Mineral Exploration: Search for the Mechanism of Vascular Calcification and Beyond
The 2003 Jeffrey M. Hoeg Award Lecture

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Abstract—Research in the area of vascular calcification has grown rapidly in the past decade, and there is a greater understanding of its active regulatory mechanisms. This brief review covers the ideas presented in the 2003 Jeffrey M. Hoeg Award lecture, including the concepts that bone tissue forms in the artery wall in patients with atherosclerosis, that vascular cells undergo osteoblastic differentiation, that bone morphogenetic protein and matrix GLA protein regulate vascular calcification in opposition, that inflammatory cytokines and lipids promote vascular cell calcification but inhibit osteoblastic cell differentiation, that these same factors promote differentiation of bone-resorbing osteoclasts, and that the artery wall may contain osteoclast-like cells with the potential to resorb calcium mineral. The review closes with a mention of therapeutic possibilities and an evolutionary paradigm to explain the reciprocal responses of vascular and bone mineralization to inflammation. (Arterioscler Thromb Vasc Biol. 2003;23:H18546–H18558.)

Key Words: calcification • vascular • smooth muscle • differentiation • bone

Approximately 10 years ago, I had the pleasure of working with Dr Jeffrey Hoeg and sharing his enthusiastic, collaborative approach to the science of vascular biology. This presentation, dedicated to his memory, provides an account of the events leading to development of a relatively new field in vascular biology, the study of vascular calcification, which also has become a new field in bone biology. This review addresses evidence that vascular cells have the potential for osteoblastic differentiation and that chronic inflammation regulates bone cell differentiation and may account for the epidemiological links among lipids, vascular calcification, and osteoporosis.

Vascular calcification first came to my attention in my studies of atherosclerotic plaque mechanics. Together with Drs Craig Hartley and Avi Jain, I was recording instantaneous balloon pressure and volume during human coronary angioplasty. The real-time stress-strain curves showed fracture patterns in most of the stenotic lesions, but not in those without plaque calcification. Such mechanical events were reproduced in calcified human arteries from autopsy and calcified rabbit aortas in vivo, but not in noncalcified aortas, even when advanced atheromatous disease was present. This suggested that calcification was required for mechanically detectable plaque fracture during angioplasty. The pathologist who reviewed the autopsy specimens pointed out, as an aside, that the calcium deposits in some of the arteries not only contained complete bone tissue but also bone marrow and fat tissue and that these were not unusual findings. The mystery of how a new tissue, bone, could form within another tissue, the artery wall, captured my imagination.

In some respects, the field of vascular calcification is not new but rediscovered. In the 1700s and 1800s, pathologists observed that sclerotic arteries underwent ossification and not merely calcification. But for most of the 20th century, vascular calcification was widely dismissed as a passive, degenerative, irreversible, end-stage process of aging. Ironically, the same terms had been used to discount atherosclerosis itself in the early 1900s. The prevalent view had been that calcium and phosphate simply crystallized out of solution into plaque, similar to mineral deposition in plumbing. A few early findings pointed to a relationship between vascular calcification and osteogenesis, including the demonstration of matrix vesicles, similar to those in bone, in calcified human aorta; the identification of the mineral as hydroxyapatite, not merely amorphous calcium phosphate; and the demonstration that microvascular pericytes produce mineralized matrix in vitro. With development of clinical electron beam computed tomography, it became apparent that coronary calcification is not an end-stage event but is widespread even in early disease and predictive of cardiac events. Calcium deposits also affect the aorta, peripheral vessels, and cardiac valves. Even the pulmonary artery and veins may calcify when exposed to abnormally high pressures. Whether the association with risk merely reflects a correlation with plaque burden or whether mineralization directly affects plaque stability is currently controversial. Interestingly, patients with end-stage renal
disease undergoing hemodialysis rapidly develop advanced, diffuse mineralization and have high cardiovascular event rates despite relatively mild atheromatous burden. This suggests that vascular calcification itself contributes to cardiovascular risk, possibly through effects on plaque stability.

Bone in the Artery Wall

Most calcium deposits in arteries are amorphous, and whether these are precursors to ossified plaque is not established. Just as fatty streaks are believed to precede mature atherosclerotic plaques, the connection is based on occurrence of lesions containing transitional stages. The progression from amorphous mineral to ossification follows the same stages as embryonic endochondral ossification. The earliest stage is an acellular, mineralized matrix. This matrix is partially replaced with osteoid, which undergoes remodeling as neoangiogenic vessels invade. Finally, mature bone tissue forms as the osteoid mineralizes (Figure 1).

Similar steps are required for embryonic development and maturation of bone tissue in the skeleton. In the embryonic skeleton, most bones form by endochondral ossification. First, a scaffold of acellular mineralized matrix is produced by chondrocytes. As the chondrocytes undergo physiological maturation, they undergo hypertrophy and then apoptosis, leaving behind matrix vesicles and apoptotic bodies that nucleate calcium phosphate crystals, producing calcified cartilage. Next, this acellular calcified cartilage is remodeled by chondroclasts and invaded by neoangiogenic vessels. Pericytes accompanying the neovessels differentiate into osteoblasts and deposit osteoid around the vessel channels. When osteoid undergoes mineralization, mature bone tissue is formed, complete with vascular canals and marrow spaces.

Human atherosclerotic lesions that contain mature bone tissue are usually anchored to amorphous mineralized matrix. A smaller number also contain cartilage tissue, suggesting that the amorphous mineral serves as a scaffold for the mature bone. Thus, it is expected that amorphous calcium mineral deposits be more common than mature bone, just as fatty streaks are more common than mature atherosclerotic plaque.

Osteoblastic Differentiation of Vascular Cells

Studies of the cellular and molecular mechanisms of vascular calcification have been possible because of recently developed in vitro models. Each of these models has confirmed that vascular cells undergo osteoblastic differentiation. Schor et al first showed in vitro vascular calcification in nodules produced by microvascular pericytes. Giachelli et al used immature vascular smooth muscle cells and found expression of osteopontin. Bostrom et al showed, by dilutional cloning, that a subpopulation of bovine aortic smooth muscle cells is responsible for mineralization. The mineralization in these cells occurs in 3D aggregates that resemble the calcified nodules that form on human calcific aortic valves. Based on the cloning results, ~10% to 30% of cells from smooth muscle cell cultures have this capacity, and they retain it through passaging. These clonal derivatives have been named calcifying vascular cells, and they express osteoblastic differentiation markers with a time course similar to that described by Stein and Lian for rat osteosarcoma cells. These findings support the concept that vascular calcification is mediated, at least in part, by osteoblastic differentiation of a subpopulation of artery wall cells. Several regulatory mechanisms have been proposed (Figure 2).
In vivo, Bostrom et al. demonstrated expression of the potent bone differentiation factor, bone morphogenetic protein-2 (BMP-2), in human calcified plaque as well as in calcifying vascular cells. A variety of bone matrix proteins and regulatory factors have now been demonstrated in human calcified plaque, including osteocalcin, bone sialoprotein, osteonectin, collagen I, alkaline phosphatase, Mxs-2, and Cbfa-1. Importantly, Doherty et al. showed that cultured vascular pericytes also have osteogenic and chondrogenic potential when implanted subcutaneously in diffusion chambers.

Matrix GLA Protein and Bone Morphogenetic Protein
A major regulator of vascular calcification was discovered by bone biologists who generated a mouse deficient in matrix GLA protein (MGP), a component of cartilage. Although the skeletal phenotype was not dramatic, the homozygous mice had complete ossification of the aorta and its branches; the smooth muscle cells of the aortic medial layer were replaced with chondrocytes in calcified cartilage. This finding suggested that MGP inhibits vascular calcification, an unexpected result because it was previously shown that MGP expression is higher in calcified human plaque. One explanation is that MGP may be induced in response to calcification as a negative-feedback mechanism. Although some evidence suggests that MGP acts by direct inhibition of calcium crystal formation, it is difficult to account for the replacement of aortic smooth muscle with calcified cartilage on that basis alone. Other evidence suggests that MGP regulates differentiation by inhibiting BMP-2. BMP-2, which is produced by endothelial cells, regulates whether mesenchymal progenitor cells differentiate along smooth muscle, chondrocyte, osteoblastic, or adipocytic lineages. Higher levels of BMP-2 activity favor chondrogenic differentiation. Thus, unopposed BMP-2 activity would explain the findings in the MGP-deficient mice.

Mesenchymal progenitor cells that would normally differentiate into smooth muscle cells of the aortic media develop instead into chondrocytes because of unopposed BMP-2 activity from the endothelium.

Inflammatory Lipids and Vascular Calcification
Epidemiological studies indicate a relationship between hyperlipidemia and vascular calcification. Coronary calcification correlates with serum LDL independently of age and inversely with HDL. Cardiac valve calcification progression also correlates with serum LDL. Vascular calcification also colocalizes with atherosclerosis. In animals, a high-fat diet induces bone matrix vesicles and renal glomerular calcification. Although regression of calcification has not been definitively established, lipid-lowering treatment seems to inhibit coronary calcification in monkeys and in humans.

Inflammatory stimuli promote vascular cell calcification in vitro. Inflammatory lipids, such as minimally oxidized LDL and isoprostaglandin E2, as well as inflammatory cytokines, such as tumor necrosis factor-α and activated monocyte-macrophages, promote expression of the osteoblastic marker, alkaline phosphatase, and mineralization. In vivo, hyperlipidemia promotes aortic calcification in mice.

Inflammatory Lipids and Osteoporosis
Clinical studies suggest a relationship between serum lipid levels and osteoporosis. Some retrospective studies suggest that lipid-lowering treatment reduces fractures, but prospective studies have not confirmed this. Clinical studies also show that a relationship between aortic calcification correlates with osteoporosis that is independent of age, supporting the possibility that inflammatory lipids promote both vascular calcification and osteoporosis.

Osteoblastic Differentiation
Paradoxically, the same inflammatory lipids and cytokines that promote osteoblastic differentiation of vascular cells have the opposite effect on osteoblast precursors from bone. It is not intuitively obvious that preosteoblasts would be exposed to inflammatory lipids from the blood. However, bone is a vascular organ, and osteoblast precursor cells are located immediately adjacent to the subendothelial space of arteries in the cortical bone Haversian canals and of sinusoidal vessels in the trabecular bone marrow spaces. Just as lipid deposit in the subendothelial space of atherosclerotic arteries, they also deposit in the subendothelium of osteoporotic bone. In mice, prolonged hyperlipidemia suppresses differentiation of bone marrow preosteoblasts and diverts differentiation toward the adipogenic lineage.

Osteoclastic Differentiation
Osteoporosis results not only from depressed osteoblastic activity but also from increased osteoclastic resorptive activity. Like macrophages, osteoclasts derive from monocytes under the influence of monocyte-colony stimulating factor, but they also require receptor activator of nuclear factor-κB ligand. These factors are normally produced by the osteoblasts or marrow stromal cells adjacent to osteoclasts. It is well-known that chronic inflammation promotes osteoclastic activity, leading to inflammatory osteolysis of rheumatoid arthritis and osteomyelitis. Inflammatory lipids also enhance osteoclastic activity in vitro. In vivo, because oxidized lipids are known to trigger endothelial release of monocyte chemoattractant protein-1 and monocyte-colony stimulating factor, deposition of oxidized lipids in the bone’s subendothelial spaces would be expected to trigger both recruitment and differentiation of monocytes. However, in the presence of receptor activator of nuclear factor-κB ligand in the bone environment, instead of becoming macrophages and foam cells, the monocytes would differentiate into osteoclasts. By such a mechanism, hyperlipidemia may contribute to osteoporosis.

Osteoclastic Potential in the Artery Wall
Calcified human atherosclerotic lesions contain cells with histological features of osteoclasts, including immunoreactivity for cathepsin K and tartrate-resistant acid phosphatase. If functional osteoclast-like cells are present within the artery wall, it is conceivable that interventions that activate vascular...
osteoclastic cells have potential use as therapies to reverse or prevent vascular calcification.

**Vascular Calcification in End-Stage Renal Disease**

Why dialysis or end-stage renal disease is a risk factor for vascular calcification remains uncertain, but several possible contributing factors have been proposed, including high phosphate levels, vitamin D supplementation, warfarin, high calcium-phosphate products, and secondary hyperparathyroidism. Jono et al have provided evidence that the high serum phosphate levels seen in dialysis patients may trigger the sodium-dependent phosphate cotransporter Pit-1, which signals through Cbfa-1 to induce osteoblastic differentiation of vascular cells. Other evidence suggests a role for the calcium-sensing receptor. Another potential mechanism is inadequate levels or activity of mineralization inhibitors, such as fetuin-A, which is reduced in patients undergoing dialysis, or MGP. Warfarin is used in these patients to maintain patency of arteriovenous shunts, but it may promote vascular mineralization by blocking vitamin K-dependent carboxylation of the matrix GLA protein. It seems that γ-carboxylation is required for MGP to inhibit mineralization by binding BMP-2. Thus, the role of dietary vitamin K is under active investigation.

**Teleological Speculation**

The opposite responses of artery versus bone mineralization to chronic infection may seem to violate nature’s conservation of mechanisms. One possible explanation lies in the strong evolutionary pressure of chronic infection. Cell walls of bacteria and other microbes are rich in lipids that are oxidatively modified by radicals released by activated leukocytes participating in the immune response. Oxidized lipids may accumulate in tissues around the infection. In prolonged infectious processes, when the primary immune response fails, the connective tissue environment seems to undergo protective physical changes as a secondary response. For example, in the skin, a scar or callous forms at a site of repetitive injury. Similarly, in bone, mineral matrix undergoes lysis at sites of chronic osteomyelitis. This inflammatory osteolysis is mediated by inflammatory cytokines that inhibit osteoblastic bone formation and promote osteoclastic resorption. This response would serve to eliminate the substrate on which the microbial infection is thriving. Conversely, in chronic infection or inflammation of soft tissue, such as tuberculosis, helminthic infection, or foreign body reaction, the soft tissue is often remodeled into ectopic cartilage or bone, under the influence of inflammatory cytokines. These responses to chronic infection may be inappropriately activated in the inflammatory response to hyperlipidemia. It is well-known that dietary lipids also accumulate and undergo oxidation in tissues. The resulting modified lipids and lipoproteins may mimic microbial lipids and trigger osteolysis and ectopic bone. Thus, lipid deposition in the subendothelial matrices of arteries and bone tissue may account for the hardening of soft tissue and softening of hard tissue that we associate with age.

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**References**


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