EVIDENCE FOR LINKAGE BETWEEN HL-A HISTOCOMPATIBILITY GENES AND THOSE INVOLVED IN THE SYNTHESIS OF THE SECOND COMPONENT OF COMPLEMENT*

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In a previous report (1), a family with C2 deficiency in the homozygous and the heterozygous states was described. The propositus was of special interest because the complete absence of C2 was associated with some manifestations of systemic lupus erythematosus. Further studies of this family included analysis of possible genetic linkage of the deficiency with known genetic markers. Determinations of HL-A antigens provided evidence that linkage with this system was present.

Methods and Materials

Serum samples were obtained from clotted blood, quickly frozen, and stored at −60°C. Samples were thawed once. C2 determinations were performed by both hemolytic titration and radial immunodiffusion (1). The heterozygous individuals were recognized by their level of C2 which was close to one half the normal.

HL-A typing was done on lymphocytes isolated from the peripheral blood by Ficoll-Isopaque gradient centrifugation. A two-stage microtoxicity assay procedure was used. 120 typing sera were used and 28 antigens were typed for. The typing sera were obtained from the New York Blood Center and the Transplantation Immunology Branch Serum Bank of NIAID (2). Unidirectional mixed leukocyte cultures (MLC) were performed according to Hartzman et al. (3). Briefly, lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation. 3×10^5 X-irradiated (3,000 rad) stimulating cells and 1.5×10^5 in 0.2 ml of RPMI 1640 medium supplemented with streptomycin, penicillin, and 20% heat-inactivated normal human serum were 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% CO_2 humidified atmosphere, 2 μCi of [^{3}H]thymidine was added to each culture 16 h before harvesting. The lymphocytes were harvested and processed for liquid scintillation counting.

Results and Discussion

The pedigree of the S family is depicted in Fig. 1. The propositus, II2, is a woman with manifestations of systemic lupus erythematosus and homozygous for C2 deficiency. HL-A analysis also indicated homozygosity for HL-A10,W18. This was checked in two laboratories with different typing sera. To verify this further, unidirectional MLC were carried out using her cells as the stimulator and those of her daughter as the responder. It was clear that her daughter's leukocytes did

* This investigation has been supported by U. S. Public Health Service Grants nos. RR-102, AM 09792, HL09011, and HL08630.

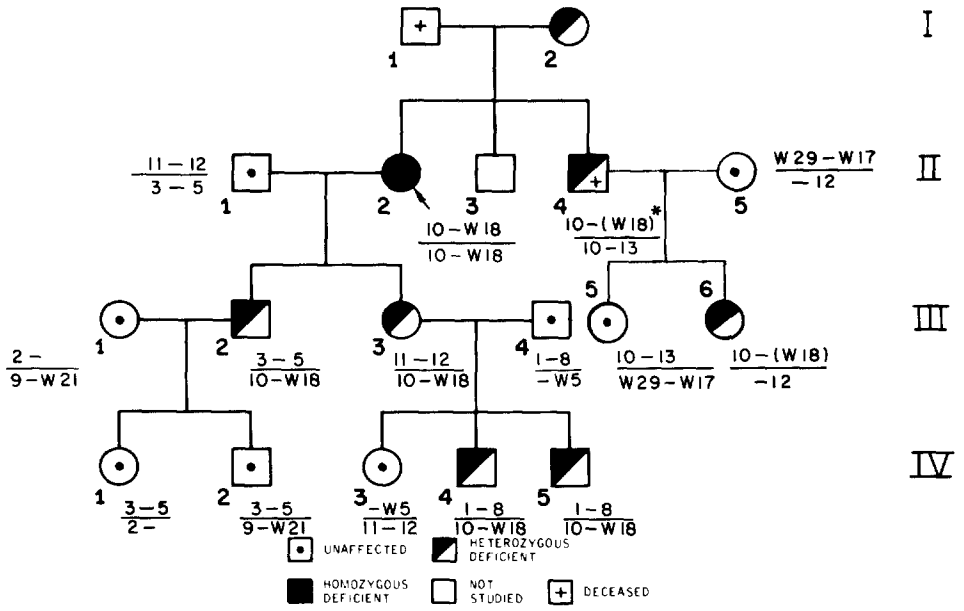

FIG. 1. Pedigree of the S family. The heterozygous and homozygous C2-deficient cases are indicated by the solid black symbols and the HL-A type is given in adjoining space.

not respond to her's while her cells responded to the stimulation of her daughter's cells. When the lymphocytes of an unrelated individual were used as the stimulator, lymphocytes of both the propositus and her daughter responded well. These results strongly support the argument that the propositus, II2, is homozygous for HL-A10,W18. HL-A typing of III6 showed typical reactions with HL-A10 and HL-A12 specific antisera. She also was assigned W18 because her lymphocytes reacted with some but not all of the W18 antisera utilized. The HL-A assignement for II4 was made from the typing results of his family members and is indicated in Fig. 1 with an asterisk.

There are three double backcross matings in this family. Among the seven informative children, no apparent recombinants were found. Statistical analysis with the likely assumption that I1 and I2 were heterozygous for HL-A10,W18 and C2 deficiency indicates that the odds for close linkage between C2 and HL-A loci is greater than 100 to 1. This assumption appears reasonable since both C2 deficiency and the HL-A10,W18 haplotype are quite uncommon (1, 4). No family history of consanguinity was obtained despite a special search. However, the fact that I1 and I2 have come from nearby small upstate New York communities and both have the rare combination of HL-A10,W18 and C2 deficiency would favor that they have a common ancestor. If this is indeed the case, the closeness of the linkage is greatly strengthened because it would indicate no recombinations over a number of generations.

The exact recombination frequency and the question of association between a particular HL-A haplotype and the C2 deficiency gene should become estab-

lished when more C2-deficient families become available. However, at the present time, only a few such families have been described. Some evidence for an association between total complement levels and the histocompatibility complex has been reported in the mouse (5), but this work was preliminary and did not identify the components of the system.

The accumulated evidence in the S family indicated that the C2 deficiency resulted from some structural gene alteration involving the C2 gene itself. No C2 was detected antigenically or in hemolytic titration in the propositus and the level of C2 was close to one half the normal in the heterozygous individuals. The possibility, however, still remains that a regulator or another gene defect in the synthetic pathway of C2 might be involved.

The histocompatibility complex has been shown to be linked to some of the immune response genes (6), and parts of the complement system have been implicated in the initial events of an immune response (7). Linkage between C2 deficiency and HL-A loci is therefore of special interest and relevance. Furthermore, C2 deficiency specifically has been associated with diseases such as systemic lupus erythematosus, dermatomyositis, and purpura (1, 8) and these diseases may stem from deranged immune responses resulting from the deficiency. Thus, some direct or indirect relationship to the immune response genes might be present. The exact nature of these relationships obviously remains to be resolved but the addition of a component of the complement system to the other important components controlled by the genes of the HL-A or H-2 complex appears significant.

Summary

HL-A analysis of a family with C2 deficiency revealed evidence for close linkage between the C2 defect and the histocompatibility HL-A loci. The propositus was homozygous both for C2 deficiency and the HL-A haplotype 10,W18. Among seven children of three double backcross matings, no recombinants were found. The possible significance of such linkage is discussed.

We thank Dr. C. Jersild for HL-A typing of selected members of the family, especially typing for the Ty antigen, and Dr. M. Polley for some of the C2 determinations.

Received for publication 15 July 1974.

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