-deficient patient (no. 61) and one of the two children (no. 62) both of which were heterozygous for C2. Furthermore, the homozygous C2-deficient patient (no. 61) carried this haplotype. The C2 levels of the remaining individuals and their HL-A typing in the family are presented in Table IV. Either normal or elevated levels were present.

Discussion

Family studies of the paternal members of a patient associated with C2 deficiency and systemic lupus erythematosus (SLE) indicated that the C2 deficiency was inherited as an autosomal recessive trait. Hemolytic C2 levels in

TABLE IV
Normal or Elevated Hemolytic C2 Levels of Individuals and Their HL-A Typing

No. of individual*	C2 levels‡	HL-A§	No. of individual*	C2 levels‡	HL-A§
12	1,827 N	N/Q	49‖	1,765 N	F/G
19	1,350 N	B/C	57	1,386 N	X/d
21	2,500↑↑	ND	59	1,134 N	X/Y
24‖	2,291↑	d/Y	63	1,490 N	O/R
30‖	2,686↑↑	b/c	66	1,386 N	Z/a
32‖	1,860↑	H/D	68‖	1,178 N	O/c
33‖	1,580 N	ND	69	1,764 N	M/H
34	1,235 N	H/D	70	1,386 N	ND¶
35‖	2,686↑↑	K/L	71‖	2,325↑	ND
37‖	2,975↑↑	I/J	72‖	3,160↑↑	ND
38	2,710↑↑	D/H	74‖	2,790↑↑	ND
39‖	1,896↑	D/H	75	1,084 N	S/W
42	2,260↑	ND	79‖	1,401 N	V/Q
43	1,575 N	N/S	81	1,750 N	P/O
44‖	2,133↑	T/Q	85‖	2,760↑↑	E/F
47	2,770↑↑	N/O	92	1,700 N	ND

* See Fig. 1.
‡ See footnote (§) of Table I.
§ See Fig. 2.
‖ Mean of determinations on three separate samples.
¶ ND, not done.

the heterozygous-carrier state were in general 50% or less of normal values. HL-A typing of this family indicated that the homozygous C2-deficient patient inherited HL-A10,W18 from his mother and HL-A2,4A2* from his father. Two out of four siblings from this family were heterozygote for C2 and these siblings carried one of these haplotypes.

94 family members were accounted for and it was demonstrated that 28 members carried the 2,4A2* haplotype and of these 27 members were also heterozygote with respect to C2 levels. One member carrying this haplotype (no. 36), 2,4A2* had elevated C2 levels suggesting a recombination between the C2 gene and the HL-A. The HL-A2,4A2* is an infrequent haplotype, the gene frequency being 0.173% in a normal unrelated Danish population.

A - 2,4A2*	P - 3,-	e - 10,W5
B - 1,8	Q - 11,W5,315	f - W25,W18
C - 9,W10	R - 2,W27	
D - 9,W16	S - 3,W18	
E - 11,W5	T - 3,13	
F - 3,W5	U - 2,W18	
G - 3,W21	V - 3,7	
H - W19,W5,315	W - 2,W10	
I - 2,W16	X - 29,12	
J - 2,W5	Y - 1,27	
K - 2,12	Z - 11,W10,315	
L - 29,17	a - 2,8	
M - -,W2	b - 2,5,315	
N - 2,27	c - 3,5	
O - 2,13	d - 9,7	

FIG. 2. Fig. 2 represents the haplotypes in Fig. 1. "*f*" representing HL-AW25, W18 is the same as HL-A10,W18.

The common occurrence of this low frequency haplotype 2,4A2* in our pedigree strongly indicates that this particular haplotype is inherited in this family. The haplotype 10,W18, which is expressed more frequently in the general population, was evident in the mother and two siblings of our homozygous C2-deficient SLE patient. It is of particular interest that this haplotype 10,W18 was expressed in association with C2 deficiency and lupus-like syndrome by Fu et al. (6). Studies of HL-A antigens in two families with hereditary Clr deficiency indicate that a gene controlling serum levels of this component does not segregate in linkage with the HL-A region although in one family, the homozygous Clr-deficient patient was homozygous for HL-A2, W10 haplotype.[1] In the other family, the homozygous Clr-deficient individuals (brother and sister) are currently under investigation for HL-A typing by us.[1]

The influence of determinants within the MHR on disease susceptibility and/or resistance has been the subject of intense investigation in both the experimental animals and in man. The inclusion of a third modality (i.e., the C system) in the immunological host defense, to the HL-A region, within which genetic control of immune responses are located are of significance for understanding the relationship between certain diseases, HL-A antigens, and C deficiencies. Differences in disease susceptibility could be explained by the possible occurrence of genetic polymorphism within several of the C components.

Summary

Hereditary C2-deficiency has been shown to be transmitted as an autosomal recessive characteristic. Recent evidence indicates that some genetic factors involved in the control of the complement (C) system in both man and mice are governed by genes localized within the major histocompatibility region. This study describes a large pedigree of the paternal family of a C2-deficient patient with systemic lupus erythematosus. It is shown that this condition is transmitted as an autosomal recessive trait, the heterozygous carriers having approximately half normal levels of C2. Furthermore, this trait was shown to be inherited in close linkage with an infrequent HL-A type, 2,4A2*. The maternal, C2-defective chromosome was shown to be transmitted by HL-AW10,W18 haplotype. This same haplotype was described in a similar study by Fu et al. (6) to be associated with C2 deficiency. Finally, a third haplotype HL-A2,W18 carrying a defective C2 gene was demonstrated in a part of this pedigree.

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[1] N. K. Day et al. Manuscript in preparation.

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