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Xenogeneic Osteogenin, a Bone Morphogenetic Protein, and Demineralized Bone Matrices, Including Human, Induce Bone Differentiation in Athymic Rats and Baboons

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Summary

Subcutaneous implantation of xenogeneic demineralized bone matrix does not initiate endochondral bone differentiation. Dissociative extraction in 4 M guanidine-HCl or 6 M urea has shown that the apparent species-specificity of intact bone matrix resides in its insoluble immunogenic component, since there is homology in solubilized osteogenic proteins amongst mammals. To further investigate the species-specificity and cross-species reactivity of bone matrix components, baboon and human demineralized bone matrix (DBM) and bovine osteogenin, purified greater than 50000-fold and with an apparent molecular mass of 28–42 kilodaltons, were implanted in the subcutaneous space of athymic and euthymic rats and into the *rectus abdominis* of 16 baboons *(Papio ursinus)*. Baboon DBM was also implanted in athymic and euthymic mice. Alkaline phosphatase activity and histology of implants harvested at day 11 and 30 showed that baboon and human DBM induced endochondral bone differentiation both in athymic rats and baboons. Bovine osteogenin in conjunction with baboon insoluble collagenous matrix induced extensive bone differentiation in athymic rats and baboons. Baboon and human DBM did not induce bone differentiation in euthymic rats and, in athymic mice, baboon DBM failed to induce bone differentiation, determining instead the recruitment of multinucleated giant cells. The results indicate that in rodents bone differentiation induced by intact bone matrix is species specific and that T-cell functions are not a requirement for bone induction, although immunologically competent rats block bone differentiation from xenogeneic matrix. Bone differentiation induced by human DBM in baboons suggests that intact bone matrices may not be species-specific amongst primates. These findings, by demonstrating bone induction in nonhuman primates using bovine osteogenin and intact human bone matrix, may help to design alternative osteogenic delivery systems other than allogeneic collagenous matrix for craniofacial and orthopedic applications in man.

Key words: alkaline phosphatase, athymic rodents, bone induction, bone matrix, osteogenin, primates.

Introduction

Intramuscular and subcutaneous implantation of *allogeneic* demineralized bone matrix results in local differentiation of endochondral bone by induction (Urist, 1965; Reddi and Huggins, 1972; Reddi, 1981). Subcutaneous implantation of *xenogeneic* bone matrix does not promote bone differentiation. Instead, the implanted matrix is sur-

rounded by multinucleated giant cells and lymphocytes suggesting rejection by the recipient (Sampath and Reddi, 1983). Dissociative extraction of the bone matrix (Sampath and Reddi, 1981) and reconstitution of rat inactive insoluble collagenous bone matrix residue with xenogeneic solubilized protein fractions of <50 kilodaltons has been shown to restore the biological activity of the xenogeneic osteogenic proteins when implanted in the subcutaneous space of allogeneic recipient rats (Sampath and Reddi, 1981; Sampath and Reddi, 1983). These findings demonstrate that there is homology in the osteogenic proteins from human, monkey, bovine and rat extracellular bone matrices (Sampath and Reddi, 1983). Bone differentiation by intact bone matrix is, however, species-specific, and the apparent specificity of xenogeneic bone matrices is due to immunogenic and inhibitory components in the insoluble collagenous bone matrix and solubilized protein fractions of >50 kilodaltons (Sampath and Reddi, 1983).

The allogeneic insoluble collagenous matrix of bone after dissociative extraction with 4 M guanidine-HCl or 6 M urea has been shown to optimize the induction of endochondral bone differentiation when reconstituted with osteogenin, a protein initiator of bone differentiation (Sampath et al., 1987; Luyten et al., 1989). Osteogenin, in conjunction with insoluble collagenous matrix, initiates a developmental cascade of morphogenetic events culminating in local differentiation of endochondral bone *in vivo* (Luyten et al., 1989; Reddi et al., 1989). Thus, the insoluble collagenous matrix plays a crucial role for the expression of the biological activity of osteogenin and for modulating, as carrier of insoluble antigenic determinants, the immunological response of the recipient animal.

The homozygous mouse and rat mutant "nude" (nu/nu) has a congenital thymus aplasia, resulting in a deficient T-lymphocyte system (Pantelouris, 1968; Festing, 1978). Because of the lack of T-cell-mediated immune response, the athymic rat has been used as an animal model to test the bone inductive potential of xenogeneic crude bone matrix preparations (Aspenberg and Andolf, 1989) and the athymic mouse as a recipient of transformed osteogenic cell lines (Ostrowski et al., 1975). We have studied the induction of bone in the subcutaneous space of athymic rats using human and baboon demineralized bone matrices and baboon insoluble collagenous matrix reconstituted with bovine osteogenin. Whilst the results in rodents indicate that bone differentiation induced by intact extracellular matrix is apparently species-specific, we report that human demineralized bone matrix-induced bone differentiation in extraskeletal sites of adult baboons, suggesting that intact bone matrix may not be species-specific amongst primates.

Materials and Methods

Animals

Rodents. Forty-six rats and mice (38–48 days old) were subdivided as follows: 4 athymic nude mice (FM1 nu/nu), 4-euthymic mice (FM1), 18 athymic nude rats (Fisher 344 Rnu/nu) and 14 euthymic rats (6 Fisher 344 and 12 Long-Evans).

Primates. Sixteen clinically healthy outbred Chacma baboons *(Papio ursinus)* were selected from the primate colony of the University of the Witwatersrand, Johannesburg. Criteria for selection were normal hematological and biochemical profiles (Melton and Melton, 1982). In 8 animals, skeletal maturity was confirmed by radiographic evidence of closure of the distal epiphyseal plate of the radius and ulna. Animals were caged individually in rooms kept under slight negative pressure (-25 KPa) with controlled ventilation (18 filtered air changes/hour), temperature (22 ± 2 °C), humidity ($40 \pm 10\%$) and photoperiod (lights on 0600 to 1800). Before any procedures were initiated, all research protocols were approved by the Animal Ethics Committee of the University.

Purification of bovine osteogenin

Extracts of bovine cortical demineralized bone matrix in 6 M urea were loaded sequentially onto hydroxyapatite-Ultrogel (IBF Biotechnics) adsorption chromatography and heparin-Sepharose (Pharmacia LKB) affinity chromatography columns, washed and eluted as described (Sampath et al., 1987). Heparin-bound protein fractions eluted with 500 mM NaCl were concentrated and exchanged with 4 M guanidine hydrochloride (Gdn-HCl), 50 mM Tris, pH 7.4 and loaded onto tandem HR Sephacryl S-200 gel filtration columns (Luyten et al., 1989). Eluted fractions were bioassayed for osteogenic activity in the subcutaneous space of additional Long-Evans rats (28–36 days old) after reconstitution with 25 mg of rat insoluble collagenous matrix, the inactive residue obtained after dissociative extraction of rat demineralized bone matrix with 4 M guanidine-HCl (Sampath and Reddi, 1981). Implants were harvested at day 11 and processed for alkaline phosphatase activity determination and histological analysis (data not shown). Bovine osteogenin in 4 M guanidine-HCl, purified greater than 50000-fold with respect to initial crude urea extract and with an apparent molecular mass of 28–42 kilodaltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Luyten et al., 1989), was used for implantation in athymic rats and baboons.

Preparation of demineralized bone matrices

Muscle, periosteum and marrow were mechanically removed from diaphyses harvested from subadult baboons. Diaphyseal segments were washed extensively in cold

sterile deionized water, defatted in two changes of absolute ethanol for 8 h in the cold and dehydrated in ethyl ether overnight. Cortical segments were pulverized and sieved to a discrete particle size of 74–420 μm, demineralized in 0.5 N HCl at room temperature, washed twice in cold sterile deionized water, dehydrated in two changes of absolute ethanol and dried overnight after a wash in ethyl ether. Human demineralized bone matrix (human DBM) was prepared as described for baboon demineralized bone matrix (baboon DBM) and was a gift from Dr. R. Katz, National Institute of Dental Research. Rat demineralized bone matrix (rat DBM) was prepared using diaphyseal bone of adult Long-Evans rats as described (Reddi and Huggins, 1972).

Implant preparation and reconstitution

Aliquots of baboon DBM were dissociatively extracted at room temperature for 16 h in 20 volumes of 4 M Gdn-HCl, 50 mM Tris, pH 7.4 containing 100 mM α-amino-*n*-caproic acid, 5 mM benzamidine HCl, 0.5 mM phenyl methasulphonyl fluoride and 5 mM N-ethyl maleimide as protease inhibitors. After a wash in 0.5 M Tris, pH 7.4, dehydrated baboon insoluble collagenous bone matrix (baboon ICBM), 25 mg per implant, was reconstituted with 50 μg of bovine osteogenin fractions. One mg of chondroitin-6-sulfate (Seikaguko Kogio, Japan) and 0.5 mg of acid-soluble baboon type I collagen were added to each sample as carriers (Muthukumaran et al., 1988). Additional implants were prepared by reconstituting 25 mg of bovine ICBM with 50 μg of bovine osteogenin fractions. For the preparation of pellets for baboon implantation, 100 mg of baboon ICBM were reconstituted with 150 μg of bovine osteogenin fractions as described (Ripamonti et al., 1991). Two mg of chondroitin-6-sulfate and 4 mg of baboon type I collagen were added to each sample. Type I collagen was prepared from baboon ICBM as described (Miller and Rhodes, 1982). Briefly, pepsin-extracts of baboon ICBM in 0.5 M acetic acid were dialyzed against 0.1 M acetic acid in a Spectropor tube with 10 000 MW cut-off. After dialysis against 0.05 M sodium phosphate dibasic and centrifugation at 16 500 × g, the precipitate was redissolved in 0.5 M acetic acid and redialysed extensively against sodium phosphate dibasic. The final precipitate was lyophilized and collagen type I was dissolved in 0.5 M acetic acid with a concentration of 5 mg per ml. Implants of baboon DBM, human DBM and baboon ICBM were prepared as described above, 25- and 100-mg aliquots for implantation in rodents and baboons respectively. Two mg of chondroitin-6-sulfate and 4 mg of baboon type I collagen were used for the 100-mg preparations. Implants of rat DBM for implantation in allogeneic animals were prepared using 0.5 mg of acid-soluble type I collagen extracted from rat tail tendon. After ethanol precipitation, the pellets were lyophilized in a SpeedVac concentrator (Savant) and stored in the cold until implantation.

Bioassay in rodents

FM1 mice were anesthetized with an intraperitoneal injection of 0.2 ml ketamine-HCl and 0.1 ml xylazine-HCl in 0.7 ml sterile deionized water. Fisher rats were anesthetized with an intramuscular injection of 0.4 mg fentanil and 20 mg droperidol (0.02 ml/100 g). Long-Evans rats were ether-anesthetized. Using sterile operative techniques, a total of 76 matrix preparations were bioassayed for osteogenic activity in the subcutaneous space at bilateral sites over the pectoralis fascia of athymic and euthymic rats. Four athymic and 4 euthymic rats were implanted subcutaneously with 4 matrix preparations per animal. Athymic mice received one implant per animal. Skin incisions were closed in one layer using atraumatic 4-0 resorbable sutures. After surgery, athymic animals were housed in flexible sterile isolators. Implants were harvested at day 11. After selection of histological material, implants were homogenized in 2 ml of ice-cold 3 mM sodium bicarbonate containing 0.15 M NaCl, pH 7.8. The homogenate was centrifuged at 4500 × g for 15 min. and the alkaline phosphatase activity of the supernatant was used as an index of bone formation by induction (Reddi and Huggins, 1972; Reddi and Sullivan, 1980). Remaining tissues were fixed in Bouin's fluid, dehydrated through a graded series of ethanol, infiltrated and embedded in Historesin (LKB, Bromma). Sections, cut at 2 μm, were stained with toluidine blue. Two athymic rats were killed at day 6 yielding 2 baboon and 2 human DBM implants for early morphological examination.

During experiments, results indicated that baboon DBM failed to induce bone differentiation in the subcutaneous space of athymic mice, eliciting instead the recruitment of lymphocytic and multinucleated giant cells. We were interested in determining whether osteogenesis would occur after transplantation of xenografts of viable bone into the subcutaneous space of athymic FM1 mice. To investigate whether athymic mice could "tolerate" vital osteogenic tissue as opposed to devitalized extracellular matrix, 8 calvarial segments (20 × 5 mm), harvested from a baboon fetus of 23–25 weeks of gestation period (Hendrickx, 1971), were grafted in the subcutaneous space of 4 additional FM1 nu/nu athymic mice. Xenografts with surrounding adhering tissues were harvested at day 21 after transplantation. Tissues were fixed in 70% ethanol or formol calcium and embedded in Historesin. Sagittal sections, cut at 4 μm, were stained with a modified Goldner's trichrome stain. To further characterize osteogenesis in grafted fetal calvariae, alternate serial sections were used for enzyme histochemical demonstration of alkaline phosphatase activity. Alkaline phosphatase was used as marker enzyme for osteoblastic activity and investigated as

described (Franklin and Martin, 1980). Naphthol-As-BI-phosphate buffered with Tris-HCl at pH 9.0 was used as a substrate. The azo-coupled dye was fast red T. R.

Bioassay in baboons

Food was withdrawn from the animals on the evening before surgery with access to water *ad libitum*. On the day of surgery, the animals were immobilized by intramuscular ketamine-HCl (8 mg/kg) and anesthetized with intravenous thiopentone sodium (15 mg/kg). Maintenance was achieved by halothane vapor in 100% oxygen after orotracheal intubation. Using sterile operative techniques, 52 matrix preparations were implanted bilaterally in ventral intramuscular pouches created by sharp and blunt dissection in the *rectus abdominis* of 16 baboons. Whilst superiorly and laterally surrounded by muscular tissue, implants were resting directly over the peritoneal fascia. Incisions were closed by repairing in layers the fasciae and the superficial tissues with atraumatic resorbable sutures. Post-operative pain was controlled by intramuscular buprenorphine-HCl (0.3 mg). Individually housed animals were monitored and fed as described (Ripamonti, 1991; Ripamonti et al., 1991). Implants were harvested at day 30 after surgery. After selection of histological material, implants were subjected to alkaline phosphatase activity determination as described earlier. Selected histological material was fixed in Bouin's fluid, decalcified in formic acid-sodium citrate solution and embedded in Historesin or in celloidin paraffin wax. Sections, cut at 4 μm, were stained with toluidine blue.

Results

Rodents

At harvest, the implants were firmly attached to the fascia and the underlying pectoralis muscles. The alkaline phosphatase activity of the various matrix preparations in athymic and euthymic animals is presented in Table I. In athymic rats, subcutaneous implantation of baboon ICBM reconstituted with bovine osteogenin had resulted in firm and enlarged highly vascularized red ossicles. In all instances, histological analysis showed a reproducible pattern of extensive endochondral bone differentiation, vascular invasion and remodelling of the newly formed ossicles (Figs. 1 A and B). Baboon DBM and to a lesser extent human DBM (Table I), induced endochondral bone differentiation in the subcutaneous space of athymic rats (Fig. 2 A). Although a difference existed between the means, this difference was not statistically significant (Table I). Histological analysis of day 6 baboon and human DBM specimens showed distinct differentiation and phenotypic transformation of hypertrophied metachromatic mesenchymal cells in contact with the implanted matrix (Fig. 2 B).

Table I. Alkaline phosphatase activity in implants of various matrix preparations harvested at day 11 in euthymic and athymic animals. Levels of significance were determined using the SAS (Statistical Analysis System, 1985) general linear models procedure for multiple comparisons with correction for unbalanced data.

Group	Alkaline phosphatase U/mg protein Euthymic	Athymic
	Fisher 344	Fisher 344R nu/nu
baboon DBM	0.16 ± 0.02 (8)	1.17 ± 0.31[a] (8)
human DBM	0.11 ± 0.03 (4)	0.62 ± 0.17[a] (8)
bovine osteogenin and baboon ICBM		2.82 ± 0.22[b] (12)
	Long-Evans	
rat DBM	1.87 ± 0.32[a] (8)	
baboon DBM	0.31 ± 0.09 (8)	
bovine osteogenin and bovine ICBM	0.9 ± 0.1 (8)	
	FM1	FM1 nu/nu
baboon DBM	0.48 ± 0.09 (4)	0.34 ± 0.09 (4)

The alkaline phosphatase activity of the supernatant after homogenization of implants was determined with 0.1 M *p*-nitrophenylphosphate (Sigma) as substrate at pH 9.3 and 37 °C for 30 min. One unit of enzyme liberates one micromole of *p*-nitrophenol under the assay conditions. Alkaline phosphatase is expressed as units of activity per mg protein. Protein concentration in the supernatant was measured by the method of Lowry et al. (1951). DBM: demineralized bone matrix, ICBM: insoluble collagenous matrix after 4 M Gdn-HCl extraction of baboon demineralized bone matrix. In parenthesis, the number of specimens per group in euthymic and athymic animals. Values are given as means ± SEM.

[a] $P < 0.01$ *vs.* control

[b] $P < 0.01$ *vs.* baboon and human DBM

In athymic mice, baboon DBM did not induce endochondral bone differentiation. Instead, the implanted matrix was surrounded by multinucleated giant cells (Fig. 3). Bone was never observed in specimens harvested from control euthymic rats and mice, although an island of cartilage was seen in an implant of baboon DBM harvested from a Long-Evans rat (not shown).

Xenografts of fetal baboon calvaria showed distinctive and florid osteogenesis when implanted in the subcutaneous space of athymic mice. Newly deposited osteoid seams lined by contiguous layers of osteoblasts had formed particularly along the pericranial surfaces of the calvarial grafts (Fig. 4 A). Bone forming surfaces were intensively alkaline phosphatase positive, correlating with osteoid deposition by contiguous layers of osteoblasts on the surface of nascent mineralizing matrix (Fig. 4 B). Recipient highly cellular and vascularized mesenchymal tissue had penetrated the diploic spaces at the margins of the calvarial segments. Differentiation of osteoclasts and resorption lacunae were seen on the endosteal surfaces.

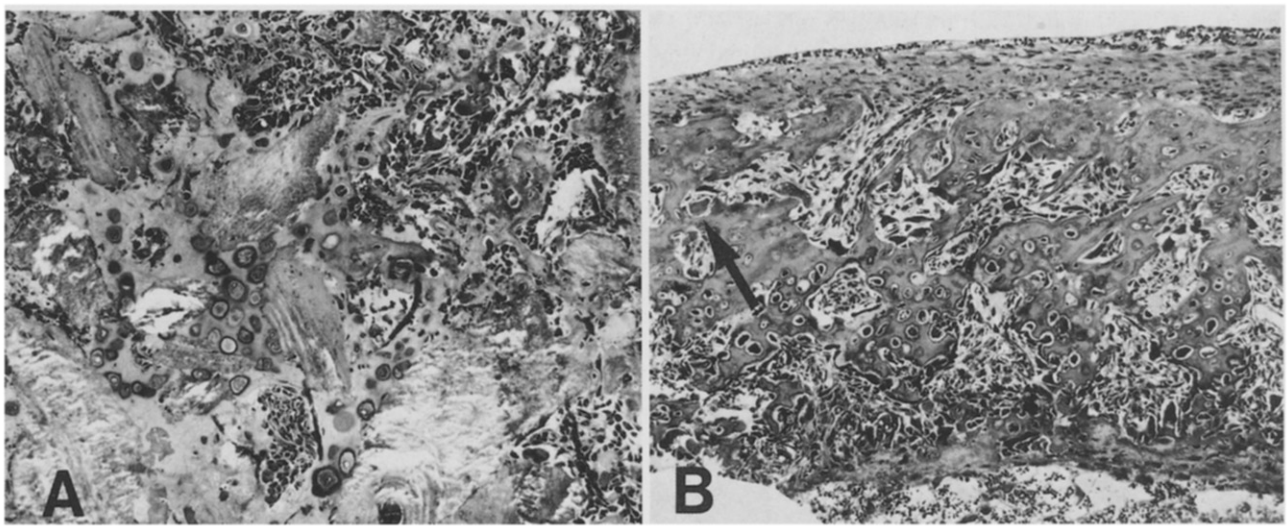

Fig. 1. Endochondral bone differentiation in implants of baboon ICBM reconstituted with bovine osteogenin harvested from athymic rats on day 11. (A) Cartilage and bone formation in direct apposition to the implanted soluble matrix. (B) Extensive bone differentiation, remodelling and formation of an ossicle. Note the extensive chondrolysis and vascular invasion. Osteoblastic activity is coupled to osteoclastic activity (arrows) on resorbing surfaces (toluidine blue, original magnification × 200).

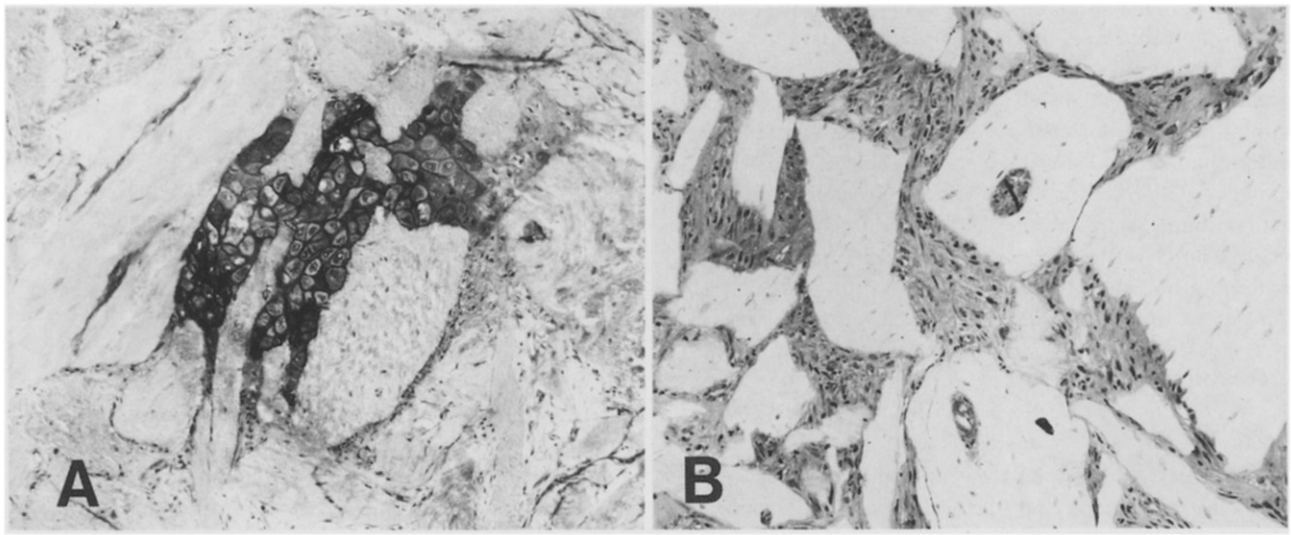

Fig. 2. (A) Differentiation of large islands of cartilage in an implant of baboon DBM harvested from an athymic rat at day 11. (B) Transformation of mesenchymal cells invading an implant of human DBM harvested from an athymic rat at day 6 (toluidine blue, original magnification × 200).

Primates

The alkaline phosphatase activity of the various matrix preparations is presented in Table II. Extraskeletal implantation of baboon ICBM reconstituted with bovine osteogenin resulted in florid bone differentiation, incorporation and dissolution of the implanted ICBM carrier (Fig. 5 A), and in high alkaline phosphatase activity (Table II) as described previously (Ripamonti et al., 1991). Extraskeletal implantation of baboon DBM also resulted in bone differentiation by induction, yielding implants with a relatively high alkaline phosphatase activity (Table II). Human DBM was also effective in inducing bone differentiation as assessed histologically (Fig. 5 B) and biochemically (Table II). Histological analysis showed the formation of bone in direct contact with the implanted demineralized matrix and the differentiation of trabecular-like structures of newly formed bone lined by contiguous layers of osteoblasts (Fig. 5 B). No new bone formed in implants of baboon ICBM in the absence of osteogenin.

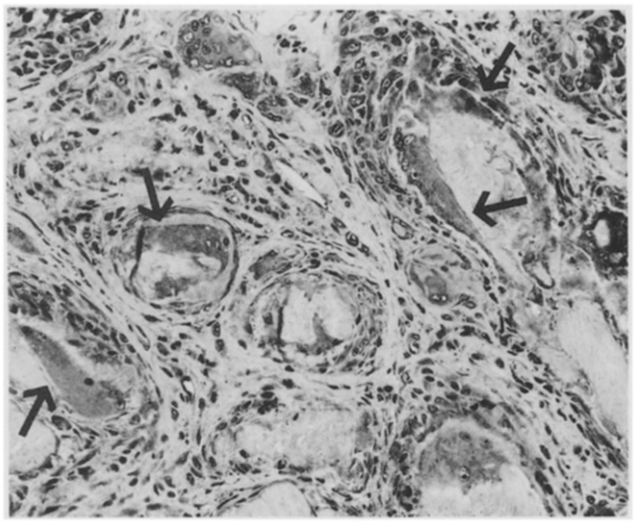

Fig. 3. Differentiation of multinucleated giant cells (arrows) surrounding baboon DBM implanted in athymic mice (toluidine blue, original magnification × 180).

Discussion

The rodent subcutaneous space is the established model to investigate postfetal endochondral bone differentiation using demineralized bone matrix (Reddi and Huggins, 1972; Reddi, 1981) or purified osteogenic proteins (Sampath et al., 1987; Luyten et al., 1989; Reddi et al., 1989). When implanted subcutaneously in allogeneic rats, demineralized bone matrix induces a reproducible sequence of events culminating in local differentiation of cartilage, bone and bone marrow (Reddi and Anderson, 1976; Reddi, 1981; Reddi, 1982).

The athymic rat is a valuable model to study bone differentiation induced by xenogeneic bone matrix preparations, including human demineralized bone matrix (Aspenberg and Andolf, 1989). The results of the present study demonstrate that the sequential developmental cascade leading to endochondral bone differentiation in athymic rats can be elicited by pulverized bone matrix preparations and osteogenin, purified greater than 50000-fold from bovine bone matrix. In a previous study in athymic rats, bone formed in implants of human matrix harvested 42 days after implantation (Aspenberg and Andolf, 1989). However, the present results cannot be compared to that study as the duration of the experiments are different. Histological analysis of the present material showed endochondral bone differentiation in intact bone matrices 11 days after implantation. Transformation and differentiation of hypertrophied metachromatic mesenchymal cells was seen as early as 6 days, whilst extensive bone differentation, remodelling and ossicle formation characterized the implants of baboon insoluble collagenous matrix reconstituted with bovine osteogenin. Collectively, these experiments demonstrate that T-cell functions are not a requirement for bone induction, although immunologically competent euthymic rats block the developmental cascade of bone differentiation. Interestingly, no bone formed in athymic mice implanted with baboon demineralized bone matrix. The lack of bone formation may be explained by the lack of responding undifferentiated mesenchymal cells in

Table II. Alkaline phosphatase activity in implants of various matrix preparations harvested at day 30 from 16 baboons.

Group	Alkaline phosphatase (U/mg protein)
human DBM	0.98 ± 0.22 (22)
baboon DBM	1.35 ± 0.19 (8)
baboon ICBM	0.31 ± 0.09 (8)
bovine osteogenin and baboon ICBM	2.05 ± 0.41 (8)

DBM: demineralized bone matrix, ICBM: insoluble collagenous matrix. In parenthesis the number of specimens per group. Values are mean ± SEM.

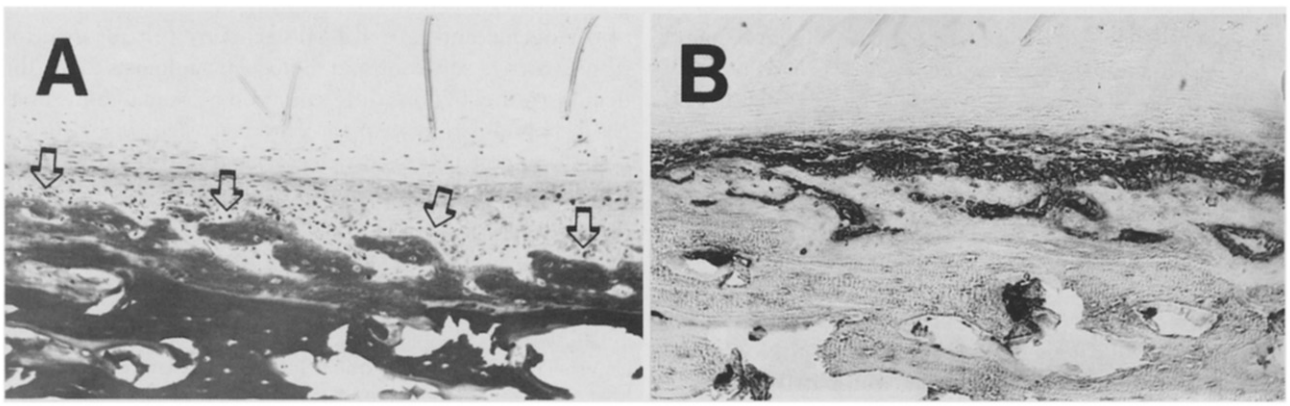

Fig. 4. Osteogenesis in fetal baboon calvariae grafted in the subcutaneous space of athymic mice. (A) Osteoid seams lined by contiguous layers of osteoblasts (arrows) on the pericranial surface (modified Goldner's trichrome stain, original magnification × 200). (B) Intensive alkaline phosphatase activity on the surface of nascent mineralizing matrix (original magnification × 200).

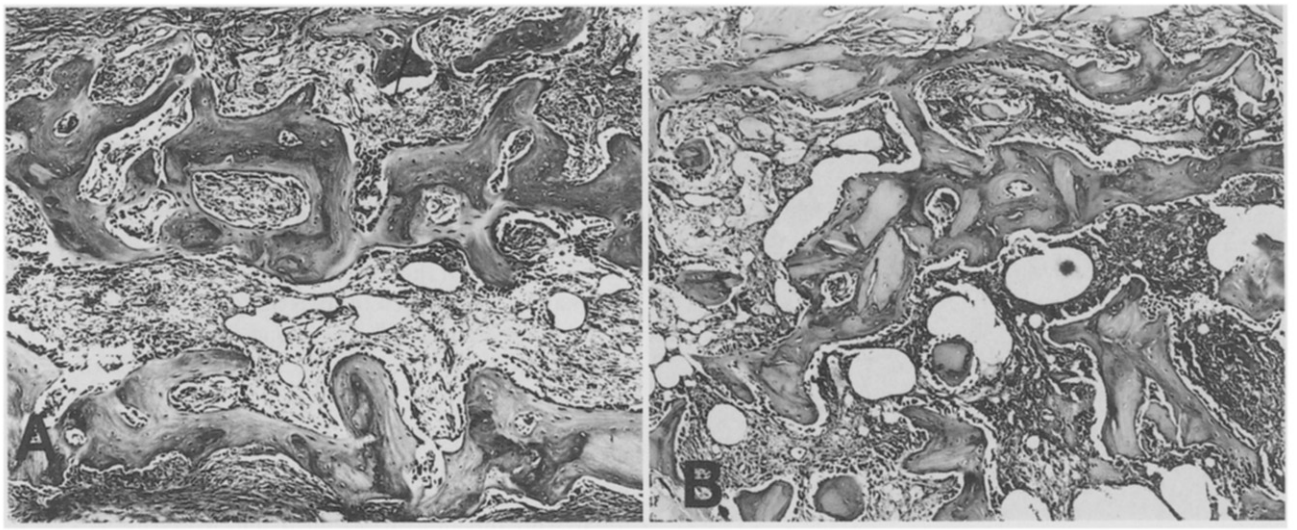

Fig. 5. (A) Extensive bone formation in an implant of baboon ICBM reconstituted with bovine osteogenin and implanted extraskeletally in a baboon. (B) Bone differentiation in human DBM implanted in the *rectus abdominis* of a baboon and harvested at day 30. Note the prominent vascular invasion and the differentiation of contiguous layers of osteoblasts lining newly formed bone matrix (toluidine blue, original magnification × 120).

the subcutaneous space of the mouse, since intramuscular implantation of demineralized bone matrix in euthymic mice (Urist, 1965) or transformed osteogenic cell lines in athymic mice (Ostrowski et al., 1975) results in bone differentiation. Osteogenesis, most likely from pre-existing vital osteoblasts within the grafts, did result, however, in baboon fetal calvaria xenografted in the subcutaneous space of athymic mice, demonstrating that athymic mice do not reject viable mineralized bone matrix.

The use of primates is crucial for the investigation of osteogenic preparations to be ultimately used in man (Ripamonti, 1990; 1991; Ripamonti et al., 1991). The present study demonstrates bone differentiation by induction in baboons using baboon and human demineralized bone matrices. The induction of bone in preparations of human matrix is noteworthy and suggests that intact bone matrices, as opposed to rodents, may not be species-specific amongst primates.

An entirely new family of protein initiators that regulate cartilage and bone differentiation *in vivo* has been recently characterized (Wozney et al., 1988; Luyten et al., 1989). The amino acid sequence of native osteogenin is identical to the amino acid sequence deduced from the cDNA clones of one of the human bone morphogenetic proteins (BMP), BMP-3 (Luyten et al., 1989; Wozney et al., 1988). Optimal biological activity of osteogenin and human recombinant BMP-2A requires the use of allogeneic insoluble collagenous bone matrix (Luyten et al., 1989; Ma et al., 1990; Reddi et al., 1989; Wang et al., 1990), the residue obtained after dissociative extraction of intact bone matrix (Sampath and Reddi, 1981; Sampath and Reddi, 1983). The insoluble collagenous matrix may act as delivery system avoiding premature dissolution of osteogenin at site of implantation and may provide an optimal substratum for anchorage-dependent cell attachment to the matrix (Reddi et al., 1989). Thus, the optimal osteoinductive potency of xenogeneic organic collagenous substrata reconstituted with osteogenin and related BMPs may be conveniently tested in the subcutaneous space of the athymic rat before clinical application in man.

The need for *human* insoluble collagenous matrix for therapeutic osteogenesis in man may limit the clinical application of the phenomenon of bone induction, since the insoluble component prepared from human matrix may retain transmittable viruses such as human immunodeficiency virus and hepatitis B virus. The delivery of bone inductive extracts in purified xenogeneic collagenous preparations has been described previously, albeit in rodents (Deatherage and Miller, 1987; Moore et al., 1990). Whilst the identification of inorganic biocompatible nonimmunogenic substrata capable of restoring the biological activity of osteogenin and related BMPs remains a major goal for reconstructive surgeons and molecular biologists alike, the demonstration of bone differentiation by human bone matrix in baboons may help to construct osteogenic delivery systems of collagenous matrices prepared from alternative primate sources for the controlled therapeutic initiation of osteogenesis.

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