Journal of the American Heart Association

EDITORIAL

A Potential New Link Between Inflammation and Vascular Calcification

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Inflammation and inflammatory cytokines are so closely associated with atherosclerosis and vascular calcification (VC) that the latter conditions may be more aptly termed *atheroscleritis* and *calcific vasculitis*. The association between inflammation and ectopic calcification is also observed prominently in diabetes,[1] calcific tendinitis,[2] and inflammatory bowel disease.[3] One possible exception is in the case of chronic kidney disease, where inflammation is less dominant but calcification is extreme. The calcification in chronic kidney disease also differs in location—the medial layer of the artery wall, unlike in atherosclerosis,[1] where inflammation and calcification occur primarily on the opposite side of the elastic lamina—in the neointima. While circulating levels of systemic biomarkers of inflammation are known to correlate with atherosclerosis and increased cardiovascular risks, more recent studies have also revealed the important roles of local inflammatory cytokines in regulating cell phenotypic transition, osteogenic differentiation, and vascular calcification.[1] Colocalization of inflammation with calcification, based on markers for both macrophages and osteogenesis, was shown in the atherosclerotic aortas of hyperlipidemic apolipoprotein $E^{-/-}$ mice.[4] Clinical evidence that inflammation precedes calcification was provided by ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography scans of >100 patients in a longitudinal study, where uptake of ^{18}F-labeled glucose (an indicator of metabolic activity likely to be inflammation) preceded radiographically detectable calcification in the aortic valve ring.[5] However, it remains possible that radiographically undetectable calcium crystals may precede and even cause inflammation, given the example of gout, where it is well established that calcium pyrophosphate crystals cause painful inflammation. In any case, whatever the cause, no pharmacological treatments have been clearly established to reduce VC in humans.

See Article by Hao et al.

In this issue of the *Journal of the American Heart Association* (*JAHA*), Hao et al[6] provide evidence that expression of interleukin-29 is upregulated in calcified carotid arteries from patients with coronary artery disease and chronic kidney disease. The authors also show that expression of interleukin-28a, the human interleukin-29 paralogue in mice, and its receptor interleukin-28Ra are increased in the calcified aortic tissues of the vitamin D_3–induced mouse model of VC. Notably, all type III interferons, including interleukin-29 and interleukin-28a, bind to the same heterodimeric complex that comprises interleukin-28Ra and interleukin-10R2 subunits.[7] Using vascular smooth muscle cells and ex vivo rat aortic ring cultures in high phosphate or osteogenic media, Hao et al[6] demonstrate that interleukin-29, which is known to activate the Janus kinase–signal transducer and activator of transcription signaling pathway,[8] promotes osteogenic differentiation and calcification. Conversely, Hao et al[6] showed that small-molecule inhibitors antagonizing interleukin-29 and its downstream Janus kinase–signal

Key Words: Editorials ■ cytokines ■ osteogenic differentiation ■ smooth muscle cells ■ vitamin D

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transducer and activator of transcription-3 signaling pathway attenuate these processes. Although presumably from the inflammatory cells, it would be of great interest to identify the source of interleukin-29.

The Janus kinase–signal transducer and activator of transcription signaling pathway is activated by several other cytokines and growth factors,[9] and it regulates many cell functions, including tissue repair. Interleukin-29 is the most potent and abundant proinflammatory cytokine belonging to the type III interferon-gamma family, produced by maturing dendritic cells and macrophages.[7,10] Interestingly, interleukin-29 has been shown to play a role in pro- as well as anti-cancer effects by regulating proliferation and apoptosis.[10] A prior study, showing activation of signal transducer and activator of transcription-3 during the spontaneous osteoblastic differentiation of vascular smooth muscle cells and induction by interleukins, such as interleukin-6,[11] is consistent with the findings of Hao et al,[6] who further delineate the downstream regulation of interleukin-29 on acceleration of vascular smooth muscle cell calcification via induction of osteogenic markers, bone morphogenetic protein-2 (BMP-2) and Runt-related transcription factor 2, and downregulating smooth muscle markers alpha-smooth muscle actin and smooth muscle protein 22α.

Vitamin D Model

In this study by Hao et al,[6] and several others, rodents treated short term with high doses of vitamin D have been used as a model to study the mechanisms of VC. This model has been questioned on the basis that hypervitaminosis D may induce calcification through an entirely different mechanism, and over a longer time scale than operates in human calcific disease. For instance, inflammatory cells are not usually described in such models. The present finding of Hao et al,[6] that the inflammatory cytokine interleukin-29 has an in vivo role in this model, suggests that at least 1 inflammatory cytokine may be involved.

Mechanical Considerations

Ectopic calcification is a natural component of tissue injury response. For instance, it is activated in tuberculosis, foreign body reactions, breast cancer, and helminthic parasitic diseases. This may represent an ultimate form of immune defense, where unwelcome organisms and objects that resist acute and chronic conventional humoral and cellular immune defenses may be eventually subject to containment by a wall of ectopic bone mineral. Thus, on a plain film, a hydatid cyst on radiograph may look like an ostrich egg, and the parasite may be destroyed by loss of nutrients. In the artery wall, accumulations of partially oxidized lipid nanoparticles (low-density lipoprotein) may closely resemble bacterial organisms that have resisted conventional immune defenses, and vascular calcification may represent an incomplete and unsuccessful effort of the surrounding cells to produce enough mineral to encompass the lipid deposits. Unfortunately, the presence of rigid mineral deposits within the soft tissue concentrates the ordinary circumferential and longitudinal mechanical stresses, which may result in debonding because of compliance mismatch. This separation of the mineral from the soft tissue may result in intraplaque or intramural hemorrhage or plaque rupture, any of which can cause myocardial infarction.

Potential Anti-Inflammatory Therapeutics

Some treatments that block inflammatory pathways and cytokines have been reported to ameliorate cardiovascular events, such as the anti-inflammatory colchicine as well as inhibitors of tumor necrosis factor-alpha and interleukin-1-beta, but not low-dose methotrexate.[12–15] However, none of these clinical studies has identified the cardioprotective effect as attributable to any action on VC. It is possible that the effects of anti-inflammatory agents are independent of VC, or are despite promotion of VC, For example, anti-inflammatory agents may confer their benefit by preventing plaque rupture through prevention of inflammatory cell infiltration and their release of destructive metalloproteinases in the fibrous cap. Alternatively, they may confer cardioprotective benefits despite promoting calcification, such as with statins, which are known to have anti-inflammatory effects and reduce cardiovascular events, but concurrently accelerate progression of coronary artery calcification.

Potential Other Therapeutics

Some approaches are available to directly block VC without affecting inflammation. Potential targets include both positive and negative regulators of calcification that are produced by vascular cells—the positive ones, including tissue nonspecific alkaline phosphatase, BMP-2, Runt-related transcription factor 2; and the negative including klotho, matrix gamma-carboxyglutamic acid protein, pyrophosphate, and osteoprotegerin.[1] Deficiency of klotho leaves the master osteoinductive transcription factor BMP-2/Runt-related transcription factor 2 signaling pathway unopposed, thus driving calcification.[16] Predicting the outcome of treatment with inhibitors is complicated by the negative feedback mechanisms involved in the signaling processes, especially with respect to BMP-2.[17] Therapies targeting these regulatory factors are still under investigation, but they have not yet been shown to slow VC progression in humans. For instance, vitamin K supplementation has been proposed as a treatment on the basis of 3

lines of evidence: Vitamin K activates matrix gamma-carboxyglutamic acid protein, which is a known inhibitor of BMP-2; the matrix gamma-carboxyglutamic acid protein–deficient mouse develops extensive vascular calcification; and warfarin, a widely used anticoagulant that blocks vitamin K activation of matrix gamma-carboxyglutamic acid protein, accelerates VC.[18] Unexpectedly, double-blinded clinical trials of vitamin K supplementation failed to hinder progression of aortic valve calcification in patients with aortic stenosis[19] or of VC in patients with chronic kidney disease.[18] Another potential therapeutic approach under investigation is inhibition of tissue nonspecific alkaline phosphatase. In the preclinical animal model, apolipoprotein $E^{-/-}$ mice, a small-molecule inhibitor of tissue nonspecific alkaline phosphatase was shown to prevent atherosclerotic calcification without impacting skeletal bone; in addition, it reduced atherosclerosis and serum levels of cholesterol and triglycerides by decreasing dephosphorylation of phosphocholine in the liver.[20]

VC is associated with substantial morbidity and mortality, yet there is insufficient evidence of causality to ensure that blocking it would prevent cardiovascular events any more than correcting earlobe creases would.[21] Development of agents that prevent or reverse cardiovascular calcification will be important for determining the true consequences of VC, but their use as therapeutic agents may need to wait for establishment of a causal link to cardiovascular events and for exclusion of potential adverse effects on skeletal bone.

ARTICLE INFORMATION

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Sources of Funding

This work was funded by grants from the National Institutes of Health; Heart, Lung, and Blood Institute to HL137647, HL151391 (YT and LLD), and Tibor Fabian Research Award at UCLA (XC).

Disclosures

None.

REFERENCES

1. Demer LL, Tintut Y. Inflammatory, metabolic, and genetic mechanisms of vascular calcification. *Arterioscler Thromb Vasc Biol*. 2014;34:715–723. doi: 10.1161/ATVBAHA.113.302070
2. Mihmanli I, Karaarslan E, Kanberoglu K. Inflammation of vertebral bone associated with acute calcific tendinitis of the longus colli muscle. *Neuroradiology*. 2001;43:1098–1101. doi: 10.1007/s002340100644
3. Mantaka A, Galanakis N, Tsetis D, Koutroubakis IE. Abdominal aortic calcification in patients with inflammatory bowel disease: does anti-tumor necrosis factor alpha use protect from chronic inflammation-induced atherosclerosis? *Intest Res*. 2022;20:495–505. doi: 10.5217/ir.2022.00017
4. Aikawa E, Nahrendorf M, Figueiredo JL, Swirski FK, Shtatland T, Kohler RH, Jaffer FA, Aikawa M, Weissleder R. Osteogenesis associates with inflammation in early-stage atherosclerosis evaluated by molecular imaging in vivo. *Circulation*. 2007;116:2841–2850. doi: 10.1161/CIRCULATIONAHA.107.732867
5. Abdelbaky A, Corsini E, Figueroa AL, Fontanez S, Subramanian S, Ferencik M, Brady TJ, Hoffmann U, Tawakol A. Focal arterial inflammation precedes subsequent calcification in the same location: a longitudinal FDG-PET/CT study. *Circ Cardiovasc Imaging*. 2013;6:747–754. doi: 10.1161/CIRCIMAGING.113.000382
6. Hao N, Zhou Z, Zhang F, Li Y, Hu R, Zou J, Zheng R, Wang L, Xu L, Tan W, et al. Interleukin 29 accelerates vascular calcification via JAK2/STAT3/BMP2 signaling. *J Am Heart Assoc*. 2022. doi: 10.1161/JAHA.122.027222
7. Wang JM, Huang AF, Xu WD, Su LC. Insights into IL-29: emerging role in inflammatory autoimmune diseases. *J Cell Mol Med*. 2019;23:7926–7932. doi: 10.1111/jcmm.14697
8. Maher SG, Sheikh F, Scarzello AJ, Romero-Weaver AL, Baker DP, Donnelly RP, Gamero AM. IFNalpha and IFNlambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol Ther*. 2008;7:1109–1115. doi: 10.4161/cbt.7.7.6192
9. Hu X, Li J, Fu M, Zhao X, Wang W. The JAK/STAT signaling pathway: from bench to clinic. *Signal Transduct Target Ther*. 2021;6:402. doi: 10.1038/s41392-021-00791-1
10. Kelm NE, Zhu Z, Ding VA, Xiao H, Wakefield MR, Bai Q, Fang Y. The role of IL-29 in immunity and cancer. *Crit Rev Oncol Hematol*. 2016;106:91–98. doi: 10.1016/j.critrevonc.2016.08.002
11. Parhami F, Basseri B, Hwang J, Tintut Y, Demer LL. High-density lipoprotein regulates calcification of vascular cells. *Circ Res*. 2002;91:570–576. doi: 10.1161/01.RES.0000036607.05037.DA
12. Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriaga E, et al. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med*. 2019;380:752–762. doi: 10.1056/NEJMoa1809798
13. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, et al. Antiinflammatory therapy with Canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914
14. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, Pinto FJ, Ibrahim R, Gamra H, Kiwan GS, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med*. 2019;381:2497–2505. doi: 10.1056/NEJMoa1912388
15. Kirchgesner J, Nyboe Andersen N, Carrat F, Jess T, Beaugerie L, Group Bs. Risk of acute arterial events associated with treatment of inflammatory bowel diseases: Nationwide French cohort study. *Gut*. 2020;69:852–858. doi: 10.1136/gutjnl-2019-318932
16. Lin Y, Sun Z. Klotho deficiency-induced arterial calcification involves osteoblastic transition of VSMCs and activation of BMP signaling. *J Cell Physiol*. 2022;237:720–729. doi: 10.1002/jcp.30541
17. Nguyen KQ, Olesen P, Ledet T, Rasmussen LM. Bone morphogenetic proteins regulate osteoprotegerin and its ligands in human vascular smooth muscle cells. *Endocrine*. 2007;32:52–58. doi: 10.1007/s12020-007-9007-0
18. Levy-Schousboe K, Frimodt-Moller M, Hansen D, Peters CD, Kjaergaard KD, Jensen JD, Strandhave C, Elming H, Larsen CT, Sandstrom H, et al. Vitamin K supplementation and arterial calcification in dialysis: results of the double-blind, randomized, placebo-controlled RenaKvit trial. *Clin Kidney J*. 2021;14:2114–2123. doi: 10.1093/ckj/sfab017
19. Diederichsen ACP, Lindholt JS, Moller S, Ovrehus KA, Auscher S, Lambrechtsen J, Hosbond SE, Alan DH, Urbonaviciene G, Becker SW, et al. Vitamin K2 and D in patients with aortic valve calcification: a randomized double-blinded clinical trial. *Circulation*. 2022;145:1387–1397. doi: 10.1161/CIRCULATIONAHA.121.057008
20. Bessueille L, Kawtharany L, Quillard T, Goettsch C, Briolay A, Taraconat N, Balayssac S, Gilard V, Mebarek S, Peyruchaud O, et al. Inhibition of alkaline phosphatase impairs dyslipidemia and protects mice from atherosclerosis. *Transl Res*. 2022:S1931-5244(22)00144-X. doi: 10.1016/j.trsl.2022.06.010
21. Pasternac A, Sami M. Predictive value of the ear-crease sign in coronary artery disease. *Can Med Assoc J*. 1982;126:645–649.