DETERMINATION OF H-2 GENOTYPE IN MICE OF CC57BR AND CC57W STRAINS

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Mice of lines CC57BR (BR) and CC57W (W)* were isolated by N. N. Medvedev by inbreeding between the offspring of crossing a BALB/c (C) female with a CC57BL (B) male [2]. The genetic structure of the H-2 locus of the mice of the parent lines is known: line BALB/c belongs to the family of lines with a genotype H-2^d/H-2^d, and most of the C57BL sublines have a genotype H-2^b/H-2^b [3,7]. The hybrids (CxB)F1 were evidently heterozygous at the H-2 locus, i.e., they had the structure H-2^b/H-2^d. In subsequent generations dissociation took place at these genes until, as a result of inbreeding, the mice of lines BR and W became homozygous, having inherited a particular H-2 genotype. It is most likely that the mice BR and W have a genotype H-2^b or H-2^d; a less likely suggestion is that a mixed, cross-over H-2 genotype has appeared in the mice of these lines, as will be discussed below.

In the present report we describe the results of experiments to determine the H-2 genotype in BR and W mice by means of F1 hybrids [12,15] with a pair of coisogenous lines C57BL/10Sn (B10) and C57BL/10-H-2^d (B-H-2^d) and with corresponding tumors. The significance of the investigations in relation to the genetics of tissue compatibility has been discussed in detail in the literature [1,4,10].

EXPERIMENTAL METHOD

Mice of inbred lines CC57BR 47 inbred generation (F47), CC57W (F 47), C57BL/10Sn (F72), and C57BL/10-H-2^d (G12F3G6F35G2F2) [3], hybrids of the first generation between these lines (BRxB10)F1, (BrxB-H-2^d)F1, (WxB10)F1, and also tumors S913 of mice B10 and tumors MX2 and D586 of mice B-H-2^d were used in the experiments. Tumor S913 has been transplanted from mice B10 for many years and is a leukemia induced by roentgen rays [14]. Tumors MX2 and D586 were induced by methylcholanthrene and are sarcomas. At the beginning of the experiments they were passed through 5 generations of B-H-2^d mice. The tumors were injected subcutaneously into experimental mice aged 11-13 weeks.

The two co-isogenous lines of B10 mice (genotype BB H-2^b/H-2^b) and B-H-2^d mice (genotype BB H-2^d/H-2^d) differ from each other only at the H-2 locus, and all the remaining genes are common to both. Therefore, the result of transplantation of tumor S913 from B10 mice into mice of the isogenous resistant line B-H-2^d is determined exclusively by the H-2 locus and is independent of the other genes of tissue compatibility [11]. If B-H-2^d mice (genotype BB-H-2^d/H-2^d) are crossed with BR mice (genotype NN H-$2^?$/H-$2^?$), the hybrids (B-H-2^d x BR)F1 obtain from their B-H-2^d parent all the genes of line B10 except the H-2 locus, and will have the genotype B/N H-2^d/H-$2^?$. The H-2 locus of mice consists of several pseudoalleles, each of which affects the result of tissue transplantation. Hence, the tumor S913 of mice B10 may grow from hybrids (B-H-2^2 x BR)F1 only if the locus H-2 of line BR contains all those genes of the H-2 system by which line B-H-2^d differs from line B10. The H-2 locus of line BR may thus be compared with the H-2 locus of line B10 (H-2^b) and the H-2 genotype of line BR determined. By using hybrids (B10 x BR)F1 and tumor MX2 or D586, hybrids (B-H-2^d x W)F1 and tumor S913, and hybrids (B10 x W)F1 and tumors MX2 and D586, the H-2 loci of the lines BR and B-H-2^d, W and B10, and W and B-H-2^d may be compared. This method is called the F1 hybrids test [1,12,15].

The reliability of the results of an experiment with F1 hybrids is checked in two ways, for there are two sources of error. One of these is that the tumor used for the experiment with F1 hybrids possesses the power of growth in mice

*In the text and table abbreviations are used to denote the lines and sublines of mice used in these experiments.

Results of Experiments to Determine the H-2 Genotype of Mice of Lines CC57BR and CC57W by Means of the Locus of F1 Hybrids with Mice of Lines C57BL/10Sn and C57BL/10-H-2^d

Expt. No.	Donor of tumor	H-2 type of donor	Tumor	Host of tumor	Line tested	Results of transplantation of tumor: dying mice	Results of transplantation of tumor: surviving mice	H-2 locus of tested line
1	B10[1]	H-2^b	S913	(BRxB-H-2^d)F1	CC57BR	25	0	H-2^b
2	"	"	"	(BRxB10)F1	"	5	0	—
3	» »	» »	» »	(WxB-H-2^d)F1	CC57W	25	0	H-2^b
4	» »	» »	» »	(WxB10)F1	"	5	0	—
5	» »	» »	» »	B-H-2^d	—	0	9	—
6	B-H-2^d	H-2^d	MX2	(BRxB10)F1	CC57BR	0	10	He H-2^d
7	"	"	"	(WxB10)F1	CC57W	0	19	He H-2^d
8	» »	» »	» »	B-H-2^d	—	5	0	—
9	» »	» »	D586	(BRxB10)F1	CC57BR	0	12	He H-2^d
10	» »	» »		(BRxB-H-2^d)F1	"	5	0	—
11	» »	» »	» »	(WxB10)F1	CC57W	0	11	He H-2^d
12	» »	» »	» »	(WxB-H-2^d)F1	"	3	0	—
13	» »	» »	» »	B10	—	0	8	—

* The names of the lines in the table are abbreviated. The full names are given in the text.

incompatible with it as regards H-2 locus [13,16]. Such a tumor is unsuitable for genetic investigations. In order to test the suitability of the tumor for experiments of the category under review, it must be transplanted into H-2-incompatible mice of an isogenous, resistant line [15]. In other cases the tumor may be taken from a genetically compatible host of the F1 hybrid [14,16]. In order to demonstrate that the negative results of transplantation are not erroneous, suitable control experiments must be performed. For this purpose the tumors used for the experiment with the F1 hybrids, for example D586, were transplanted into F1 hybrids obtained by crossing mice of the donor and the test lines, for example (W × B-H-2^d)F1. Such hybrids are compatible with the D586 tumor in respect to all their genes, for these genes are derived from the B-H-2^d parent and possess the same degree of heterosis as the (W × B10)F1 hybrids used in the main experiment.

EXPERIMENTAL RESULTS

The results of these experiments are shown in the table. The hybrids (BR × B-H-2^d)F1 (experiment No. 1) and (W × B-H-2^d)F1 (experiment No. 3) were injected with tumor S913(H-2^b), and all died from the tumor. Control mice (BR × B10)F1 (experiment No. 2) and (W × B10)F1 (experiment No. 4) died from the tumor after the same time interval as the mice in experiments Nos. 1 and 3. Meanwhile, tumor S913, to be injected into mice B-H-2^d, was taken from all these mice on the 12th-13th day (experiment No. 5), which demonstrates that this tumor is suitable for the particular experiments.

It follows from experiments Nos. 1-5 that mice BR and W possess the genotype H-2^b.

The hybrids (BR × B10)F1 (experiment No. 6) and (W × B10)F1 (experiment No. 7) were resistant to tumor MX2(H-2^d). The same tumor killed the B-H-2^d mice (experiment No. 8). Tumor D586 (H-2d) was taken from the hybrids (BR × B10)F1 (experiment No. 9) and (W × B10)F1 (experiment No. 11) and killed the control mice (BR × B-H-2^d)F1 (experiment No. 10) and (W × B-H-2^d)F1 (experiment No. 12). Mice B10 (experiment No. 13) were resistant to this tumor. It follows from experiments Nos. 6-13 that mice BR and W do not belong to the group of lines with the genotype H-2^d.

However, in the hybrid mice BALB/c × C57BL(H-2^b/H-2^d), giving origin to lines BR and W, a crossover of chromosome IX could be produced in the region of the H-2 locus. The genotype of the offspring of this heterozygous crossover line is mixed: they may carry the determinants H-2^b and H-2^d [5]. As a result of the crossover, it is theoretically possible for several new H-2 genotypes to be formed. Some of these combinations of antigens of the H-2 system cannot be differentiated from combinations of H-2^b by means of the method used in this investigation.

We know that the H-2 locus of mice may undergo mutation, although this is not likely [6]. It is obvious that changes in the H-2 locus of BR and W mice as a result of mutation may be very diverse, and not all the newly formed genotypes can be differentiated from the $H\text{-}2^b$ genotype by means of the experiment with F1 hybrids.

It is not within the scope of this article to examine the subsequent analysis of these theoretically possible cases. For the final elucidation of the genetic structure of the H-2 locus in BR and W mice it will be necessary to use a serological method [8,9], which is complementary to genetic methods and also increases the reliability of the results of determination of the H-2 genotype of mice of inbred lines.

Hence, there is every reason to suppose that mice of lines CC57BR and CC57W have an $H\text{-}2^b$ genotype. The accuracy of the results may be increased by means of a serological method of determination of the antigens of the H-2 system.

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SUMMARY

Histocompatibility of the H-2 locus of the CC57BR and CC57W mice was identified with the help of F1 hybrids, one of the parents of which was C57BL/10Sn or $C57BL/10\text{-}H\text{-}2^d$. Both strains were found to carry $H\text{-}2^b$.

LITERATURE CITED

1. N. N. Medvedev, Uspekhi sovr. biol. 50, 1(4), 77 (1960).
2. N. N. Medvedev, Vopr. onkol., 9, 23 (1961).
3. N. N. Medvedev, Vopr. onkol., 7, 120 (1962).
4. O. S. Frankfurt, Patol. fiziol., 1, 74 (1961).
5. D. B. Amos, P. A. Gorer, and Z. B. Mikulska, Proc. roy. Soc., 144, 369 (1955).
6. P. R. F. Borges and B. J. Kvedar, Cancer Res., 12, 19 (1952).
7. The Committee on Standardized Genetic Nomenclature for Mice, Cancer Res., 20, 145 (1960).
8. P. A. Gorer, S. Lyman and G. D. Snell, Proc. roy. Soc. B., 135, 499 (1948).
9. P. A. Gorer and Z. B. Mikulska, Cancer Res., 14, 651 (1954).
10. L. W. Law, Advanc. Cancer Res., 2, 281 (1954).
11. G. D. Snell and J. Genet, 49, 87 (1948).
12. Idem, J. nat. Cancer Inst., 14, 691 (1953).
13. G. D. Snell, E. Russell, E. Fekete et al., Cancer Inst., p. 485.
14. G. D. Snell, Cancer Inst., 20, 787 (1958).
15. Idem, Cancer Inst., 21, 843 (1958).
16. Idem, in the book: The Physiopathology of Cancer, p. 293, New York (1958).
