# Strong and Weak Histocompatibility Gene Differences in Mice and Their Role in the Rejection of Homografts of Tumors and Skin * †

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SUSCEPTIBILITY and resistance to homografts, like compatibility and incompatibility in the case of blood transfusions, is genetically determined.[7, 13, 25] The genes producing the individual differences, which in turn cause homograft rejection, have been called *histocompatibility genes.*[16] While our knowledge of histocompatibility genes is in a primitive state compared with our knowledge of blood group genes, it has of recent years been possible to detect and precisely characterize some of them in the mouse.[1, 2, 8, 11, 17-22, 26]

Three genetic loci, *H-1*, *H-2* and *H-3*, have been identified to date. *H-2* is a complex locus showing some similarities to the locus in man which determines the Rh blood types. At least 10 alleles or alternative forms are known. *H-1* and *H-3* are less fully analyzed. Besides being a histocompatibility gene, *H-2* is also a blood group gene.[8]

One method for the study of histocompatibility genes in the mouse is the production and analysis of *isogenic resistant* or *IR* lines.[16, 22] By appropriate genetic methods, pairs of lines have been developed which are essentially identical or isogenic except for a difference at one histocompatibility locus. This single difference makes either member of the pair resistant to grafts from the other member. One of the lines is always a standard inbred strain (e.g., C57BL/10, C3H/He, DBA/2); the other has been synthesized by a series of crosses which introduce, onto the background of the inbred strain, a gene for graft resistance originally present in the other parent of the initial cross. The two strains constitute a *coisogenic pair.* Or, since several IR lines may be produced on a single genetic background (e.g., that of inbred strain C57BL/10) a group of lines may all be coisogenic, though some of these may differ at two loci rather than one.

It should be emphasized that complete isogenicity cannot be achieved in lines synthesized by crossing. A chromosome segment of indeterminate length bearing a foreign histocompatibility gene, rather than the isolated gene itself, is always introduced onto the pure strain background. Occasionally the chromosome segment may carry, in addition to the histocompatibility gene, some gene producing a known and recognizable effect, as, for example, a modified coat color. Possibly in some cases two or more histocompatibility genes may be present, though so far we have no evidence of this. Despite these qualifications, it can be stated both on theoretical grounds and on the basis of extensive tests that the degree of isogenicity achieved in most of

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TABLE I. *Inbred and Isogenic Resistant (IR) Strains and Their Genotypes*

| Inbred Strain | | IR Strain | | Loci at Which the Two Strains Differ |
|---|---|---|---|---|
| Designation | Genetic Formula | Designation* | Genetic Formula | |
| C57BL/10** | *H-2$^b$ H-3$^a$ a* | B10.D2 | *H-2$^d$ H-3$^a$ a* | *H-2* |
| C57BL/10** | *H-2$^b$ H-3$^a$ a* | B10.LP | *H-2$^b$ H-3$^b$ A* | *H-3 A* |
| C3H** | *H-2$^k$ H-1$^a$ C* | C3H.K | *H-2$^k$ H-1$^b$ c* | *H-1 c* |
| DBA/2 | *H-2$^d$ H-1$^a$ C* | | | |

* The part of the symbol before the period indicates (sometimes in abbreviated form) the strain providing the inbred background. The part of the symbol after the period indicates (sometimes in abbreviated form) the other parent in the original cross. Thus strain B10.D2 was derived from an initial cross between strains C57BL/10 and DBA/2.

** The sublines of C57BL/10 and C3H used were C57BL/10ScSn and C3H/HeDiSn.

our IR lines renders them both suitable and highly useful for a wide variety of experiments.

Twenty-eight isogenic resistant or IR lines have now been produced, constituting, with their inbred mates, 28 coisogenic pairs. Most of these lines are now available in sufficient quantity for use in experimentation.[27]

During the development of the IR lines, differences in degree of resistance to tumor grafts were noted. This led to the concept that there are "strong" and "weak," or "major" and "minor," histocompatibility genes. With suitable IR lines established and available in quantity, this concept has now been put to precise tests.

## MATERIALS AND METHODS

Six strains of mice were used (Table I). Three of these, C57BL/10, B10.D2 and B10.LP, form a coisogenic trio, with C57BL/10 and B10.D2 differing at the *H-2* locus, and C57BL/10 and B10.LP differing at the *H-3* locus. The gene *H-3* is closely linked with the gene *A* which determines agouti coat color. In the production of IR line B10.LP, both genes were introduced onto the C57BL/10 background. Lines C57BL/10 and B10.D2 are thus black, line B10.LP agouti or gray. C3H and C3H.K are a coisogenic pair differing at *H-1* and the closely linked albino (*c*) loci. Strain C3H has a gray coat, strain C3H.K an albino coat. To provide a skin donor differing from C3H.K at the *H-2* locus, inbred strain DBA/2 was used. This is not coisogenic with C3H or C3H.K, and hence presumably differs from these strains at numerous loci besides *H-2*. It is known to differ at the *c* (albino) locus, and probably also at *H-1*.

Two transplantable tumors were used. C1498, originally classified as a myeloid leukemia, arose in 1941 in a mouse of strain C57BL/6.[6] Though native to subline 6 of strain C57BL, it has always grown equally well in the closely related subline 10, and is commonly used interchangeably in the two sublines. In routine practice it is carried in our laboratory by subcutaneous implantation by the sieve method.[24] It was employed in two experiments, one at transplant generation 454, the other at transplant generation 495. A gradual increase in capacity to kill mice of foreign strains has been noted.[23] B12 is an ascites tumor found by Dr. Borges and Miss Foerster in strain C57BL/10 in 1953. It has been carried regularly by intra-abdominal inoculation and was in transplant generations 60 and 72 when used.

Cell suspensions of C1498 were prepared from the solid, subcutaneous growth by the sieve method.[24] Since B12 is an ascites tumor, suspensions consisting mostly of single cells could be drawn directly from the abdominal cavity. From the ini-

tial cell suspensions, serial dilutions such that 0.1 ml. of fluid contained 625,000, 25,000, 1,000 or 40 cells were prepared. These were implanted subcutaneously in the case of C1498, intra-abdominally in the case of B12, 0.1 ml. per mouse. Some animals also received doses of 125,000, 5,000 and 200 cells, with results in conformity with those for the other dose levels, but these are not included in our tables as they add nothing to the data given.

All animals were between 49 and 74 days of age (only one animal was over 70 days) at the time of tumor implantation. Following implantation, animals were examined weekly and tumor development and the occurrence of deaths recorded.

In testing the survival of skin homografts between mice of the coisogenic trio, C57BL/10, B10.D2 and B10.LP, a slight modification of the method described by Billingham and Medawar [3] was employed. Fitted Thiersch grafts of ear skin 5 to 7 square millimeters in area were made to the lateral or dorso-lateral region of the trunk of the host. Rather than stripping skin from the ear, the whole ear was cut off, split, and the collagen layer removed. By utilizing the whole ear, four to six grafts could be made from one ear, and eight to 12 animals grafted from a single donor. Donors and recipients were male mice three to five months of age.

The graft bed was prepared as recommended by Billingham and Medawar and the graft fitted carefully in place. Small drops of collodion were placed over two diagonally opposite corners, and the graft covered with a small Vaseline®-impregnated gauze bandage. A cohesive rubber bandage was then applied.[5]

Grafts were checked on the sixth day, and the animals rebandaged. Bandages were removed seven or eight days after grafting. Grafts were examined every day under a dissecting microscope and the general appearance of the graft recorded until sloughing had occurred. Medawar [14] has described in detail the changes in the gross appearance of the graft during the course of the homograft reaction. In general, the appearance of ulceration of the graft was taken as the end point of graft survival. In a few instances, no ulceration was recorded before the appearance of a blackish scab; when this happened, the survival time was arbitrarily set as the previous day. Such observations are admittedly crude and their use in determining graft survival time has been criticized.[4] However, such observations can be made with sufficient care and accuracy to give an indication of the relative times of the homograft reaction in different donor-host combinations.

The difference in hair conformation between ear skin and body skin, and the existence of pigment differences between donor and host in every combination except C57BL/10 and B10.D2, made it impossible to confuse regenerated skin of the host with that of the donor.

In grafts between C3H and C3H.K, slightly larger pieces of ear skin were used, and the collagen layer was not removed. Checks were somewhat less frequent, and many of them were made without the use of a dissecting microscope. The methods were otherwise the same.

## RESULTS

The results of implanting the two tumors, C1498 and B12, both C57BL origin, in mice of strain C57BL/10 and two of its isogenic resistant sublines are shown in Table II. As expected, both tumors grew progressively in all C57BL/10 animals when the dose was adequate. There was 100 per cent mortality from both tumors among mice receiving 625,000, 25,000, or 1,000 cells. With a dose of 40 cells, both tumors gave some negatives.

At the other extreme, all mice of IR strain B10.D2 survived, except for one receiving 625,000 cells of C1498. The difference at the *H-2* locus which distinguishes

TABLE II. *Per Cent Mice Dying from Tumor Homografts, as Influenced by the Tumor Used, the Genetic Locus at Which Donor and Host Differ, and the Number of Cells Administered*

| Tumor (C57BL Origin) | Host | Locus at Which Tumor and Host Differ | Per Cent Deaths (Number of Mice in Parentheses) | | | |
|---|---|---|---|---|---|---|
| | | | 40 Cells | 1,000 Cells | 25,000 Cells | 625,000 Cells |
| C1498 | C57BL | no difference | 95 (22) | 100 (20) | 100 (10) | 100 (10) |
| | B10.LP | *H-3* | 86 (7) | 90 (20) | 100 (17) | 100 (10) |
| | B10.D2 | *H-2* | | 0 (10) | 0 (9) | 10 (10) |
| B12 | C57BL | no difference | 11 (9) | 100 (10) | 100 (5) | |
| | B10.LP | *H-3* | | | 8 (12) | 0 (5) |
| | B10.D2 | *H-2* | | | 0 (10) | 0 (10) |

this donor-host combination evidently constituted a genetic gap which, with rare exceptions, neither tumor could cross.

The response of IR strain B10.LP was intermediate, with a clear difference according to the tumor used. When implanted with C1498, B10.LP mice responded almost like the C57BL/10 mice to which the tumor is native. Doses of 625,000 or 25,000 cells killed all mice of both strains; with doses of 1,000 and 40 cells, B10.LP gave a few more survivors than C57BL/10. This result hints at some degree of resistance in strain B10.LP, but if C1498 alone had been used, the genetic difference between C57BL/10 and B10.LP would have been difficult or impossible to establish. However, when B12 was used as the test tumor, the difference between the strains became readily apparent. Most B10.LP's survived doses which killed all C57BL/10's.

Thus while neither tumor crossed the *H-2* genetic gap, one, though not the other, crossed the *H-3* gap. Whether this difference in capacity to grow when homografted in strain B10.LP should be attributed to different characteristics of the two tumors or to the different routes of implantation is uncertain, but we are inclined to lay most emphasis on the tumor. It is noteworthy that C1498, the tumor which did consistently cross the *H-3* gap, is the older of the two and has been noted as increasing in virulence during past transfers.[22]

The skin grafting experiments brought out in even more striking fashion the fact that whereas a difference between donor and host at the *H-2* locus presents a major obstacle to homograft survival, a difference at the *H-3* locus presents a minor, or at least a lesser, obstacle.

Billingham *et al.*[4] have reported that skin homografts made between various unrelated strains of mice show mean survival times ranging, according to the particular donor-host combination, from 8.5 $\pm$ 0.2 to 11.0 $\pm$ 0.3 days. In our experiments, grafts from B10.D2 to C57BL/10 showed a mean survival time of 8.5 $\pm$ 0.8 days (Table III). Our criteria of graft survival may not have been the same, or perhaps as critical (since grafts were not sectioned), as those employed by Billingham *et al.* Nevertheless it is apparent that a difference at the *H-2* locus causes essentially as rapid graft rejection as do the multiple differences (usually including an *H-2* difference) which exist between ordinary inbred strains.

With the coisogenic strain-pair differing at the *H-3* and the agouti loci the situation was quite otherwise. Eight grafts from B10.LP to C57BL/10 survived an average of 31.1 $\pm$ 2.5 days. Ten reciprocal grafts, from C57BL/10 to B10.LP, survived an average of 24.6 $\pm$ 1.8 days. There was no

TABLE III. *Number of Mice with Skin Homograft Surviving for Specified Periods, as Influenced by the Genetic Locus at Which Donor and Host Differ*

| | Days Survival | | | | | | | | | Total Number of Mice | Mean Survival Time |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7–9 | 10–12 | 13–15 | 16–18 | 19–21 | 22–24 | 25–27 | 28–30 | >30 | | |
| Donor and host differ at *H-2* locus | | | | | | | | | | | |
| B10.D2 to C57BL/10 | 13 | 2 | 2 | | | | | | | 17 | 8.5 ± 0.8 |
| Donor and host differ at *H-3* locus | | | | | | | | | | | |
| B10.LP to C57BL/10 | | | | | 1 | | | 2 | 5 | 8 | 31.1 ± 2.5 |
| C57BL/10 to B10.LP | | | | 1 | 1 | 6 | | | 2 | 10 | 24.6 ± 1.8 |

overlapping between the length of survival of these grafts and of the grafts between B10.D2 and C57BL/10. The maximum survival time of the latter was 13 days, the minimum time of the former was 18 days.

The homograft reaction in C57BL/10 hosts was more intense when strain B10.D2 was the donor than when strain B10.LP was the donor. In the former instance, grafts were as a rule sloughed within 48 hours of the appearance of ulceration; in the latter, ulceration was sometimes not followed by sloughing until 7 to 10 days had elapsed.

Evidently graft survival is much longer and the homograft reaction generally weaker, where a coisogenic donor and host differ at the *H-3* locus than when they differ at the *H-2* locus.

Essentially the same results were obtained with the coisogenic pair C3H and C3H.K, differing at the *H-1* and the albino loci. Survival time was again very much prolonged, though in this case more variable than with the C57BL/10–B10.LP combination. Whether this variability was due to residual heterozygosity in the C3H.K strain, to different amounts of collagen on the unscraped grafts, to more variable technique, or to some other cause, is unknown. In any case, the difference in survival between the grafts from C3H to C3H.K and the control grafts from DBA/2 to C3H.K was pronounced and unmistakable. Seven out of 10 C3H skin grafts were still surviving at 32 days, and one survived to 52 days. On the other hand, at least 2 grafts had sloughed by 10 days. In a second group of 10 mice, one graft was still surviving at 112 days when the host died of chronic nephritis. Some of these grafts seemed to go through periods of partial breakdown followed by recovery. The survival of the control grafts fell within the usual range of eight to 12 days.

## DISCUSSION

A *strong* histocompatibility locus may be defined as a locus such that a difference between donor and host at this locus will prevent the progressive growth of nearly all tumor homotransplants and cause the rapid rejection of skin homografts. The *H-2* locus in the mouse is, in the sense of this definition, a strong locus. In this paper we report that two tumors, when transplanted between donor and host, $H\text{-}2^b$ and $H\text{-}2^d$ respectively, but otherwise isogenic or genetically identical, did not give progressive growth. While exceptions do occur [10, 15] this rule holds with the great majority of tumors transplanted between coisogenic strain-pairs differing at the *H-2* locus.[28] We also show that skin grafts made between members of this same coisogenic strain pair are rapidly rejected.

A *weak* histocompatibility locus may be defined as a locus such that a difference between donor and host at this locus will permit the progressive growth of various tumor homotransplants and fail to cause the rapid rejection of skin homografts. The

*H-1* and *H-3* loci are, in the sense of this definition, weak histocompatibility loci. The results with tumor grafts here reported show only that one of two transplantable tumors rejected by mice with an *H-2* difference will grow in mice with an *H-3* difference. More data would be desirable, and will be forthcoming. The results with skin grafts were unequivocal; homografts survived far beyond the usual eight to 11 days where coisogenic strains differing at the *H-1* or *H-3* loci were used as donor and host.

The "strength" or "weakness" of histocompatibility loci is relative. Loci will doubtless be found that are "weak" relative to *H-1* and *H-3*.

As a strong locus, *H-2* is probably in a class by itself. We have noted elsewhere [20, 23] its unique role in determining resistance to tumor transplants. This finds a parallel in the strength of the isoantigens associated with this locus, as revealed in studies using classical serological procedures.[9, 11] Extensive tests have disclosed no other mouse isoantigen capable of evoking such high titers of antibodies.

Lehrfeld and Taylor [12] have found that, in the rat, small grafts 1 to 4 square milimeters in area will survive more than twice as long as large grafts 100 square millimeters or more in area. Although our grafts were only slightly larger than the small grafts of these authors, we found no evidence of prolonged survival due to graft size. Possibly this is because a graft of 5 to 7 square millimeters represents a "large" dose for a species as small as the mouse.

The significance of these findings for the problems of clinical homografting lies in the fact that they show that homograft survival can be notably prolonged with only a partial matching of the genotypes of donor and host. However, the degree of partial matching possible with controlled genetic stocks of mice might be difficult to duplicate at present in man.

Matching of blood groups should be of some significance. However, our knowledge of the relation of histocompatibility genes to blood group genes is still in a primitive state. Only in the case of the *H-2* locus in the mouse has the identity of a blood group gene and a histocompatibility gene been proven.[8, 9] Only further research can clear up the numerous problems that remain unsolved.

## SUMMARY

This study is based on the use of special stocks of mice differing by known histocompatibility genes (defined as genes determining susceptibility and resistance to transplants). One pair of stocks differed at the histocompatibility-1 or *H-1* genetic locus, a second pair at the *H-2* locus, a third pair at the *H-3* locus. Using these stocks, it was shown that an *H-2* difference is more effective in causing the rejection of homografts of tumor or skin than are *H-1* or *H-3* differences. Skin homografted between mice differing at the *H-2* locus survived an average of 8.5 days, skin homografted between mice differing at the *H-1* or the *H-3* loci survived an average of 24 or more days. On the basis of these results, a distinction is drawn between *strong* and *weak* histocompatibility loci. *H-2* is a strong locus, *H-1* and *H-3* are, relatively speaking, weak loci. It is concluded that a prolongation of survival of homografts may be brought about by a considerable, but nevertheless not complete, matching of the histocompatibility genotypes of donor and host.

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