Bone Marrow Reconstitution Using a Block Grafting Technique

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SUMMARY

Experiments in rabbits with radiation-induced bone marrow aplasia have demonstrated that intramedullary block grafting is a feasible procedure. This technique requires surgical interference and offers no advantage over intravenous reconstitution. We therefore suggest that it has no value in therapeutic bone marrow transplantation.


The transplantation of bone marrow from HL-A identical and MLC non-reactive donors into patients with aplastic anaemia results in permanent recovery of marrow function in approximately 50% of cases. The currently accepted technique for such reconstitution is the intravenous infusion of a monocellular suspension, and the use of this method in animals and man has been well documented. However, a number of cells are unavoidably damaged during preparation of the suspension, and there may thus be some selective loss of stem cells. Furthermore, a large number of the infused cells are sequestered in the lung following administration of the graft and, while this is likely to be transient, their exact fate remains unknown. These observations have suggested a need to explore alternative methods for reconstitution. It seems logical that if the marrow could be replaced directly into the bone marrow cavity in particle form, both of the above limitations would be avoided. In addition, it is theoretically possible that if the architecture is intact and cell relationships are therefore undisturbed, recovery may be significantly enhanced.

This study was undertaken to explore the feasibility of such an intramedullary grafting technique, using lethally irradiated rabbits as a model.

METHODS

Rabbits were selected both for easy manipulation and for the ready availability of bone marrow from the femora. The animals had an average body weight of 2 kg. A dose of 1200 rads was delivered to the horizontal midplane from a cobalt source, and this invariably resulted in death of the control animal from bone marrow damage between 4 and 12 days after irradiation, without causing significant gastro-intestinal or other radiation-induced disturbance. It was important to deliver the radiation with the anaesthetised rabbit lying on its stomach, since in the lateral positions the femora were at different heights in the radiation field, and this resulted in an uneven distribution of damage to the marrow.

To determine the adequacy of a marrow graft from the femur in preventing death after lethal irradiation, one femur, or both, was shielded with lead during irradiation, and the recovery of peripheral blood values and bone marrow was measured.

The technique for removing the femoral marrow prior to intravenous infusion was then developed in the rabbit. A small hole was drilled at the level of the greater trochanter to admit a 20-gauge needle, and a slightly larger hole was drilled at the lower end of the bone into which a Teflon tube of 1.0 mm internal diameter fitted tightly. The contents of the femur were then washed out with tissue culture medium (TC 199, containing 20% autologous serum and 25 units of preservative-free heparin), by gentle pressure on a syringe containing the medium and attached to the 20-gauge needle. The marrow was converted into a monocellular suspension by the method previously described. After exposing the rabbits to 1200 rads total body irradiation, the autologous suspension was reinfused into the marginal vein of the ear (group I). An aliquot of the marrow was retained for measurement of colony formation in soft agar, for determination of cell viability, and for differential counting.

The critical question of intramedullary reimplantation was then systematically explored. In initial experiments the femur was de-roofed and the marrow was curretted out (group II). After irradiation, the autologous marrow was re-packed into the femoral cavity en bloc, and the excised bone fragment was replaced and secured with either internal mechanical fixation or acrylic cement.

In the third group of animals (group III), a larger hole (0.5 cm in diameter) was drilled at the lower end of the femur to avoid the de-roofing procedure. The marrow was removed by applying pressure with the syringe and a 20-gauge needle at the upper end of the femur, so that a solid marrow core was extruded intact. The animal then received lethal whole-body irradiation, and the block of marrow was reintroduced retrogradely into the now vacant femoral cavity via the lower hole. This procedure prevented the femoral fractures which occurred in group II.

In a further group of animals (group IV) the marrow was removed as described above, but was converted into coarse particles by passage through a screen of 0.6 mm...
RESULTS

The effect of the varying doses of radiation on survival is shown in Fig. 1. The L.D.50 was 1200 rads, and this point was clearly defined. It is particularly significant that this dose level caused total and irreversible bone marrow aplasia without other complications from the irradiation. Specifically, haemorrhage of the gastro-intestinal tract or pulmonary changes were not demonstrable.

All 12 animals in the control group died from overwhelming infection within a period of 4-12 days. Post-mortem examination showed that the organs were heavily infected with micro-organisms, which were prominent within the vascular system. None of the recognised short-term effects of irradiation were demonstrable at post-mortem examination.

![Graph](image)

**Fig. 1.** The relationship of radiation dosage to survival. The L.D.50 is 1200 rads delivered to the horizontal mid-plane of the rabbit.

In the control animals haemoglobin levels of between 10 g/100 ml and 12 g/100 ml were recorded. The average platelet count was 12 000/µl but, significantly, bleeding was not a problem, and leucocyte counts varied between 500/µl and 1 000/µl, without any granulocytes.

In the animals where one or both femora had been shielded (Fig. 2), peripheral blood values all returned to normal. The rise was quicker where both femora had been shielded, although during the 30 days of observation the values never returned to completely normal levels.

Of the 8 animals that received the standard irradiation, followed immediately by intravenous infusion of autologous marrow (group I), 6 survived the procedure with permanent recovery of marrow function (Table I). The 2 rabbits in this group that died, did so from infection before engraftment had occurred.

![Graph](image)

**Fig. 2.** The relationship between leucocyte count and post-irradiation interval. Irradiated animals (control group) survived 4-12 days without reconstitution. Shielding of both femora led to a more rapid return of the leucocyte count to normal than when one femur was shielded. After 30 days the leucocyte count had not reached the basal level.

<table>
<thead>
<tr>
<th>Group</th>
<th>Method of reconstitution</th>
<th>Survivors</th>
<th>% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nil</td>
<td>0/12</td>
<td>0</td>
</tr>
<tr>
<td>Group I</td>
<td>Intravenous</td>
<td>6/8</td>
<td>75</td>
</tr>
<tr>
<td>Group II</td>
<td>Block</td>
<td>0/4</td>
<td>0</td>
</tr>
<tr>
<td>Group III</td>
<td>Large fragments</td>
<td>2/8</td>
<td>25</td>
</tr>
<tr>
<td>Group IV</td>
<td>Small fragments</td>
<td>3/6</td>
<td>50</td>
</tr>
</tbody>
</table>

Aliquots of the monocellular suspension which were studied in the laboratory revealed a viability of intact cells in excess of 95%, and bone marrow differential cell counts were in close agreement with those previously reported. Colony formation in soft agar was used to predict the likelihood of engraftment, but, apart from a broad correlation with nucleated cell counts, was found to have limited value when used in this context.

None of the 4 animals in group II, in which the femur had been de-roofed, survived for more than 12 days. These animals died from overwhelming infection, and post-mortem examination showed extensive necrosis of the replaced marrow.

Of the 8 animals in group III, in which the marrow was replaced en bloc into the femur by the retrograde route using a syringe, 2 survived. In these animals normal bone marrow function was demonstrated, but large areas of marrow necrosis were evident in biopsy specimens. Among the animals that died, the striking feature was infection, with a great deal of phagocytic activity in which macrophages were ingesting the necrotic bone marrow.

In group IV, 3 of the 6 rabbits recovered, with adequate bone marrow function. Fig. 3 shows the recovery of the leucocyte count in groups I and IV. Significantly, the rate of increase was no faster after intramedullary than after...
intravenous reconstitution, and the animals in both these groups showed a more rapid haematological recovery than those in the group in which one or both of the femora had been shielded.

![Graph](image)

**Fig. 3. Comparison of the leucocyte count in intravenously grafted animals (group I) and in those receiving intramedullary grafts of small particles (group IV).** Note that the rate of recovery is not significantly different.

**DISCUSSION**

This radiation model of bone marrow aplasia has been found practical because LD₃₀ is precisely defined, and because the bone marrow aplasia is irreversible and is not complicated by other side-effects of irradiation. This model is ideal for studying immediate autologous reconstitution, since chemotherapeutically induced marrow aplasia has the disadvantage that some of the chemotherapeutic agent may circulate for a varying period of time, and so modify the response of engrafted marrow.

The cause of death in the animals which were irradiated but not reconstituted was infection, with a survival period of 4-12 days. This correlates with an absolute loss of granulocytes and monocytes and reflects severe bone marrow damage. The finding that when one or both femora were shielded all the animals survived, established that the amount of marrow contained in one femur (11.0 x 10⁵ nucleated cells) is adequate for reconstitution. A slightly more rapid rise occurred with a larger graft, obtained by using both femora (Fig. 2). However, other studies have established that a graft of 1.7 - 4.0 x 10⁵ nucleated cells per kilogram body weight is adequate, and that larger grafts appear to confer no significant further benefit. If the weight of our animals is taken into account, these observations accord well with published data.

The technique in which the femur was de-roofed was abandoned, since neither internal fixation nor acrylic cementing was suitable for stabilising the weight-bearing bone. The mortality from infection in some animals in this group was thought to be influenced by femoral fractures with local haematoma formation.

In the group where the entire marrow core was replaced intact immediately after irradiation, the low survival rate is clearly explained by the extensive necrosis of the graft which occurred, and which was demonstrated at post-mortem. The necrosis probably resulted from an inability to vascularise the graft sufficiently rapidly to sustain cell survival. In the 2 animals which survived, superficial colonisation with haematopoietic tissue occurred, but the unpredictability of this occurring contra-indicates the use of large blocks.

Of significance are the results in the animals in which intramedullary grafting was carried out using small particles. In this group, the survival rate approached that of the animals that were autologously transplanted by the intravenous route. Histological examination of the bone marrow in these animals proved that it was entirely normal. Death in this group was related to infection at a period before engraftment had taken place. As in the previous group, necrosis was observed, and although it was patchy in distribution, it is thought to have limited the delivery of nutrients essential for rapid engraftment. This finding raises a question about particle implantation as opposed to monocellular reconstitution where, we assume, nutrition would not be in balance.

Of particular interest is the finding that the grafting of 2.4 x 10⁵ nucleated cells from one femur led to more rapid recovery of peripheral blood values than when either one femur, or both, was shielded. With the shielding of the femur, normal architecture is retained, and a rapid return of bone marrow function could be expected. This paradoxical delay in recovery may be explained by the systemic effect of irradiation, the so-called abscopal effect.

These studies have led us to conclude that in the experimental animal intramedullary block grafting is feasible, but offers no advantage over the intravenous route. When these findings are extrapolated to man, the benefits of the intravenous route are further emphasised, since the problems of a surgical procedure in the thrombocytopenic and agranulocytic patient will pose a formidable challenge.

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**REFERENCES**