How to Chew Up Cells
Lessons for the Atherosclerotic Plaque

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Autophagy Links Inflammasomes to Atherosclerotic Progression

Razani et al


Macrophage Autophagy Plays a Protective Role in Advanced Atherosclerosis

Liao et al


Defective organelles as well as dead cells need to be taken care of. These are the tasks for 2 biological processes: autophagy and efferocytosis. Both occur in the atherosclerotic plaque, but their modus operandi and role in the disease process have remained unclear. Two recent studies show that efficient macrophage autophagy is needed for optimal efferocytosis and that defective autophagy leads to oxidative stress, inflammasome activation, and expansion of the plaque’s necrotic core. Therefore, autophagy may play a crucial role in the resolution of vascular inflammation and prevent the progression of atherosclerosis.

The atherosclerotic plaque is not a silent graveyard for foam cells. It is a battlefield, with living, proliferating, and differentiating cells as well as dying ones. Even death itself is handled by living cells, acting as undertakers to make sure that remnants of the dead ones are properly eliminated and not left to rot in the open in the extracellular space of the plaque. However, normal processes for handling cell death are threatened by the metabolic challenge caused by cholesterol accumulating in the artery wall. Recent studies suggest that this disturbance may play a crucial role by leading to necrosis and inflammation.

The cell death process has been described in innumerable histopathologic reports of human disease. Careful microscopic studies by John Kerr, Andrew Wyllie, and their colleagues in the years around 1970 clarified that cell death is a moderated line of events which includes controlled cell death (ie, apoptosis) for some of the cells. Further studies by John Sulston, and Horvitz unraveled a whole set of death genes that cause apoptosis during development. Subsequent work by many laboratories has identified apoptosis as a major process in growth, development, pathology, and homeostasis. Brenner, Sulston, and Horvitz received the 2002 Nobel Prize in Physiology or Medicine for their discoveries of genetic programs of development and death.

Apoptosis plays a crucial role in the resolution of inflammation. Large numbers of neutrophils and macrophages die when combating an infection, and the remnants are phagocytized by other macrophages. One hundred thirty years ago, Ilya Mechnikov (Nobel Prize 1908) observed when studying starfish in Messina, Sicily, that neutrophils are removed from an inflamed site by macrophages while the former cells are still intact. This phenomenon has been termed efferocytosis, and it is of importance by preventing secondary necrosis that could lead to persistent inflammation rather than its resolution. In modern times, an array of molecules have been identified that mediate resolution by acting on apoptosis, efferocytosis, and fibrosis.

Efferocytosis during resolution of inflammation involves several molecules including lactadherin (also called milk fat globule-EGF factor 8, Mfge8), which forms a bridge between phosphatidylycerine on the surface of apoptotic cells and αvβ3 integrin on phagocytes. By bridging between the apoptotic and the phagocytizing cell, it promotes efferocytosis. This process prevents the development of autoimmune reactions to components of apoptotic cells.

Apoptosis is known to occur frequently in the atherosclerotic plaque, in macrophages, smooth muscle cells, and other cell types. Secondary necrosis leads to buildup of the plaque’s necrotic core, a structure that acts as a diffusion barrier hampering elimination of accumulating lipids. Furthermore, the thrombogenic properties of lipids in the ne-
crotic core make this structure a nidus for thrombosis once the plaque cap has fissured. Therefore, efferocytosis is important by eliminating apoptotic cells and preventing secondary necrosis. When atherosclerosis-prone \textit{Ldlr}^{-/-} mice were made deficient in lactadherin, they displayed increased accumulation of dead cells, large necrotic cores, and accelerated atherosclerosis.

Apoptosis has a counterpart at the subcellular level. Autophagy is the mechanism by which cellular components such as organelles are sequestered and degraded. It was described 50 years ago, but, as with apoptosis, genetic approaches in simple organisms, in this case, yeast, were needed to dissect the autophagic process and identify its mediators. More than 30 \textit{Atg} genes are now known to control the different aspects of autophagy. Because of such fine-tuning, autophagy is involved in several different processes including nutritional adaptation, cancer, and inflammatory diseases.

Pioneering studies by De Meyer, Kockx, and their colleagues identified autophagosomes and protein markers of autophagy in atherosclerotic plaques. However, the role of autophagy in lesion development has remained unknown. This spring, 2 papers published back-to-back in \textit{Cell Metabolism} provide mechanistic insights into the role of autophagy in atherosclerosis.

Both studies use genetic strategies to assess autophagy in the lesions of hypercholesterolemic mice. Razani et al bred \textit{Apoe}^{-/-} mice with animals carrying a macrophage-specific defect in \textit{Atg}5, a protein needed in the formation and expansion of autophagic vacuoles. This defect promoted the formation of large lesions rich in macrophage foam cells. Liao et al used a similar Cre-lox strategy and crossed mice with a macrophage-specific \textit{Atg}5 defect to \textit{Ldlr}^{-/-} mice. Their careful lesion analysis revealed that the macrophage defect not only led to increased lesion size but to a significant expansion of the necrotic core and to an increased proportion of apoptotic cells. These data suggested that efferocytosis could be hampered. Indeed, cell culture studies showed decreased phagocytic clearance of apoptotic cells by macrophages deficient in \textit{Atg}5.

Atherosclerotic lesions of mice with defective autophagy showed that signs of increased oxidative stress and autophagy inhibition led to increased NADPH oxidase activity in cultured macrophages. When autophagy was inhibited, p47phox of the NADPH oxidase complex was associated with lysosomes to an increased extent. Such oxidative stress could obviously have important effects on cellular pathophysiology, for instance, by damaging organelle membranes and by activating intracellular enzymes.

A cytosolic protein complex termed the inflammasome has been identified as an important transducer of metabolic stress to inflammation. The NLRP3 inflammasome consists of a pattern recognition detector of the NOD-like receptor (NLRP) type, an adaptor/transducer, and the caspase-1 enzyme that processes the proform of interleukin-1β into bioactive proinflammatory cytokine. The NLRP3 inflammasome can be activated by intracellular crystals such as cholesterol or uric acid microcrystals, in a process that possibly involves oxidative stress.

Defective autophagy could conceivably lead to accumulation of cholesterol derived both from accumulating membrane structures and from internalized lipoproteins. In addition, defective autophagy can lead to intracellular accumulation of mitochondria that are the cell’s major producer of oxygen radicals and also to increased NADPH oxidase–dependent oxidative stress. Razani et al in their report show augmented inflammasome activity, with increased interleukin-1β production, in macrophages with defective autophagy. Furthermore, they provide direct evidence that cholesterol crystals inhibit autophagic processing.

Taken together, the new data establish a link between cholesterol accumulation, defective autophagy, inflammation, and growth of the plaque’s necrotic core. They support the notion that efficient autophagy is instrumental in the resolution of vascular inflammation and point to therapeutic possibilities involving promotion of autaphagic signals. In this context, it is of interest that the mammalian target of rapamycin (mTOR) pathway suppresses autophagy; therefore rapamycin/sirolimus and other mTOR inhibitors may prevent expansion of the necrotic core and thus lesion growth, in addition to their inhibitory effect on restenosis. Whether stimulation of autophagy will also prevent atherothrombosis and its clinical manifestations remains to be determined.

Exciting experimental and clinical studies lie ahead.

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References
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