Homologous Skin Transplantation from F_1 Hybrid Mice to Parent Strains.* (24843)

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Transplantation of normal and neoplastic tissues in highly inbred strains of mice is ruled by basic principles of inheritance. Tumors as well as normal tissues taken from animals of an inbred strain can be successfully transplanted into the same individual (autologous graft) or into members of the same strain (isologous grafts). In addition, by crossing mice of 2 different strains, the resulting F_1 hybrids accept transplants of tissue taken from members of either parental strain. Conversely, tissues taken from F_1 individuals and transplanted to members of either parental strain are always unsuccessful and result in a homograft rejection(1,2,3). However, incidental observations made recently in our laboratories seem to indicate that using certain "F_1 to parent" donor host combinations and under certain well defined conditions, such as transplantation in young recipient animals, skin from F_1 hybrid individuals can be successfully transplanted onto members of at least one of the parental strains. It is the purpose of this report to document these observations.

Method. Highly inbred mice of the Z,† A, BALB/C, Ce, C57 B1 (Subline 1) and their reciprocal hybrids were used. In a first set of experiments, skin taken from F_1 hybrid mice was transplanted to groups of mice of each parental strain, as follows: (A x Z) F_1 onto A and Z mice; (BALB/C x Ce) F_1 onto both BALB/C and Ce: (Z x C57 B1) F_1 onto Z and C57 B1 and (Z x Ce) F_1 or (Ce x Z) F_1 onto Z and Ce parental strains. Donors as well as recipient animals were divided into subgroups according to age at time of skin graft. These ages are indicated in Table I. In these experiments, donors of grafts and recipients were always of same sex. Method used for skin transplantation has been described(4). Essentially, it consisted of transfer of full-thickness abdominal skin graft from donor to back of recipient. In each instance the graft was turned 180° to facilitate determination of subsequent success or failure of graft. In such grafts hair on successful graft grows in a direction opposite to that on skin of host. Grafted animals were kept under observation for at least 5 months following operation. Mice were housed individually in plastic boxes and fed Purina Fox Chow and tap water.

Results. The results are recorded in Table I. When skin from (A x Z) F_1 hybrids was transplanted to 26-38 days old mice of the Z strain, and to A mice 25 days of age, none accepted the hybrid skin. The same was true when (BALB/C x Ce) F_1 skin was transplanted to mice of BALB/C or Ce strains and when (Z x C57 B1) F_1 skin was grafted onto mice of either C57 B1 or Z parental strains.

Similar negative results were obtained when (Z x Ce) F_1 skin was grafted onto Ce mice. However, when skin from this hybrid was grafted onto Z mice of 26 to 47 days of age, 28 of 34 animals (82%) accepted the graft which remained in place at least 5 months. Of this group, 9 animals were then retransplanted with skin from the same F_1 hybrids, when they reached approximately 5 months of age. This was done by replacing previous graft with another piece of hybrid skin. All of these recipient mice also accepted the second skin graft. On the other hand, when the recipient Z mice were 55 to 61 days of age at time of first grafting, only 12 of 31 (39%) accepted the graft. In contradistinction, none of 19 animals of the same parent strain (Z), ranging from 83 to 119 days in age, accepted this (Z x Ce) F_1 skin. It is interesting to note that the reciprocal hybrid skin resulting from the cross between Ce mothers and Z fathers, transplanted onto young Z mice, was also successful in 8 of 11 mice tested.

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† "Z" is used to simplify designation of mice of the C3H strain originally obtained from Dr. John J. Bittner's Laboratory.

TABLE I. Incidence of Successful Skin Grafts from F_1 Hybrid Mice to Individuals of Either Parent Strain.

Donor hybrid	Age in days	Recipient parent strain	Age in days	No. of mice accepting skin grafts*	No. of mice accepting 2nd skin graft*
(A × Z)F_1	37–100	Z	26– 38	0/12	
		A	25	"	
(BALB/C × Ce)F_1	37– 42	BALB/C	27– 67	0/14	
		Ce	26	0/10	
(Z × C57 Bl)F_1	33– 90	C57 Bl	28– 38	0/12	
		Z	25– 43	0/19	
(Z × Ce)F_1	29– 95	Ce	33– 56	0/25	
		Z	26– 47	28/34	9/9
		Z	55– 61	12/31	
		Z	83–119	0/19	
(Ce × Z)F_1	28	Z	32	8/11	

* No. +/No. grafted.

In view of these results, a second series of experiments was performed consisting of exchange of skin grafts between members of both Z and Ce parent strains.

The results of this experiment are recorded in Table II. It is clear that when Ce mice were used as donors and Z mice as recipients, rejection of the homograft regularly occurred regardless of age of recipient. Likewise, Ce mice always rejected Z skin.

Induction of acquired tolerance to (Z x Ce) F_1 skin homograft in weanling Z mice. Heretofore, all instances of immunological tolerance have been induced by injection of cells from prospective donor to prospective recipient prior to birth or in the immediate neonatal period(5-10). Since most Z mice ranging from 26 to 47 days of age accepted skin transplants from (Z x Ce) F_1 hybrids, whereas older animals, particularly those in 83 to 119 days age group always rejected this skin (Table I), it was decided to determine whether or not intravenous administration of viable spleen cells, taken from (Z x Ce) F_1 donors and injected intravenously into weanling mice of the Z strain, would induce permanent state of tolerance to the F_1 hybrid.

TABLE II. Homologous Skin Grafts between Mice of Z and Ce Strains.

Donor strain	Age in days	Recipient strain	Age in days	No. of mice accepting skin grafts*
Ce	60-180	Z	23-80	0/29
Z	44- 80	Ce	31-80	0/23

* No. +/No. grafted.

The experiment was performed in the following way: Weanling female and male mice of the Z strain from 21 to 25 days of age were injected intravenously into tail vein with 15-20 million viable spleen cells taken from (Z x Ce) F_1 adult donors. Donors were killed under ether, the spleen removed and cells to be injected prepared according to technic previously described(4). Spleen cells for each injection were suspended in 0.25 cc of Ringer-Locke's saline solution.

TABLE III. Induction of Tolerance to (Z × Ce)F_1 Skin Homograft in Weanling Z Mice Injected Intravenously with (Z × Ce)F_1 Spleen Cells.*

Host strain	Age (days) when inj. with F_1 spleen cells	Age (days) when grafted with F_1 skin	No. of mice accepting skin grafts†
Z	21-25	100-130	14/15
"		83-119	0/19

* Each mouse received 15 to 20 million viable cells.

† No. +/No. inj.

The injected mice, together with a group of non-injected controls, were set aside until they reached from 100 to 130 days of age, at which time they were grafted with skin from (Z x Ce) F_1 donors of approximately the same age. In all instances recipient and donor mice were of the same sex. After grafting, both experimental and control mice were kept under observation for no less than 5 months.

The results are recorded in Table III. Of 15 Z mice injected immediately after weaning with F_1 spleen cells, 14 or 93% accepted the corresponding F_1 graft. In contradistinction,

all 19 control animals of approximately the same age rejected the skin grafts in the usual way.

Discussion. The results of these experiments confirm that in most instances the skin taken from F_1 hybrid individuals resulting from crossing 2 different strains of mice can not be successfully transplanted onto animals from either parent stock. However, when skin taken from (Z x Ce) F_1 hybrid was grafted onto young Z mice 26 to 47 days of age, a high incidence of successful takes was achieved. On the other hand, when skin from the same donor hybrid was grafted to older recipients of parent strain, the incidence of successful grafts decreased markedly, and in fully mature animals grafts failed altogether. The differences in incidence of successful takes in the 3 groups studied are highly significant on a statistical basis. Similarly, the reciprocal F_1 hybrid skin taken from (Ce x Z) F_1 animals also was accepted in most cases when transplanted onto young mice of the Z strain.

It is further of interest that mice accepting the first homologous skin graft remained susceptible to a second transplant of the same skin performed at an age when animals of the same strain not subjected to a previous graft regularly rejected the homologous skin, suggesting that persistence of initial graft induced in recipients some degree of tolerance to the skin of homologous hybrid strain.

Further evidence for this hypothesis was obtained in the observation that injecting weanling Z mice with viable spleen cells from adult (Z x Ce) F_1 produced immunological tolerance, permitting the parent Z strain recipients to accept skin from hybrid donor at any age.

It is difficult with present concepts to offer a satisfactory explanation of these results. It seems possible, however, to postulate that. of strain combinations studied, Ce and Z mice differ from each other by weak histocompatibility genes(11,12) which are, however, sufficiently strong to permit regular rejection of skin homotransplants between the 2 parent strains. However, when strains are crossed. the histocompatibility differences between hybrid and parent strain may be lessened by genetic mechanisms such as those postulated by Fox(13). This might make it possible for the recipient strain to accept homografts or develop tolerance to the hybrid more easily than would be the case between parent strains. The observation that in these combinations the young recipients of parent strain accept such homografts from donors of the hybrid strain, whereas older recipients do not, would be best explained by the hypothesis that young animals, having not reached complete immunological maturity, are more susceptible to both homotransplantation and to development of immunological tolerance than are older animals of the same strain.

Summary. 1) Using several "F_1 hybrid to parent" strain combinations homotransplantation of skin from F_1 hybrid mice onto members of both parent strains has been attempted. 2) Results showed that while most "F_1 to parent" homologous skin grafts were unsuccessful, skin taken from (Z x Ce) F_1 or (Ce x Z) F_1 grafted onto mice of Z parent strain 26 to 47 days of age, was successfully established in most cases. Moreover, older recipients of same strain, particularly those ranging from 83 to 119 days in age, rejected the F_1 skin in all instances. 3) Exchange of skin homografts between mice of Z and Ce strain was regularly unsuccessful regardless of recipient's age at time of grafting. 4) Acquired immunological tolerance to (Z x Ce) F_1 homologous skin grafts was achieved in weanling Z mice injected intravenously with viable spleen cells taken from adult F_1 donors.

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Reduced Growth Hormone Content in Anterior Pituitaries of Rats on Protein-Free Diets.*† (24844)

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Diets inadequate in protein or lacking specific essential amino acids retard skeletal growth. Several investigators(2-10) have measured tibia length and width of epiphyseal cartilage plates to assess growth retardation during such deficiencies in the rat. Frandsen *et al.*(8) found that in complete absence of dietary protein, the tibial proximal epiphyses were sealed from shaft by bone, known to occur after hypophysectomy(11). To determine whether growth arrest in absence of dietary protein was due to decreased synthesis of growth hormone, anterior pituitaries of protein-deficient rats were bioassayed for growth hormone activity and studied histologically. An attempt was also made to measure growth-promoting potency of blood from such animals to gain additional information on pituitary function.

Methods. Adult female rats (Long-Evans strain) weighing approximately 200 g were maintained 5 weeks on purified diet free of protein, though complete in all other known dietary essentials.‡ The animals had lost an average of 65 g. Groups of control rats of similar age and body weight were maintained either on purified diet containing 24% casein‡ or on stock diet XIV(13) and during same period gained 40-50 g. Bioassay results for control groups were in such close agreement that they have been combined in the Tables. Anterior pituitaries from protein-deficient and control rats were dissected, weighed, ground, suspended in isotonic saline with 2% butanol, and stored at 4°C. Total doses ranging from 1/32 to ½ anterior lobe were injected subcutaneously into immature female hypophysectomized rats. Injections were given once daily for 4 days, followed by autopsy 24 hours after last injection. The right tibia was stained with silver nitrate(14). Width of uncalcified portion of proximal epiphyseal cartilage was measured with ocular micrometer and cartilage widths of 200 μ or more, *i.e.*, increase of at least 40 μ over uninjected control widths, were considered evidence for growth-stimulating activity. Plasma from protein-deficient and control rats was bioassayed by same method(14). Blood was collected for 4 consecutive days from the aorta in heparinized syringes and centrifuged under mineral oil 30 minutes at 3200 rpm. Plasma for each day was pooled and injected subcutaneously

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‡ The protein-free diet contained sucrose 88%, hydrogenated cottonseed oil 8%, modified salts No. 4 (12) 4%. Crystalline vit./kg of diet were: vit. B_{12} 0.05 mg, *d*-biotin 0.6 mg, 2-methyl-1,4-naphthoquinone, 10 mg, thiamine HCl 10 mg, pyridoxine HCl 10 mg, pteroylglutamic acid 11 mg, riboflavin 20 mg, p-aminobenzoic acid 20 mg, niacin 40 mg, *d*-calcium pantothenate 100 mg, inositol 800 mg, choline chloride 1000 mg. The control diet contained sucrose 64%, alcohol-extracted casein 24%, hydrogenated cottonseed oil 8%, modified salts No. 4(12) 4% and vit./kg of diet listed above with B_{12} omitted. All rats received weekly a fat-soluble vit. supplement of 800 U.S.P. units vit. A, 115 chick units vit. D, 6 mg synthetic alpha-tocopherol and 650 mg corn oil (Mazola).