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The Genetic Control of Histocompatibility Iso-Antigens

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Summary. This study provides evidence in mice of three strain combinations, that the classical law of transplantation that one gene ⇌ one antigen is applicable. It fails to confirm the suggestion that genic interaction may operate between the histocompatibility loci.

INTRODUCTION

Little and his colleagues (Little, 1914; Little and Tyzzer, 1916; Little and Johnson, 1922) suggested that the F_1 hybrid of a cross between two inbred strains displayed all the transplantation antigens of the parents' strains and no others. This led Haldane (1933) to postulate that one dominant histocompatibility gene directed the production of one transplantation antigen. The results of many more recent experiments have supported the Little–Haldane theory (Snell, 1953) and attempts to demonstrate a more complex gene–antigen relationship such as genic interaction (Martinez, Shapiro and Good, 1959) or allelic suppression among histocompatibility loci (Goodman 1965) have failed (Fig. 1).

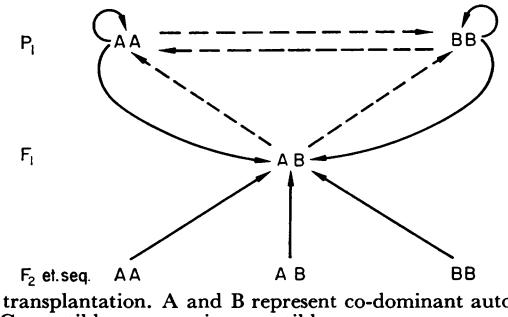

Fig. 1. Classic rules of transplantation. A and B represent co-dominant autosomal alleles at H-locus in diploid adults. →, Compatible; - - - >, incompatible.

This simple relationship is rather unexpected particularly in view of the demonstration in several species of the interaction between blood group loci to produce new antigens, e.g. *Drosophilia* (Fox, 1958), doves (Irwin, 1966a, b), cattle (Stormont, Owen and Irwin, 1951), rabbit (Cohen, 1956) and human (Watkins, 1966). Further the demonstration of gene or gene product interactions between the 'Tl_a' (thymus leukaemia) locus and the 'D' end of the *H*-2 histocompatibility locus in mice (Boyse, Stockert and Old, 1969) would support the existence of more complex histocompatibility gene–antigen relationships (Haldane, 1956; Fox, 1958).

Hildemann and Cooper (1967) examined the fate of skin grafts exchanged between F_1

hybrids and succeeding hybrid generations (F_n) in a hybrid of two inbred mice strains (C57BL/b male × A/Jax female). According to the classical genetic rules (Snell, 1953) the F_2, F_3 and F_n generations can display only those antigen specificities present in the F_1 hybrids, unless a mutation has occurred at a histocompatibility locus. Therefore, grafts from F_n donors to F_1 recipients should be uniformly successful. Some grafts may be rejected due to antigens on the X or Y chromosomes (Snell and Stimpfling, 1966).

However, Hildemann and Cooper (1967) showed that some F_2 grafts on F_1 recipients and a larger proportion of F_3 grafts on F_1 recipients were rejected between day 20 and day 100. They suggested that their results might be explained by the cumulative effect of point mutations and gene product interactions at a large number of weak histocompatibility loci, although their strains differed at the strong *H*-2 locus.

In view of the important implications of these findings, particularly in the fields of tissue typing and tolerance, we decided to examine the possibility that this was a general phenomenon, and might be a non-*H*-2 effect, using mice strains which do not differ at the *H*-2 locus.

MATERIALS AND METHODS

The four inbred mouse strains, (C3H female × CBA male) and (C57BL female × C57L male), and their hybrids were used. The C3H and CBA strains possess the same $H\text{-}2^k$ allele but differ at at least eleven weak *H*-loci (mean survival time skin grafts C3H→CBA: 16·3±0·30 days). The C57BL and C57L strains possess the same $H\text{-}2^b$ allele but differ at more than nine weaker *H*-loci (mean survival time skin grafts BL→L : 14·2±0·40 days).

The four inbred strains of mice have been maintained in the Department of Surgery for 4 years by strict sib-mating with back-crossing every third generation to eliminate any latent heterozygosity that might have arisen by spontaneous mutation. Prior to this the CBA, A, C57BL and C57L strains were maintained by Dr Krohn in the Department of Anatomy, Birmingham and the C3H mice were SPF stock from the Laboratories Animal Centre, Carshalton. Isografts are exchanged between members of each inbred strain every year, to check the homozygosity of each strain. The hybrids were bred from random matings of the parental animals. The F_1 recipients were never more than one generation removed from the parents of the R_2 (back-cross), F_2, F_3 and F_4 donors. All the mice were earmarked and caged in groups of ten and donors and hosts were observed for at least 200 days.

Full thickness tail skin grafts placed on beds on the right side of the thorax were held in place by plaster of Paris bandage using our standard method (Barnes and Krohn, 1957). The graft size varied from 20 to 50 mm^2. All grafts were between mice of the same sex to avoid rejections due to the *H*-Y locus.

The plaster bandages were removed on day 10 and the grafts were inspected on alternate days during the first month and thereafter weekly until the graft had been rejected or had survived for more than 200 days. Second set grafts were inspected daily from day 7. The distinctive phenotypic characteristics of tail skin were useful to identify the survival of grafts on like coloured hosts. The following features of the grafts were recorded: time of hair regeneration; density of hair pelt; scaling or scabbing of the surface; ulceration and contour of the dermal papillae.

Some of the grafts that survived for more than 200 days were biopsied and studied histologically while others were observed for the life time of the recipient.

RESULTS

Skin grafts exchanged between the parent strains were rejected after about 2 weeks whereas grafts exchanged within the inbred strains, or their F_1 hybrids survived permanently (Table 1). An indication of the genetic disparity of the strains used is shown by the survival of grafts of parental strain skin on F_2 hybrids recipients (Table 2).

TABLE 1

CONTROL EXPERIMENTS

		Mean survival time (days) ± SE
C3H × CBA		
P_1	P_2	15·3 ± 1·99
P_2	P_1	13·4 ± 0·88
F_1	F_1	All > 500
C57BL × C57L		
P_1	P_2	14·2 ± 0·40
P_2	P_1	All < 25
F_1	F_1	All > 500

TABLE 2

EVIDENCE OF GENETIC HETEROZYGOSITY

	Proportion of skin grafts surviving:		No. of *H*-antigens*
	> 100 days	> 200 days	
C3H × CBA			
$P_1 \rightarrow F_2$	7/89	1/89	$N \simeq 12$
$P_2 \rightarrow F_2$	0/11		
C57BL × C57L			
$P_1 \rightarrow F_2$	12/141	7/141	$N \simeq 10$
$P_2 \rightarrow F_2$	16/121	13/121	
	No. of grafts	Graft survival (days)	
C3H × CBA			
$F_1 \rightarrow F_2$	8	< 37	
$F_1 \rightarrow F_3$	9	13–27	
$F_1 \rightarrow F_4$	9	12–25	

*N the number of independently segregating genic factors is given by the function $(3/4)^n$ for grafts from $P \rightarrow F_2$ (Barnes and Krohn, 1957).

TABLE 3

TEST FOR GENIC INTERACTION AT NON-*H*-2 LOCI

Skin grafts (C3H × CBA)	Survival time (days)	
$F_1 \rightarrow F_1$ male	10/10	> 200
$F_1 \rightarrow F_1$ female	10/10	> 200
$F_2 \rightarrow F_1$ male	10/10	> 260
$F_2 \rightarrow F_1$ female	10/10	> 260
$F_3 \rightarrow F_1$ male	41/42*	> 200
$F_3 \rightarrow F_1$ female	53/53	> 200
$F_4 \rightarrow F_1$ male	9/9	> 250
	9/9	> 80
$F_4 \rightarrow F_1$ female	9/9	> 250
$R_2 \rightarrow F_1$ female	12/12	> 200

*Graft rejected at 47 days (see text).

In the first series of experiments the C3H × CBA hybrids were studied (Table 3). The F_1 hybrids accepted skin from other F_1 individuals permanently. In 154 experiments involving the grafting of F_2, F_3, F_4 and R_2 skin to F_1 recipients all but one graft survived in perfect condition for more than 200 days. The one exception was an F_3 male graft to an F_1 male host which was rejected at 47 days. The host was regrafted from the same donor at 55 days and the second graft was rejected by 69 days in a typical accelerated fashion.

In the second series of experiments 119 (C57BL × C57L)F_1 hosts received skin from F_3 and R_2 donors (Table 4). Two grafts were rejected in the F_3 to F_1 male combination at 90 and 100 days, respectively. Second set grafts were rejected in an accelerated fashion after 16 and 19 days, respectively.

TABLE 4

TEST FOR GENIC INTERACTION AT NON-*H*-2 LOCI

Skin grafts (C57BL × C57L)	Survival time (days)	
$F_1 \rightarrow F_1$ male	7/7	> 200
$F_3 \rightarrow F_1$ male	20/20	> 200
	22/24*	> 120
$F_3 \rightarrow F_1$ female	15/15	> 120
	11/11	> 200
	14/14	> 20
$R_2 \rightarrow F_1$ male	14/14	> 120
$R_2 \rightarrow F_1$ female	21/21	> 120

*Two grafts rejected at 90 and 100 days (see text).

DISCUSSION

In the present study of 273 F_2, F_3, F_4 and R_2 grafts to F_1 hosts, only one (CBA × C3H)F_3 male graft and two (C57L × C57BL)F_3 male grafts were rejected by their respective F_1 male hybrid hosts. The control data demonstrates the homozygosity of the strains used and shows that the F_2, F_3, F_4 and R_2 hybrid generations are genetically dissimilar. Therefore, the results in the (C3H × CBA) and (C57BL × C57L) hybrid combinations which possess the same *H*-2 allele, fail to confirm Hildemann and Cooper's (1967) results. They support the classical Little–Haldane theory that one histocompatibility gene determines one transplantation antigen and provide no evidence for any more complex gene–antigen relationship.

Thus, it may be assumed that an allele *H*-3A at a hypothetical *H*-3 locus will always determine the presence of the antigen *H*-3a on the cell membrane irrespective of the presence of alleles *H*-13A or B on the same, or *H*-16A or B on another hypothetical chromosome (Fig. 2).

Three F_3 to F_1 male grafts were rejected, unlike the isografts which were uniformly successful. These rejections were too infrequent to be explained on the basis of X-linked histoincompatibility or by genic interaction. The most plausible explanation of them is a spontaneous antigen gain mutation at a weak *H*-locus. This frequency suggests a mutation rate of 0·8 per cent/zygote for (CBA × C3H)F_3 males and 1·14 per cent/zygote for (C57L = C57BL)F_3 males which agrees well with the 1·35 per cent/zygote mutation rate estimated by Bailey and Kohn (1965) from isografts in a (BALB/c × C57BL/b)F_1 female population.

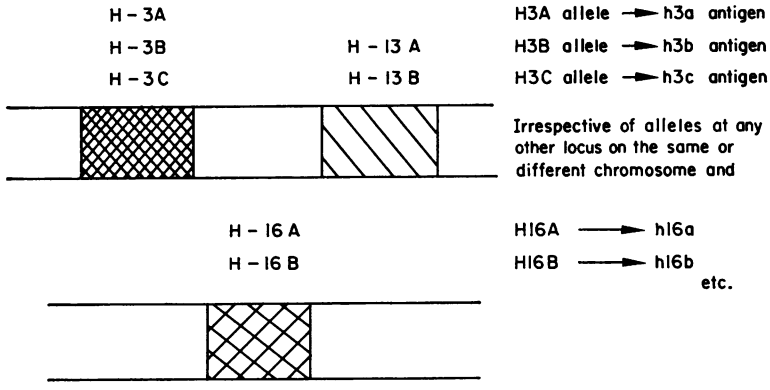

FIG. 2. The one gene ⇌ one antigen theory.

Before considering why the present results differ from those of Hildemann and Cooper (1967), mention must be made, of the failure to demonstrate X-linked histocompatibility of F_3 and F_4 male skin on F_1 male hosts in either strain combination. X-linked histocompatibility, which was first demonstrated by Bailey (1963), has been demonstrated in only C57BL hybrids. It is not surprising, therefore, that we failed to find X-linked incompatibility in the (CBA × C3H) hybrids. However, we might have expected to find X-linked rejections in the (C57BL × C57L) hybrids. The failure may be due to a very weak allogenic difference at the *H*-X locus, imperfect penetrance of the *H*-X gene or both strains may share the same *H*-X allele. Y-linked incompatibilities were not observed as all donors and hosts were of like sex.

There appear to be a number of possible explanations of the difference between Hildemann and Cooper's (1967) and the present results:

(1) *Breeding*

Hildemann and Cooper provided extensive control data to show that their strains were highly inbred and homozygous at *H*-loci. Our control data confirm that the strains are highly inbred. It is therefore highly unlikely that the difference is due to breeding errors. In experiments of this type with several hybrids that are genetically different and phenotypically identical it would be all too easy to make a breeding error.

(2) *Graft tissue*

In the present study, tail skin was grafted heterotopically to the right side of the chest whereas in Hildemann and Cooper's study, chest skin was grafted orthotopically. There is evidence that tail skin grafted orthotopically is particularly responsive to weak allogenic differences (Bailey, 1966a), but there is no evidence to suggest a difference in the response to body skin or tail skin, grafted to chest beds. More recently we have grafted body skin to chest beds and this survived as well as the tail skin.

(3) *Graft size*

In certain weak histoincompatibility differences in mice large skin grafts (50–80 mm^2) survive longer than smaller (20–30 mm^2) skin grafts (Lapp and Bliss, 1966; Zanzella, Rief, Buenuiaie, Sakumar and Deterling, 1968) and very large grafts may survive permanently (Lapp and Bliss, 1966). Although the size of our grafts varied from 20 to 50 mm^2 there was no difference in survival of the larger and small grafts. Hildemann and Cooper's grafts were of similar size (1·2–1·5 cm in diameter).

(4) *Observation period*

It is possible that incompatibilities have been missed in the present experiments because we have failed to observe the grafts over a long enough period.

This is unlikely as we have shown recently that observation for 200 days together with histological examination of 200 days grafts gives a better guide to compatibility than gross observation alone (Barnes and Cooper, 1969). In the present experiments, none of the grafts examined histologically at 200 days showed histological evidence of abnormality. Moreover, the rejections observed by Hildemann and Cooper occurred mainly during the second and third month.

(5) *Mutation*

We agree with Hildemann and Cooper that the spontaneous mutation rate would need to be impossibly high to explain the large number of F_3 grafts they observed to be rejected by F_1 female hosts.

(6) *Virus infection*

Bailey (1966b) postulated that antigen gain mutations at *H*-loci may result from the incorporation of viral genomes into parental germ cells, paralleling lysogeny in bacteria. Hildemann and Cooper suggested the possibility that viral infection accounted for their results and this has been supported by Snell (personal communication 1968). This theory does not adequately explain the facts for two reasons. Firstly, it requires the assumption that the virus selectively infects certain F_2 and F_3 mice but none of the parental and F_1 hybrid mice. Secondly, incompatibility due to virus induced antigen gain mutations would only occur as a temporary phenomenon during the infection of a colony. In the conventionally bred colonies used, a virus would become established throughout the colony in less than the 6 years of Hildemann's study.

(7) *Complex gene–antigen relationships*

Hildemann and Cooper (1967) and later Hildemann (1968) postulated that their results were due primarily to genic interaction and secondarily, to point mutations at a very large number of weak (i.e. non-*H*-2) *H*-loci. In this study we have used strain pairs which do not differ at the *H*-2 locus. Assuming Hildemann and Cooper's explanation is correct, it would have been reasonable to find similar rejections in the hybrids studied. Our results suggest that weak *H*-loci are not involved.

An alternative explanation is that of genic interaction within the *H*-2 region. Genic action in the *H*-2 region is very complex (Allen, 1955; Amos, Gorer and Mikulska, 1955; Shreffler, Amos and Mark, 1956; Gorer and Mikulska, 1959; Shreffler, 1964; Stimpfling and Richardson, 1965; Shreffler, 1966). Four examples of recombination between the $H\text{-}2^a$ and $H\text{-}2^b$ alleles (e.g. those alleles found in the A/Jax and C57BL/b strains) to form new alleles have been described (Gorer and Mikulska, 1959; Stimpfling and Richardson, 1965; Shreffler, 1966). Therefore, it is conceivable that in Hildemann and Cooper's (C57BL/b × A/Jax)F_2 and F_3 animals there could be recombinants between the $H\text{-}2^a$ $H\text{-}2^b$ alleles thus altering their weaker antigenic specificities which are known to exist (Snell and Stimpfling, 1966). This could cause weak allogenic differences between the F_2 and F_3 generation and F_1 hybrids.

Although Hildemann (1968) has been able to confirm his original findings in the (C57BL male × A female) hybrids we have so far shown no incompatibilities with the reciprocal hybrid (C57BL female × A male) (Barnes and Cooper, unpublished).

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