

Bones, Blood Vessels, and the Immune System: What's the Link?



Since the discovery of the tumor necrosis factor (TNF) family members osteoprotegerin (OPG), osteoprotegerin ligand (OPGL), and RANK (receptor activator of nuclear factor- κ B), much has been learned regarding the role of these 3 molecules in regulating the skeletal and immune systems. In recent years their role as potential mediators of vascular function has generated much interest. In particular, there has been much research into the role of this molecular pathway in vascular calcification. Calcification is a normal physiologic process in certain tissues, but is pathologic in others. Calcification is part of the normal homeostatic balance in bone. On the other hand, calcification of extracellular matrix in tissues that do not normally mineralize can lead to serious pathology. In this issue of *The Journal*, Simonini and colleagues investigate serum OPG concentrations in patients with Kawasaki disease¹ (KD), the most common cause of multisystem vasculitis in children and now the number one cause of acquired heart disease in the developed world². These authors report that children with KD had significantly higher levels of OPG in their serum compared to other children, including those with fever due to infectious causes and those with systemic lupus erythematosus, as well as healthy nonfebrile controls. In the small number of children who developed coronary artery lesions as a result of KD, the authors found a higher serum level of OPG compared to those without aneurysms.

Osteoprotegerin ligand, also known as RANK ligand (RANKL), is a critical regulator of bone remodeling via its key role in promotion of osteoclastogenesis³⁻⁶. OPG is a soluble decoy receptor for OPGL, neutralizing its ability to bind with RANK and induce a signal. This signaling pathway also plays an important role in the immune system, with OPGL functioning in costimulation, T cell–dendritic cell communication, and dendritic cell survival^{7,8}. In addition to its activity in the RANK system, OPG also binds as an antagonist to TRAIL (TNF related apoptosis-inducing lig-

and), another member of the TNF superfamily. TRAIL is produced by cells of the immune system and participates in the regulation of cell cycling, inducing cell death via interaction with TRAIL receptors⁹. Thus OPG can act as an antagonist in 2 independent molecular systems.

The OPG/OPGL/RANK pathway as a molecular link between the immune system, bone metabolism, and vascular biology was first suggested in mice with genetic modifications in these genes. In addition to early onset osteoporosis, OPG knockout mice develop medial calcification of their muscular arteries, including the renal and aortic arteries. These are sites of abundant endogenous OPG expression in normal animals^{10,11}. These results pointed to OPG as a factor possibly linking bone loss and vascular calcification, and implied a potential role in vascular protection¹². Interestingly, the ligand and receptor, which are normally undetectable in normal murine arteries, were expressed abundantly in the calcified arteries of OPG knockout mice. OPG rescue experiments revealed differences in the regulation of bone and vascular calcification. Continuous administration of exogenous OPG to mice during mid-gestation through to adulthood prevented vascular calcifications, whereas transient injections of OPG only during the postnatal period could not reverse vascular calcification, but were able to change the osteoporotic bone phenotype¹³.

Vascular endothelial cells are important cellular mediators of the inflammatory response, and may be one of the links between the immune system and vascular pathology including intravascular calcification. Vascular calcification can involve differentiation of osteogenic cells from both vascular smooth muscle cells and calcifying vascular cells¹². A number of molecules are involved in regulating the development of these cells, including members of the OPG/OPGL/RANK pathway. Their well described roles in the immune response and in bone metabolism may also have important secondary effects on the vascular system in

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addition to its direct activity on vascular cells to influence their differentiation¹².

Vascular calcification, especially calcification of the arteries, often occurs with atherosclerosis, advancing age, and certain medical disorders including renal disease and diabetes mellitus and some genetic diseases¹⁴. Interestingly, serum OPG levels are correlated with increasing age, diabetes, and chronic renal failure¹⁵⁻¹⁷. It is not clear if circulating levels of OPG are involved directly in promoting vascular calcification or are a protective response to counteract the calcification process¹². OPG plays a role in both the RANK and TRAIL receptor signaling pathways, with the potential of mediating vascular pathology both dependent on, and independent of, OPGL. As well, OPG mediates integrin-dependent survival of endothelial cells by blocking TRAIL receptor signaling, directly opposite to its effect in the RANK pathway, which, when inhibited from binding OPGL, favors programmed cell death in osteoclastic precursors^{9,18}.

OPG functions as a decoy receptor for OPGL, competing with RANK for binding with OPGL; thus evaluation of OPG levels must go hand in hand with OPGL levels, as the balance of the 2 will determine which physiologic activity prevails. This evaluation may not be a simple one, as OPG is secreted as a homodimer, not a molecular trimer, unlike others in the TNF molecular family including OPGL¹⁹. OPGL is made by activated T cells and its expression is upregulated by many soluble factors affecting bone resorption, including the proinflammatory cytokines interleukin 1 (IL-1) and TNF- α ²⁰. T cells express a cell-surface, membrane-bound OPGL, which is cleaved by metalloproteinases into a soluble form²¹. Interestingly, TNF- α and IL-1 upregulate both the ligand (OPGL) and its antagonist (OPG)²². In addition to proinflammatory cytokines, OPG is strongly upregulated by platelet derived growth factor, and also by stimulation with basic fibroblast growth factor or angiotensin II, all important regulators of vascular pathology^{12,23}. One of the major inhibitors of OPG production and activity is transforming growth factor- β ²⁴.

Calcification of the coronary arteries as a result of KD is a well recognized but infrequent longterm complication of coronary artery aneurysms^{25,26}. Calcifications of the coronary arteries can be seen on plain radiographs, as well as during coronary artery angiography. Ring shaped calcification may be associated with severe stenosis of the affected arterial segment²⁵. Severe coronary artery calcifications can occasionally be seen by chest radiograph, and attempts at earlier interventions to visualize microcalcification using high resolution computer tomography are under way²⁷. Stenosis of affected coronary artery segments in KD is often associated with severe vascular calcification, which differs from atherosclerosis²⁸ and may have important clinical implications. Rotational ablation appears to have a higher longterm success rate compared to balloon angioplasty in

patients with calcified coronary artery stenosis secondary to KD²⁸.

A persistent inflammatory infiltrate and associated vascular changes in the heart can be found in children many years after acute KD. Inflammatory cells consisting of lymphocytes are found in endomyocardial biopsies in 43% of children post-KD. Fifty-four children were biopsied with a median time of biopsy at 7 years, with a range of 2 months to 23 years. An even higher percentage (63%) had marked microvascular changes involving the capillaries, arterioles, and small arteries. These percentages included patients with persistent coronary artery lesions and those in whom the abnormalities resolved²⁹. The persistent elevation of serum OPG reported in the study by Simonini and colleagues, even at 3 months after onset of KD, lends support to mounting evidence that inflammation and vascular damage persist long after resolution of the clinical signs and symptoms of disease, and are more sensitive than traditional laboratory markers of inflammation in the peripheral blood. Increased investigation of the OPG family of molecules may open the door to understanding the link between our immune system, bones, and blood vessels.

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