MIXED LYMPHOCYTE CULTURE DETERMINANTS AND C2 DEFICIENCY: LD-7a ASSOCIATED WITH C2 DEFICIENCY IN FOUR FAMILIES*

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Recent studies (1, 2) have demonstrated a genetic linkage between the HL-A system of the major histocompatibility complex (MHC) and deficiency of the second component of complement (C2). In the first case studied (1) the propositus who was homozygous for C2 deficiency also was homozygous for the HL-A haplotype 10,W18. The latter subsequently has been encountered in association with C2 deficiency in three additional families (2, 3), an extraordinary incidence as compared to that in the general population. In addition, however, several other HL-A haplotypes have been encountered. The present studies were carried out to document these relationships further with particular emphasis on studies with mixed lymphocyte culture (MLC) reactions to determine the MLC genes involved and to map the location of the C2 gene in relation to the MHC.

Methods and Materials

Serum samples were obtained from clotted blood, quickly frozen, and stored at -60°C. Samples were thawed once. C2 determinations for members of the C and G families were performed by radial immunodiffusion. In addition, the C2 level of the propositus from family C was also measured by hemolytic titration (4). The heterozygous individuals were recognized by their level of C2 which was close to one-half of the normal. The levels of C2 in members of the S and K families were reported previously (1, 2).

HL-A typing was done either by a two-stage microcytotoxicity assay procedure (1) or a one-stage assay method (2). Unidirectional mixed leukocyte cultures were performed by two methods. Method A is a slight modification of the procedure described by Hartzman et al. (5). Briefly, lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation. 1.5 x 10⁶ X-irradiated (3,000 rad) stimulating and 1.5 x 10⁶ responding cells in a total vol of 0.2 ml of RPMI 1640 medium supplemented with streptomycin, penicillin, and 20% heat-inactivated normal human serum were

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Abbreviations used in this paper: C2, second component of complement; LD, lymphocyte-defined; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; RR, relative response; SR, stimulation ratio.
mixed in the wells of Falcon microtiter plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). Each culture was set up in triplicate. After 6 days of incubation at 37°C in 5% CO₂ humidified atmosphere, 2 μCi of [³H]thymidine was added to each culture 16 h before harvesting. The lymphocytes were harvested and processed for liquid scintillation counting. Method B is a procedure reported by Jörgensen and Lamm (6). Briefly, lymphocytes were isolated by Lymphoprep (Nyegard, Oslo) gradient centrifugation; 5 × 10⁴ responding lymphocytes and 5 × 10⁴ stimulating lymphocytes were cultured in a final vol of 150 μl RPMI 1640 medium supplemented with bicarbonate, Hepes buffer, streptomycin, penicillin, glutamine, and 15% pooled, normal human serum. The stimulating lymphocytes were X-inactivated by 1,250 rad total irradiation (125 rads per minute). The cultures were performed in plastic microtiter plates (no. 1-221-24-1, Cooke Laboratory Products, Alexandria, Va.) and were set up in triplicates. After 120 h of incubation at 37°C in 5% CO₂ humidified atmosphere, the cultures were labeled with 0.025 μCi of [²-¹⁴C]thymidine in 25 μl RPMI 1640 ([²-¹⁴C]thymidine, spec act 50 mCi/mmol, New England Nuclear, Boston, Mass.). After 24 h of additional culture, the lymphocytes were harvested on Whatman glass filter paper GF/C and processed for liquid scintillation counting.

Results of MLC testing are expressed as the mean of triplicate cultures in counts per minute (cpm). Stimulation ratios (SR) are calculated from the formula SR(A) = ABx/AAx. Relative response (RR) is calculated from the formula RR(A) = (ABx - AAx)/Reference Response - AAx), where Reference Response is the response of A against an unrelated normal control or control pool (7). Application of lymphocyte-defined (LD) homozygous test cells (LD typing cells) as stimulating cells in the MLC test to define LD specificities demonstrate that the responses obtained fall into two distinct groups: (a) Individuals who do not carry the LD specificity defined by the LD typing cell: RR 36% - 100% and (b) Individuals carrying the LD specificity defined by the LD typing cell: RR 0-35%. This last group can be subdivided into a group expressing relative MLC identity: RR 0-10% and another group defining “LD-likeliness” with RR 11-35%.

SD and LD Homozygous Test Cells. 12 LD homozygous test cells were included in the study. The panel of typing cells represent MLC determinants with a cumulated gene frequency of 0.718. The test cells included the common LD specificities LD-7a, LD-8a, LD-W5a, LD-12a, LD-W15a which are in genetic linkage disequilibrium with the FOUR locus serologically defined antigens HL-A 7, 8, W5, 12, and W15. The LD-7a homozygous test cell (CM) is HL-A 3,7 homozygous, and mutually nonresponsive in MLC test with the original LD-7a test cells (Histocompatibility Workshop, Copenhagen, 1975, no. 12003) (8). The LD-7a gene frequency in the North American Caucasian population is 0.11. The same LD specificity has a gene frequency of 0.10 in the Dutch population (9) and 0.066 in the Danish population (10). The LD-7a determinant is in genetic linkage disequilibrium with the FOUR locus antigen HL-A7 (11), the Δ-value being 0.036 (10) and 0.077 (9).

Results

Families with C2 Deficiency. The S family was the first family where studies showed the linkage between HL-A histocompatibility genes and those involved in the synthesis of C2 (1). A part of the S family is shown in Fig. 1a. The propositus, L. S., is homozygous for C2 deficiency and the HL-A 10, W18. Similar linkage was also found in the large K family (2). In Fig. 1b, a part of the K family is depicted. The propositus, R. K., is homozygous for C2 deficiency but heterozygous for the HL-A haplotypes 2,4A2* and 10,W18. After these initial studies, two additional Caucasian families, C and G, have recently been encountered at The Rockefeller University, New York. Their pedigrees are depicted in Fig. 2. The propositus, J. C, in family C, is a woman with discoid lupus erythematosus who is homozygous for C2 deficiency. Clinical details of this case will be reported elsewhere (4). The propositus is heterozygous for the HL-A haplotypes 9,5 and 10, W18. She obtained
FIG. 1. Parts of pedigrees of (a) Family S and (b) Family K. The heterozygous and homozygous C2-deficient cases are indicated by the solid black symbols and the HL-A type is given in adjacent space.

FIG. 2. (a) The pedigree of Family C and (b) the pedigree of Family G. The heterozygous and homozygous C2-deficient cases are indicated by the solid black symbols and the HL-A type is given in adjoining space. The HL-A assignment for I1 and I2 in Family C were made from the typing results of their family members and are indicated by double asterisks.

the HL-A haplotype 10,W18 from her mother. The father is unavailable for typing. There is no history of consanguinity. The propositus in the G family is healthy and heterozygous for C2 deficiency. Her father and her son are also heterozygous for C2 deficiency. In this family, the gene for C2 deficiency appears to be linked to the HL-A haplotype 10,W18.

Identical MLC Locus Associated with C2 Deficiency. As reported previously (1) the propositus, L. S. of the S family, is homozygous for the MLC (LD) locus. A part of the S family pedigree relevant to this discussion is shown in Fig. 1 a and the MLC homozygosity of L. S. is documented in Table I. L. S. failed to
stimulate the cells of her children G. S. and C. D., while her children's cells stimulated her cells well.

Although the propositus, J. C., in the C family, is heterozygous for the loci of the HL-A system, she is homozygous for the MLC locus as shown in Table II. None of her three children responded to her cells while she responded to their cells. MLC homozygosity of J. C. is also shown in the MLC reactions between her children, K. C. and M. C. Both K. C. and M. C. inherited the same HL-A haplotype 3,7 from their father, F. C. Although they inherited different HL-A haplotypes from their mother, J. C., they were nonreactive in MLC. This indicates that identical MLC determinants are associated with the HL-A haplotypes 9,5 and 10, W18. In addition, J. C. (from the family C) and L. S. from the family S were mutually nonreactive in MLC.

In the family K(2), the propositus R. K., as shown in Fig. 1 b, is heterozygous for the HL-A loci. In Table III is shown the results of MLC testing performed.

### Table I

**MLC* Reactions Among Members of the S Family Showing the Propositus, L. S., to be Homozygous**

| Responding cells: | Stimulator cells |
|-------------------|-----------------|
| HL-A genotypes    | L. S. x | A. S. x | G. S. x | C. D. x |
|                   | cpm‡    | SR$    | cpm    | SR     | cpm    | SR     | cpm    |
| L. S., (mother): 10,W18/10,W18 | (238) — 10,834 45 25,343 108 9,575 40 |
| A. S., (father): 11,12/3,5 | 5,491 11 — (485) 47,229 97 24,606 50 |
| G. S., (child): 3,5/10,W18 | 1,351 1 64,090 33 (1,922) — 75,835 39 |
| C. D., (child): 11,12/10,W18 | 2,660 2 61,473 41 71,473 48 (1,501) — |

* MLC reactions were carried out by Method A.
‡ cpm indicates the means of cpm of triplicate samples.
§ SR is the stimulation ratios (ABx/AAx) and is expressed as the nearest integer.
‖ Indicates homozygous C2 deficiency.

### Table II

**MLC* Reactions Among Members of the C Family and L. S. Showing J. C., the Propositus, Homozygous and MLC Identical to L.S.**

| Responding cells: | Stimulator cells |
|-------------------|-----------------|
| HL-A genotypes    | L. S. x | J. C. x | F. C. x | K. C. x | S. C. x | M. C. x |
|                   | cpm‡    | SR$    | cpm    | SR     | cpm    | SR     | cpm    |
| L. S.: 10,W18/10,W18 | (1,059) — 1,259 1 21,588 21 17,184 17 8,188 8 4,782 5 |
| J. C. (mother): 9,5/10,W18 | 1,065 2 (888) — 25,131 36 16,556 24 19,302 28 15,139 22 |
| F. C. (father): 3,7/2,W22 | 26,614 67 29,760 75 (397) — 31,941 81 31,494 79 23,972 60 |
| K. C. (child): 3,7/10,W18 | 1,514 3 1,941 3 23,972 40 (692) — 30,240 50 1,077 2 |
| S. C. (child): 2,W22/9,5 | 1,159 2 946 1 15,909 22 20,064 28 (714) — 18,780 26 |
| M. C. (child): 3,7/9,5 | 1,671 2 629 1 33,254 47 3,955 6 54,872 78 (702) — |

* MLC reactions were carried out according to Method A.
‡ See Table I.
§ Indicates homozygous C2 deficiency.
### Table III

**MLC* Reactions of Members of the K Family and L. S. Showing the Propositus, R. K., Homozygous, and MLC Identical to L. S.**

| Responding cells: HL-A genotypes | L. S. | R. K. | Ro. K. | L. K. | Rt. K. | Rh. K. | Ri. K. |
|----------------------------------|------|------|-------|------|-------|-------|-------|
|                                  | cpm  | SR   | cpm   | SR   | cpm   | SR   | cpm   | SR   |
| L. S. †: 10, W18/10, W18         | 164  | 41   | 2,050 | 18  | 3,530 | 22  | 3,450 | 22  |
| R. K. (father): 2, 4, A2*, 2, 13  | 334  | 2    | 529   | 17  | 2,530 | 15  | 2,852 | 17  |
| R. K. (mother): 2, 4, W18, 2, W27| 1,897| 6    | 445   | 1   | 323   | 13  | 4,069 | 14  |
| R. K. (child): 2, 13, 10, W18    | 2,240| 6    | 592   | 2   | 3,773 | 9   | 3,427 | 9   |
| Rh. K. (child): 2, 13, 12, 2, W27| 1,980| 11   | 820   | 5   | 364   | 2   | 3,748 | 22  |
| Ri. K. (child): 2, 4, A2*, 2, W27| 1,622| 24   | 53    | 4   | 4,185 | 29  | 4,052 | 28  |

*RR < 35% obtained with LD homozygous test cells indicate typing responses.
† Indicates homozygous C2 deficiency.

### Table IV

**MLC* Responses among Members of the G Family and L. S. Showing that those Family Members with HL-A 10, W18 Share Similar MLC Determinants with L. S.**

| Responding cells: HL-A genotypes | L. S. | A. G. | R. G. | N. G. | M. G. | E. G. | P. G. |
|----------------------------------|------|------|------|------|------|------|------|
|                                  | cpm  | SR   | cpm  | SR   | cpm  | SR   | cpm  | SR   |
| L. S. †: 10, W18/10, W18         | 160  | 51   | 12,266| 59  | 7,174| 34  | 9,067| 43  |
| A. F. (grandfather): 1, W5/10, W18| 2,330| 7    | (337)| 36  | 11,620| 34  | 11,784| 34  |
| R. G. (grandmother): 2, W5/3, 12| 31,740| 64   | 24,743| 50  | 1,488| 5   | 23,933| 49  |
| N. G. (maternal aunt): 1, W5/3, 12| 25,094| 37   | 17,555| 26  | 1,546| 2   | 17,342| 26  |
| M. G. (mother): 10, W18/3, 12   | 3,469| 7    | 17,865| 38  | 21,521| 46  | 23,840| 51  |
| E. G. (father): 9, W16/9, 16, 12| 34,877| 17   | 60,801| 30  | 50,144| 25  | 45,128| 22  |
| P. G. (child): 9, W16/10, W18   | 1,248| 3    | 12,456| 40  | 14,521| 47  | 15,586| 51  |

* See Table I.
† Indicates homozygous C2 deficiency.
‡ Indicates homozygous C2 deficiency.

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*MLC reactions were carried out by Method A.
† See Table I.
‡ Indicates homozygous C2 deficiency.
between the family members of family K and the C2-deficient patient L. S. (family S). The lymphocytes of L. S. and of the propositus R. K. (family K) are mutually nonresponsive in MLC tests. L. S. and R. K. do not stimulate the lymphocytes from family members in family K with the HL-A haplotypes 2,4A2* and 10,W18. These family members are also heterozygous for C2 deficiency. The C2 normal family member Rh. K., who has inherited the other two parental HL-A haplotypes 2,13 and 2,W27, shows a normal MLC response when stimulated by the cells of L. S. and R. K. Proof of the LD homozygosity of the R. K. cells is given by the MLC combinations Ro. K. x Ri. K. (2,4A2*/2,13 x 10,W18/2,13) and by the combinations L. K. x Ri. K. (10,W18/2,W27 x 2,4A2*/2,W27).

In the other C2-deficient family, i.e. family G, the propositus is heterozygous for HL-A loci and C2 deficiency. The members of the G family stimulated L. S. cells well (Table IV). However, those with the HL-A haplotype 10,W18 (A. G., M. G., and P. G.) failed to respond to L. S. in the MLC reaction while the other three members of the family responded well.

Thus, it appears that the three C2-deficient homozygous individuals from different families are mutually nonreactive in MLC and the C2-deficient heterozygotes did not respond to the cells of the C2-deficient homozygotes. This point is further documented in a separate experiment shown in Table V. Here it is shown that the MLC gene associated with the C2 deficiency gene is LD-7a. In this experiment, values for the responses were calculated as RR. The cells from the three C2-deficient homozygotes and a known LD-7a homozygote, CM without C2 deficiency, were mutually nonreactive. In this testing lymphocytes from three known LD-7a heterozygous individuals with normal C2 levels (M. T., T. K., and E. S.) were included. The lymphocytes from the three homozygous C2-deficient patients (L. S., R. K., and J. C.) functioned as LD homozygous test cells when stimulating the LD-7a heterozygous cells, and the mutual MLC identity between the three cells and the LD-7a homozygous test cell (CM) identified the LD-7a determinant as the appropriate LD specificity. The three C2 homozygous cells did not show typing responses with LD homozygous test cells.

### Table V

**MLC* Reactions of C2-Deficient Homozygotes**

| Responding cells | C. M. x | L. S. x | R. K. x |
|------------------|---------|---------|---------|
| **HL-A genotypes** | **LD-genotype** | **cpm†** | **SR‡** | **RR¶** | **cpm** | **SR** | **RR** | **cpm** | **SR** | **RR** |
| C. M.: 3,7/3,7 | 7a/7a | (212) | — | — | 1,656 | 7.8 | 13 | 2,346 | 11.1 | 20 |
| L. S.: 10,W18/10,W18 | 314 | 2.3 | 2 | (134) | — | — | 707 | 5.3 | 7 |
| R. K.: 2,4A2*/10,W18 | 1,172 | 9.3 | 14 | 823 | 6.5 | 9 | (126) | — | — |
| J. C.: 9,6/10,W18 | 471 | 4.7 | 4 | 375 | 3.8 | 3 | 170 | 1.7 | 0 |
| M. T.: 9,7/1,TY | 2,404 | 4.6 | 19 | 4,369 | 8.8 | 41 | 1,840 | 3.5 | 13 |
| T. K.: 3,7/1,8 | 7a/Un‡ | 937 | 2.1 | 4 | 3,546 | 8.0 | 27 | 3,792 | 8.6 | 29 |
| E. S.: 1,2,W5,W18 | 7a/Un | 638 | 3.1 | 4 | 1,696 | 8.5 | 16 | 802 | 4.0 | 6 |
| R. D.: 2,10,5,MK | Un/Un | 8,398 | 23.2 | 84 | 4,459 | 12.3 | 42 | 4,458 | 12.3 | 42 |

* MLC reactions were done by Method B.
† See Table III.
‡ "Pool" indicates a pool of cells from four unrelated individuals.
¶ Indicates homozygous C2 deficiency.
§ Un indicates unknown LD genotypes which are not 7a.
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(L. S., R. K., J. C.) and 7a and non 7a Individuals

The relative response of 41% by the LD-7a heterozygous cells from M. T. when they were stimulated by the L. S. cells, was the only discordant observation. The L. S. cells have been included as an L. D. homozygous test cell to stimulate a panel of randomly selected normal Caucasian blood donors in New York City. Of 42 individuals tested so far, seven showed typing responses to the test cells, giving a gene frequency of 0.087. This is a slightly lower gene frequency than the one for the LD-7a test cell (0.110). The studies seem to indicate that the L. S. test cell may express minor LD inclusions on the major LD locus in addition to the LD-7a specificity (12).

Cross-over Between the HL-A Loci and the C2 Gene. HL-A and LD typing of the family members in family C (Fig. 2a) together with C2 level determinations seem to supply additional information concerning the mapping of the C2 gene in relation to the MHC. The mother (J. E.) of patient J. C. is C2 heterozygous. Her two siblings H. B. (10, W18/1, W17) and W. L. (W19, W18/1, W17) have normal C2 levels. They are mutually nonreactive in MLC testing and do not carry the LD-7a determinant as shown by the vigorous response to the LD-7a test cell (CM) and to the lymphocytes from the two C2 homozygous deficient patients L. S. and J. C. (Table VI).

Their sister J. E., who has the HL-A haplotype 10, W18 in common with H. B., does carry the LD-7a determinant on one haplotype. The positive MLC response of the LD-7a test cell (CM) to her lymphocytes demonstrate, however, that J. E. is LD-7a heterozygous. Her diseased child J. C. does not respond to the stimulation of the maternal cells. The observation that the two siblings H. B. and W. L. are mutually nonresponsive in MLC, together with the above observation, indicates that the two MHC haplotypes 10, W18 and W19, W18 must have some LD components in common but differ on others. Thus, H. B. might represent a recombinant, but less likely possibilities could be considered.

Discussion

Four C2-deficient families from different geographic locations and with diverse national origins were studied in this investigation. The proposita from three
### Table VI

**Additional MLC* Responses Among Some Members of the C Family and 7a Individuals, Showing the Reaction of H. B. a Possible Recombinant of MLC and HL-A Loci**

| Responding cells: HL-A genotype | Stimulating cells | L. S. x | C. M. x | J. C. x | J. E. x | H. B. x | W. L. x | R. S. x |
|---------------------------------|------------------|---------|---------|---------|---------|---------|---------|---------|
|                                 | cpm† | SR‡     | cpm      | SR      | cpm     | SR      | cpm     | SR      |
| L. S. & 10, W18/10, W18         | (157) | —       | 367      | 1       | 174     | 1       | 3,295   | 21      | 6,347   | 40      | 3,449   | 22      | 3,971   | 25      |
| C. M. I; 3,7/3,7                | 1,420 | 2       | (710)    | —       | 1,510   | 3       | 61,928  | 87      | 85,962  | 120     | 77,380  | 108     | 55,409  | 78      |
| J. C. § I (child): 9,5/10, W18  | 248   | 1       | 574      | 1       | (443)   | —       | 1,870   | 4       | 11,420  | 26      | 6,658   | 15      | 7,673   | 17      |
| J. E. (mother): 10, W18/10, W18 | 619   | 2       | 7,431    | 19      | 1,126   | 3       | (390)   | —       | 17,737  | 45      | 8,490   | 22      | 12,966  | 33      |
| H. B. (sunit): 10, W18/10, W18  | 16,114| 52      | 109,602  | 352     | 41,558  | 133     | 21,833  | 71      | (309)   | —       | 437     | 1       | 17,379  | 56      |
| W. L. (uncle): 1, W17/10, W18   | 17,379| 31      | 89,588   | 163     | 32,236  | 59      | 14,962  | 27      | 678     | 1       | (548)   | —       | 16,256  | 39      |
| R. S. § (unrelated)             | 12,226| 21      | 100,119  | 170     | 31,080  | 63      | 24,908  | 42      | 24,964  | 42      | 16,726  | 28      | (588)   | —       |

* MLC reactions were done by Method A.
† Homozygous C2 deficiency.
‡ Homozygous LD-7a individuals and CM is a 7a homozygous test cell (Histocompatibility Workshop Cell HWC no. 3001) which is mutually nonresponsive with the original 7a-test cell HWC no. 12003.
§ His HL-A typing shows HL-A 3,10,W5,W16.
families are homozygous for C2 deficiency and those of the fourth family are heterozygous for C2 deficiency. HL-A typing studies of these families reveal that the haplotype 10,W18 is encountered in all four individuals. Since one of them (L.S.) is homozygous for the HL-A haplotype 10,W18, this haplotype is present in five instances. Other HL-A haplotypes encountered in these families associated with C2 deficiency are HL-A 9,5, HL-A 2,4A2*, and HL-A2, W18 (1-3). The haplotype HL-A 10,W18 has a gene frequency of less than 1% (13). The prominence of this haplotype in association with C2 deficiency appears to represent a remarkable linkage disequilibrium between these two loci. The more accurate estimate of this phenomenon will be available as more C2-deficient families are being typed for HL-A antigens.

Family studies in MLC reactions indicate that the MLC locus is even more closely linked to the genes of C2 deficiency. Particularly striking was the finding that the three C2-deficient homozygotes of the three families were mutually nonreactive in the MLC reactions. In addition, the heterozygous C2-deficient individuals in the fourth family were nonreactive to the stimulation by the one of these homozygotes tested. Thus, these data indicate that the seven MHC involved in the C2 deficiency of the four families possess an apparently identical MLC gene. Further MLC typing experiments revealed that LD-7a is involved in all these instances. LD-7a is relatively common in the Caucasian population with a gene frequency of 0.11 in the North American Caucasian population and is in positive genetic linkage disequilibrium with the FOUR locus antigen HL-A7 (9-11). The exact gene frequency of C2 deficiency in the Caucasian population is not known but it is reasonable to assign an estimate of 0.01-0.02 (14). Thus, the association of LD-7a with C2 deficiency also appears to show linkage disequilibrium. More family studies are needed to determine if other LD determinants are involved in C2 deficiency.

The association of LD-7a with C2 deficiency in these families deserves further comment. This MLC determinant has been shown to be associated with patients with multiple sclerosis (15). Furthermore, the patients with multiple sclerosis have some suppression of cell-mediated immunity (16, 17). The three homozygotes in this study have manifestations of systemic lupus erythematosus (SLE). It is relevant that similar suppression of cell-mediated immunity has also been demonstrated in SLE (17). Perhaps, the associated immune response genes predispose these homozygotes to have the lupus erythematosuslike syndrome and possibly healthy homozygotes with C2 deficiency might possess different immune response genes. As further studies involving these C2-deficient individuals progress, a clear answer to this questions should be obtained.

One individual of the C family in these studies might represent a recombinant within the MLC locus and the C2 gene. She has the HL-A haplotype 10,W18, associated with C2 deficiency in other members of this family, but she is neither LD-7a nor a C2-deficient individual. This finding would place the genes governing C2 deficiency close to the LD genes which have been placed outside the second and the first HL-A locus. As reported in previous studies (2), an additional possible recombinant between the FOUR and C2-deficient loci is found in the K family. If this is indeed the case there are two recombinants resulting from 50 meiotic events in the four families and the recombination frequency between the FOUR HL-A locus and that of C2 deficiency is approximately 4%. This frequency is ex-
traordinarily high in view of the marked linkage disequilibrium between these two loci. The reasons for this paradoxical phenomenon are obscure. It is estimated that the recombination frequency between the FOUR HL-A locus and the major MLC locus is less than 1% (18). These very preliminary and indirect estimations would suggest placing the locus governing C2 deficiency outside the major MLC locus as well as the HL-A loci. One of the chromosomes encountered in this study would have the relevant genes in the following order: C2 deficiency, 7a, W18, HL-A10; the genes of the major histocompatibility complex and C2 deficiency on chromosome 6 might be assigned the following order: C2 deficiency, MLC (major), HL-A (FOUR locus), HL-A (LA locus). Further studies certainly are required to substantiate these relationships.

In addition to C2 deficiency, the locus for factor B of the alternate complement pathway is closely linked to the HL-A histocompatibility locus (19). In this case, the structural gene for factor B is probably involved. Although it is likely that C2 deficiency results from some structural gene alteration involving the C2 gene itself, the possibility still remains that a regulator or another gene defect in the synthetic pathway of C2 might be involved. In the mouse, the accumulated evidence suggests that the S region in the H-2 complex controls complement activity either directly by containing the structural gene for one or more components or indirectly by a regulatory pathway (20). Serum C3 levels have been shown to vary from strain to strain and the gene responsible for this variation is linked to the H-2 complex (21). The possibility also exists that the Ss protein of the mouse determined by the S region is itself a complement component (22). Evidence exists that it is not C3 or Factor B. However, the possibility that it might represent C2 remains open. The positioning of the C2 deficiency gene mentioned above is not directly parallel to the Ss position of the mouse but there are a number of known differences in this genetic region between the mouse and human genes (18).

Summary

Four families with C2 deficiency were studied. Among eight HL-A haplotypes involved with C2 deficiency, five were HL-A 10, W18. Three homozygotes for C2 deficiency from different families were mutually nonreactive in mixed lymphocyte cultures (MLC) and the heterozygotes from the fourth family failed to react to the homozygous cells. It appeared that identical MLC determinants were associated with all the genes from the different families that related to C2 deficiency. Further experiments identified the MLC determinant, LD-7a, as being involved. These results suggest marked linkage disequilibrium between the genes for C2 deficiency and the major histocompatibility complex (MHC). Studies of possible recombinants have offered tentative evidence for the positioning of the locus for C2 deficiency with respect to other segments of the MHC.

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Note added in proof: Two additional families with C2 deficiency subsequently have been studied. In one instance the C2 deficiency was not associated with LD-7a. Thus, in a total of six families, five showed the association.
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