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Growth and Morphogenetic Factors in Bone Induction: Role of Osteogenin and Related Bone Morphogenetic Proteins in Craniofacial and Periodontal Bone Repair

Ugo Ripamonti and A. Hari Reddi

Bone Cell Biology Section, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892

ABSTRACT: Bone has considerable potential for repair as illustrated by the phenomenon of fracture healing. Repair and regeneration of bone recapitulate the sequential stages of development. It is well known that demineralized bone matrix has the potential to induce new bone formation locally at a heterotopic site of implantation. The sequential development of bone is reminiscent of endochondral bone differentiation during bone development. The collagenous matrix-induced bone formation is a prototype model for matrix-cell interactions *in vivo*. The developmental cascade includes migration of progenitor cells by chemotaxis, attachment of cells through fibronectin, proliferation of mesenchymal cells, and differentiation of bone. The bone inductive protein, osteogenin, was isolated by heparin affinity chromatography. Osteogenin initiates new bone formation and is promoted by other growth factors. Recently, the genes for osteogenin and related bone morphogenetic proteins were cloned and expressed. Recombinant osteogenin is osteogenic *in vivo*. The future prospects for bone induction are bright, and this is an exciting frontier with applications in oral and orthopaedic surgery.

KEY WORDS: extracellular matrix (ECM), growth and morphogenetic factors, inductive interactions, bone matrix, endochondral bone.

I. INTRODUCTION

One of the most exciting advances in bone cell biology has been the recognition of the extracellular matrix (ECM) of bone as a multifactorial repository of locally active growth and morphogenetic factors that modulate the function of bone cells.[1-4] While in most cases their role *in vivo* remains to be established definitively, it is clear that growth and morphogenetic factors isolated from the ECM of bone are potential molecular mediators of bone differentiation, maintenance, and repair. A remarkable example of inductive interactions between the ECM of bone and responding cells is the phenomenon of bone formation by induction.[5,6] Subcutaneous implantation of demineralized bone matrix results in a sequential developmental cascade of biochemical and morphogenetic events culminating in local differentiation of endochondral bone.[7] The origins of this research lie in the studies of Huggins, Lacroix, Urist, Reddi and others who first used biological assays to study the bone-inductive properties of the ECM of bone and other tissues, including tooth matrix and uroepithelium, and then stimulated many groups to contribute to the growing knowledge about the molecular and cellular signals involved in endochondral bone differentiation.

The growing appreciation that the ECM of bone is a rich source of cellular modulators underlies increasing interest in their role in repair and regeneration of the bone-bone marrow organ. These developments have arisen from a desire to

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understand fundamental developmental processes; the control of cell differentiation, and the generation of form. One expectation of this research is the discovery of new regulatory agents with novel biological and therapeutic potential. The molecular dissection of the ECM of bone has permitted the identification of an entirely new family of protein initiators that regulate differentiation of cartilage and bone *in vivo*.[8-10] Osteogenin, a protein initiator of bone differentiation, has been purified recently from bovine bone matrix and the amino acid sequence of several tryptic peptides was determined and found to be unique.[10] The amino acid sequence of bovine osteogenin is identical to the amino acid sequence deduced from the cDNA clones of one of the human bone morphogenetic proteins (BMP), BMP-3.[9]

The whole research into osteogenic initiators is at a very exciting stage as it is likely in the next few years that answers to questions of basic research that have therapeutic implications for the regeneration of bone in man will be found. This review surveys the recent advances in the regulation of bone development by osteogenin and related bone morphogenetic proteins. Using the postnatal bone development models to obtain a conceptual framework of the cellular and molecular mechanisms regulating endochondral bone differentiation, we provide evidence for the potential therapeutic application of osteogenin for the architectural reconstruction of bone in man based on cell biology of matrix-cell interactions.

II. THE POSTNATAL BONE DIFFERENTIATION MODEL: A DEVELOPMENTAL CASCADE

There is a direct relationship between growth and differentiation processes in early development and regeneration processes; fracture repair may be considered to recapitulate events that occur in the normal course of embryonic bone development. The tissue response elicited by subcutaneous implantation of demineralized bone matrix is reminiscent of embryonic bone development. However, unlike the epiphyseal growth plate, where a continuum of cartilage and bone differentiation is observed, in the matrix-induced implants single cycle of endochondral bone formation is evident.[2,7] The sequential developmental cascade includes:[6,7,11-15] activation and migration of undifferentiated mesenchymal cells by chemotaxis; anchorage-dependent cell attachment to the matrix via fibronectin; mitosis and proliferation of mesenchymal cells; differentiation of cartilage; mineralization of the cartilage; vascular invasion and chondrolysis; differentiation of osteoblasts and deposition of bone matrix; and finally mineralization of bone and differentiation of hemopoietic marrow in the newly developed ossicle.

Identification of osteogenic proteins in mammalian bone matrix has been a difficult task due to the low abundance of osteogenin and related BMPs tightly bound to the organic and inorganic components of the ECM of bone. Progress is recent years has been aided by four important technical developments: the development of a functional bioassay in the subcutaneous space of the rat to monitor the specific biological activity of osteogenic proteins;[7,16,17] the development of specific purification schemes involving heparin affinity chromatography;[8] the use of electroendosmotic elution techniques after preparative sodium dodecyl sulfate gel electrophoresis to achieve final purification homogeneity;[10] and finally the use of recombinant DNA methodologies for the cloning and expression of several members of the BMP family.[9,18]

A role for osteogenin and cell-substratum interactions in the initiation of chondroblastic and osteoblastic cell differentiation has been extensively demonstrated both *in vivo* and *in vitro*.[4,10,19,20] Osteogenin, isolated from the extracellular matrix of bone by heparin affinity chromatography and purified to homogeneity by electroendosmotic elution, in conjunction with insoluble collagenous bone matrix, initiates the developmental cascade of morphogenetic events culminating in local differentiation of endochondral bone *in vivo*.[4,10] Instrumental to the purification of osteogenin was the discovery that the bone differentiating activity could be dissociatively extracted from the ECM of bone and reconstituted with the inactive insoluble collagenous bone matrix residue, restoring the biological activity of the extracted soluble osteogenic fractions.[16] The bioassay by reconstitution of bioac-

tive fractions obtained during the different steps of the purification procedures, is a functional assay, and has permitted purification of native osteogenin from the ECM of bovine bone.[4,10]

The reproducible initiation of cartilage and bone in the rat extraskeletal site using demineralized bone matrix or osteogenin permits the dissection of the first wave of endochondral bone development and mineralization,[4,7] and enables a systematic study of endogenous and exogenous growth factors in bone development by the operational dissection of the major steps in the sequential developmental cascade.[2]

III. CHARACTERIZATION, AMINO ACID SEQUENCE, AND MOLECULAR CLONING OF OSTEOGENIN AND RELATED BONE MORPHOGENETIC PROTEINS

Osteogenin and related BMPs are members of the transforming growth factor-β (TGF-β) gene family. TGF-β is a multifactorial regulator of cellular growth in developing systems,[21,22] and it is a prominent component of the ECM of bone.[23-25] While TGF-β molecules are most abundant in the ECM of bone, their *in vivo* role in bone regulation is not clear. The TGF-β gene family is rapidly emerging as one of the most important regulatory growth and differentiating factor superfamily. The TGF-β family includes five distinct forms:[26] the hormones activin and inhibin;[27,28] the Mullerian-inhibiting substance;[29] the *Drosophila melanogaster* decapentaplegic complex;[30] the Vg-1 gene product of *Xenopus laevis;*[31] and the Vg-1-related murine protein Vgr-1.[32] In addition, several subsets of BMPs are all in the TGF-β superfamily of molecules.[9] The biologically active native osteogenin (BMP-3),[10] the human recombinant BMP-2A,[33] and BMP-2B[18] share limited homology with TGF-β molecules.

The induction of bone in the rodent extraskeletal site demonstrates that native osteogenin (BMP-3) and human recombinant BMP-2A and 2B orchestrate endochondral bone differentiation in postnatal tissues, in a pattern highly reminiscent of the embryonic bone development. An emerging body of evidence is now indicating that osteogenic proteins may be involved in inductive events that control pattern formation during embryonic development.[34,35] The recent discovery of specific binding sites for osteogenin in the developing rat embryo with the highest concentration in bone, cartilage, and surrounding connective tissues, indicates that in addition to the regeneration of bone, osteogenin may have a role in skeletal differentiation morphogenesis.[36] BMP-2A RNA was localized in condensing precartilagenous mesenchyme, and in the osteogenic zones of developing bones in older mouse embryos, indicating that BMP-2A may also regulate cartilage and bone formation during embryogenesis.[34] It is likely, however, that BMP gene products are also involved in morphogenetic processes outside the developing skeletal system. Thus, high levels of BMP-2A transcripts have been localized in developing mouse hair and whisker follicles, limb buds, tooth buds — including the dental papilla and the odontoblastic layer, and in the cranofacial mesenchyme, particularly in regions of precartilaginous mesenchymal condensations (Meckel's cartilage and nasal cartilage), and the mesenchyme of the palatal shelves.[34,37]

IV. ANGIOGONESIS, VASCULAR INVASION, AND OSTEOGENESIS

Bone induction is the result of the combinatorial action of osteogenin and the collagenous matrix.[4,10,38] It is likely that after the initiation of the first wave of bone differentiation osteogenin, including the commitment and the clonal expansion of osteoprogenitor stem cells, the osteogenic cascade may be promoted and maintained by a variety of growth factors, including TGF-β.[4] Indeed, TGF-β was detected from day 9 onward after subcutaneous implantation of bone matrix in rats.[39] The increased concentration correlated with the onset of angiogenesis and calcification of cartilage. TGF-β appeared to be compartmentalized in the mineral phase of the newly formed bone matrix and this may be a mechanism for storage of the latent or processed growth factor.[4,39]

Vascular invasion is a prerequisite for bone formation during endochondral bone differentia-

tion.[40,41] Angiogenesis is of paramount importance in fracture healing.[42,43] In the matrix-dependent bone induction model, angiogenesis is correlated with chondrolysis and concomitant osteogenesis. These observations have prompted a number of investigations into the interaction of osteogenin with other components of the ECM in an attempt to elucidate the combinatorial regulatory role of basement membrane components and molecules of the TGF-β superfamily. These studies have very recently demonstrated that both osteogenin and TGF-β bind to type IV collagen.[44,45] In addition, osteogenin binds also to type I and type IX collagens.[44] These findings provide a conceptual framework for the supramolecular assembly of the ECM of bone, and provide novel insights into the regulatory role of growth factors in the solid state. It is possible that type IV collagen and other matrix components around the endothelial cells of the invading capillaries may bind growth and differentiating factors, and present them locally in an immobilized form to responding mesenchymal cells and osteoprogenitors to initiate osteogenesis.[44] In view of the affinity of both TGF-β molecules and osteogenin for type IV collagen, Paralkar et al.[44,45] have proposed that the biological actions of members of the TGF-β superfamily molecules are regulated by a complex interaction with ECM components, and that type IV collagen may function as a delivery system by sequestering both initiators and promoters involved in endochondral bone differentiation, as well as in other inductive phenomena.

Novel information on the concept of the instructive role of matrix components in morphogenesis has been provided recently by Vukicevic et al.,[46] who investigated the interaction of rat primary calvarial cells and the mouse osteoblast-like cell line MC3T3-E1 with basement membrane components. The results of these studies have shown that osteoblastic cell lines recognized components of basement membranes, i.e., laminin and type IV collagen, undergo profound morphological changes when cultured on a reconstituted basement membrane gel.[46] Taken together, these findings provide evidence for the crucial role of basement membrane components of invading blood vessels in osteogenesis. First, macromolecules of the ECM may bind growth and differentiating factors, protect them from proteolytic degradation, modulate a controlled slow release, and finally, orient them in an optimal conformation to locally initiate bone formation.[44,45] Second, they modulate the phenotypic differentiation of osteoblastic cells. Thus, the instructive role of the ECM may be modulated by the affinity of the matrix components for soluble growth and differentiating factors in the solid state, resulting in tissue patterning in embryonic development,[1,47] and locally regulating wound healing and a variety of contact-dependent physiological processes, including the matrix-dependent endochondral bone differentiation model.[44,45]

V. OSTEOGENIN AS THERAPEUTIC INITIATOR OF OSTEOGENESIS: A PRIMATE CALVARIAL MODEL

The purification of osteogenin and related BMPs, and the operational dissection of the matrix-dependent bone induction model have permitted substantial progress in the elucidation of the molecular and cellular mechanisms involved in endochondral bone differentiation. However, the morphogenetic potential of osteogenin and related BMPs is solely based on work in rodents.[4,10,33] Information concerning bone induction in primates is a prerequisite for the exploration of potential therapeutic applications for the regeneration of bone in man.[48] There is a growing interest in novel bone substitutes incorporating osteogenic proteins for craniofacial and periodontal applications in an effort to promote the controlled initiation of bone formation, and to reduce the harvest of autogenous bone and associated morbidity. The creation of a nonhuman primate model, using species comparable to man with respect to bone regulatory mechanisms and bone remodeling, would closely replicate bone repair and the biological fate of bone substitutes in man, accelerating the pace of clinical trials.

We have studied the healing potential of calvarial defects in a series of adult individuals of genus *Papio* (baboon), establishing a critical size defect (CSD)-dependent nonunion of the baboon calvaria,[49] i.e., a defect that does not repair spontaneously with bone, requiring a graft of viable bone or alternative substitutes to heal.[50] The lim-

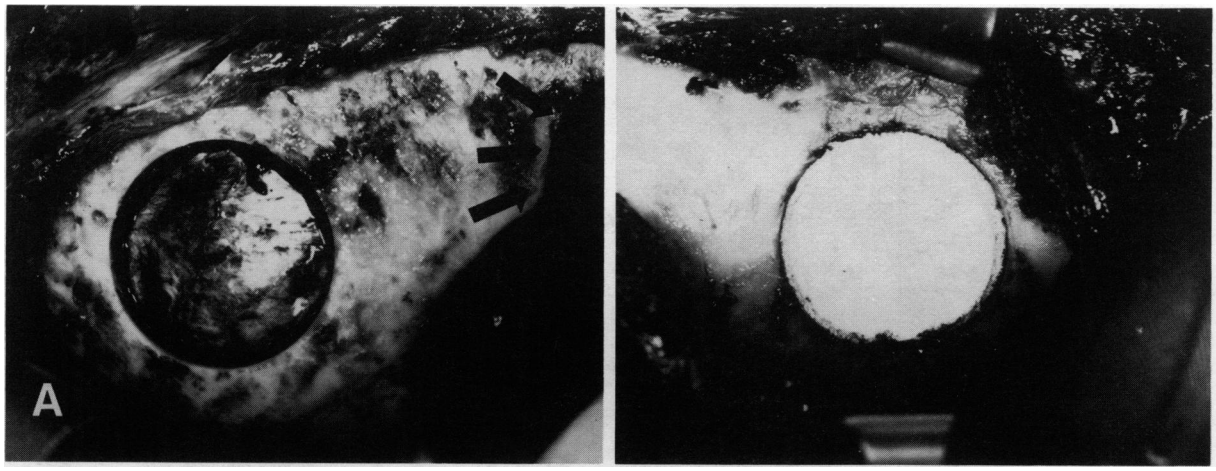

FIGURE 1. Surgical exposure of the right calvaria in an adult male baboon, (A) Preparation of full-thickness circular defects, 25 mm in diameter. Arrows indicate the location of the second defect trefined in the anterior region of the calvaria. (B) Insertion of a 25-mm disc of insoluble collagenous matrix reconstituted with baboon osteogenin.

ited regenerative capacity of the membranous calvarial bones provides an ideal model to study the osteogenetic potential of bone substitutes independent of the weight-bearing component. A considerable sexual dimorphism exists in the *Papio* species, the adult males being almost twice the size of the females. Because of the relatively large calvaria, four circular cranial defects, each 25 mm in diameter, can be surgically prepared in an adult male without compromising the vascular support of the intervening bone (Figure 1A). This allows the simultaneous comparison within the same animal of bone substitutes with a control defect and a graft of autogenous bone without animal-to-animal variation, as systemic factors may influence healing.[49] In 48 adult male baboons, calvarial defects were implanted with a graft of autogenous bone harvested from the iliac crest, and with different osteoconductive and osteoinductive substrata. These included baboon insoluble collagenous bone matrix reconstituted with osteogenin (Figure 1B), isolated and purified from baboon bone matrix,[51,52] baboon demineralized bone matrix, and insoluble collagenous matrix without osteogenin. Forty-eight defects were left ungrafted to monitor the spontaneous regeneration potential of the adult baboon calvaria. Before calvarial implantation, osteogenin, both isolated and purified from baboon and bovine bone matrix and with biological activity in rats (Figures 2A and B), were tested for biologic activity in the *rectus abdominis* of an additional 16 baboons. The extraskeletal implantation permits the unequivocal histological investigation of bone formation by induction, avoiding possible ambiguities of the orthotopic site (Figure 3).

Specimens with surrounding recipient calvariae were harvested at 1, 3, 6, and 9 months after surgery, and histomorphometry was performed on semithin undecalcified sections at 7 μm, cut from plastic-embedded specimen blocks. At 3 and 9 months, the amount of bone in control defects was less than 14 and 20%, respectively (Figure 4A). At 1 and 3 months, new bone formation in autogeneic bone grafts was less than 8 and 24%, respectively (Figure 4B). Reconstitution of insoluble collagenous matrix with baboon osteogenin-induced copious amounts of new bone as early as 30d (Figure 5A), and at 3 months, bone formation was extensive culminating in complete regeneration of the craniotomy defects (Figure 5B). In implants of demineralized bone matrix bone formed with an intervening phase of endochondral development and at 30 d large islands of cartilage had differentiated within the internal and central regions of the demineralized bone matrix implants (Figures 6A and B). Cartilage formation in defects of membranous calvarial bone in adult primates is the phenotypic evidence for bone development by induction, as

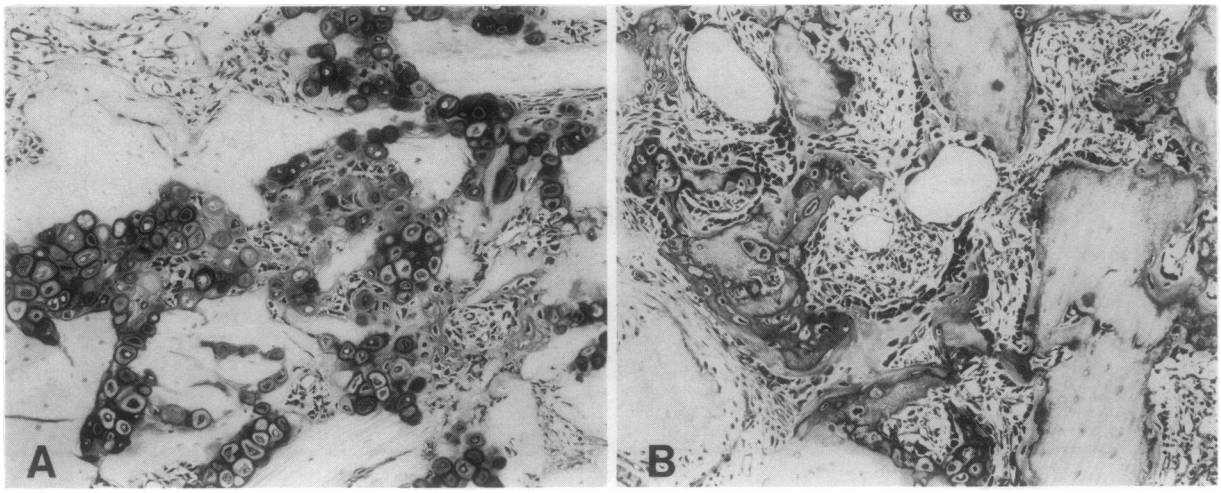

FIGURE 2. Endochondral bone differentiation in implants of rat insoluble collagenous matrix reconstituted with baboon osteogenin fractions obtained after heparin-Sepharose, hydroxyapatite-Ultrogel, and S-200 Sephacryl chromatography (A), and baboon osteogenin purified to homogeneity after electroendosmotic elution (B). Reconstituted implants were inserted in the subcutaneous space of 28 to 36-d-old Long Evans rats, and harvested 11 d after implantation (Toluidine blue stain on 2-μm sections cut from JB4 plastic-embedded specimens).

opposed to a merely passive osteoconductive pattern of bone deposition from the margins of the craniotomy defect.

VI. CARRIERS AND DELIVERY SYSTEMS FOR THE BIOLOGICAL EXPRESSION OF OSTEOGENIN AND RELATED BMPS

The above-mentioned results in primates have demonstrated that the association of osteogenin with the complementary substratum of the collagenous matrix can be exploited to construct delivery systems for the rapid and controlled initiation of bone morphogenesis. The restoration of biological activity after dissociative extraction and reconstitution of osteogenin with insoluble collagenous matrix[16] suggests that components of the ECM of bone act as carriers for the functional expression of osteogenin. Previous results have shown that the collagenous bone matrix provides an optimal substratum for anchorage of cells and subsequent proliferation and differentiation.[7] In addition, the collagenous matrix may prevent premature diffusion and dissolution of osteogenin at the site of surgical implantation. This allows a spatially controlled osteogenesis, restricting bone differentiation in predetermined surgical sites. While the reconstitution with insoluble collagenous matrix is a requirement for optimum delivery of biological activity,[38] the collagenous matrix, with its potential problems of antigenicity and viral contamination, may limit the utilization of osteogenin as a potential widespread therapeutic agent. A major goal of the combined efforts of biomaterial scientists and reconstructive surgeons is the development of delivery systems and substrata capable of restoring the optimal expression of the biological activity of osteogenin in the absence of the organic substratum of the collagenous matrix.

By exploiting the biological principle of centripetal mesenchymal tissue ingrowth,[53] porous biomaterials appear to be best suited for the construction of delivery systems for osteogenin. We have investigated the osteogenic potential of osteogenin combined with porous hydroxyapatite replicas obtained after hydrothermal conversion of calcium carbonate exoskeletons of corals.[54-59] The results have shown that osteogenin, adsorbed on hydroxyapatite substrata, induced *in vivo* differentiation of the osteogenic phenotype in mesenchymal cells populating the tridimensional porous framework of the hydroxyapatite substratum when implanted extraskeletally in rodents[83] (Fig-

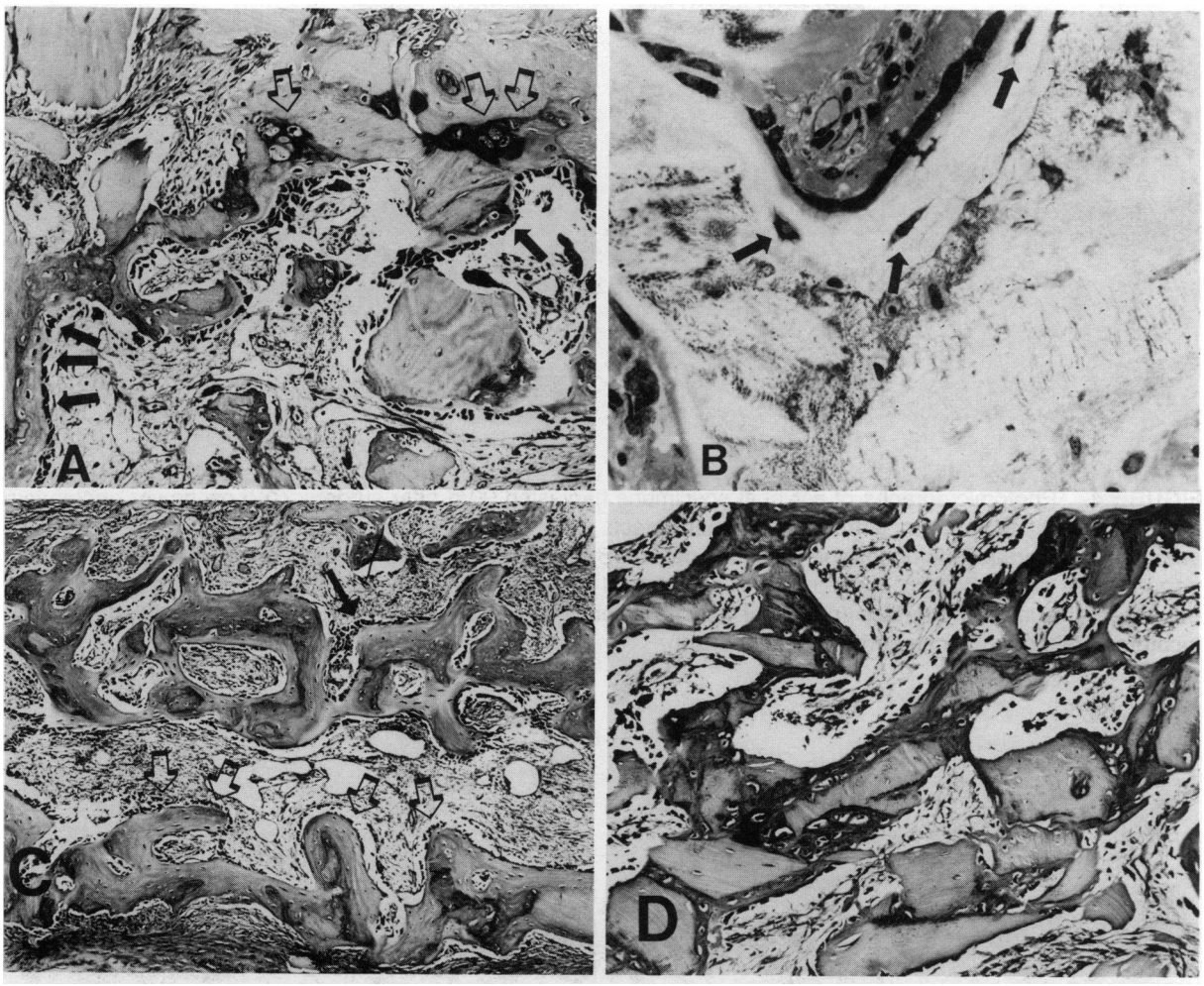

FIGURE 3. Bone differentiation in 30-d implants of baboon insoluble collagenous matrix reconstituted with baboon (A and B), and bovine (C) osteogenin fractions obtained after S-20 Sephacryl gel filtration and implanted extraskeletally in baboons *(rectus abdominis)*. (A) N of cartilage (open arrow) invading the insoluble collagenous matrix. (B) HIgh-power view showing osteoblasts lining newly formed bone with embedded osteocytes (arrowheads). (C) Extensive bone formation, remodeling, and dissolution of the implanted collagenous matrix. Arrows indicate contiguous layers of osteoblasts lining newly formed trabeculae of induced bone. (D) Bone differentiation after extraskeletal implantation of baboon demineralized bone matrix in an adult baboon.

ure 7). Interestingly, intramuscular implantation of porous hydroxyapatite replicas in baboons resulted in bone formation within the porous spaces (Figures 8A and B), irrespective of the osteogenic stimulus of osteogenin,[60,61] underscoring the importance of primates when investigating the osteogenic potential of bone substitutes for clinical application in man.[62] The realization that the biological activity of osteogenin can be restored and delivered by inorganic porous substrata to obtain predictable phenotypes with complete host acceptance and incorporation, will help to design appropriate delivery systems for the controlled initiation and promotion of bone morphogenesis for craniofacial and periodontal applications.

VII. CRANIOFACIAL AND PERIODONTAL APPLICATIONS

The architectural and functional reconstruction of the craniofacial skeletal tissues lost as a consequence of disease and trauma is a formidable challenge for modern surgery, and neces-

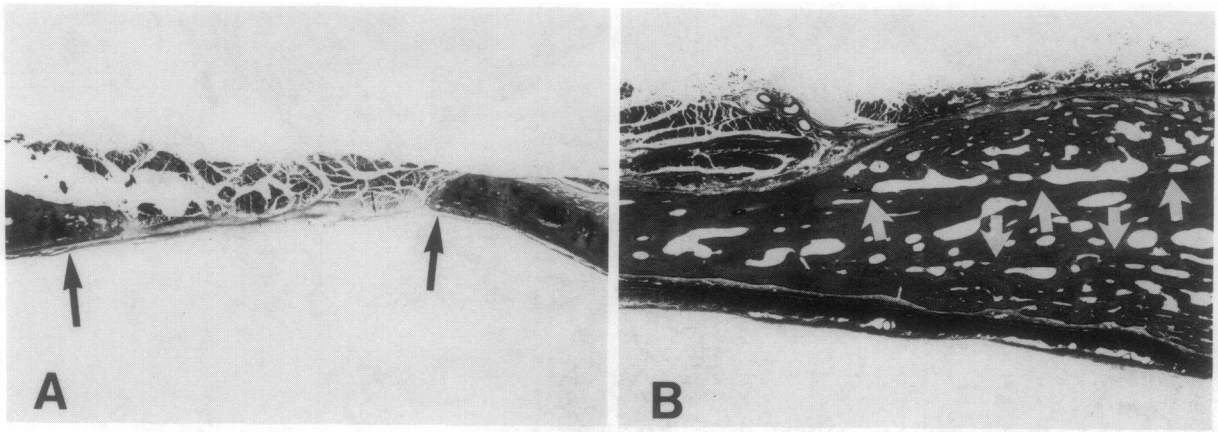

FIGURE 4. Photomicrographs of calvarial specimens harvested 90 d after surgery. (A) Low-power photomicrograph of a section from an untreated calvarial defect. Arrows indicate the original margins of the craniotomy preparation. Minimal bone deposition within the defect. (B) Photomicrograph of an autogeneic bone graft harvested from the iliac crest showing pericranial and endocranial bone deposition (arrowed) along the implanted scaffold near the calvaria-graft interface (right) but absent toward the central region of the implant (modified Goldner's trichrome stain on undecalcified sections at 7 μm).

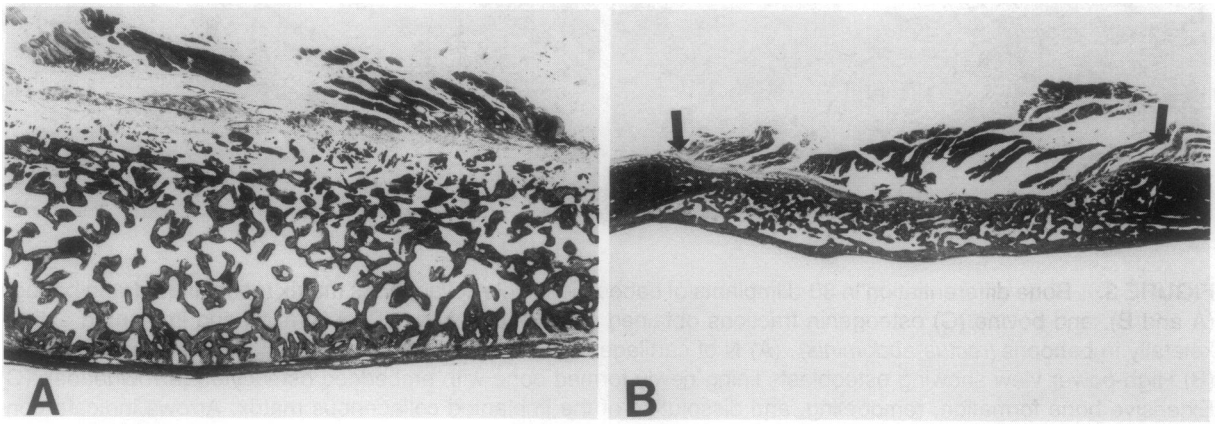

FIGURE 5. Photomicrographs of calvarial specimens of insoluble collagenous matrix reconstituted with baboon osteogenin fractions. (A) Central region of a section prepared from a specimen 30 d after implantation. Extensive bone formation, rapid incorporation and dissolution of the implanted matrix, and formation of solid trabeculae of mineralized bone. Low-power photomicrograph of a section of osteogenin-reconstituted baboon insoluble collagenous matrix harvest 90 d after implantation in a calvarial defect. Arrows indicate the margins of the craniotomy preparation. Extensive bone formation with complete regeneration of the defect.

sitates the combined efforts of molecular and cellular biologists, biomaterial scientists, and reconstructive surgeons. The use of osteogenin and related BMPs in reconstructive procedures is a fertile area of applied research and holds realistic potential for the therapeutic regeneration of bone, including the correction of congenital and acquired craniofacial anomalies.[63]

Regeneration of the periodontal tissues following destructive episodes of inflammatory-infective periodontal diseases is an even more challenging problem, since repair processes must involve not only the affected alveolar bone, but other crucial components, i.e., the periodontal ligament and the root cementum. The final goal is the formation of new connective tissue attach-

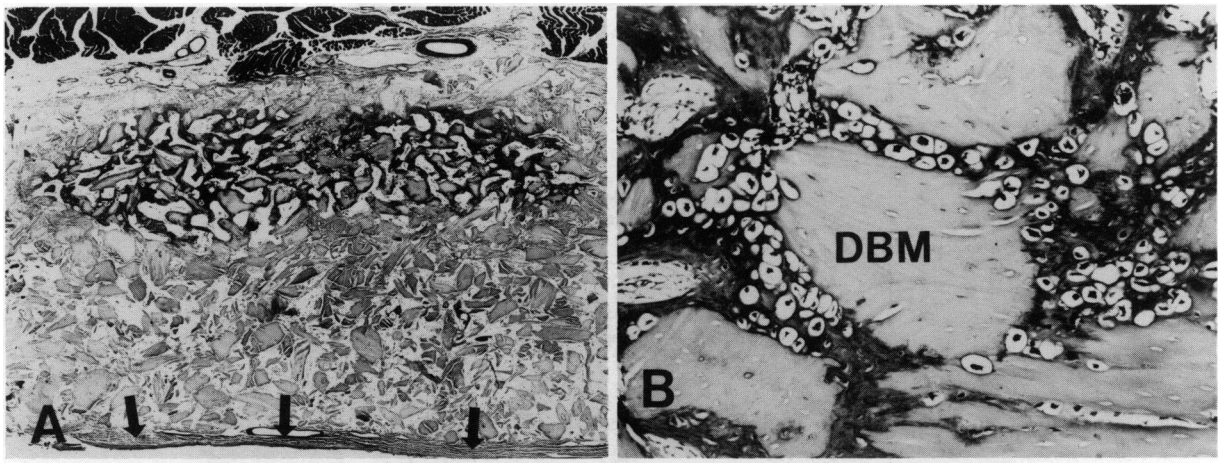

FIGURE 6. Photomicrographs of a section of a calvarial specimen of baboon demineralized bone matrix harvested 30 d after implantation. (A) Low-power photomicrograph showing endochondral bone differentiation in the central region of the implant. Arrows indicate the dural layer. (B) High-power view illustrating the intervening cartilage phase after implantation of demineralized bone matrix (DBM) in calvarial defects.

ment with functional orientated periodontal ligament fibers inserted into newly formed cementum, and the organization of a competent gingival unit. Provided the root surface is adequately decontaminated, three major biological problems are faced by practicing periodontists and researchers alike:[64] establishment of connective tissue attachment to highly mineralized, almost impermeable cemental or dentinal surfaces after root planing; promotion of osteogenesis; and inhibition of apical migration of gingival epithelial cells. The latter problem has been addressed by the development of strategies that mechanically guide and control tissue regeneration by selectively excluding epithelial cells to participate in the healing process adjacent to the prepared root surfaces.[65,66] More recently, the concept of biochemically mediated tissue regeneration has emerged as a potential therapeutic approach to the reconstruction of the periodontal unit.[67-72] Exposure of dentinal collagen type I after citric acid[73,74] or tetracycline-HCl[67] surface demineralization has been followed by the exogenous application of attachment factors such as fibronectin and laminin,[68-71,75] to enhance fibroblast migration, attachment, and collagen synthesis, and to inhibit apical proliferation of junctional epithelial cells. Promising preliminary results have been obtained using a combination of platelet-derived growth factor (PDGF) and insulin-like growth factor in a canine periodontal wound model.[76] The use of PDGF in periodontal surgery is appealing since it has been demonstrated that supplementation of PDGF resulted in enhancement of cartilage and bone formation in conditions where bone induction was subminimal.[77] This may have important therapeutic implications in the treatment of impaired bone formation in the aged, particularly in instances of periodontal disease.

So far, osteogenin and human recombinant BMPs have not been tested as potential initiators of osteogenesis for the experimental treatment of periodontal conditions. However, a number of studies using crude bone matrix preparations have suggested that demineralized freeze-dried allogeneic bone has therapeutic potential for bone and connective tissue regeneration in humans.[78-82] From a therapeutic point of view, future research should focus on the delivery of osteogenin and related BMPs in combination with collagenous matrix or porous biomaterials to regulate bone formation in periodontal regenerative procedures. With the complexity of the periodontal disease process, it is likely that enhanced bone resorption may be coupled with inhibited bone deposition,[83] particularly when related to cyclic periods of disease activity. In conditions of limited bone formation, local delivery of osteogenin and related BMPs may initiate the osteogenetic

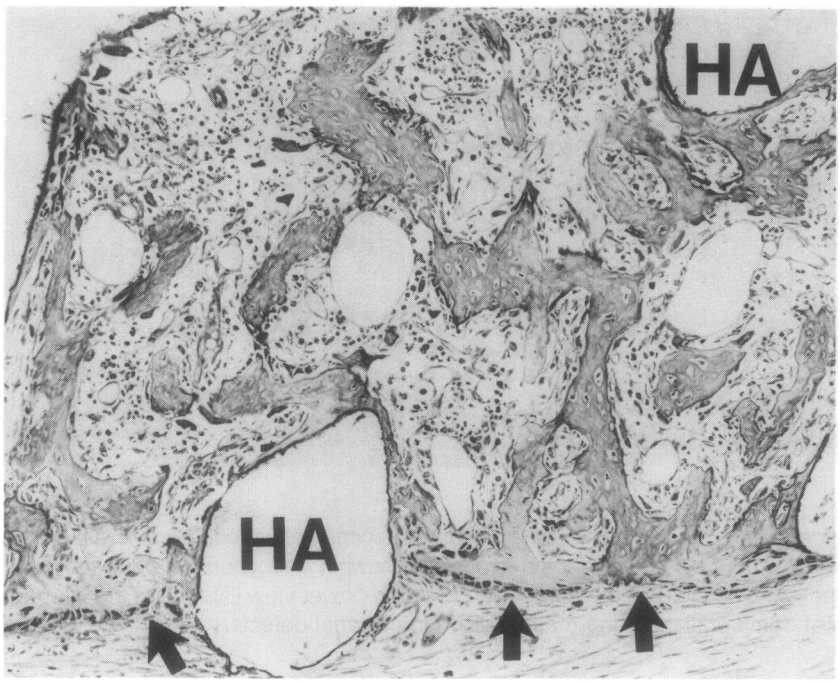

FIGURE 7. Bone differentiation in an implant of porous coralline hydroxyapatite (HA) and bovine osteogenin 11 d after implantation in the subcutaneous space of 28- to 36-d-old Long Evans rats. Arrows indicate contiguous layers of osteoblasts. Plastic-embedded section at 2 μm stained with toluidine blue.

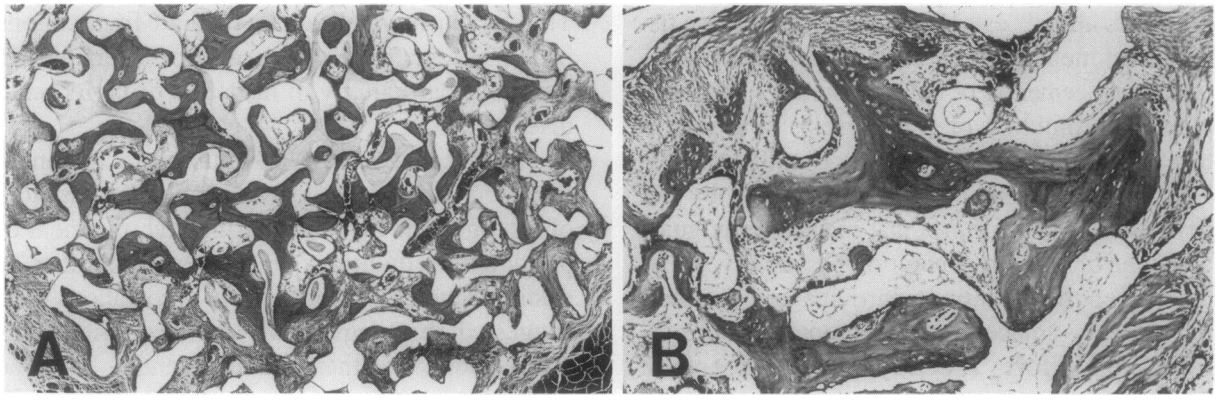

FIGURE 8. Photomicrographs of bone differentiation in porous hydroxyapatite specimens implanted in the *rectus abdominis* of adult baboons. (A) Control hydroxyapatite implant without osteogenin. Note the extensive bone deposition, remodeling, and organization of lamellar bone 6 months after surgical implantation. (B) Bone differentiation in the porous spaces of a porous hydroxyapatite and bovine osteogenin specimen harvested 90 d after implantation. Paraffin-embedded sections at 5 μm stained with toluidine blue.

stimulus necessary for the regeneration of the periodontal unit.

VIII. CONCLUSIONS

The molecular biology and biochemistry of protein initiators of bone differentiation are becoming increasingly defined. Osteogenin and related BMPs are members of the TGF-β supergene family, and are involved in a number of physiological events that include embryonic development, skeletal tissue maintenance and repair. The interaction of osteogenin with other ECM components has permitted a conceptual design of the possible regulatory role of growth and initiating factors in the solid state. This wealth of knowledge can now be applied to controlling the rapid initiation of bone formation for craniofacial and periodontal conditions in man.

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