A GENE LOCUS CONCERNED WITH HEMOLYTIC COMPLEMENT IN MUS MUSCULUS[1]

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THERE is present in the serum of virtually all vertebrates a group of proteinaceous substances known collectively as complement. An impressive body of evidence indicates that complement plays a significant role in enhancing the defenses of the vertebrate against microbial invasion. It may also be of some importance in mediating certain allergic or hypersensitive responses (OSLER 1961, 1958). Doubtless because of the complexity of the complement system, the exact function of individual complement components remains unknown. Indeed, there is no certainty as to the number of substances or individual chemical species which are required for the manifestation of any particular complement activity. By simple techniques of fractionation and recombination, four components, identified as C'1, 2, 3, and 4, were recognized many years ago. Recently, several additional components, and a dependence upon calcium and magnesium for activity, have been recognized (OSLER 1958; MAYER, OSLER, BIER and HEIDELBERGER 1946).

The amount of complement present in a particular serum is most conveniently and classically measured by its ability to cause the lysis of antibody coated cells, e.g., sheep red-blood cells coated with rabbit antibody. When measured in this way it has been found that there is a more or less characteristic amount of complement present in the serum of each species of animal. It has long been known that the mouse is particularly poor in complement. This overall deficiency in complement activity measurable in mouse serum has been variously attributed to a deficiency of one or another of the complement components (RITZ 1911; BROWN 1943; RICE and CROWSON 1950; MUSCHEL and MOTO 1956), or to the presence of an inhibitor of complement activity (BORSOS and COOPER 1961). A few reports demonstrate that the deficiency in complement measurable in mouse serum is not an absolute one, and with special techniques the small amount of complement present in mouse blood can be measured (MCGHEE 1952; WILLIAMS and WEMYSS 1961; ROSENBERG and TACHIBANA 1962). We wish in the present report to present the genetic data which indicate that two closely related strains of mice differ in their complement activity because of a single gene difference.

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The observations we report herein recall similar ones made on a complement-deficient strain of guinea pigs many years ago (MOORE 1919; HYDE 1923; PARSONS 1926). The complement-deficient strain of guinea pigs was shown to differ from other guinea pigs by a simple recessive mutation which in the homozygous condition caused the guinea pigs to be deficient in titratable hemolytic complement. The strain was said to be less resistant to certain kinds of stress than the wild type and was ultimately lost. By contrast, the circumstances within which one of the complement negative strains of mice arose suggest that the lack of complement activity may be of selective advantage in mice, at least in certain special conditions.

MATERIALS AND METHODS

The strains of mice used in the present study are: C57BL/10Sn; C57BL/10-*H-2*^{d}(B10.D2); and DBA/2J. Three "sublines" of the strain B10.D2 have been studied. Two of these are from the same backcross generation but have been carried for two years by brother × sister mating in different laboratories and are identified in the present report as B10.D2SnHz (old line) G12F20+ and B10.D2Sn (old line) G12F20+. The third subline differs from the first two in that it was obtained after five additional backcross generations to the ancestral line C57BL/10Sn, and is identified as B10.D2Sn (new line) G12F3G6F2G4F— (SNELL 1958, and personal communication 1962). In addition to the parental types listed above, we have studied the F_1 hybrid B10.D2SnHz × C57BL/10Sn, both backcrosses, i.e., F_1 × B10.D2 (old) and F_1 × C57BL/10 and the F_2 generation.

A sensitive hemolytic test measuring mouse complement has been previously described in detail (ROSENBERG and TACHIBANA 1962). Briefly, the test used was as follows: A 2½ percent suspension of sheep red-blood cells, labelled with radioactive chromium, was sensitized with a high concentration of rabbit amboceptor at ice-bath temperature. Then, to 0.05 ml sensitized cell suspension were added 0.1 ml mouse serum or five drops of fresh blood directly from the tail artery. The test mixture was incubated for 60 minutes at 37°C. The reaction was stopped by the addition of 2 ml of cold veronal-buffered saline. The tubes were centrifuged, supernatants were separated, and the percent lysis of the cells was estimated by counting the radioactivity in the supernatant and in the cells. Appropriate controls were included with each batch of tests. In the present report, we score mice as either being positive for hemolytic complement or negative for hemolytic complement. A negative result indicates the absence of measurable lysis of the indicator red cells. Each mouse was at least six weeks of age when first tested, and was tested on at least two occasions. The results of the two tests, usually done once with serum and once with whole blood, were consistent.

For the purpose of determining whether the locus controlling complement activity is linked to the *H-2* locus, the *H-2* phenotypes were determined by methods described previously (HERZENBERG and HERZENBERG 1961).

RESULTS

The data in Table 1 show that of the strains ancestral to B10.D2, one, C57BL/10Sn, is hemolytic complement positive and the other, DBA/2J, is negative. Of the three sublines of B10.D2 tested, the two "old lines" are complement negative. The third subline, which has undergone five additional backcrosses to C57BL/10Sn, is complement positive. We may assume from this that the complement-negative gene (or genes) of the "old lines" B10.D2 came from DBA/2J and was finally replaced by the complement positive C57BL/10Sn gene(s).

The results of appropriate crosses to test a genetic hypothesis are given in Table 2. The F_1 progeny of a cross between a complement positive and complement negative strain are complement positive. The backcross to C57BL/10 yields only positive progeny. The progeny from a F_1 backcross B10.D2 (old line) to the parental complement negative strain, as well as the individual F_2 mice, segregate for complement phenotype. The ratio of positive to negative animals in each of these crosses is not significantly different from the ratio expected if a single, segregating locus controls complement activity in these strains. The positive allele is dominant, and a dosage effect has not as yet been recognized.

We propose that this locus be designated *Hc* (hemolytic complement), and that the alleles now known be called Hc^1 for complement positive and Hc^0 for complement negative.

The B10.D2 strain was developed by successive selections for a strong histo-

TABLE 1

Hemolytic complement activity of several inbred mouse strains

	Strain	No. tested	Complement type
1	C57BL/10Sn	16	positive
2	DBA/2J	39	negative
3 a	B10.D2/SnHz, "old line" (G12F20+)	200+	negative
b	B10.D2/Sn, "old line" (G12F20+)	100+	negative
c	B10.D2/Sn,"new line" (G12F3G6F2G4F)	100+	positive

TABLE 2

Segregation of Hc

Cross	Genotypes	No. of positive (Hc^1/–) animals/total	Chi-square*
F_1†	($Hc^0/Hc^0 \times Hc^1/Hc^1$)	32/32	...
F_2	$Hc^0/Hc^1 \times Hc^0/Hc^1$	44/64	1.02 (0.5>P>0.3)
Backcross to positive	$Hc^0/Hc^1 \times Hc^1/Hc^1$	21/21	...
Backcross to negative	$Hc^0/Hc^1 \times Hc^0/Hc^0$	19/33	0.48 (0.5>P>0.3)

* Hypothesis of one segregating locus.
† B10.D2/SnHz (old line) × C57BL/10Sn.

compatibility difference between C57BL/10 and the developing B10.D2, while reaching towards coisogenicity of the two strains in the rest of the genome (SNELL 1958). Unlinked DBA/2 genes would be expected, in this scheme, to be lost with a probability of ½ in each backcross generation. Linkage between *H-2* and *Hc* was estimated. Individual backcross and F_2 mice were scored for both *Hc* and *H-2* alleles. As the data in Table 3 show, close linkage was not observed. These results were substantiated in family studies of backcrosses of *Hc* and *H-2* typed F_2 mice.

DISCUSSION

The symbols chosen to represent the locus and the recognized alleles controlling complement activity allow systematic expansion of this nomenclature if and when substances determined by these alleles are identified. Immunochemical evidence has been obtained which identifies the product of Hc^1 and suggests that Hc^0 has no recognizable product (ERICKSON, TACHIBANA, HERZENBERG and ROSENBERG 1963).

The existence of complement-lacking strains or individuals, in apparent good health and genetic stability, stands in contrast to the extensive evidence which implicates complement as a significant factor in immune phenomena. Such strains as those identified in the present report should prove useful in studies of the functions of the elusive substances identified at present only as components of the complement system. Furthermore, sera from mice genetically lacking one or another component of complement can serve as readily available reagents for assaying these components.

The negative allele, designated Hc^0, presumably derives from the DBA/2J strain used for the first cross to C57BL/10 in the production of the B10.D2 strain. This negative allele persisted through six backcross generations and many inbreeding generations. Close linkage to the *H-2* locus would serve to explain the persistence of this allele, but the genes appear to be unlinked. Were this the only instance of complement deficiency in mice, we would assume that chance provides a satisfactory explanation of this persistence. However, several other ob-

TABLE 3

Independent segregation of genes at the Hc *and* H-2 *loci*

Cross	Genotypes crossed	Complement positive			Complement negative		
		$\frac{H\text{-}2^b}{H\text{-}2^b}$	$\frac{H\text{-}2^b}{H\text{-}2^d}$	$\frac{H\text{-}2^d}{H\text{-}2^d}$	$\frac{H\text{-}2^b}{H\text{-}2^b}$	$\frac{H\text{-}2^b}{H\text{-}2^d}$	$\frac{H\text{-}2^d}{H\text{-}2^d}$
Backcross	$\frac{Hc^1, H\text{-}2^b}{Hc^0\ H\text{-}2^d} \times \frac{Hc^0, H\text{-}2^d}{Hc^0\ H\text{-}2^d}$	..	9	10	..	8	6
F_2	$\frac{Hc^1, H\text{-}2^b}{Hc^0\ H\text{-}2^d} \times \frac{Hc^1, H\text{-}2^b}{Hc^0\ H\text{-}2^d}$	5	9	4	5	6	1

Note: X^2 test for independent segregation: 0.5>P>0.3 backcross.
X^2 test for independent segregation: 0.2>P>0.1 F_2.

servations, now being more fully investigated, support the hypothesis of a selective advantage for either the heterozygote or the complement negative homozygote. It may be noted that the Hc^0 genotype appears to be fairly widely distributed in both highly and partially inbred mice.

SUMMARY

Hemolytic complement activity in certain inbred strains of mice has been shown to be dependent on a gene locus for which the symbol *Hc* is proposed. Two alleles of this locus have been identified. Hc^1 is present in C57BL/10Sn and one subline of B10.D2, and Hc^0 (hemolytic complement deficient) is present in DBA/2J and two sublines of B10.D2.

LITERATURE CITED

BROWN, G. G., 1943 The complementary activity of mouse serum. J. Immunol. **46**: 319–323.

BORSOS, T., and M. COOPER, 1961 On the hemolytic activity of mouse complement. Proc. Soc. Exptl. Biol. Med. **107**: 227–232.

ERICKSON, R. P., D. K. TACHIBANA, L. A. HERZENBERG, and L. T. ROSENBERG, 1963 A single gene controlling hemolytic complement and a β-mobility serum antigen in the mouse. Federation Proc. **22**: 612.

HERZENBERG, L. A., and L. A. HERZENBERG, 1961 Association of H-2 antigens with the cell membrane fraction of mouse liver. Proc. Natl. Acad. Sci. U. S. **47**: 762–767.

HYDE, R. R., 1923 Complement-deficient guinea-pig serum. J. Immunol. **8**: 267–286.

MAYER, M. M., A. G. OSLER, O. G. BIER, and M. HEIDELBERG, 1946 The activating effect of magnesium and other cations on the hemolytic function of complement. J. Exptl. Med. **84**: 535–547.

MCGHEE, R. B., 1952 Presence of complement in serum of the mouse. Proc. Soc. Exptl. Biol. Med. **80**: 419–420.

MOORE, H. D., 1919 Complementary and opsonic functions in their relation to immunity. A study of the serum of guinea pigs naturally deficient in complement. J. Immunol. **4**: 425–441.

MUSCHEL, L. H., and T. MUTO, 1956 Bactericidal reaction of mouse serum. Science. **123**: 62–63.

OSLER, A. G., 1958 Quantitative studies of complement fixation. Bacteriol. Rev. **22**: 246–261.

OSLER, A. G., 1961 Functions of the complement system. Advan. Immunol. **1**: 132–194.

PARSONS, E. I., 1926 Studies in complement splitting with special reference to the activation of yeast absorbed and complement deficient guinea pig serum. J. Immunol. **12**: 47–80.

RICE, C. E., and C. N. CROWSON, 1950 The interchangeability of the complement components of different animal species. II. In the hemolysis of sheep erythrocytes sensitized with rabbit amboceptor. J. Immunol. **65**: 201–210.

RITZ, H., 1911 Ueber Antikörperbildung und Anaphylaxie bei weissen Mausen. Z. Immunitäts forsch. **9**: 321–344.

ROSENBERG, L. T., and D. K. TACHIBANA, 1962 Activity of mouse complement. J. Immunol. **89**: 861–867.

SNELL, G. D., 1959 'Histocompatibility' genes of the mouse. II. Production and analysis of isogenic resistant lines. J. Natl. Cancer Inst. **21**: 843–875.

WILLIAMS, C. A., and C. T. WEMYSS, 1961 Changes produced in mouse plasma proteins by acute bacterial infections. J. Exptl. Med. **114**: 311–324.