

## Capping it off

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### See related article, pp. 753–762

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Atherosclerosis progresses silently for decades before producing sudden catastrophic clinical events. During the late preclinical phase, when there is no end organ damage, there can be major savings in terms of health care costs and a major reduction in morbidity and mortality, if lesions likely to produce a clinical event can be identified and treated. Indirect measures of inflammation, such as C-reactive protein (CRP), are often employed as an indicator of risk for cardiovascular events.<sup>1</sup> Although CRP is sensitive, it is not very specific. Adding other measurements, such as circulating levels of monocyte/macrophage colony stimulating factor (M-CSF) increases the predictive value of the assays to identify patients at risk for major cardiovascular events.<sup>2</sup> To determine the specific organs at risk, it is necessary to add an imaging procedure to survey the vasculature. Over the past 20 years, criteria to identify atheroma, especially in the carotid arteries, have been developed using multiple modalities, including magnetic resonance imaging,<sup>3</sup> computed tomography,<sup>4</sup> intravascular ultrasound,<sup>5</sup> and radionuclide imaging.<sup>6</sup> The clinical challenge, however, is not just localizing the disease, but characterizing the lesion. Several radiopharmaceuticals have been designed to localize in atheroma based on specific attributes of the lesion.<sup>7,8</sup> The distribution of these agents can be readily depicted on combined images of anatomy and pathophysiology with hybrid PET-CT, SPECT-CT, or fusion with magnetic resonance images.<sup>9</sup>

Inflammation is found throughout the life of atheroma. Since macrophages consume exogenous glucose to meet their energy needs, the radiolabeled glucose

analog, 2-fluoro-2-deoxy-D-glucose (FDG), has been used to visualize inflammation in the lesions.<sup>10</sup> A high prevalence of vascular FDG uptake was seen<sup>11–13</sup> especially in patients with elevated biomarkers. Since inflammation is present throughout the life of the lesion, from inception to rupture, and is only extinguished as lesions calcify,<sup>14</sup> it may not be a suitable marker to identify patients at imminent risk for vascular events. The same can be said for lipoprotein cholesterol,<sup>15</sup> oxidized lipoprotein cholesterol,<sup>16,17</sup> or identification of cellular elements such as macrophages and T-cells (see Figure 1). As a result, imaging lipids, inflammation, or cells may be very useful to define the presence and location of lesions, but not as a marker of vulnerability.

A major component of atheroma is the cap, providing the barrier separating the lesion from the flowing blood. In atheroma, vascular smooth muscle cells (VSMC) are stimulated to proliferate (by platelet-derived growth factor, fibroblast growth factor, and transforming growth factor  $\beta$ ) and migrate<sup>18</sup> (from their normal location in the media, through the internal elastic lamina into the intima<sup>19</sup>). The chemokines and cytokines causing VSMC migration and stimulation are produced by injured endothelial cells, tissue macrophages, and lymphocytes in the lesion. The factors causing migration of the VSMCs also stimulate both the proteolysis and production of collagen, elastin, and proteoglycans that form the cap of the atheroma. The maturity of the collagen and thickness of the cap are the result of a balance between production of proteoglycans by VSMC and their catabolism by a series of matrix metalloproteinases (MMPs). MMPs are zinc dependent endopeptidases capable of degrading extracellular matrix protein, cleaving cell surface receptors, and inducing apoptosis (through production of *fas* ligand).<sup>20</sup> This family of 23 proteinases, discovered 47 years ago as the agents responsible for tail resorption during frog metamorphosis,<sup>21</sup> can be produced by all cells in the wall<sup>22</sup> of the blood vessel. MMPs play a dual role in atheroma, they allow controlled remodeling of the vessel and migration of VSMC to stabilize the lesion on the one hand, and in excess cause thinning of the cap and contribute to plaque rupture.<sup>23</sup> Activated macrophages appear to be the major source of MMPs in atheroma.<sup>24</sup>

Macrophages, programmed to ingest and digest cholesterol in the plaque, gorge themselves on lipoprotein-cholesterol complexes and toxic oxidized low

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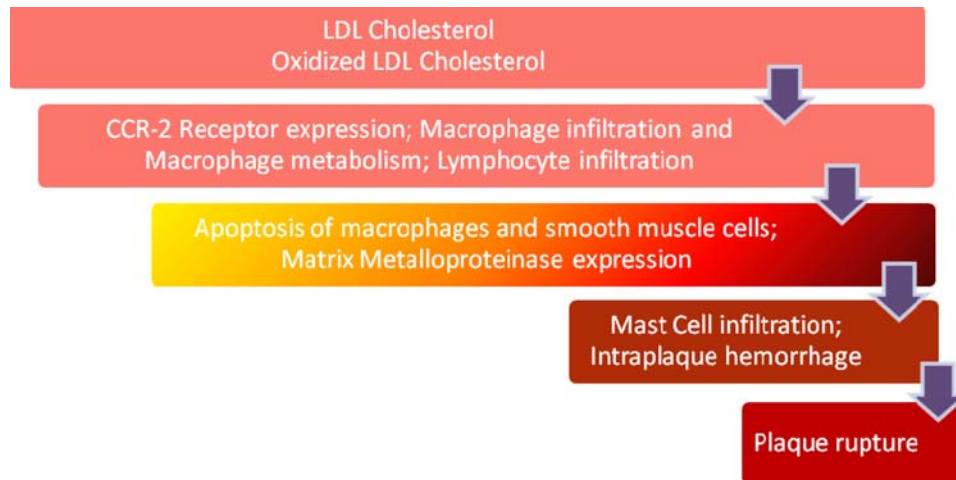
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J Nucl Cardiol 2009;16:686–8.

1071-3581/\$34.00

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doi:10.1007/s12350-009-9124-7



**Figure 1.** Major steps in the progression of atheroma from inception of the fatty streak (*top row*) to plaque rupture (*bottom row*).

density lipoprotein-cholesterol.<sup>25</sup> These cells become stressed, causing them to release MMPs and undergo apoptosis. Both apoptosis and the release of MMPs appear to identify a later stage in the evolution of the lesion than *pan atheroma* markers, such as inflammation or lipoprotein-cholesterol or oxidized lipoprotein cholesterol. Based on their important role in the evolution of vulnerable atheroma, several groups have developed imaging approaches to MMPs.<sup>26-28</sup>

In this issue of the Journal, Haider et al.<sup>29</sup> describe a very elegant series of experiments documenting the distribution of a radiolabeled MMP inhibitor and radiolabeled annexin-V, a marker of apoptosis in the cholesterol fed rabbit/injured aorta model of atheroma. The investigators observed a high level of apoptosis and MMP expression (particularly MMP-9) in the untreated animals with late stage atheroma. Eliminating cholesterol from the diet or maintaining the diet and treating with fluvastatin decreased apoptosis and MMP expression in the lesions. Figure 2 of the Haider manuscript highlights the relationship of these two markers. While the distributions are similar, they are not identical, suggesting that each marker is contributing different information about the lesion.

In addition to demonstrating the geographic relationship of apoptosis and expression of MMP, the investigators went one step further, to demonstrate the mechanism involved in MMP expression. A monocytic leukemia cell line, THP-1 cells, was transfected to over-express caspase-1. Caspase-1 is a member of the cysteine protease family of enzymes associated with apoptosis. Caspase-1 activation promotes secretion of proinflammatory cytokines.<sup>30</sup> Caspase production occurs

when cells are stressed. If the cells are severely stressed, caspase levels are high enough to induce the apoptotic cascade, with externalization of the membrane phospholipid phosphatidylserine, initiation of DNAases, and orderly packaging of cellular elements into apoptosomes for phagocytosis. Lower levels of stress cause lower concentrations of caspase, leading to externalization of phosphatidylserine, but not the initiation of the full catastrophic proteolysis associated the cell destruct sequence. The investigators observed a 25-fold increase in the production MMP-9 in the transfected cells. This observation documents the relationship of this initiator of apoptosis to the production of MMP. In addition the investigators also demonstrated increased MMP-9 production when THP-1 cells were incubated with oxysterol, cholesterol-5 $\beta$ , and 7-ketocholesterol.

The combination of imaging apoptosis and MMP production is a major step toward the goal of imaging vulnerable plaque. It is likely that additional steps will be required to achieve the goal. One approach may include specific cellular imaging of mast cells (Unfortunately, there are no methods currently available to selectively label these cells.). The presence of mast cells correlates with the vulnerable lesion. Activated mast cells release cytokines, growth factors, and proteolytic enzymes such as tryptase and chymase. Mast cells colocalize in the rupture prone shoulder region of the plaque, and at perivascular sites associated with intraplaque hemorrhage.<sup>31</sup> As a result of these observations, it is likely that selective imaging of activated mast cells, especially when combined with imaging of apoptosis and MMP expression, will provide a specific indicator of advanced, rupture prone lesions.

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