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Role of Vascular Endothelial Cells in Bone Biology

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Abstract Bone development and remodeling depend on complex interactions between bone-forming osteoblasts, bone-degrading osteoclasts, and other cells present within the bone microenvironment. Balanced control of bone formative and degradative processes is normally carefully maintained in the adult skeleton but becomes uncoupled in the course of aging or in various pathological disease states. Systemic regulators of bone metabolism and local mediators, including matrix molecules, cytokines, prostaglandins, leukotrienes, and other autocrine or paracrine factors, regulate the recruitment, differentiation, and function of cells participating in bone formation and turnover. Although some of these interactions are now understood, many yet remain to be elucidated. Recent studies have begun exploring in detail how vascular endothelial cells and their products function in bone physiology. The findings are revealing that bone vascular endothelial cells may be members of a complex communication network in bone which operates between endothelial cells, osteoblasts, osteoclasts, macrophages, stromal cells, and perhaps other cell types found in bone as well. Therefore, multiple systemic and locally produced signals may be received, transduced, and integrated by individual cells and then propagated by the release from these cells of further signals targeted to other members of the bone cell network. In this manner, bone cell activities may be continuously coordinated to afford concerted actions and rapid responses to physiological changes. The bone microvasculature may play a pivotal role in these processes, both in linking circulatory and local signals with cells of the bone microenvironment and in actively contributing itself to the regulation of bone cell physiology. Thus, skeletal homeostasis and the coupling observed between bone resorption and bone formation during normal bone remodeling may be manifestations of this dynamic interactive communication network, operating via diverse signals not only between osteoblasts and osteoclasts but between many cell types residing within bone.

Key words: bone, endothelial cells, osteoclast, osteoblast, cytokines, free radicals

In recent years, it has become increasingly evident that the endothelium functions as more than a simple semipermeable barrier between the blood and the vascular smooth muscle. In fact, the endothelium is properly regarded these days as a highly active endocrine organ central to the local control of vascular regulation through the release of both vasoconstrictor and vasodilatory substances. In addition to regulating macromolecular permeability, endothelial cells also secrete an array of immunoregulatory and neuromodulatory factors that regulate hematopoiesis, hemostasis, differentiation and proliferation of T and B lymphocytes, recruitment of leukocytes at sites of inflammation and tissue injury, coagulation, angiogenesis, and osteoblast-mediated bone formation [Streeten and Brandi, 1990; Ryan, 1990]. In turn, endothelial cells are themselves responsive targets for local regulatory factors and particularly for circulating hormones owing to their direct contact with the blood. That the vasculature plays an important role in bone development and its remodeling is highlighted by the prerequisite for vascularization that precedes osteogenesis and by the special intimate physical relationship which exists both during embryogenesis and growth between the vasculature and osteoblasts that form bone as well as osteoclasts that resorb bone [Favus, 1993]. This closeness affords bone vascular endothelial cells, which may release a diverse complement of regulatory molecules, the opportunity to serve as a local source of bone-modulating factors and to pass on systemic signals that could function in controlling bone cell recruitment, development, and activity.

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VASCULAR ENDOTHELIUM AND BONE DEVELOPMENT

It has long been appreciated that the bone vasculature is important in the development,

remodeling, and repair of bone, but only recently have the molecular mechanisms involved begun to be elucidated [Favus, 1993]. Both intramembranous and endochondral ossification occur in association with blood capillaries. Intramembranous ossification is characterized by capillary ingrowth into a mesenchymal region of embryonic connective tissue, followed by mesenchymal cell differentiation into osteogenic cells that secrete collagen and mineral to produce woven bone. This woven bone is later remodeled into mature lamellar bone. Early in endochondral long bone development, embryonic mesenchymal cells differentiate into chondroblasts that secrete a cartilagenous matrix kept avascular by antiangiogenic substances. As the cartilage model enlarges through continued cell proliferation and matrix deposition, chondrocytes embedded in the central core become compressed, hypertrophy, and calcify the surrounding matrix, ultimately leading to their death and the local degradation of matrix by chondroclasts to form lacunae. Blood vessels and associated perivascular osteoprogenitor cells invade the hypertrophying calcified cartilage region, coalesce to form capillaries, and establish an environment suitable for the differentiation of the stroma into osteoblasts. The osteoblasts proceed to deposit initial bone upon the remaining calcified cartilage spicules. During this process, a tight collaboration apparently exists between the eroding chondroclasts and invading capillary endothelial cells [Lewinson and Silberman, 1992], and osteoclasts are found at the tips of the sprouting capillaries [Streeten and Brandi, 1990]. Eventually, bone is serviced by a nutrient artery and an elaborate vascular network of vessels, capillaries, and blood sinusoids [De Saint-Georges and Miller, 1992]. This vascular network in embryonic tissues appears to coordinate and direct limb development, may serve as a scaffold for bone-forming osteoblasts [Caplan et al., 1983; Drushel et al., 1985], and potentially enables degraded bone components to be passed from the site of resorption via polarized osteoclasts to directly apposed endothelial cells which through the process of transcytosis could deliver them into the bloodstream [Travis, 1993]. Furthermore, since the endothelium is of a mesenchymal origin, exists in close contact with the bone marrow, and shares certain surface antigens in common with bone marrow cells, it has been proposed that the endothelium may be directly capable of giving rise to osteoblasts and hematopoietic progenitor cells [Islam et al., 1992; Trueta, 1963]. These observations as well as the close physical relationship between blood sinusoids and osteoblasts [Pritchard, 1956], the initial formation of osteoclasts in response to specific stimuli from stem cells located adjacent to capillaries [Trueta, 1963], the close proximity of osteoclasts and bone vascular endothelial cells [Jaffe, 1930; Soskolne, 1979], the array of known modulators released from endothelial cells [Ryan, 1990; Mantovani et al., 1990; Seelentag et al., 1987; Yanagisawa et al., 1988; Palmer et al., 1987; Zaidi et al., 1993; Falasca et al., 1993], and the osteopenia or bone necrosis that results from inflammation or disruption of the vascular network [Burkhardt, 1987] all suggest that bone and vascular endothelial cell interactions may play a central role in regulating bone remodeling [Streeten and Brandi, 1990; Zaidi et al., 1993].

Presently, there is only a poor understanding of the interrelationships between angiogenesis, the vascular network, bone formation and remodeling, and immunoregulation in the maintainence of skeletal homeostasis. We clearly need to learn more about the fundamental cellular, biochemical, and molecular mechanisms that govern vascular endothelial cell interactions with other bone cells in order to understand how endothelial cells function in bone development, remodeling, and repair.

VASCULAR ENDOTHELIAL CELLS PRODUCE POTENTIAL BONE ACTIVE MODULATORS

Vascular endothelial cells synthesize and secrete an array of soluble mediators either constitutively or in response to induction stimuli such as injury or inflammation. Among these are growth factors and cytokines such as fibroblast growth factor (FGF), interleukin-1 (IL-1), interleukin-6 (IL-6), and colony stimulating factors (CSFs) of the G, GM, and M subtypes, arachidonic acid metabolites like prostacyclin, small peptides like endothelin-1, and gaseous and free radical messengers like nitric oxide and superoxide anions [Ryan, 1990; Mantovani et al., 1990; Seelentag et al., 1987; Yanagisawa et al., 1988; Palmer et al., 1987; Zaidi et al., 1993; Falasca et al., 1993]. These regulatory molecules have been found in other studies to control the recruitment, proliferation, differentiation, function, and/or survival of various cells including bone-forming osteoblasts and bone-resorbing osteoclasts [Favus et al., 1993; Gowen, 1992; Alam et al., 1992a; Zaidi et al., 1993]. Owing to the close

proximity of vascular endothelial cells to developing and mature osteoblasts and osteoclasts, and their production of such known bone-regulating substances, vascular endothelial cells may be a prime source for the local modulation of bone cell development and activity.

VASCULAR ENDOTHELIAL CELLS RESPOND TO KNOWN BONE MODULATORS

In addition to serving as a source for a wide assortment of mediators, endothelial cells also represent targets for these and other bone active modulators, including systemic hormones in particular owing to their direct contact with circulating blood. Thus, vascular endothelial cells cloned from fetal bovine bone (BBE-1) have been found to respond to parathyroid hormone (PTH), progesterone, estrogen (E_2), insulin-like growth factors types I and II (IGF-I, IGF-II), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and endothelial cell growth factor [Streeten et al., 1989]. Pulmonary artery endothelial cells possess high affinity binding sites for glucocorticoid hormones which are associated with bone loss, and their exposure to dexamethasone leads to the diminished release of prostacyclin [Crutchley et al., 1985]. Dexamethasone has also been found to *disrupt* the normal control of microcapillary invasion into the growth plate cartilage zone of rabbits [Brown et al., 1990]. IL-1, a pleiotropic mediator that is involved in the initiation of the tissue inflammatory/injury response and acts as a potent bone-resorbing agent, activates endothelial cells via autocrine/paracrine pathways to elicit their production of cell surface adhesion proteins, prostacyclin (PGI_2), plasminogen activator inhibitor (PAI), and CSFs [Rossi et al., 1985; Nachman et al., 1986; Seelentag et al., 1987]. IL-1 also causes a reduction in endothelial cell growth in vitro and in vivo, in plasminogen activator activity, and in FGF receptor levels [Cozzolino et al., 1990; Maier et al., 1990]. Another potent bone-resorbing agent, tumor necrosis factor (TNF), causes changes in endothelial cells similar to those engendered by IL-1 [Kettlehut et al., 1987], and both TNF and IL-1 independently induce the synthesis of another bone-resorbing and angiogenic cytokine, IL-6, in endothelial cells [Mantovani et al., 1990]. CSFs can act via autocrine/paracrine circuits to induce endothelial cell migration, proliferation, and plasminogen activator activity [Bussolini et al., 1989; Kozima et al., 1989]. As in other systems, mixed effects may be obtained with the multifunctional mediator transforming growth factor β (TGFβ), dependent upon such parameters as its concentration or the presence of other factors. Thus, TGFβ increases endothelial cell incorporation of fibronectin into the extracellular matrix but inhibits endothelial cell proliferation in response to growth-promoting factors or wounding and decreases their adhesive capacity for blood neutrophils [Gamble and Vadas, 1988]. Although some reports cite the angiogenic nature of TGFβ [Ryan, 1990], others have found that it prevents vascular invasion of capillary cells into collagen matrices [Muller et al., 1987]. Vascular endothelial cells also appear responsive to arachidonic acid metabolites, neuropeptides, and free radicals. For example, prostaglandin E_2 and leukotriene C_4, but not leukotrienes B_4 or D_4, stimulate angiogenesis, endothelial migration, and capillary tube formation [Kanayasu et al., 1989], proliferation of human umbilical vein endothelial cells is stimulated by the bone-resorptive inhibitor and vasoactive neuropeptide calcitonin gene-related peptide (CGRP) with an accompanying increase in cAMP [Haegerstrand et al., 1990], and oxygen radicals induce adhesion proteins on endothelial cells for neutrophil binding and extravasion, regulate the expression of endothelial nitric oxide synthase (the enzyme responsible for the production of nitric oxide from L-arginine), and combine with nitric oxide to abrogate its vasodilatory effects [Patel et al., 1991; Ryan, 1990]. Clearly, many of the same systemic and local mediators that are known to affect osteoblasts, osteoclasts, and bone remodeling derive from and/or are active upon endothelial cells as well, leading to the hypothesis that bone vascular endothelial cells may be essential contributing members of the intricate and multitiered communication pathways operative in bone that link diverse cell types via diffusible signalling molecules, and perhaps by cell contact-mediated mechanisms as well.

COMMUNICATION NETWORKS LINKING VASCULAR ENDOTHELIAL CELLS, OSTEOBLASTS, OSTEOCLASTS, AND BONE REMODELING

A prodigous literature exists that describes our current understanding of the complex, overlapping, synergistic or antagonistic, and temporal actions of individual hormones and local mediators on osteoblasts, osteoclasts, bone mar-

row stromal and hematopoietic cell populations, and bone remodeling both in vivo and in vitro, and this information has been well summarized in recent reviews [for example, Gowen, 1992]. The following discussion is focused therefore only on selected potential intercellular communication pathways in bone that highlight mechanisms for vascular endothelial cell involvement in bone physiology.

Bone vascular endothelial cells may contribute to the regulation of both bone-formative and bone-resorptive processes via modulation of the precise complement of mediators they release either constitutively or in response to various stimuli (developmental, tissue injury, inflammatory, physical, or other). Thus, endothelial cells implanted into diffusion chambers with fetal calvarial cells greatly enhance their bone-forming ability [Villanueva and Nimni, 1990], and bovine aortic endothelial cells have been shown to synthesize a potent bone active mitogen which may promote bone formation [Guenther et al., 1986]. In addition, endothelial cells produce angiogenic-promoting FGFs which elicit mitogenic responses (and depressed differentiative properties) in calvarial organ cultures and bone nodule formation associated with osteoblast-like cells in bone marrow cultures [Rodan, 1992]. Endothelin-1, a potent vasoconstictor first identified as a product of vascular endothelial cells, has been shown to stimulate cell proliferation and inositol phosphate signalling in MC3T3-E1 and UMR-106 osteoblast-like cells [Tatrai et al., 1992], inhibit osteoclast bone resorption through a reduction in osteoclast cellular motility [Alam et al., 1992b], and function both as a catabolic (resorption) and anabolic (collagen synthesis) agent in bone organ cultures [Tatrai et al., 1992]. Endothelial cells also release the cytokine IL-1, a multifunctional inflammatory mediator whose levels appear high in the synovial fluids and circulation of rheumatoid arthritic and osteoporotic patients, respectively. IL-1 is a marked stimulator of osteoclast progenitor proliferation, differentiation, and bone-resorptive activity, while it has been reported to inhibit osteoblast-mediated bone formation and to induce osteoblasts to produce M-CSF, thereby enhancing osteoclast precursor growth and differentiation [Gowen, 1992]. Furthermore, IL-1 autocrine (or paracrine) action upon endothelial cells has been described as resulting in their release of the short-lived arachidonic acid metabolite prostacyclin which has been shown to stimulate osteoclast activity [Rossi et al., 1985], and of IL-6 [Mantovani et al., 1990] and CSFs [Seelentag et al., 1987], which might enable endothelial cells to participate in the regulation of osteoclast proliferation and differentiation from hematopoietic precursor cells [Gowen, 1992]. Lastly, endothelial cell release of other short-lived messenger molecules, namely reactive oxygen species and nitric oxide (NO), may help to control osteoclast activity since oxygen radicals appear to enhance resorption, whereas NO has been shown to interfere with osteoclast bone resorption [Alam et al., 1992a; Zaidi et al., 1993] and inhibitors of the enzyme responsible for NO synthesis potentiate osteoclast bone resorption [Kasten et al., in press]. Nor is such communication envisioned to be undirectional; osteoblasts, and recently osteoclasts, have been reported to similarly release cytokines and other regulatory molecules that could affect endothelial cells. At a level beyond the local bone microenvironment, all of these signalling events are subject to the overall influences of systemic modulatory hormones, such as estrogen, progesterone, PTH, and glucocorticoids, which may target any or all of these cells to modify their synthesis, secretion, and/or responsiveness to local cytokines and other immunoregulatory stimuli.

CONSIDERATIONS FOR THE FUTURE

To fulfill its mechanical and metabolic functions, bone needs to be highly sensitive to fluctuating physiological demands while at the same time maintaining a homeostatic balance. As a consequence of these sometimes opposing forces, the cells associated with bone must be able to rapidly respond in coordinated fashion to changes in systemic and local cues. The bone vascular endothelium may serve a pivotal role in this regard, being in direct contact with circulating blood on the one hand and closely associated with osteoblasts, osteoclasts, and perhaps other cells of the bone microenvironment on the other. Apart from its essential role in bone development, whether the bone vascular endothelium actually represents both a source and target for diverse bone-acting systemic and hormonal modulators or gives rise to osteoblasts or hematopoietic precursor cells are central questions in need of resolution for understanding the role of the bone microvasculature in the complex processes of normal bone remodeling and repair, as well as its possible participation in pathological bone disorders. It is conceivable that the endo-

thelium functions as an active bidirectional conduit for circulating hormonal signals like estrogen, PTH, vitamin D_3, and glucocorticoids to bone cells while conveying bone-degradative products (signals?) from the resorption site into the bloodstream. Furthermore, if indeed bone vascular endothelial cells respond to hormonal (estrogen, PTH, glucocorticoids) and local bone-acting mediator signals, as well as release various cytokines, small molecules, or other modulators that can influence bone cell recruitment, growth, differentiation, and physiology, then the bone vascular endothelium must be considered an integral component of the bone cell communication network. These issues need to be carefully addressed experimentally and the molecular mechanisms of interdependent intercellular interactions defined in detail. Such interactions are further complicated by emerging evidence which suggests that vascular endothelial cells, osteoblasts, osteoclasts, and marrow stromal populations are heterogeneous in nature with respect to their morphology, physiology, and functional attributes. Normal skeletal homeostasis and local bone coupling (formation/resorption) are therefore likely to be manifestations of this dynamic interplay involving systemic regulators of bone metabolism and local mediators, operating amongst a community of highly interactive and diverse bone cell types. Perturbations of this communication network involving the bone microvasculature may be implicated in the imbalances observed between bone formation and resorption in association with such conditions as osteoporosis, periodontal disease, Paget's disease, and other pathological bone disorders.

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