SKIN GRAFTING BETWEEN SUB-LINES OF INBRED STRAINS OF MICE

By Berenice Kindred*

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Summary

Skin grafts made between sub-lines of inbred strains of mice revealed no simple histocompatibility mutants. Some of the grafts were rejected, but the basis of this rejection is obviously complex.

I. Introduction

It has been well established that sub-lines of inbred strains of mice have a tendency to deviate (Green 1953; Deol *et al.* 1957; Carpenter, Grüneberg, and Russell 1957; Bailey 1959). This deviation could be due to fixation of alleles which were segregating at the time the sub-lines were separated or, more probably, to mutations which have arisen since the lines were separated (Lyon 1959; Deol *et al.* 1960). Bailey (1959) has estimated the rates of differentiation for six metric traits of the skeleton in sub-lines of C57BL/6 and BALB/cAn. In the latter strain he calculates that the number of generations of 10 mice each required to produce a significant difference ranges from six for the length of the ulna to 45 for a particular skull measurement. The estimates for C57BL/6 are very much greater. In this Laboratory, five inbred strains have each been divided into five sub-lines and it was decided to make reciprocal skin grafts between members of the sub-lines within each inbred strain to determine if divergence with regard to histocompatibility had taken place.

The rate of histocompatibility divergence is completely unknown so that the number of generations necessary to produce a difference could only be guessed. It was also hoped to collect some information on the relative frequencies of strong and weak histocompatibility genes. As 15 has been estimated as the minimum number of genes responsible for a homograft reaction (Barnes and Krohn 1957), and only one really strong locus, H-2, has been found, it seemed likely that weak reactions would be more common.

II. Materials and Methods

Five inbred strains of mice were used: A, 101, CBA, C3H, and DBA. Each of these had been divided into five sub-lines which, at the beginning of the experiment, had been maintained as separate lines for about 10 generations.

In the first set of experiments, reciprocal skin grafts about 1 cm^2 were made between pairs of mice from different sub-lines. As the number of available mice was limited, grafts could not be made between all sub-lines so one was chosen and grafts were made between it and the others. Later, however, a system of grafting line

* Division of Animal Genetics, C.S.I.R.O., Animal Genetics Laboratory, Department of Zoology, University of Sydney.

1 → line 2 → line 3 → line 4 → line 5 → line 1 was used since fewer animals were required. Grafts between males and females were avoided.

So that the graft could be easily identified, all grafts were rotated and, where paired mice were used, back skin was grafted to the belly and vice versa. This allowed the length and thickness of the hair as well as direction of hair growth to be used to differentiate between the graft and the surrounding skin.

The technique used was that of sub-pannicular grafting, i.e. the panniculus adiposus and the panniculus carnosus were included in the graft. This technique is much quicker than separating the dermis from these layers and, in rats at least, there is no difference in number of takes or time of survival with the two methods (Ballantyne and Converse 1957). All mice were kept for 180 days before the grafts were finally scored as surviving.

TABLE 1

RESULTS OF GRAFTS MADE BETWEEN MEMBERS OF INBRED SUB-LINES

Type of Graft	Strain CBA		Strain 101		Strain C3H		Strain DBA		Strain A	
	No. Grafted	No. Rejected	No. Grafted	No. Rejected	No. Grafted	No. Rejected	No. Grafted	No. Rejected	No. Grafted	No. Rejected
1 on 2	11	0	14	0	14	0	5	0	11	2
2 on 1	9	0	13	0	14	1	5	0	18	1
1 on 3	11	0	13	0	*		9	0	22	0
3 on 1	16	0	13	1	*		9	1	15	5
1 on 4	11	0	14	0	10	0	5	0	14	0
4 on 1	12	0	13	0	13	1	4	0	10	2
1 on 5	12	0	11	0	11	0	5	0	29	0
5 on 1	10	0	12	0	14	0	5	0	22	2
1 on 1	8	0	12	0	11	0	0	0	19	3

* Line 3 of this strain was lost.

III. RESULTS

All the grafts which were rejected appeared healthy at first and grew hair but were destroyed after periods varying from 47 to 130 days. Grafts which survived for long periods underwent a protracted breakdown similar to that described by Eichwald, Slimser, and Wheeler (1957) for the destruction of grafts due to the male-specific incompatibility factor. Firstly, the hair fell from the graft area, then small scabs appeared and these coalesced to cover the graft; when the scab came off, only scar tissue remained. This process would cover a period of up to 30 days.

The number of grafts made and rejected in the first experiments is shown in Table 1. Rejections among sub-lines were encountered in all lines except CBA. The number of grafts varies because space was not available to keep single animals in cages, and they frequently fought when the dressings were removed, thus damaging the grafts which had to be discarded. Mortality was also high in some strains, particularly A and DBA.

In A strain, a relatively high proportion of grafts from any other line on line 1 was rejected, but most of the reciprocal grafts remained perfectly healthy. The time for rejection was quite uniform, the first signs appearing 50–60 days after grafting. It should be noted that of 19 grafts of 1 on 1, three were rejected, indicating genetic diversity even within that subline.

As the mice were usually about 12–16 weeks old when grafted, they were somewhat aged by the time the grafts were rejected. Attempts to test for an accelerated rejection of a second graft usually led to the death of the mouse, but in the two cases where such tests were performed successfully the second graft was not rejected. The hair was lost and some scab developed two weeks after grafting but the grafts recovered. This may be simply an effect of age (Medawar and Sparrow 1956) or it could be due to the fact that the second graft was taken from a member of the same line as the original donor but not from the original donor itself.

TABLE 2

RESULTS OF GRAFTS FROM STRAIN A LINE 3 TO THE PROGENY OF STRAIN A LINE 1 ANIMALS WHICH HAD REJECTED GRAFTS

Type of Mating	No. of Grafts	No. Dead	No. Rejected
Rejector × rejector	12	7	4
Rejector × non-rejector	16	6	0
Rejector × line 3	12	0	0
Sib × sib (of rejector)	15	5	2

Unfortunately, it was only in A strain that male and female mice which rejected grafts were available for mating, but again, owing to age and a marked tendency for females to die at parturition, only small numbers of progeny were obtained. Mice which rejected grafts were mated to other rejectors, to sibs, or to animals from another A strain sub-line and their progeny were grafted with skin from line 3 (line 3 on line 1 produced the highest number of rejected grafts). The results of these grafts are given in Table 2. They are by no means clean cut. The occurrence of rejection is increased, but even when two animals which rejected grafts were mated, some of their progeny were capable of accepting grafts. The usual sensitivity of A strain to anaesthetic seemed to be exaggerated in these mice, and of 12 progeny born to parents both of which had rejected grafts only five survived the grafting operation.

The number of grafts rejected in the second series is given in Table 3. Once again, rejection was encountered in all lines except CBA. In this case a direct test of the genetic basis of rejection was attempted. In a cross between two different inbred lines, skin from animals of either parent strain will grow on all F_1 animals except those produced by a parent which differs from the rest of its strain in one or

more histocompatibility genes. The rejections listed in Table 3 could be due to such differences either in the donors or recipients of the grafts. Therefore, these mice were

TABLE 3

RESULTS OF FURTHER GRAFTS BETWEEN SUB-LINES USING A DIFFERENT PATTERN OF GRAFTING OPERATIONS

Type of Graft	Strain CBA		Strain 101		Strain C3H		Strain DBA	
	No. Grafted	No. Rejected	No. Grafted	No. Rejected	No. Grafted	No. Rejected	No. Grafted	No. Rejected
1 on 2	12	0	32	1	41	0	12	1
2 on 3	17	0	25	1	*	*	8	0
3 on 4	14	0	27	0	26	2	23	0
4 on 5	9	0	27	0	35	2	19	1
5 on 1	3	0	29	0	48	4	7	0

* Line 3 of this strain was lost.

mated with animals from two or more different strains, and their F_1 progeny were grafted with skin from normal members of the parental strain. Uniform rejection would have been expected.

TABLE 4

RESULTS OF GRAFTS FROM AN INBRED STRAIN TO THE PROGENY OF A MEMBER OF THAT STRAIN WHICH WAS THE DONOR OR RECIPIENT OF A REJECTED GRAFT, THE OTHER PARENT BEING AN UNRELATED INBRED

Type of Mating	No. Grafted	No. Rejected
DBA* × CBA	28	1
DBA* × C57	6	1
101* × C57	4	2
C3H* × CBA	24	2
C3H* × C57	12	3
C3H* × 101	13	2
C3H* × DBA	5	0
A* × CBA	10	2
A* × C57	3	3
A* × 101	22	9

* Donors or recipients of rejected grafts.

As Table 4 shows, this expectation was not fulfilled. Some grafts were rejected and some accepted without any apparent pattern. The genetic basis of graft rejection within the inbred lines does not appear to be simple.

IV. Discussion

It is clear that heritable incompatibilities have arisen within some of the sublines of our inbred strains. The increase in the frequency of rejections among the progeny of mice which have themselves rejected grafts demonstrates that these incompatibilities are inherited even though clear-cut segregation ratios were not obtained. The failure of the expected second set rejection may be due to one of the reasons given, but the possibility exists that the incompatibility in A strain at least may not be of the typical immunological type. It is conceivable that the surrounding cells play a part perhaps by some process concerned with the healing in of the graft.

The pattern of rejection in A strain suggested at first a homozygous deletion of a histocompatibility gene occurring in some animals of line 1, causing these animals to lack an antigen present in the rest of the strain so that they would reject the grafts but would not bear an antigen which would cause the reciprocal graft to be rejected. The poor viability and fertility of these mice fitted in quite well, but since progeny of two such animals may reject grafts, this hypothesis is untenable.

Since histocompatibility genes usually appear to act as straightforward semidominants whether a strong locus like the H-2 is considered (Snell 1953) or the whole genotype, it was surprising to find that the inherited incompatibilities which occurred were not of the usual straightforward nature. However, Fox (1958) has suggested that the usual sort of result has been obtained in mice because the techniques used are particularly suited to detect them and has predicted that more ambiguous effects would be found upon closer examination. The incompatibilities found here are probably due to weak histocompatibility genes and either the effects of these genes are influenced by environment or the interaction of two or more genes is required for the response. The first possibility is less likely despite the well-established susceptibility of inbreds to environmental variation (Wright 1949; Robertson and Reeve 1952) because grafts tended to be rejected at roughly the same time within a strain; in strain A, for example, grafts which had not begun to break down at 70 days remained healthy as long as 180 days. If more than one gene is involved, large numbers of weak histocompatibility genes must exist since mutations in established inbred strains must have occurred and the long survival time and low frequency of rejected grafts preclude contamination as an explanation. The interaction of genes in the production of histocompatibility antigens was mentioned as a possibility by Billingham and Medawar in 1951 but they dismissed it as unlikely to be of importance and since then little attention has been given to the idea. It is possible that many and perhaps even all genes which have other functions could also participate in the production of such weak antigens. Fox (1958) has indeed shown that in *Drosophila melanogaster* the antigenic properties of the vermilion and ruby genes depend on the residual genotype.

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