Dog bone less osteogenetic than rat bone

Bone-matrix transplants in nude rats

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Demineralized bone matrix and bone-matrix gelatin prepared from cortical rat bone, and from cortical and cancellous canine bone were implanted into muscle pouches of nude rats for 6 weeks. Evaluation was done by histology, histomorphometry, and determination of alkaline phosphatase. Rat matrix consistently induced new bone and high phosphatase levels. Canine matrix induced but small amounts of bone and lower phosphatase levels, with cortical matrix somewhat more inductive than cancellous matrix; demineralized cancellous bone matrix from the dog was the only material tested not showing any inductivity. Irrespective of bone type or species, gelatin had clearly higher induction capacity than demineralized bone matrix.

Bone matrix is routinely prepared from cortical bone (Urist 1965) because cancellous bone has lower inductive capacity (Nade and Burwell 1977). Experiments with bone matrix mainly deal with small animals, whereas there is a paucity of information regarding higher mammals. We have compared the osteoinductivity of rat and canine bone matrix, and of matrix of cortical and cancellous canine bone. To overcome the antigeneic dog/rat problem, athymic, nude rats were used as hosts.

Materials and methods

Demineralized bone matrix was obtained from diaphyseal Sprague-Dawley rat bone, diaphyseal canine bone (femur, tibia, humerus), and from cancellous canine bone (vertebral bodies). The bones were cleansed of soft tissue and of bone marrow by water-jet irrigation, minced, freed from fat (chloroform/methanol 1 + 1), decalcified (0.6 N HCl 24 h), and lyophilized.

To produced bone-matrix gelatin the demineralization was followed by extraction (3 x 24 h) with 2 M $CaCl_2$, 0.5 M EDTA, and 8 M LiCl (Thielemann et al. 1982). The lyophilized material was frozen in liquid nitrogen, ground in a micromill, and sieved to a particle size of 400–1,000 µm (Thielemann et al. 1982).

Experimental animals. The experiments were carried out in 32 eight-week-old male, nude rats (HAN: RNU rnu/rnu, Zentralinstitut für Versuchstierzucht, Hannover, FRG). They were kept under a laminar air stream and fed a standard diet and tap water ad libitum.

Implantation. The implantation was heterotopic, i.e., into a muscle pouch of the lateral abdominal wall following blunt dissection. Each animal received two 50-mg implants, both from only one donor species (rat or dog). Anesthesia was ensured by ether inhalation.

Histologic techniques. The implanted specimens were excised and divided into two parts of equal size with a razor blade. One half of the specimen served for determination of alkaline phosphatase activity and the other half was fixed and embedded in paraffin. Four-micron sections taken in the proximity of the razor-blade cut near the center were stained with hematoxylin-eosin and with aniline blue according to Heidenhain's method.

Histomorphometry. A grid large enough to cover the entire cross section of the specimen was interpolated into the field of vision in a Reichert-Zetopæn microscope. The frequency of intersections of the grid and newly formed bone, cell and fat marrow, as well as implant remnants was determined and correlated with the total count. The figures are reported as a percentage of the total count.

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Table 1. Test protocol and results of demineralized bone matrix (D) and bone-matrix gelatin (B) of cortical (co) (rats or dogs) and cancellous (ca) origin (dogs) evaluated for osteogenesis after 6 weeks in the ectopic bed of nude rats

Donor	Implant	Bone	Incidence	Histomorphometry			n	AP	
				A	B	C			
Rat	B	co	7/7		ne			ne	
Dog	D	co	2/6		ne			ne	
Dog	B	co	7/7		ne			ne	
Rat	D	co	5/5	19	19	49	6	3.5	*1.7**
Rat	B	co	5/5	24	38	27	6	8.4	*7.3**
Dog	D	co	0/5	0	0	66	5	1.0	*0.5**
Dog	B	co	3/6	3	0.5	75	6	2.5	*1.6**
Dog	D	ca	0/6	0	0	55	6	0.3	*0.6*
Dog	B	ca	2/6	2	0.2	59	6	0.5	*0.5*
Muscle nude rat							9	0.2	*0.1*

Incidence (specimens with new bone formation/number of specimens).
Histomorphometry (percentage of total score for: A new bone, B bone marrow, and C implant material).
AP alkaline phosphatase (U/gWW *SD*), ne not evaluated, * $P < 0.02$.

Determination of alkaline phosphatase. Tissue specimens of about 300 mg were homogenized in 1 mL H_2O for 1 min at the maximum speed of a tissue homogenizer, and water was added up to 2 mL. The supernatant following centrifugation at 4,500 rpm for 10 min was diluted 1:5 with H_2O, and was processed like a plasma specimen to determine alkaline phosphatase levels on the Hitachi 705 (Kit Boehringer, Mannheim, FRG). The calculation was done in International Enzyme Units per gram wet weight (U/gWW). Analyses of alkaline phosphatase data were performed with nonparametric Kruska-Wallis statistics followed by individual Mann-Whitney rank-sum tests. An error probability of 5 percent was chosen as the level of significance.

Results

The rat transplants induced massive new bone formation within 42 days. At that time, osteoblasts were still seen. The bone was osteoid-rich and exhibited fatty and hemopoietic marrow. New bone formation was observed to be more pronounced after gelatin than after matrix implantation as indirectly confirmed by the differences in alkaline phosphatase levels (Table 1).

Cortical dog matrix evoked very small osseous foci in only 2 out of 11 specimens, while cortical gelatin stimulated new, albeit moderate, bone formation in 10 out of 13 specimens; matrix initiated a foreign-body reaction with polynuclear giant cells, which was not observed with gelatin. Histologically and morphometrically, implants of cortical origin could not be differentiated from cancellous implants irrespective of the type of preparation. The phosphatase level was higher with cortical than with cancellous implants. In all the series except those with cancellous dog matrix, the phosphatase levels were above those in normal muscle.

Discussion

Demineralized matrix regularly produces new bone upon allogeneic implantation (Urist 1965), also in athymic animals (Aspenberg et al. 1988b, Sato and Urist 1985). Because nude rats do not possess immunogenic capability, the different amount of newly formed bone in implants from Sprague-Dawley rats or dogs must be due to solely differences in the osteogenic capacity of the implanted matrix.

Our experiments lasted for 42 days, a period that may be relatively long to observe bone induction in small animals. Because in Sprague-Dawley rats the inductivity reaches its peak around the 10th postoperative day, as confirmed by alkaline phosphatase levels and histology (Reddi and Anderson 1976), a marked osteoneogenesis is not expected to occur from the 42nd day onwards. The calcium content showed only a moderate increase during the seventh and eight postoperative weeks in the experiments recently published by Aspenberg et al (1988b). Because the higher immunogenicity of cancellous matrix (Urist 1980) did not interfere with our experiments, the different levels of alkaline phosphatase could be due to a lower effective BMP content of cancellous bone. The most striking result of our experiment was the finding that matrix prepared from rats has a markedly higher inductive capability than that from dogs. Bone and bone marrow covered 62 percent of the cross-sectional area in gelatin implants from rats, as compared with only 4 percent in dog implants. The lower inductivity could partly be explained by the different relative ages of the animals (Sprague-Dawley rat 100 days, dog 1.5 to 3 years), because the inductive potency of the matrix decreases with age (Syftestad and Urist 1982). Another possible explanation is a species difference of the BMP content of bone, although Sampath and Reddi (1983) have shown that there is a homology as regards bone-inductive proteins of mammals.

In contrast to rodents, heterotopic osteoinduction cannot be achieved experimentally in higher vertebrates (Sato and Urist 1985, Aspenberg et al. 1988a, Lindholm et al. 1988), nor in humans (Aspenberg et al. 1988a). We were unable to demonstrate demineralized bone matrix or bone-matrix gelatin-dependent osteoinduction in adult mongrel dogs within 8 weeks. Our experiments provide further evidence that osteoinduction is less pronounced in higher vertebrates.

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