Mortality and Skin Transplantability in X-Irradiated Mice Receiving Isologous, Homologous or Heterologous Bone Marrow.*† (22582)

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The protective effect of intravenous administration of bone marrow or spleen cell suspensions after an otherwise lethal dose of x-irradiation is well established. Such protection is usually associated with rapid hematopoietic recovery. Early observations usually involved short term protection using cell suspensions from the same strain of animals as the irradiated recipient. Later it was reported that some degree of irradiation protection could be obtained with homologous‡ and even heterologous marrow(1-4). Barnes and Loutit(5-6) failed to obtain protection with heterologous spleen or marrow, and have called attention to the importance of evaluating protection with homologous marrow beyond the usual 30 day end-point. It is well established that most tissues of one inbred strain of mice may ordinarily be transplanted with success to all other members of the same inbred strain, but not to members of another strain, unless that strain contains all of the histocompatibility genes of the donor strain (7). Thus, tissue may be transplanted successfully from either inbred parent strain to the F_1 hybrid, but not vice versa. Main and Prehn have reported that in mice protected with marrow from an F_1 hybrid of the irradiated strain, it was subsequently possible to successfully homograft skin from the other (foreign) parental strain. Such skin grafts sloughed in mice protected with isologous marrow(8). The present study was undertaken to determine the relative degree and duration of protection afforded by isologous, homologous, and heterologous sources of bone marrow in irradiated mice, and to extend the observations of Main and Prehn on skin homografting.

Materials and methods. Bone marrow preparation: Mice of the following inbred strains were employed: CBA, Strong A, Cb, C_{57}, C_3H, Db and their F_1 hybrids A x Db and Cb x CBA. The Cb and Db strains are the Balb c and Dba strains without the mammary tumor inciter. Mice received total body x-irradiation in groups of 6 under the following conditions: 200 KVP, 15 Ma, 40 cm target distance, 0.5 mm Cu and 1.0 mm Al filters, HVL = 1.3 mm Cu, 88-101 r/minute dose rate. Calibration with a Victoreen dosimeter placed in the mouse chamber was carried out each day the machine was used.§ In preparing bone marrow suspensions from mice, 6 bones from each young adult donor mouse (femora, humeri, tibias), were scraped free of muscle, the marrow cavity was broken open at both ends, and a tight fitting hypodermic needle on a 1 ml syringe was inserted into one end. The bone was then submerged in a tube of saline solution (.8% NaCl, .02% KCl) and the marrow flushed from the bone by drawing saline in and out of the syringe. Saline was used at the rate of .5 ml per donor mouse. The washings were pooled and the small amount of coarse particles allowed to settle out by gravity. The remaining super-

* This investigation was supported by a research grant from the Nat. Cancer Inst., U.S.P.H.S.

† Preliminary report presented at April, 1956 meeting of the Am. Assn. for Cancer Research (*Proc. Am. Assn. Can. Res.*, 1956, v2, 153).

** The author is indebted to Drs. A. Kirschbaum and H. B. Andervont for the inbred strains of mice used; to Dr. R. Shalek for assistance in dosimetry, and to M. A. Flores, B. Morris and A. Liebelt for technical assistance.

‡ The terms autologous, isologous, homologous, and heterologous are used as defined by Snell(7): autologous—from same individual; isologous—from another individual of same inbred strain (or identical twin); homologous—from individual of different genotype but of same species; heterologous—from different species.

§ X-ray facilities and calibration were generously provided by the Radiation Physics Department of the M. D. Anderson Hospital and Tumor Institute.

natant fluid was essentially a single-cell suspension. Cell counts made by the method for white cells in peripheral blood gave counts on the order of 50,000 cells per cu mm. A sample of each cell suspension was spun down, smeared and stained for microscopic examination. The suspension was administered (.5 ml per mouse) via the tail vein, under sodium amytal anesthesia, within 1 to 3 hours after irradiation. Aseptic technic was used throughout. *Rat marrow* was similarly collected from 5 Sprague-Dawley rats and suspended in 16 cc of saline. Each irradiated mouse received .5 ml. *Guinea pig marrow* was similarly collected from each of 5 pigs, 8 days old, using 13 ml of saline. The suspension was spun down at 200 x G for 10 minutes, the supernatant fluid discarded, the cells resuspended in saline, and the process repeated. The cell count on the material injected was 70,350 per cu mm. Volume injected was .5 ml. *Rabbit marrow* (4 ml) was collected by aspirating the marrow cavities of 6 bones from a 5.8 kg albino male. This was dispersed in 10 ml saline, and fat and debris removed by centrifugation. Saline was added to bring the volume of the cell suspension up to 13 ml. The volume injected per mouse was .5 ml with a cell count of 13,550 per cu mm. One batch of isologous mouse marrow was fractionated into a washed cell fraction and a pooled supernatant plus washings fraction. This batch was prepared initially with 4 ml of saline for 6 donor mice. The cells were spun down at 200 x G, the supernatant pipetted off and the cells resuspended in 1.5 ml saline. This was repeated a total of 3 times. The fourth time the cells were resuspended in 7 ml saline and .5 ml injected per irradiated mouse. The 4 supernatants were pooled and freed of cellular contamination by centrifuging at 1000 x G for 20 minutes, the sediment discarded, and the supernatant spun again at 7000 x G for 5 minutes. This final supernatant fluid was injected (.5 ml per irradiated mouse). Another batch of isologous mouse marrow was prepared in the usual way except that distilled water was used instead of saline to flush the marrow from the bones, in order to lyse the cells (no intact cells could be found in the smear). *Skin grafts*: Irradiated mice that were well-protected by isologous or F_1 hybrid, or foreign strain mouse marrow were skin-grafted in from 33 to 161 days post-irradiation. Under sodium amytal anesthesia the hair was removed from the dorsum of the mouse with electric clippers. A piece of skin approximately 2 cm square was removed and replaced with a piece of skin of the same size from the same strain, or a foreign strain, or an F_1 hybrid of the irradiated strain. Interrupted silk sutures were used. No dressing or bandage was applied. The mice were individually penned until the graft had sloughed or was definitely healed. When skin from the same strain was grafted in an unirradiated control the graft was an autograft; the piece of skin removed was turned 180° and sutured back into the bed from which it came. Cortisone treatment consisted of 5 mg per day of cortisone acetate intraperitoneally in 2 divided doses, morning and evening, for 11 days. Bone marrow suspension from the F_1 hybrid was injected at 1, 3 and 13 days following the last injection of cortisone.

Results. The 21-day LD_{50} was approximately 600 r. At 770 r mortality was 100% for 136 mice, the longest survival being 12 days. At this level of irradiation, which was selected as a standard against which to measure protection, the peripheral leukocyte count fell to a few hundred cells per cu mm by the third day and remained low until death. Platelets became difficult to find in the blood smears by the fifth day. Body weight declined progressively until death. Mice dying before the seventh day showed no hemorrhage, but after the eighth day hemorrhage of the gastrointestinal tract and brain was a very common autopsy finding. *Mice receiving isologous bone marrow* after irradiation showed the same severe depression of circulating leukocytes by the third day, followed by a rapid return to the normal range as early as the seventh day. Mortality by the 12th day, when all of the controls were dead, was only 13%, and there was very little further mortality as late as 100 days post-irradiation (Table I). Protective activity was exhibited by the washed cell fraction but not by the cell free supernatant fraction or by the lysed cell

TABLE I. Cumulative Mortality in Mice Receiving 770r followed by Isologous, F_1 Hybrid, Foreign Strain, and Heterologous Bone Marrow.

Source of marrow	Irradiated mouse strain	No. of mice	Marrow donor, strain or species	% mortality after indicated No. of days 12	21	30	60	100
Same strain of mice	A	12	A	0	0	0	0	0
	Cb	11	Cb	55	55	55	55	64
	CBA	12	CBA	0	0	0	0	0
	CBA	12	CBA	0	0	0	17	19
	All combined	47		13	13	13	17	19
F_1 hybrid mouse	A	10	A × Db	30	50	50	80	90†
	A	12	A × Db	0	0	8	8	16‡
	A	10	A × Db	40	40	60	80	90
	CBA	12	Cb × CBA	0	0	0	0	0§
	All combined	44		16	20	27	39	45
Foreign strain of mice	A	12	Db	0	17	42	100	100
	CBA	12	C_3H	8	8	8	33	33‖
	CBA	12	A	8	8	25	75	83
	Cb × CBA	11	A	18	27	36	45	*
	C57	7	A	0	0	0	100	100
	All combined	54		8	15	28	69	
Foreign species	Cb	31	Rat	45	71	77	94	97
	CBA	24	Rabbit	83	96	96	96	96
	A	24	Guinea pig	83	100	100	100	100

* Has not yet reached 100 days post-irradiation.
† Of 3 mice skin grafted at 43 days, 1 died at 57 days, 1 at 90 days.
‡ Of 8 mice " " " 74 to 77 days, all lived beyond 100 days.
§ All 12 mice skin grafted at 30 to 56 days.
‖ Of 6 mice skin grafted at 71 to 72 days, all lived beyond 100 days.

suspension. *Marrow from an F_1 hybrid or foreign strain* of mice likewise resulted in rapid recovery of circulating leukocytes, and protected against mortality just as effectively as isologous marrow until the 21st day. Between the 21st and 60th day however, mortality increased steadily and significantly in the foreign marrow group, in spite of circulating levels of red cells, white cells and platelets within the normal range (with the exception of one mouse that showed a terminal erythropenia). Late death in these mice was usually preceded by progressive loss of body weight. The only apparent gross pathology at autopsy was pulmonary congestion and consolidation. *Marrow from F_1 hybrids* was, on the average, intermediate in protective activity between isologous and foreign strain marrow. Variation between groups receiving F_1 marrow, however, was considerable. Two such groups showed excellent protection as late as 100 days post-irradiation. In the peripheral blood of the mouse, lymphocytes normally outnumber polymorphonuclear leukocytes in the ratio of about 2 to 1. In the mice surviving 770 r as a result of receiving either isologous, F_1 hybrid, or foreign strain mouse marrow, this ratio was reversed. *Irradiated mice receiving guinea pig or rabbit marrow* showed 83% mortality by the 12th day and were all dead by the 21st day except one mouse. This mouse received rabbit marrow and is still alive at 234 days. It survived in spite of a very slow recovery of circulating white cells (950 cells/cu mm at 16 days, 2800 at 22 days; 10,500 at 29 days). At no time were granulocytes characteristic of the rabbit seen in the peripheral blood of this mouse. (Mouse and rat neutrophils are essentially agranular, while rabbit and guinea pig neutrophils have characteristic granules which distinguish them morphologically from those of the mouse.) *Irradiated mice receiving rat marrow*, although not as well protected as those receiving mouse marrow, showed significant protection on the 12th day. By the 21st day mortality in the rat marrow group had increased significantly, and by 60 days only 2 mice were still alive. These results with rat marrow are very similar to those reported by Cole *et al.*(3). *Skin grafts.* Of 34 autografts in unirradiated control mice, 3 sloughed, presumably because of

TABLE II. Results of Skin Grafting.

Host strain	X-ray dose (r)	Marrow donor	Skin donor	Day post-r when grafted	Takes		Sloughs	
					No.	Days duration to date of writing	No.	Days duration
A	None	None	A	—	7	142–162	2	13–14
CBA	"	"	CBA	—	24	57–140	1	<27
"	"	"	C_3H	—	0		4	17–19
"	"	"	Cb	—	0		9	<21
A	880	A	A	33	2	253	0	
"	770	"	A × Db	161	0		5	25–33
"	"	"	Db	161	0		6	22–40
"	"	A × Db	A × Db	43–77	1	Dead at 48	2	<21
					4	143–260		
"	"	"	Db	74–77	2	143	2	<24
CBA	660–770	Cb × CBA	Cb	35–56	15	57–79	3	13–16
"	770	C_3H	CBA	71	2	140	0	
"	"	"	C_3H	71–72	4	139–140	0	

inadequacies of technic (Table II). Of 13 homografts in unirradiated control mice, all sloughed. Of 22 irradiated mice protected with marrow from an F_1 hybrid and homografted with skin from the other parent strain, only 5 sloughed. The other 17 grafts, after an initial shedding of hair, regrew hair characteristic of the donor skin and are still in good condition 57 to 143 days after grafting.

Of 54 mice protected with foreign strain marrow, only 6 CBA mice that received C_3H marrow remained in good enough physical condition for long enough to warrant skin grafting. Of these, 2 were grafted with isologous skin, and 4 with homologous skin from the same strain that donated the bone marrow. All 6 grafts took and have persisted for 139 to 140 days. It may be significant that the CBA and C_3H strains are closely related in origin, and that tumors arising in one strain sometimes transplant to the other.

In irradiated mice protected with isologous marrow and homografted with skin from a foreign strain or an F_1 hybrid, all 11 grafts sloughed, indicating a specificity of the tolerance of skin homografts in irradiated marrow-protected mice. Only if the marrow used for protection came from the same foreign strain as the skin donor, or from an F_1 hybrid of the skin donor strain, was tolerance of the foreign skin exhibited.

Of the irradiated mice included in the mortality data, 29 mice that appeared well-protected were skin grafted during the 100-day period for which mortality data is presented. Skin grafting did not significantly alter the mortality data. Only 2 of the skin grafted mice died during the 100-day period.

Forty-five mice were started on cortisone treatment followed by F_1 hybrid bone marrow. Twenty-three mice survived the treatment. From 17 to 61 days after the last injection of cortisone, 12 of these mice were grafted with skin from the F_1 hybrid and 9 with skin from the other parent strain. In all 21 cases the grafts sloughed.

Discussion. The closer the genetic relationship, the greater is the protective activity of the marrow. The importance of evaluating the longevity of protection beyond the 21- or 30-day endpoint is apparent from the results obtained with rat marrow and foreign strain mouse marrow. Successful homografting of skin after protection with marrow from an F_1 hybrid of the irradiated and skin donor strains, but not after protection with isologous marrow, agrees with the results of Main and Prehn(8). That F_1 marrow is not unique in this respect is indicated by the successful homografting of skin, in the 4 cases in which this was tried, after protection with marrow from the same foreign strain as the skin donor.

There is increasing evidence supporting the concept that the tissue homograft reaction is an immunological response to foreign tissue antigens(7,9,10,11). Homologous mouse bone marrow in addition to permitting sur-

vival by virtue of hematopoietic recovery, appears also to have induced immunologic tolerance of tissue antigens from the marrow donor source. Main and Prehn(8) have called attention to the possibility that the mechanism of the latter effect may be similar to the induced immunological tolerance of foreign strain skin homografts in mice that have been injected as fetuses with tissue suspensions from the skin donor strain(12). The initial failure to induce tolerance to skin homografts by cortisone treatment and F_1 hybrid marrow does not eliminate this possibility. Pertinent to the question of whether survival of foreign strain marrow and skin after irradiation results from an induced immunological tolerance, in the sense of Billingham, Brent and Medawar, is the following: After protection with homologous or heterologous marrow against an otherwise lethal dose of x-irradiation, does the host's antibody producing tissue survive in a functional sense, or is it completely replaced by the comparable system of the marrow donor? Only in the first case must one postulate an altered immunological specificity of the surviving host system.

It has recently been shown that in irradiated mice and rats protected with homologous marrow, and in irradiated mice protected with rat marrow, the introduced cells survive and repopulate the irradiated host's otherwise depleted hematopoietic tissues(13-16). The failure of heterologous marrow to afford as great protection as any kind of mouse marrow in the first 2 or 3 weeks after irradiation may be related to an inability of heterologous marrow in a foreign host to perform all of the vital functions performed by homologous or isologous marrow. On the other hand, while rat marrow persists and proliferates in the irradiated mouse, it is possible that guinea pig and rabbit marrow, because of a greater genetic dissimilarity, does not. The failure to find granular leukocytes characteristic of the rabbit in the blood smears of the one rabbit-marrow-injected mouse that did survive indefinitely suggests this possibility.

The late mortality in irradiated mice receiving foreign strain mouse marrow is of interest, since it occurs in spite of 21-day protection equal to that with isologous marrow. Does this represent a delayed immunological response of the host to the tissue of the foreign strain as Barnes and Loutit(6) and Cole *et al.*(15) have suggested to explain late mortality in mice protected with homologous or heterologous bone marrow? The essentially normal blood counts before late death of mice receiving foreign strain mouse marrow, and the long duration of tolerance to skin homografts do not accord with this hypothesis. Perhaps more likely is the opposite possibility. The evidence for survival of marrow cells from a foreign strain or foreign species, and of skin from a foreign strain, indicates an induced immunological tolerance for tissues from the foreign marrow donor, on the part of any surviving antibody producing tissue of the irradiated host. But is there adequate reason to expect that the reverse has been accomplished? If the introduced foreign strain mouse or rat marrow survives and proliferates and produces cells capable of antibody production, this antibody producing system, by virtue of its foreign origin, should be immunologically active against all of the tissues of the irradiated host. Only the antibody producing systems of the same inbred strain or of an F_1 hybrid should not be immunologically active against the irradiated strain.

Summary. The degree and duration of protection afforded by isologous, homologous and heterologous sources of bone marrow administered after lethal doses of x-irradiation in mice was directly proportional to the closeness of genetic relationship of the marrow donor to the irradiated mice. Uninjected controls were all dead by the 12th day. Mouse marrow from the same strain or from an F_1 hybrid of the irradiated strain, or from a foreign strain, all gave excellent and essentially equal protection as late as 21 days post-irradiation. While mice receiving marrow from the same strain remained well protected beyond 100 days, those receiving marrow from a foreign strain showed considerable late mortality between the 21st and 60th days. Mortality in mice receiving F_1 hybrid marrow was intermediate at 60 days. Guinea pig and rabbit

marrow elicited a very low degree and duration of protection. Rat marrow permitted 55% of the irradiated mice to survive beyond the longest survival of the untreated controls. The duration of protection however was short, only 6% at 60 days. Irradiated mice protected with marrow from an F_1 hybrid of the irradiated strain showed a high degree and long duration of tolerance of skin homografts from the F_1 hybrid or from the other parent strain. Irradiated mice protected with foreign strain mouse marrow showed a high degree and long duration of tolerance for skin homografts from the same foreign strain. Irradiated mice protected with marrow from the same strain did not tolerate skin homografts from a foreign strain or from an F_1 hybrid.

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Received May 25, 1956. P.S.E.B.M., 1956, v92.

I^{131} and P^{32} Accumulation in Anuran Thyroid Gland in Normal and Accelerated Metamorphosis.* (22583)

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The application of radioactive tracers to studies of amphibian development, though infrequent, reveals that the tadpole thyroid can concentrate radioactive iodine and that this process may be influenced by thyroid stimulating hormone (TSH). Employing autoradiographic and histologic methods, Gorbman and Evans(1) demonstrated that radioiodine accumulation in the developing thyroid of Hyla larvae began soon after folliculation appeared in the gland. Autoradiographs became progressively darker as the thyroid increased in size and the colloid increased in amount. Dent and Hunt(2) found marked localization of I^{131} in autoradiograms of the thyroid from Hyla, Bufo, and Rana larvae, with no diminution in thyroidal radioactivity observed throughout metamorphic and post-metamorphic processes. Money, Lucas, and Rawson (3) measured the turnover of I^{131} in normal, thiouracil, and TSH treated *Rana pipiens* tadpoles. Radioactivity of the area of tissue bearing the thyroid ("block dissection") approximated 10-20% of the administered radioactivity. No substantial difference was found between metamorphosing and non-metamorphosing tadpoles. Surprisingly, the administration of TSH usually decreased rather than increased thyroidal I^{131} collection. In the main, the results of these experiments with radioactive tracers do not conform to those

* This investigation was supported in part by research grant, A-432, from the N.I.H., Public Health Service.