Rabbit bone matrix induces bone formation in the athymic rat

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Rabbit and rat bone matrix were implanted in athymic rat muscle, and the bone yield was measured as total calcium content after 4, 6, and 8 weeks. Matrix from both species induced equal amounts of new bone in the athymic rat. In rabbit and normal rat, the xenogenic matrix induced little or no bone formation. Thus, in the case of rabbit and rat, bone induction is species specific due to immunogenic mechanisms. The athymic rat can be used to measure inductive properties of bone matrix from different species.

The ability of demineralized bone matrix to induce bone formation has been used in numerous experiments and even for the treatment of skeletal defects in man (Glowacki et al. 1981, Urist 1980, Harakas 1984). This property of bone matrix is species specific according to some authors (Sampath and Reddi 1983), whereas others claim it is not (Glowacki and Mulliken 1985).

Sampath and Reddi (1983) suggested that the species specificity of bone induction could be due to immunogenic rejection of xenogenous matrix. We tested this hypothesis by using athymic rats. This mutant strain has no thymus function. In consequence, no T-lymphocytes are developed that can initiate the immunogenic rejection of xenogenic material. If bone induction by bone matrix were species specific due to immunogenic mechanisms only, the athymic rat could prove useful for assaying human bone matrix preparations intended for clinical use.

Material and methods

Matrix preparation

Twenty-five female Sprague-Dawley rats were killed by intraperitoneal injection of Pentothal. The femoral diaphyses were excised, and the periosteum and marrow were removed. Further preparation was performed under sterile conditions. The bone was defatted in chloroform-methanol (1:1) for 2 h, rinsed in methanol, decalcified in 0.6 N HCl for 48 h, rinsed in water, and lyophilized. To avoid that a tubular shape would differ from the rabbit matrix pieces (see below), each femoral diaphysis was divided longitudinally. The median dry weight of the pieces was 8 (6–15) mg.

The diaphyses of all long bones from one young healthy white mongrel rabbit (2.6 kg) were prepared as above and divided into fragments matching the rat matrix in weight.

Implantation

Rabbit and rat matrix pieces were matched in pairs according to weight (± 10 percent). Each recipient animal had one such pair of pieces operated into muscle pouches in the abdominal wall.

The recipient animals were 30 athymic rats (nu/nu Rowett, Wallenberg Labortories, Lund, Sweden, about 60 days old), 30 Sprague-Dawley rats (Möllegaard, Copenhagen, Denmark, about 60 days old), and 20 white mongrel rabbits (local farm, 1.9–2.9 kg).

Evaluation

The recipient animals were killed after 4, 6, and

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8 weeks, respectively. The matrix implants were dissected out and ashed in a muffle furnace at 800° C for 24 h. The ash was dissolved in 1.5 ml 6 M HCl, evaporated in a vacuum centrifuge, redissolved, and analyzed for calcium in a DACOS machine using the methyl thymol blue reaction (Cossar and Fitzpatrick 1974).

Two-way analysis of variance was used.

Table 1. Calcium per implant weight (μmol/mg)

Recipient	Athymic rat		Normal rat		Rabbit	
Donor	Rat	Rabbit	Rat	Rabbit	Rat	Rabbit
4 weeks	1.9	1.2	2.0	0.05	0.02	1.1
	2.1	2.0	0.6	0.01	0.02	1.4
	1.9	0.6	5.2	0.26	0.07	–
	2.8	5.9	4.3	0.87	0.04	1.1
	3.0	3.1	1.8	0.13	0.02	1.4
	1.3	3.3	3.4	0.70	0.02	–
	4.8	5.0	2.6	0.02		
	2.8	1.8	3.8	0.03		
			4.8	0.04		
6 weeks	4.5	1.2	1.8	0.89	0.02	2.7
	3.4	3.7	2.7	0.24	0.01	–
	3.3	3.7	4.4	3.0	0.03	1.7
	3.0	2.6	2.3	0.08	0.03	4.3
	2.6	3.2	3.4	0.01	0.01	–
	3.2	2.6	3.9	0.07	0.02	0.7
	2.6	3.5	5.3	1.4	0.03	4.0
	4.4	5.3	7.6	0.04	0.06	–
	4.0	1.8	5.5	0.40		
	4.1	4.6	3.6	1.2		
	3.1	3.7				
8 weeks	4.9	6.6	7.3	1.1	0.04	1.2
	5.9	5.6	4.9	0.05	0.01	0.4
	5.3	5.6	6.0	2.0	0.02	1.4
	6.0	5.3	8.0	0.13	0.01	0.52
	4.1	6.8	6.1	3.8	0.03	0.02
	4.6	6.3	4.8	0.45	0.03	3.3
	3.8	3.8	4.7	0.03		
	7.4	6.6	8.8	1.2		
	5.5	5.8	8.0	3.0		
	1.2	6.2				

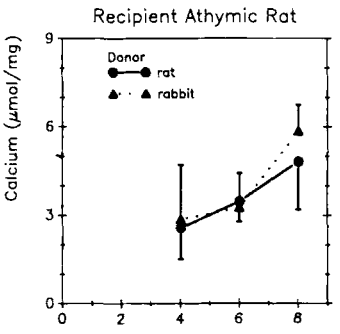

Figure 1

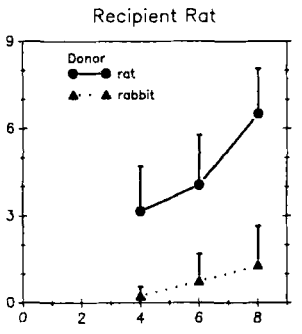

Figure 2

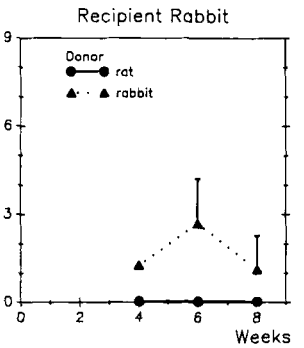

Figure 3

Figure 1. Bone-forming response of athymic rat to implanted rat and rabbit matrix, expressed as calcium content per dry weight of implanted matrix.

Figure 2. Bone-forming response of normal rat to implanted rat and rabbit matrix, expressed as calcium content per dry weight of implanted matrix.

Figure 3. Bone-forming response of rabbit to implanted rat and rabbit matrix, expressed as calcium content per dry weight of implanted matrix.

Results

In the athymic rats (Figure 1), the calcium content of rat and rabbit matrices did not differ at any time (Table 1). After 8 weeks, the calcium content indicated that about 20 percent of the matrix was replaced by new bone.

In the normal rats (Figure 2), rat matrix contained about the same amount of calcium as in athymic rats, whereas rabbit matrix contained only small amounts of calcium. However, a few rabbit matrix specimens contained more calcium, almost reaching the level of the rat matrix. The difference in response to rabbit matrix between athymic and normal rats was significant ($P < 0.001$).

In the rabbit (Figure 3) the calcium content of rat matrix was of the same magnitude as that of normal body-fluid concentration. Five rabbit matrix implants could not be found, probably due to penetration into the abdominal cavity. The calcium content of the others indicated bone formation to a lesser degree than in rat matrix in rats. In response to rabbit matrix, the athymic rats produced more bone than the rabbit ($P < 0.001$).

Discussion

The results show that bone induction by demineralized bone matrix is species specific between normal rat and rabbit, although not completely, because in a few rats some bone formation was induced by rabbit matrix. However, when immunogenic rejection is eliminated, the species specificity is eliminated, too. This accords with the results of Sampath and Reddi (1983): namely, that bone inductive proteins are homologous for several species. These authors extracted proteins from the bone matrix of several species and transferred the proteins to the inactive residue of similarly extracted rat matrix. The rat matrix was then shown to have regained bone inductive properties when implanted into rats. In vitro, a human factor (CIF-A) can induce a chondrogenic response from rat muscle mesenchymal cells (Seyedin et al. 1986). Inductive factors are active in vivo mainly if reconstituted to matrix (Sampath and Reddi 1984), although large amounts of some extracts have activity alone (Urist et al. 1983).

Heterotopic bone induction by bone matrix depends on the inductive (and/or inhibitory) properties of the matrix implant and the ability of the recipient to respond. In our study, rat and rabbit matrix were equally inductive, but the rat was a better responder. This seems to reflect a species difference in the intensity of the bone-forming response to the same stimulus, but could also be due to, for example, age differences of the donors, although both rat and rabbit donors were adolescent.

The athymic rat might become a useful standard recipient site to measure inductive capacity of bone matrix preparations from different species, including man.

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