Comparison of Autologous Marrow Injection to Shielding in Lethal Irradiation of the Mouse (35702)

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Shielding of small portions of the hematopoietic system provides protection against the lethal effects of whole-body irradiation in the midlethal dose range. Transfusion of either whole blood or transplantation of hematopoietic tissues protects to some degree in every mammalian species studied.

Manipulation of the radiation dose, time of transplant and recipient treatment with immune suppressors leads to some success in transplants between species. The probability of acceptance of an allograft is inversely related to the number of histocompatibility differences. Hence, in identical twins with no antigenic differences there should be 100% takes. Several investigators (1–4) have followed Atkinson (5) in attempting transplants between a normal individual and his whole-body irradiated leukemic identical twin. A logical extension of identical twin donor–recipient matching is the autologous transplant. The patient serves as donor and recipient with an interim treatment of radio or chemotherapy. This method has been used with varied success in a number of clinical situations (3, 6). Experience has shown that in most radiation incidents patients have received a partial body or nonuniform exposure in which there exist areas of relatively unexposed marrow. These areas can be predicted by depth-dose studies and proven by marrow aspiration. In such cases the question is how best to aid the individual in reseeding his aplastic marrow from the shielded and intact marrow pool. Rudakov *et al.* (7) have shown that the introduction of a needle puncture into one or both femurs of irradiated rats led to a decrease in mortality. This effect could be increased by flushing with saline. Weinstein (8) demonstrated a similar effect by the intramedullary mixing of marrow by a trocar inserted into the shielded rat tibia.

Several investigators (9, 10) have compared the protective effect of shielded marrow to the injection of an equivalent amount of marrow cells. However, in each case there was some question as to either the exact number of marrow cells in the shielded volume or the radiation dose delivered to the "shielded cells."

Ingram (11) recently suggested that not enough is known about the relative merits of autoreseeding by undisturbed migration as compared to the reinjection of spared marrow in the nonuniform irradiated individual. In an attempt to answer this question the following study was undertaken.

Materials and Methods. The experimental plan was to compare the mortality between groups of mice which had one leg shielded while the rest of the body was irradiated to a group similarly shielded and irradiated in which the shielded leg was amputated and marrow cells injected intravenously. It was necessary to inject as many marrow cells as were shielded, therefore, the technique (12) of grinding the whole bones was used rather than washing or blowing cells out of the marrow cavity. The selection of this technique was based on earlier studies (13) in which it was shown that nearly 50% of the stem cells (as measured by spleen colony technique in mice) remains in the shaft and chondyles of the bones after flushing out of the marrow cavities.

Eight-week-old female mice of the Brookhaven–Hale–Stoner strain were used. Throughout the experimental period they were housed 10 to a cage and given food and water *ad libitum*.

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All animals were irradiated by a 250 kVp General Electric Maxitron X-ray machine operating at 250 kVp and 30 mA. The dose rate was 118 rad/min at a TSD of 60 cm and the HVL 1.25 mm of Cu. All mice were anesthetized with sodium pentobarbital and taped to a holding board with the right leg extended under tension. The entire leg was covered with a cave-type shield as previously described (12, 14).

Amputation was performed immediately after irradiation while the animals were still anesthetized. Incisions extending the full length of the femur were made on the lateral and medial aspects of the thigh. The femur was exposed by blunt dissection, the major vessels and muscle tissue were tied using surgical silk and then severed distal to the ties. The femur and lower leg were removed and the skin flap secured using wound clips. Marrow from the femur and tibia were prepared and injected as previously described (12).

Experimental Design. Four groups of mice were used in this experiment. Their treatments were as follows: *Group 1.* The right leg was shielded during irradiation, then immediately amputated, the marrow cells harvested from the entire leg and injected into the tail vein.

Group 2. The right leg was shielded during irradiation, then immediately following irradiation the left leg was amputated. This allowed for migration of shielded elements from the right leg while submitting the animal to the stress of surgery as in Group 1. Amputation of the irradiated left leg does lead to loss of some viable stem cells, however since the D_0 for these cells is approximately 80 rads, and the lowest dose to the leg 700 rads, the surviving population is minimal.

Group 3. The right leg was shielded during irradiation and no surgery performed on either leg. When compared with Group 2, this allows a further measure of the degree of stress involved in surgery.

Group 4. These animals received whole-body irradiation without any shielding.

Results. Five exposure levels ranging from 700 to 1300 rads were tested in all four treatment groups.

Nonsurviving animals all died within the same period postexposure apparently from similar causes. With doses lower than 900 rads (the LD 100(30) for this strain) no significant difference was noted within protected Groups 1, 2, and 3, however, these groups all showed a significantly higher survival than the untreated group (Table I). For exposures of 900 to 1300 rads the reinjection treatment resulted in a significantly ($p > 0.5$) lower survival than in any of the other three groups. There was no significant difference between Groups 2 and 3.

Discussion. These results indicate that the reinjection of autologous marrow from shielded portions of animals receiving midlethal exposures does result in some degree of protection, as compared to total-body irradiated animals. However, when compared to the shielded animals without reinjection, there appears to be no advantage, and at higher dose levels there is evidence of a deleterious effect.

Our findings indicate that migration of cells from the undisturbed shielded mouse limb takes place at a rate which is more effective in protecting the animal than can be obtained by reinjecting this marrow. Extrapolation of these data to man is difficult. The clinical implication would be that harvesting and reinjection of autologous marrow in the nonuniform irradiated patient may be of no or little value. However, there are great differences in the technique used in this mouse study and marrow aspiration in man. In the former traumatic stress and sepsis are evident. In the latter asepsis is mandatory and trauma is minimal. Before rejecting the therapeutic notion completely animal studies more closely mimicking this proposed human therapy should be performed.

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TABLE I.

Radiation dose rads	Treatments											
	I			II			III			IV		
	Total no.	Dead	Mortality (%)	Total no.	Dead	Mortality (%)	Total no.	Dead	Mortality (%)	Total no.	Dead	Mortality (%)
Lethal range												
700	7	1	14	9	0	00	9	0	00	10	7	70
800	17	0	00	17	0	00	17	2	12	28	25	89
Total	24	1	4	26	0	00	26	2	8	32	22	58
Supralethal range												
900	10	2	20	10	0	00	10	0	00	18	18	100
1000	7	4	57	9	4	44	9	3	33	10	10	100
1200	5	4	80	6	2	33	6	3	50	6	6	100
1300	11	10	91	12	5	42	12	7	58	14	14	100
Total	33	20	61	37	11	30	37	13	35	48	48	100

Treatments

I Shield right leg, amputate right leg, inject marrow from right leg.
II Shield right leg, amputate left leg.
III Shield right leg.
IV No treatment other than irradiation.

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