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Polypeptide Factors Regulating Osteogenesis and Bone Marrow Repair

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Abstract Osteogenic growth polypeptides regulate bone cell function in vitro and may act in vivo in an autocrine, paracrine, or endocrine manner. Several of these polypeptides are present in the blood in an inactive form. During postablation bone marrow regeneration these factors may be activated, released from the blood clot, and together with locally produced polypeptides mediate the initial intramedullary/systemic osteogenic phase of this process. Then, the same and/or other polypeptides expressed by stromal cells have the potential to promote the second phase of regeneration that consists of osteoclastogenesis, resorption of the transient intramedullary bone, and hemopoiesis. This may be an indirect influence since these polypeptides can regulate the stromal cell expression of some of the hemopoietic factors. Clinically, the osteogenic growth polypeptides that regulate osteogenesis and hemopoiesis have a potential role in osteoporosis therapy, implant bone surgery, and bone marrow transplantation.

Key words: bone marrow, osteogenesis, bone resorption, osteogenic growth peptide, hemopoiesis, growth factors

There is an intimate relationship between bone and bone marrow, both anatomically and functionally. The cells responsible for bone formation and resorption, the osteoblasts and osteoclasts, are derived from the marrow's stromal and hemopoietic systems, respectively [Friedenstein, 1976; Kurihara et al., 1990]. Marrow derived stromal cells, from man and other species, cultured in vitro or in vivo in diffusion chambers, constitutively undergo terminal osteogenic differentiation leading to the deposition of a mineralized matrix [Ashton et al., 1980; Ashton et al., 1984; Bab et al., 1984a,b, 1986; Howlett et al., 1986; Pfeilschifter et al., 1993]. Osteoclasts are believed to arise from hemopoietic progenitors and osteoclast development appears to be under the control of the same paracrine cytokines that are responsible for granulocyte and macrophage formation [Kurihara et al., 1989; Barton and Mayer, 1989].

Under physiologic conditions, the osteogenic and osteoclastic potentials of the marrow are expressed to a limited degree during normal bone remodeling. However, bone formation and resorption of a substantially greater extent occur in instances of marrow regeneration [Amsel et al., 1969; Patt and Maloney, 1975]. Perhaps the most reproducible injury-induced experimental process of bone marrow regeneration is the postablation healing model [Amsel et al., 1969]. Marrow ablation in long bones triggers an initial local osteogenic reaction whereby the blood clot that fills the medullary cavity immediately after marrow removal is organized into primary cancellous bone. The primary bone trabeculae are then subjected to osteoclastic resorption and replacement by intact marrow. A similar sequence of events was reported following an irradiation insult to the marrow [Patt and Maloney, 1975]. Unlike repair processes in other soft tissues, the injured bone marrow undergoes complete regeneration without scar formation. In addition, this marrow healing process is also distinct from fracture repair in that the transient osteogenic phase is not preceded by cartilage formation. Interestingly, the marrow reaction to injury induced by the insertion of intramedullary metallic implants is somewhat different in that a bony, cortex-like, collar forms in direct contact with the implant [Bab and Einhorn, 1993]. It appears that the formation of

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this persistent collar represents a physiologic modification of the marrow regeneration process in the presence of a chemically inert solid substrate rather than a marrow reaction to a continuous irritation induced by an unstable prosthetic system [Bobyn et al., 1981; Engh et al., 1987]. This conclusion is further supported by the pattern of bone formation in osteogenic diffusion chamber cultures which occurs on the chamber's solid membrane filters [Ashton et al., 1980; Bab et al., 1984b, 1986]. The development of an "intramedullary cortex" of this nature provides the basis for the concept of osseointegration between the implant and the skeleton [Keller, 1991].

REGULATION OF BLOOD CLOT ORGANIZATION INTO PRIMARY CANCELLOUS BONE

The local osteogenic phase of postablation marrow regeneration is accompanied by a transient increase in bone formation at distant skeletal sites. This systemic response to marrow injury has been established in experiments that involved marrow ablation, insertion of an intramedullary nail, and other forms of mechanical injury [Bab et al., 1985; Gazit et al., 1990; Einhorn et al., 1990]. An analogous response also occurs in normal human bone marrow donors in whom an increase in serum osteocalcin and alkaline phosphatase was measured after the removal of a substantial portion of their ilial marrow [Foldes et al., 1989]. In addition, irradiation and massive bleeding elicit a similar, generalized, osteogenic reaction [Bleiberg et al., 1993; Lucas et al., 1993]. Massive bleeding also enhances the serum stimulation of bone cell proliferation [Lippiello et al., 1992]. However, it does not seam to be an essential component in the systemic response to marrow injury [Bab and Einhorn, 1993]. This response appears to be restricted to the osteomyeloid system inasmuch as liver and periosteal injuries fail to elicit a peripheral enhancement of bone formation [Gazit et al., 1990].

The notion that the systemic response to bone marrow injury is mediated by osteogenic growth factors produced at the site of regeneration and released into the circulation not only follows intuitively from the basic phenomenology, but is also strongly supported by a series of recent studies. In these studies, the conditioned medium recovered from cultured regenerating marrow, but not normal marrow, contained growth promoting activity for osteogenic cells. This activity most likely consists of several growth factors [Bab et al., 1988; Gazit et al., 1989]. One of these factors, a 14-amino acid histone H4-related osteogenic growth peptide (OGP) was recently purified to homogeneity [Bab et al., 1992]. The OGP regulates proliferation in osteoblastic and fibroblastic cell lines and osteoblastic cell alkaline phosphatase activity. It also stimulates alkaline phosphatase and matrix mineralization in human- or rabbit-derived stromal cell cultures [Robinson et al., 1993]. OGP in high abundance is present in the serum, mainly in the form of an OGP-OGP binding protein complex. A transient marked increase in serum bound and unbound OGP accompanies the osteogenic reactions to marrow ablation [Bab et al., 1992], irradiation, and bleeding [Bleiberg et al., 1993; Lucas et al., 1993]. It thus appears that increased OGP production constitutes a bone marrow reaction to stress. It will be of interest to examine the role of such an exciting, newly discovered peptide in other osteogenic processes and in metabolic bone diseases such as osteoporosis.

Studies to date have shown that insulin-like growth factor I (IGF-I) is also involved in the osteogenic phase of marrow regeneration [Suva et al., 1993]. Surprisingly, although transforming growth factor beta (TGF-β) may be an important stimulator of bone matrix synthesis [Ruoslahti, 1990] and a factor which links between bone formation and resorption [Rodan and Noda, 1991], the mRNAs for members of the *TGF-β* family, TGF-β1, TGF-β2, and TGF-β3 are only weakly expressed in the post-ablation healing system [Suva et al., 1993]. The expression of TGF-β3 mRNA as well as that of the bone morphogenetic proteins (BMP) BMP-2 and BMP-4 have been shown recently to be down regulated in another system of differentiating osteogenic cells [Gazit et al., 1993]. Despite the fact that at the mRNA level TGF-β does not change in ways suggesting that it initiates the postablation osteogenic phase, this polypeptide may still be available from the blood clot and blood circulation. The absence of BMP expression during bone marrow healing is not surprising inasmuch as the BMP is a cartilage inducer [Wozney, 1992] and the bone formed as a result of ablation develops by an *intramembranous* process. Furthermore, the BMPs are expressed in several extraskeletal tissues [Jones et al., 1991] and their physiologic role in bone metabo-

lism, which can be studied by homologous recombination in transgenic mice, is still unknown.

Unlike instances of soft tissue healing, where preexisting fibroblastic cells give rise to soft granulation tissue, in the postablation marrow regeneration model the blood clot is immediately engaged by the remaining osteoprogenitors present on the osteonal and endosteal surfaces of the cortex. This interaction initiates the unique set of events which ultimately lead to new bone formation [Amsel et al., 1969; Patt and Maloney, 1975]. Moreover, since i) inhibition of clot formation prevents both the local and systemic osteogenic responses [Gazit et al., 1990], and ii) the regeneration of new marrow appears to be dependent on the formation of intramedullary cancellous bone, we consider the interaction between the clot and osteoprogenitor cells to be a critical stage for the entire process. Similar circumstances pertain to the well-documented process of wound healing in the jaw that follows tooth extraction. Here too, the blood clot is replaced by primary cancellous bone that is subsequently remodeled and partially replaced by marrow [Huebsch and Hansen, 1969]. Jeopardizing the clot integrity, a clinical situation known as "dry socket," markedly delays the extraction wound healing [Amber, 1973].

It appears that the blood clot functions in two principal capacities. First, it releases growth *and differentiation* stimuli which induce the local osteogenic cascade of events. Although there are numerous reports on the biology of blood derived growth and differentiation polypeptides, we have been surprised at how few deal with the release of these factors from the blood clot during wound healing. The blood is replete with factors capable of stimulating osteogenic cell proliferation such as OGP [Bab et al., 1992; Bleiberg et al., 1993; Lucas et al., 1993], TGF-β [Hock et al., 1990], β_2 microglobulin (β2M) [Canalis et al., 1987], platelet derived growth factor (PDGF) [Bowen-Pope et al., 1984], and IGF [Zhang et al., 1991]. For the most part these factors are present in an inactive form. The TGF-β, for example, is a part of a larger latent molecule [Wakefield et al., 1989]. OGP, PDGF, and IGF are bound to their specific binding proteins [Raines and Roos, 1987; McCuster et al., 1988; Bab et al., 1992; Greenberg et al., 1993] and β2M is complexed to free class I histocompatibility receptors [Kozlofsky et al., 1991]. It is likely that the conditions present in the blood clot favor a transition of these factors from the inactive to an active form with their subsequent dissolution into the immediate environment. Many of these factors, including the OGP, are released by bone cells and act in an autocrine/paracrine manner [Lehnert and Akhurst, 1988; Evans et al., 1991; Rydziel et al., 1992; Bab et al., 1993], mechanisms that may increase the availability of growth stimulation during the critical phase of extremely high proliferative activity. The above listed factors probably comprise an incomplete list in the sense that other hormones and hormone fragments such as parathyroid hormone (PTH), growth hormone (GH), and glucocorticoids may also be involved in this process [Somjen et al., 1990; Barnard et al., 1991; Finkelman et al., 1992].

Second, it appears reasonable to speculate that in addition to its function in stimulating osteogenic cell growth, the clot provides "mechanical guidance" essential for the migration of osteogenic cells that express integrin receptors [Puleo and Bizios, 1991]. The mechanism involved in the cell migration is probably based on alternate attachment/detachment cycles to from glycoproteins containing RGD (Arg-Gly-Asp) sequences that bind to the integrin receptors.

The medullary osteogenic process is accompanied by endothelial budding and angiogenesis. The budding most probably originates in disrupted blood vessels in the cortex and metaphysis. In vivo diffusion chamber studies have demonstrated that while the formation of a vasculature is not essential for osteogenesis, an association between neovascularization and osteogenesis results in remodeling of the new osteogenic tissue into cancellous bone [Budenz and Bernard, 1980; Bab and Einhorn, 1993]. Until recently it was believed that the main regulators of neovascularization belong to the fibroblast growth factor (FGF) family [Thomas et al., 1985]. While these factors are abundant in bone matrix [Hauschka et al., 1986], at least the marrow ablation model does not involve any significant damage to bone per se [Bab et al., 1985]. Thus the release of members of this family immediately after the insult is not an obligatory part of the process. Instead, the endothelial budding may be regulated by molecules such as vascular endothelial growth factors (VEGF), a group of related soluble factors expressed in endothelial cells as alternatively spliced transcripts of a single gene [Houck et al., 1991]. The

VEGFs are highly expressed during neovascularization and their effect on endothelial cells is similar to that of the FGF. Like FGF [Globus et al., 1988], they may also affect osteogenic cell function.

INDUCTION OF OSTEOCLASTOGENESIS AND HEMOPOIESIS

Our histologic studies in the marrow ablation model and in vivo mineralizing diffusion chamber cultures exposed to the external environment suggest that some degree of bone resorption takes place before the formation of a complete marrow organ [Bab and Einhorn, 1993]. These studies further indicate that the hemopoietic elements, including the osteoclasts that appear following osteogenesis, are derived from circulating multipotential hemopoietic stem cells [Budenz and Bernard, 1980; Bab and Einhorn, 1993]. Timewise, there is a substantial overlap between the osteogenic and hemopoietic stages of bone marrow regeneration [Suva et al., 1993]. For example, when hemopoietic cells are first detected in the peripheral zones of the healing tissue, there are still considerable numbers of preosteoblasts undergoing terminal differentiation, especially in the center of the medullary cavity. Therefore, and in the absence of more specific information, all stromal tissue components, namely, osteoblasts, preosteoblasts and other stromal cells, as well as the bone and soft tissue extracellular matrices, should be considered potential participants in the establishment of the microenvironment essential for the development of regenerated marrow. Furthermore, since at the peak of the osteogenic phase, osteogenic cells and mineralized and unmineralized bone matrix are the dominant constituents of the medullary cavity, they appear to be the most important inducers of osteoclastogenesis and hemopoiesis.

Emerging direct evidence suggests a role for osteoblast products in the induction of osteoclastogenesis and hemopoiesis. It has been shown that soluble and bone matrix derived osteoblast products may be chemotactic for hemopoietic cells, direct their migration across the blood vessel walls, and regulate their differentiation [Malone et al., 1982]. It is now commonly held that the osteoclast develops from the common hemopoietic stem cell and is probably related to the granulocyte/macrophage lineage [Kurihara et al., 1990]. The chemotactic osteoblast products, in particular osteocalcin and type I collagen [Lian and Gundberg, 1988], may affect the osteoclast precursors immediately following their secretion. Since these factors are incorporated into the bone matrix, they may also be effective by virtue of some physical contact with the respective precursors and stimulate osteoclastogenesis following their release during bone resorption. In vitro experiments have shown that osteogenic cells produce several hemopoietic factors, such as macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), and interleukin 6 (IL-6) capable of regulating different phases of development of osteoclasts and other hemopoietic cells [Felix et al., 1989; Horowitz et al., 1989; Kurihara et al., 1991; Jilka *et al.*, 1992; Horowitz, 1993]. In addition, our unpublished preliminary results suggest that osteogenic cells express the stem cell factor (SCF). This recently reported factor is present either as a free or cell surface bound molecule and stimulates the proliferation of hemopoietic stem cells and mastocytes by interacting with the receptor encoded by the c-kit proto-oncogene [Chabot et al., 1988; Toksoz et al., 1992]. Furthermore, it has been recently reported that the SCF, in concert with other factors, affects osteoclast development [Demulder et al., 1992].

Although the fine tuning of osteogenesis and hemopoietic induction by bone is still unknown, these considerations identify some of what appear to be the major cellular and biochemical elements in the process, and provide a basis for the design of relevant clinical applications in metabolic bone diseases, hematology, orthopaedics, and oral/maxillofacial surgery.

CLINICAL CONSIDERATIONS RELATED TO THE REGULATION OF BONE MARROW REGENERATION

The multiple factors that assumingly mediate the local and systemic osteogenic reactions to bone marrow insult, including OGP, may be potentially applied for the treatment of conditions requiring stimulation of bone formation such as the healing of large osseous defects, periodontal disease, implant surgery, and particularly osteoporosis. Currently, osteoporosis drug treatment protocols can be divided into the antiresorptive modalities (e.g., estrogen, calcitonin, and bisphosphonates) and formation stimulating therapies, especially PTH and sodium fluoride [Riggs and Melton, 1992]. Clinical studies involving PTH fragments have shown an in-

crease in cancellous bone density accompanied by a significant decrease in cortical bone mass [Parisien et al., 1990]. Similarly, studies with sodium fluoride have suggested increased cancellous bone volume at the expense of the cortical bone compartment [Riggs et al., 1990]. Neither of these treatments has been associated with a significant decrease in the incidence of fractures [Riggs and Melton, 1992]. Therefore a compound such as OGP, which has been shown in experimental animals to increase bone formation and trabecular bone mass systemically, could lead to an attractive antiosteoporotic treatment if the new bone accumulated can be maintained and if it is associated with an improvement in the bone biomechanical properties and a reduced fracture incidence.

The traumatic and surgical disruption of osseous integrity is often associated with disruption of the bone marrow. One can therefore hypothesize that locally produced as well as circulating osteogenic polypeptide factors may be endogenous enhancers of certain aspects of bone repair. For example, massive periosteal injury also stimulates bone formation systemically [Einhorn et al., 1990]. Thus endogenous OGP and molecules with OGP-like activity may regulate cellular processes involved in the formation of membranous bone from the periosteum and endosteum on either side of the fracture gap. Moreover, in elective surgical procedures such as corrective angular osteotomies, spinal fusions, and joint arthrodeses, which are accompanied by the same set of biological responses, the regenerative aspects of marrow healing can be manipulated in order to improve the clinical success. This may be accomplished through further studies on the clinical use of regulatory polypeptides involved in this response as well as their synthetic analogues.

The ability of the bone marrow compartment to form intramedullary cancellous bone has a direct clinical application to modern day joint replacement surgery. Recently, there has been a significant trend towards the development of noncemented techniques for joint replacement arthroplasty [Haddad et al., 1987]. As in dental implantology, these techniques rely on osseointegration to stabilize the prosthesis. The procedure involves the insertion of a metallic implant into the medullary cavity of a long bone. This is followed by a local osteogenic reaction in which the skeleton actually grows into or onto the prosthesis. The extent of this response largely dictates the degree of success of the entire operation [Haddad et al., 1987]. Therefore, not only does this new direction for joint replacement arthroplasty depend on a process similar or identical to postablation marrow regeneration, but the ability to manipulate this process may determine the future of this approach. Ultimately, it would be ideal to regulate bone marrow regeneration in a manner that directs osseointegration to the surfaces between the implant and bone where optimal mechanical stress transfer is desired [Einhorn, 1992].

We now present some final thoughts on the potential application of osteogenic growth polypeptides to enhance the engraftment of bone marrow transplants (BMT). BMT, preceded by ablative radiochemotherapy, would be much more effective if a way is found to accelerate the process of engraftment, enhance marrow reconstruction, reduce the incidence of infection and other medical hurdles, and shorten the hospitalization period [Gabrilove et al., 1988]. The currently available experimental clinical treatment for stimulating post BMT marrow reconstruction consists mainly of the administration of recombinant human granulocyte colony stimulating factor (rhG-CSF) and/or recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) [Blazar et al., 1989]. These cytokines affect directly the proliferation of transplanted pluripotent cells already committed to the white-cell lineages [Vellenga et al., 1987] and consequently decrease the time to leukocyte and neutrophil recovery. There are, however, some major concerns regarding the therapeutic use of rhG-CSF and rhGM-CSF. Tumor and leukemic cells possess normal receptors for these cytokines [Vellenga et al., 1987] and their administration can increase the rates of tumor recurrence by enhancing the proliferation of residual host tumor cells. Another concern about using CSFs in the setting of BMT is that the CSFs, by stimulating the proliferation of relatively committed cells with no capacity for self renewal, deplete the number of earlier progenitor cells. For a similar reason, the CSFs fail to support erythropoiesis and platelet formation.

A prerequisite for hemopoiesis and therefore successful BMT is the presence of functional osteogenic and other stromal cells and tissues that comprise the hemopoietic microenviron-

ment, determine the homing of the injected stem cells from the circulation to the bone marrow, and support hemopoiesis [Watson and McKenna, 1992]. Our as yet unpublished data indicate that the OGP, administered in a manner that stimulates the stromal hemopoietic microenvironment, markedly enhances the engraftment of BMT possibly at the level of the noncommitted stem cells, thus increasing the number of reconstituted red and white cells as well as platelets. Polypeptides with an OGP-like activity may also support hemopoiesis in spontaneously occurring or induced myelosuppression, conditions that do not necessarily involve BMT. Such polypeptides may optimize the microenvironment by upregulating the production of hemopoietic stimulating cytokines in the osteoblast and other stromal lineages.

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