Inflammation Ushers in Calcification
A Cycle of Damage and Protection?

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The layman’s term “hardening of the arteries” is synonymous with vascular disease and describes the ubiquitous calcification of the intima and media of the vessel wall that occurs in atherosclerosis and aging. Vascular calcification measured and quantified by electron beam computer tomography is a powerful predictor of myocardial infarction, vascular complications such as amputation, and all-cause mortality. Despite this fact, calcification has remained a neglected pathology, particularly in the field of atherosclerosis. This neglect is due in part to the controversy surrounding whether calcification is a bystander marker of disease load or acts to induce plaque rupture and thus increase the cardiovascular event rate; it is also due to the long-held belief that vascular calcification is an end-stage dystrophic process and therefore not amenable to manipulation. However, huge progress has been made over the last 10 years in elucidating the cell biological mechanisms underlying vascular calcification.

Consensus now exists that calcification is a regulated process, orchestrated by vascular smooth muscle cells (VSMCs) and occurring stepwise in a manner analogous to physiological mineralization processes. In the normal vessel wall, VSMCs are protected from calcification by local and circulating proteins such as matrix Gla protein and fetuin-A that act as mineralization inhibitors. However, in response to insults, VSMCs may die by apoptosis and release apoptotic bodies or may be induced by signals and mechanisms that are still not well defined to release matrix vesicles. These small membrane-bound microparticles have the capacity to concentrate calcium and phosphate to allow crystal nucleation and thus act as the first nidus for mineralization. Concomitant with vesicle release, although the precise order and relationship between these 2 events remain unclear, VSMCs undergo an osteo/chondrocytic phenotypic transition and begin to express transcription factors normally associated with differentiated chondrocytes and osteoblasts such as Sox 9, Cbfal/Runx2, and osterix that regulate the expression of a cascade of mineralization regulating proteins such as alkaline phos-
calized with cholesterol crystals and were present in membrane-bound vesicles in the size range of apoptotic bodies and matrix vesicles. They concluded that infiltrating macrophages drawn to sites of lipid accumulation induce VSMC death and VSMC phenotypic transition to an osteogenic phenotype via cytokine release. This scenario was supported by further experiments using the same model very early on in plaque development leading to calcification. Statins, via their capacity to inhibit inflammation, reduced the increase in osteogenic and inflammatory coactivities with plaque progression, and this correlated with a reduction in calcification. Thus, early treatment of inflammation can ameliorate the calcification response in the vessel wall. In addition to the animal experiments, Aikawa et al applied the same methods and technologies to human carotid endarterectomy specimens ex vivo. This analysis showed the same coassociations of inflammation with OsteoSense750 throughout the lesions. Although these analyses on human lesions did not allow an accurate time course as did the mouse studies, they lend strong support to the notion that the events in the mouse lesions are mirrored in human atherosclerosis in vivo.

An additional aspect of the study by Aikawa et al was the analysis of inflammation and calcification in advanced plaques in the same apolipoprotein E knockout mouse model. Here, the results were very different. In contrast to the early plaque calcifications, in aged mice with more advanced plaques, calcification was spatially distinct from macrophage accumulation. The lack of association between these 2 factors in advanced plaques suggests perhaps that calcification is initiated by factors other than inflammation as plaques progress. Alternatively, it may reflect the possibility that once calcification is established, it has the propensity to progress by physicochemical processes, enhanced by the absence of mineralization inhibitors, the expression of which may be compromised by loss of or damage to the VSMCs that produce them. Alternatively, it may represent a continuation of active osteogenic processes by VSMCs. Indeed, Aikawa et al showed that areas of inflammation also were decreased in advanced calcified plaques, suggesting an interrelationship between inflammation and calcification, but this time a negative one. Thus, we are left with the intriguing possibility that calcification can dampen inflammation and is protective in a manner perhaps analogous to the calcific mumification of infections that occurs in other soft tissues. Thus, by ensuring their own survival and using calcification to seal off inflammation, VSMCs may be prolonging the lifetime integrity of the vessel wall. So, does any evidence exist that calcification is an adaptive/protective response? And is it possible that calcified deposits can act directly on inflammation?

The rapid initiation of osteogenic gene expression patterns in VSMCs in response to insults is highly suggestive of an adaptive response aimed at regulating mineralization. The proteins upregulated by osteogenic VSMCs include matrix Gla protein (MGP) and other “bone”–specific proteins that dampen and control the mineralization process. In addition, matrix vesicle release from VSMCs was first described as a protective mechanism against calcium overload, able to rescue VSMCs from apoptotic cell death. Moreover, vesicles released by VSMCs are loaded with calcification inhibitors, including matrix Gla protein and fetuin-A, that act to minimize their capacity to initiate calcification and facilitate their rapid phagocytosis. Potentially, elevated extracellular calcium at sites of apoptosis induces VSMC vesicle release, and in the absence of inhibitors or phagocytosis, microcalcifications result, with these events preceding the osteogenic differentiation of VSMCs. Indeed, this may be a universal initiation mechanism for both medial and intimal calcification, and it would be interesting to determine whether the technologies used in the Aikawa et al study could be adapted to investigate this idea in animal models of medial calcification.

However, other evidence argues against the notion of calcification as protective. Recent in vitro studies have shown that microcalcifications induce a proinflammatory response in macrophages, leading to a vicious cycle of macrophage infiltration, matrix breakdown, VSMC apoptosis, and plaque rupture. Interestingly, it was found that HA crystals of <2 μm induced the most proinflammatory response in macrophages, with larger particles being inert. However, these studies used naked HA, whereas in the vessel wall, HA is complexed with a number of protein components that may block inflammatory responses. Moreover, in the Aikawa et al study, calcification also colocalized with cathepsin K, an enzyme involved in bone reabsorption, suggesting that macrophages may respond differentially to HA in the vessel wall. Indeed, medial calcification occurs in the absence of macrophage infiltration, suggesting that in vivo not all calcification is proinflammatory and that this is an area that requires further investigation of the effects of calcified deposits on not only macrophages but also VSMCs.

Finally, clinical studies clearly show that not all calcifications in vivo are the same. Intravascular ultrasound has shown that spotty calcification within a plaque is more predictive of a cardiovascular event than plate-like heavy calcification. Using the knowledge obtained from the Aikawa et al study, it seems likely that these “spotty” areas represent inflammatory lesions and therefore are more likely to rupture. Plaques with advanced calcification are potentially more benign because they are no longer inflammatory. Because atherosclerosis is a progressive inflammatory disease, it is clear that as long as inflammatory stimuli are present, there will be continuous cycles of inflammation leading to macrophage infiltration and microcalcifications. If each inflammatory region is contained, potentially by macrocalcification, and the VSMC repair system is not overwhelmed, over a lifetime, this process will lead to calcified fibrous acellular plaques. If, however, inflammation is sustained, the plaque may become heavily calcified but will still be prone to rupture if an ongoing
Inflammatory process is present with associated loss of VSMCs (the Figure). Although this hypothesis is controversial, these plaques also may rupture as a result of debonding around microcalcifications or may be susceptible to increased hemodynamic stresses and therefore prone to mechanical rupture.18,19 We should also remember that, within an individual, plaques are heterogeneous, exhibiting a continuum of inflammation and calcification, with any inflamed plaque, calcified or not, vulnerable to rupture.20

In summary, the Aikawa et al study has provided important insights into the earliest cell biological mechanisms of atherosclerotic calcification in vivo and highlighted key issues that remain controversial in the field of atherosclerosis and calcification. If these technologies could be modified for use in the clinic, inflammation and microcalcification may eventually become the keys to identifying the vulnerable patient and the vulnerable plaque.

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Disclosures
None.

References


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