INVESTIGATION OF THE HOMOZYGOSITY OF MICE OF THE CONGENIC LINE B10.D2 BY A SKIN GRAFTING METHOD

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The study of the degree of homozygosity of mice of inbred and congenic* lines and of their differences from the corresponding standard inbred lines is of great importance in connection with the widespread use of inbred animals for all manner of research purposes.

In the present study the homozygosity of mice of the congenic line B10.D2 was investigated by a skin grafting method.

EXPERIMENTAL METHOD

The mice used in the investigation were of lines C57BL/10ScSn (abbreviation B10), of the seventy-third-seventy-sixth inbred generation (F73-76), H-2^b; C57L/J (L), F89-92, H-2^b, and of the congenic line B10.D2 (synonym C57Bl/10-H-2^d), G12F3G6F5G4F4-7, H-2^d, and also their hybrids.

The skin of the mice was transplanted by the method fully described earlier. Skin was not transplanted from males to females, in order to avoid the influence of the tissue compatibility factor, linked with the Y chromosome.

To study the degree of homozygosity of the mice of the congenic line B10.D2 the method of reciprocal isotransplantation of the skin of 20 males of this line was used. Each mouse acted simultaneously as donor and recipient of the skin for 3 other mice, so that all the animals were linked into a single "block."

The existence of possible genetic differences between mice B10.D2 and B10 relative to the weak genes of tissue compatibility have been investigated in experiments with hybrid mice, of which one parent was B10.D2 and the other B10 or L, i.e., a line whose H-2 allele is identical with that of B10 (H-2^b). If the mice of lines B10(H-2^b) and B10.D2 in fact differ only at the H-2 locus, and all their other genes of tissue compatibility are identical, the skin grafts of B10 will take completely (because most of the tissue compatibility genes are codominants). In half the hybrids from the back crossing F1×B10.D2 the skin of the B10 mice will be rejected quickly because of splitting at the H-2 locus, whereas in all the other BC1 mice, the B10 skin will take. However, if additional differences are present between the B10 and B10.D2 mice relative to the weak genes of tissue compatibility, the splitting in BC1 will be more complex. Some (or all) of the grafts will be rejected because of incompatibility at the weak loci at longer periods of time after the operation.

The genotype of several males of the line B10.D2 was determined from their progeny. Five B10.D2 males were crossed in individual cages with B10 females and the offspring of each male was kept separately. All the F1 hybrids were grafted with the skin of B10.D2 mice. Rejection of the grafts by the F1 hybrids was evidence of the heterozygosity of their father, and taking was evidence of homozygosity.

EXPERIMENTAL RESULTS

The results of experiments to determine the homozygosity of the B10.D2 mice are given in Table 1. Two of the 60 skin isografts (from different mice) first lost their hair (on the 35th day after operation) and

*The term "congenic resistant" line has been introduced recently [4] instead of the term "isogenic resistant" line as being more in line with modern ideas on the genotype of such mice.

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TABLE 1. Results of Grafting the Skin of Mice of Different Genotype on to Mice of Congenic Line B10.D2 and Their Hybrids

Line of skin donor	Line of recipients	Number of recipients	Number of skin grafts				Duration of observations (in days)
			rejected on the 9th-16th day	rejected after the 16th day (day of rejection in parentheses)	taking	altogether	
B10.D2	B10.D2	20	–	2 (41)	58	60	50–250
B10	(L×B10.D2)×B10·D2 BC1	26	15	4 (112–147)	7	26	112–300
B10.D2	(B10.D2×B10)×B10 BC1	20	11	3 (40–47)	6	20	56–103

TABLE 2. Results of Grafting the Skin of B10.D2 Mice on to the First Generation of Offspring of 5 B10.D2 Crossed Individually with Females of Line B10

Male No.	Number of offspring B10×B10·D2F1	Number of skin grafts			Duration of observations (in days)
		taking	rejected (day of rejection in parentheses)	altogether	
1	6	1	5(41–50)	6	70
2	14	10	4(43–61)	14	70
3	16	16	–	16	90
4	13	13	–	13	90
5	11	11	–	11	90

then were finally rejected (on the 41st day). This experiment demonstrated the variation among the line B10.D2 mice in relation to the genes of tissue compatibility.

To study the genetic differences between the mice of lines B10.D2 and B10, as mentioned above, hybrids from back crossings between mice of these lines and of line L were used. The results of such an experiment with hybrides (L× B10.D2) ×B10.D2 BC1 are given in Table 1. In this experiment the BC1 mice which rejected the grafts of B10(H-2^b) skin on the 10th-16th day after the operation were regarded as homozygotic relative to H-2^d/H-2^d; mice on which grafts of B10 skin survived were regarded as heterozygotic relative to H-2^b/H-2^d. The skin of the B mice was rejected on the 112nd-147th day by 4 of the 11 BC1 hybrids possessing the genotype H-2^b/H-2^d, demonstrating dissociation of these mice relative to an additional gene of tissue compatibility, and not H-2. Consequently, B10 mice possess a weak gene of tissue compatibility not present in the B10.D2 mice, and causing rejection of the skin by hybrids with a genotype deficient in this gene.

A similar experiment was carried out, with a slight modification, in which B10 and B10.D2 were crossed and the hybrids (B10.D2×B10)×B10 BC1 were grafted with the skin of B10.D2 mice. The skin grafts on 9 of these 20 BC1 hybrids survived longer than 16 days. These mice were regarded as heterozygotic relative to H-2^b/H-2^d. The grafts of B10.D2 skin were rejected by 3 of the 9 heterozygotic mice on the 40th-47th day after the operation. Consequently, the hybrids of this type also were dissociated relative to at least one additional gene of tissue compatibility besides H-2. In this case the cause of the rejection of the grafts by the corresponding hybrids was an unknown gene of tissue compatibility of the B10.D2 mice, evidently not present in the B10 mice.

It is possible that in the two experiments described dissociation took place at the same locus of tissue compatibility, the different alleles of which are unequally antigenic: B10.D2 mice possess an antigenically stronger allele and B10 mice a weaker allele. In all probability this locus is not linked with H-2 (method of maximal probability: $P = 0.370 \pm 0.104$; difference of P from 0.5 not significant), although the data are insufficient for a final conclusion to be drawn.

The results of the experiment to determine the genotype of the B10.D2 males from their offspring are given in Table 2. Among the F1 progeny from crossing males Nos. 1 and 2 with the B10 females, dissociation relative to the sign of compatibility with the skin of the B10.D2 mice was found. This demonstrates the heterozygosity of these males. The other three B10.D2 males (Nos. 3-5) were found to be homozygotic relative to the genes of tissue compatibility, for their F1 offspring did not reject B10.D2 skin during the period of observation (90 days).

The suggestion could also be made that the females of the B10 line were heterozygotic. However, this suggestion is much less probable, first, because the cases of rejection of the skin were distributed irregularly among the offspring of the B10.D2 males, and second, because the B10 females belonged to a highly inbred line. Special experiments showed that the mice of line B10 were homozygotic relative to the genes of tissue compatibility.

The heterozygosity of the mice of the B10.D2 subline at the weak gene of tissue compatibility must be regarded as having developed by mutation, for experiments carried out several years ago [1] showed that mice of line B10.D2 are homozygotic relative to the genes of tissue compatibility. The suggestion that the mice of the B10.D2 subline had been crossed accidentally with mice of another line is groundless, for in this case the heterozygosity would have arisen in several loci at one, and not just in one locus of tissue compatibility. This has been demonstrated for congenic lines on the genetic basis of line A [2, 3].*

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