ALLELES AT THE HISTOCOMPATIBILITY-2 LOCUS IN THE MOUSE AS DETERMINED BY TUMOR TRANSPLANTATION *

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PREVIOUSLY published data (GORER 1942; GORER, LYMAN and SNELL 1948) have shown that alleles at the histocompatibility-2 locus in the mouse have a dual effect, determining a blood group antigen and also susceptibility or resistance to certain transplantable tumors. The blood group is most easily demonstrated with sera from C57BL mice which have received one or more inoculations of A strain tumor 15091a. Such sera will agglutinate A strain red cells, indicating that the tumor and the red cells possess an antigen in common. In crosses between the A and the C57BL strains, the A erythrocyte antigen and susceptibility to A strain tumors are always found associated; hence they probably are determined by the same genetic locus. Three alleles have been recognized: *H-2,* characteristic of the A strain; *H-2^d,* found in the dba's; and *h-2,* heretofore regarded as characteristic of C57BL, CBA and P mice. The interpretation of *H-2^d* as a separate allele rests on three serological observations. Strain dba tumor P1534 will elicit antibodies in C57BL mice, but at a lower titer than those produced by 15091a. Strain dba red cells tested against anti-15091a serum give a slightly lower titer than do A red cells. Finally, this antiserum when absorbed with dba red cells still reacts in a dilution of 1 to 8 with A red cells.

Tests carried out by methods adopted to show linkage between histocompatibility genes and known marker genes (SNELL 1948) showed that *H-2* is closely linked with the locus for fused tail, *Fu.* In the experiments previously reported, though there were several animals that can have been either crossovers, or normal overlaps in the expression of fused, no unquestionable crossovers were obtained.

METHODS

This linkage has been used to detect new alleles at the *H-2* locus and to determine which allele is present in certain inbred strains. The cross used is as follows:

$$(M \times F) \times N$$

where M and N are any two inbred strains, and F is a strain carrying either *Fu,* or the closely linked gene, brachyury or *T* (DUNN and CASPARI 1945). The F parent is usually heterozygous (*Fu fu*), but only fused (or brachyury)

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F_1 are saved to cross to N. The offspring of this double cross are inoculated with an M strain tumor, and observed to determine which animals survive and which die. If fused tail shows linkage with resistance, in either the coupling or repulsion relationship, it can be inferred that strains M and N carry different alleles at the *H-2* locus. This is the basic theorem of the tests. If they carry the same allele (*e.g.*, M and N both $H\text{-}2^m fu/H\text{-}2^m fu$), mice of the test generation will have the formulae:

$$H\text{-}2^m fu/H\text{-}2^m fu \quad H\text{-}2^m Fu/H\text{-}2^m fu \quad h\text{-}2^f fu/H\text{-}2^m fu \quad h\text{-}2^f Fu/H\text{-}2^m fu$$

and, since susceptibility is dominant (LITTLE 1941), must either be all susceptible, or, if other histocompatibility loci are segregating, be part susceptible and part resistant but without linkage with fused.

It is also apparent that a linkage of fused tail with resistance proves that strains M and F carry different alleles at the *H-2* locus. If they carry the same allele, the F_1 will be $H\text{-}2^m fu/H\text{-}2^m Fu$, and linkage cannot be manifest.

The converse of these theorems is not true; the absence of linkage does not necessarily prove that either or both strain F and N carry the same allele as that carried by strain M. Thus strains F and N might carry alleles $h\text{-}2^f$ and $h\text{-}2^n$ such that $h\text{-}2^f/h\text{-}2^n = H\text{-}2^m/h\text{-}2^n$, in which case all inoculated mice would succumb (assuming no other factors for resistance). A known parallel is provided by the agouti locus in mice, where $a^t/A = A^w/A$, the light belly of a^t and A^w being dominant, though the non-agouti coat of a^t is recessive.

The transplantable tumors used were 15091a (mammary gland spindle cell carcinoma, A strain origin), P1534 (lymphoid leukemia, dba/2 origin), S621 (induced fibrosarcoma, B alb/c origin), C1498 (myeloid leukemia, C57BL origin) and S637 (induced anaplastic sarcoma, P origin). All are virulent, rapidly growing tumors. S621 and S637, the only two not previously described in the literature, were first transplanted in July 1948 and December 1948 respectively. A few tests were also made with C57BL mammary carcinoma E0771.

All inoculated animals were classified as + if they succumbed to the tumor, as – if they survived. There was temporary growth followed by regression in many of the latter group. A few of the positive animals survived much longer than the others. These were probably genetically resistant and are indicated as such by a footnote in table 1.

The fused tail gene shows some normal overlaps, particularly when the fused gene is inherited from the mother (REED 1937). To lessen this difficulty, male rather than female fused F_1 were selected as parents for the second cross. Also many of the normal tail resistant mice were tested genetically to determine if they were actually *Fu fu* instead of being crossovers. A number of such mice were found, of which three appear in one cross listed in table 1.

The inbred strains of mice used were: A, dba/2, B alb/c, C57BL/6, C57BL/10 (6 and 10 are closely related sublines) and P. The brachyury (*T*) line was kindly supplied by DR. DUNN. Two fused lines were used, the CA strain (indicated in table 1 by *Fu*) and fused-Columbia, a strain from DR. DUNN (indicated in table 1 by *Fu*/C).

RESULTS

The results are summarized in table 1. They fall into three general groups.

In the first group, of which the first two crosses in the table are typical, nearly all the normal tailed animals succumbed to the tumor while the *Fu* (or *T*) mice were resistant. Some of the normal tailed resistant (+−) animals may have been normal overlaps, others may be crossovers. There is close linkage, with the genes in the repulsion relationship. These crosses must have been segregating for at least two different histocompatibility-2 alleles.

In the second group, exemplified by the third cross in table 1, all inoculated mice, both fused and non-fused, succumbed. This establishes a presumption of, but does not conclusively prove, the identity of the allele carried by the first and either the second or third strains.

TABLE 1

Data showing linkage or absence of linkage between Fu (or T) and susceptibility (+) or resistance (−) to tumor transplants. With one exception noted in a footnote, mice were inoculated with a tumor native to the first strain listed for each cross.

Cross	++	+−	*Fu*+	*Fu*−	Conclusion
(A × *Fu*) × dba/2	12	1†	2(1§)	11	dba/2 is not *H-2*, is *H-2*^{d}
" × B alb/c	17	0	0	11	B alb/c is not *H-2*
(dba/2 × *T*) × B alb/c	10	0	12	0	B alb/c is *H-2*^{d}
(B alb/c × *Fu*) × dba/2	13	0	5	0	B alb/c is *H-2*^{d}
(A × *Fu*) × C57BL/10	5	1	0	8(3‡)	C57BL/10 is not *H-2*
(dba/2 × *T*) × C57BL/10	10	2	0	17	C57BL/10 is not *H-2*^{d}
(C57BL/6 × *Fu*/C) × A	10	0	1	10	C57BL/6 is *h-2*^{b}; *Fu*/C is not *h-2*^{b}
" × C57BL/6*	4	19	13	0	*Fu*/C is *H-2*
(A × *Fu*/C) × C57BL/10	28	0	15	1**	*Fu*/C is *H-2*
(A × *Fu*) × P	13	2	1	10	P is not *H-2*
(dba/2 × *Fu*) × P	35	3	5(2§)	33	P is not *H-2*^{d}
(C57BL/6 × *Fu*/C) × P	16(1§)	0	0	9	P is not *h-2*^{b}
(P × *Fu*/C) × C57BL/10	4	3	0	5	P is *h-2*^{p}

* Animals from this cross were inoculated with A strain tumor 15091a. In all other cases the tumor was native to the first strain named in the formula for the cross.

† Tested genetically; not a normal overlap.

‡ Tested normal overlaps.

§ Succumbed to the tumor, but probably genetically resistant on the basis of long survival.

** No palpable tumor growth at any time. Perhaps was not inoculated.

In the third group, consisting of the cross (C57BL/6 × *Fu*/C) × C57BL/6, inoculated with A strain tumor 15091a, there is linkage, but in the coupling instead of the repulsion relationship. The inoculated mice had an A strain great-grandparent, but no other A ancestors. Since all *Fu* mice succumbed to the A strain tumor, they probably carried the A strain allele, *H-2*.

DISCUSSION

The results prove the existence of four alleles at the histocompatibility-2 locus. These are: *H-2*, characteristic of strain A; *H-2*^{d}, characteristic of strain

dba/2 and B alb/c; *h-2^b*, characteristic of strains C57BL/10 and C57BL/6; and *h-2^p*, characteristic of strain P. The nature of the test is such that the identity of the allele in strains dba/2 and B alb/c cannot be regarded as beyond question. However, these stocks have been used in a number of crosses, some of them not listed in the table, and have always given identical results. Hence the presumption of identity at this locus is strong.

One of the unsolved questions in regard to histocompatibility genes is whether one allele at each locus is a complete recessive, or whether the situation is like the M, N blood types in man where each allele expresses itself in the heterozygote. The classical genetic theory of tumor transplantation (LITTLE 1941) assumes the first of these alternatives, and this alternative gives the best fit to the published data (SNELL 1948). However, because of the close similarity of histocompatibility genes to blood group genes the second alternative has considerable plausibility. The results here reported prove that, at least in the case of the histocompatibility-2 locus, the second alternative is correct. The allele *h-2^b*, previously treated as a complete recessive, actually behaves as a dominant in crosses where the tumor used is C1498 (C57BL origin). C1498 will grow if, and only if, *h-2^b* is present.

Another fact brought out in this investigation is the importance of this one locus in controlling susceptibility or resistance to old and virulent transplantable tumors. It is known that there are a dozen or more loci concerned with susceptibility and resistance to transplants (LITTLE 1941; SNELL 1948), but the tests demonstrating these were carried out with "young," recently derived tumors. It will be seen from table 1 that in all our crosses, involving a number of different strains and four transplantable tumors of diverse origin, there is no hint of any factor other than histocompatibility-2 causing resistance to transplants. The few exceptional animals are explainable as normal overlaps or crossovers (we have definite proof, to be published later, that crossovers between *Fu* and *H-2* do occur). Moreover, with the tumors included in table 1, only rarely do genetically resistant animals fail to inhibit tumor growth. In table 1, there are 4 probable exceptions out of a total of 377 mice. However, with other tumors exceptions are more common; C57BL tumor EO771 gave 6 probable exceptions out of 39 mice.

Additional stocks are being tested to determine what *H-2* allele they carry. Quite possibly these tests will reveal additional alleles.

As in every study of multiple alleles, there is a possibility that we are dealing with closely linked genes rather than true alleles.

SUMMARY

The histocompatibility-2 locus in the mouse determines susceptibility or resistance to certain transplantable tumors. By using the close linkage of this locus with the genes for fused tail (*Fu*) and brachyury (*T*), tests for new alleles have been carried out.

Four alleles have been identified: *H-2*, characteristic of strain A and necessary for the growth of strain A tumor 15091a; *H-2^d*, characteristic of strains

dba-2 and B alb/c and necessary for the growth of dba/2 tumor P1534 and B alb/c tumor S621; *h-2^b*, characteristic of strains C57BL/6 and C57BL/10 and necessary for the growth of C57BL tumor C1498; and *h-2^p*, characteristic of strain P and necessary for the growth of P tumor S637. All four alleles behave as dominants in crosses. There is no indication in the crosses here reported that any factor other than the particular *H-2* allele in question is necessary for the growth of these tumors.

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