Bone grafts in T-cell deficient rats

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Revascularization, new bone formation, and resorption of fresh syngeneic and allogeneic cancellous bone that were transplanted to an intramuscular pouch have been studied in athymic and normal rats. Revascularization was evaluated with radioactive microspheres; formation of new bone was assessed with ^{85}Sr incorporation; and resorption was measured by the graft weight reduction. Animals were killed 2, 6, or 12 weeks after transplantation. The circulation and bone formation in allogeneic grafts were greatly impaired in normal rats as compared with the athymic group and the syngeneic grafts. The allografts in normal rats had a smaller weight reduction than the allografts in athymic rats, suggesting impaired resorption. We conclude that the T-lymphocyte system is at least partly responsible for the difference between syngeneic and allogeneic bone grafts, and that the thymus-dependent primary rejection mechanism probably is important for the vitality of allogeneic bone grafts.

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Bone allografts are inferior to their autogenous counterparts with regard to bone induction properties, revascularization, and incorporation into host bone (Goldberg et al. 1985, Wilson et al. 1985, Burchardt 1987). Clinical matching of transplantation antigens between donor and host has not given a definite answer as to whether allograft inferiority is due to immunologic incompatibility (Friedlaender 1983, Burwell et al. 1985), although experimental evidence suggests that this is the case (Bos et al. 1983, Goldberg et al. 1985). Analogous to cell-mediated mechanisms in skin-graft rejection, T-lymphocyte immune responses might be important for the revascularization and incorporation of allogeneic bone grafts. The influence of the T-lymphocyte system on the acceptance or rejection of allogeneic bone grafts can be studied in congenitally athymic rats (nude, rnu/rnu). These animals are born without a functioning thymus gland, and consequently they have no T-lymphocyte function (Festing 1981). A difference in the revascularization and bone-inductive properties by allogeneic bone grafts between athymic and normal rats could be ascribed to the T-lymphocyte immune system.

We investigated the revascularization, the formation of new bone, and the resorption of heterotopic fresh syngeneic and allogeneic cancellous bone in athymic and normal rats.

Material and methods

The donors of allogeneic bone were male Wistar rats, whereas the donors of syngeneic bone were male Lewis rats. Recipients were male rats of the Lewis strain inbred with the donor Lewis rats and male Lewis athymic rats inbred with the other Lewis rats, genetically differing from these only by the nude mutation. The Wistar/Lewis combination is a major histocompatibility mismatch (Gill 1978). All the rats weighed between 180 and 200 grams. The recipients were kept 1 in each cage postoperatively and fed water and standard rat pellets ad libitum. A total of 72 rats were used as recipients and 36 rats were used as donors.

The recipients were divided into four groups with 6 rats in each group: normal syngeneic recipients, normal allogeneic recipients, athymic syngeneic recipients, and athymic allogeneic recipients. Each donor animal gave bone grafts to 1 normal and 1 athymic rat.

The donor animals were killed with an overdose of pentobarbital administered intraperitoneally, and both iliac bones were excised. The periosteum, all the soft tissues, and some of the cortical bone were removed. The rest, including bone marrow, was implanted in an intramuscular pouch in the long back musculature of the recipient animal under fentanyl anaesthesia. All the grafts were weighed to the nearest milligram, stored in 0.9 precent saline at room temperature, and transplanted within 30 minutes.

Table 1. Revascularization of heterotopic cancellous bone grafts measured with ^{141}Ce-labeled microspheres. Relative vascularization is expressed as cpm per mg in the bone graft/cpm per mg in the os ileum of the recipient rat. Significance levels of the difference from the allogeneic-Lewis group. Mean *SD*

	Normal Lewis rats				Athymic rats			
Weeks postop.	Allogeneic grafts		Syngeneic grafts		Allogeneic grafts		Syngeneic grafts	
2	0.62	*0.26*	1.34	*0.36***	1.50	*0.41****	1.43	*0.47***
6	0.71	*0.40*	2.05	*0.72***	1.34	*0.60**	1.70	*0.97**
12	0.35	*0.10*	1.02	*0.56***	0.75	*0.23****	0.79	*0.13*****

*$P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Table 2. New bone formation in heterotopic cancellous bone grafts measured with rate of ^{85}Sr incorporation. Relative bone formation expressed as cpm per mg of the bone graft/cpm per mg of the os ileum of the recipient rat. Significance levels of the difference from the allogeneic-Lewis group. Mean *SD*

	Normal Lewis rats				Athymic rats			
Weeks postop	Allogeneic grafts		Syngeneic grafts		Allogeneic grafts		Syngeneic grafts	
2	0.40	*0.11*	1.32	*0.36****	1.36	*0.23****	0.99	*0.39****
6	0.71	*0.05*	0.93	*0.22****	0.77	*0.30****	0.63	*0.12****
12	0.35	*0.14*	0.75	*0.24****	0.71	*0.19****	0.95	*0.36****

*** $P < 0.005$.

Table 3. Percentage of original weight of heterotopic cancellous bone grafts. Significance levels of the difference from the allogeneic-Lewis group. Mean *SD*

	Normal Lewis rats				Athymic rats			
Weeks postop.	Allogeneic grafts		Syngeneic grafts		Allogeneic grafts		Syngeneic grafts	
2	60	*11*	46	*15*	51	*19*	58	*20*
6	51	*12*	21	*19****	31	*14**	33	*18**
12	59	*25*	20	*22****	33	*19**	24	17***

*$P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

Three days before the recipients were killed, 1 μCi/100 g body weight of ^{85}Sr was injected intraperitoneally as strontium chloride in 0.5 mL saline (Elves 1974). They were killed 2, 6, and 12 weeks postoperatively. They were anesthetized with fentanyl. The right common carotid artery was isolated through a longitudinal midline incision, and a polyethylene catheter (PE-10, OD 0.63 mm, 2 FG, Portex United, Kent, England) was introduced into the ascending aorta through the right carotid artery (Kirkeby 1991a). The correct catheter position was confirmed at autopsy. Approximately 1 million ^{141}Ce-labeled microspheres (NenTrac, New England Nuclear, U.S.A.)—15 μm in diameter, suspended in 1 mL isotonic saline—were injected into the ascending aorta. The syringe was flushed for remaining microspheres with 1 mL isotonic saline. The animals were killed with an intraarterial overdose of pentobarbital shortly after the microsphere injection. An operating microscope was used to observe even arteriolar trapping of the microspheres in the iris of each animal.

The iliac bones and all the bone grafts were excised, and all the soft tissue was meticulously removed. All the samples were weighed to the nearest 0.1 mg (wet

weight). Total radioactivity in counts/minute (cpm) of each isotope in the samples was measured by placing the sample in the center of a Packard 5221 Autogamma scintillation spectrometer with the windows set over the highest energy peak of each isotope. Individual isotope activity of the samples was calculated with correction for background, cross-talk, and physical decay according to Heymann et al. (1977). Radioactivity in the grafts was compared as cpm in the bone graft/cpm in the os ilium.

The statistical analyses were performed with repeated analyses of variance for all animals together, and analyses of variance with the Bonferroni test at the three postoperative intervals.

Results

There was manifest ingrowth of vessels 2 weeks after transplantation in three of the groups: namely, both the athymic groups and the normal syngeneic recipients (Table 1). For these grafts the circulation already exceeded the circulation of the os ileum by 30–50 percent. The circulation was of similar magnitude after the next 4 weeks, with a declining tendency 12 weeks after transplantation (NS). In contrast, the normal allogeneic recipients had a lower blood flow in the grafts than the other three groups throughout the 12-week period. The same phenomenon was apparent regarding the mineral accretion of the grafts (Table 2). The strontium incorporation in the grafts relative to the iliac incorporation was smaller at all times in the normal allogeneic recipients than in the other three groups. All the grafts had a considerable weight reduction 2 weeks after transplantation. The grafts in the normal allogeneic group lost less weight than the other three groups. At 6 and 12 weeks, the difference was significant (Table 3).

Discussion

The grafts in this study were transplanted fresh because the immunologic reaction to frozen grafts is of a smaller magnitude (Friedlaender 1987), and effects of the T-lymphocyte immune reaction would be more difficult to detect in frozen grafts.

The biological difference between fresh syngeneic and fresh allogeneic cancellous bone grafts as regards osteogenetic activity and revascularization has previously been described by several authors. Histologic techniques (Heiple et al. 1963) and the ^{85}Sr incorporation method (Goldberg and Lance 1972) have shown that allogeneic bone induces less new bone than its syngeneic counterpart. The authors have postulated that an immunologic mechanism is responsible for the inferiority of allogeneic bone.

The revascularization of syngeneic and allogeneic bone has also been evaluated by dye injection and histologic studies (Zeiss et al. 1960). Revascularization has been shown to be slower and less extensive in allogeneic grafts, possibly because of the transplantation reaction that these grafts may evoke (Burchardt 1987). The hypothesis has also been put forward, however, that autogenous grafts possess some factor not possessed by allografts that induces rapid capillary invasion and new bone formation (Wilson et al. 1985). In our study, weight reduction was less after 6 and 12 weeks in allogeneic than in syngeneic grafts, probably due to impaired revascularization. The results confirm that the osteogenetic activity and revascularization of allogeneic bone grafts were of a lesser magnitude than in syngeneic grafts.

These parameters of biological function are dependent upon the genetic constitution of the donor and host. Why this is so has been unclear, but an immunologic mechanism has been suggested by several authors (Friedlaender 1983, Burchardt 1987). It is reasonable to make an analogy to transplant rejection mechanisms, and it has been speculated that the T-lymphocyte is the prime effector of this immune response, and thus responsible for the decreased function of bone allografts (Horowitz and Friedlaender 1987). Our results strongly support this view. The athymic rat has in effect no T-lymphocyte function, but an otherwise adequate immune system. The morphology and the mechanical, osteogenetic, and healing properties of their skeleton are indistinguishable from those of normal rats (Kirkeby 1991b). Allogeneic bone grafts in athymic animals did not show an inferior incorporation compared with syngeneic bone grafts as normal recipients show.

We conclude that the T-lymphocyte system is at least partly responsible for the difference between syngeneic and allogeneic bone grafts, and that the thymus-dependant primary rejection mechanism probably is important for the biological function of allogeneic bone grafts.

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