Hydrolytic Enzymes Released From Resident Macrophages and Located in the Intima Extracellular Matrix as Agents That Modify Retained Apolipoprotein B Lipoproteins

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Identification of the molecular mechanisms causing the focal response of arterial cells that induce atherosclerotic lesions may open new therapeutic venues for prevention of this disease. Many of the products that appear to trigger the tissue reaction originate from apolipoprotein B (apoB)-lipoproteins retained in the intima extracellular matrix. These may be key phenomena contributing to the initial and late phases of atherosclerotic plaque development. The preferential retention of apoB-lipoproteins, especially LDL, can lead to direct modifications of the labile structure of these complex particles and furthermore could provide the time required for enzymatic and nonenzymatic more profound alterations of their lipid and protein moieties. Such structural changes may produce lipid and peptide neo-epitopes, lipid hydrolytic, and oxidative products with potent biological effects. Degradation of the apoB-100 and the polar lipid surface components can also lead to aggregation and fusion of lipoprotein particles that eventually form part of the complex array of extracellular lipid aggregates that grow with the progress of lesions. The structural and enzymatic agents that could contribute to such alterations in the extracellular intima may pre-exist there, like the proteoglycans, or could be secreted by macrophages and other intima-residing cells. These modifications may be part of a physiological scavenging process for removal of undesirable lipoprotein components. However, with elevated circulating levels of apoB-lipoproteins, especially at sites where intimal thickening occurs by matrix expansion, the beneficial scavenging process may become insufficient and may turn into an atherogenic cycle. Identification of the specific pathways that modify apoB-lipoproteins in the intima and that may generate cytotoxic, pro-inflammatory or immunogenic products in "response to their retention" may be crucial in the search for sites at which the link between dyslipidemias and atherogenesis may be interrupted.

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In this issue of Arteriosclerosis Thrombosis and Vascular Biology, Hakala and colleagues present results indicating that atherosclerotic lesions of human coronaries contain abundant immunopositive lysosomal acidic lipase (LAL) and cathepsin D (CathD) in the extracellular matrix surrounding macrophage-rich regions. These enzymes are present in conditioned media from macrophages in culture and are elicited by opsonized zymosan. LAL was found to hydrolyze LDL cholesteryl esters and triglycerides, and CathD extensively degraded LDL apoB-100. Furthermore, the LAL/CathD containing media induced LDL aggregation. Such modified LDL was taken up rapidly by peritoneal mouse macrophages and human coronary arterial smooth muscle causing lipoprotein and lipid accumulation. These interesting findings support an atherogenic scenario with original twists in it (see Figure). The authors suggest that lysosomal enzymes could be released from macrophage-rich areas when the cells are exposed to LDL-immune complexes or immunoglobulins deposited there by local complement activation. Immune complexes exist in lesions, probably as a local response of the adaptive immune system to neo-epitopes appearing from modification of retained LDL, and they could trigger the chronic release of lysosomal enzymes, as suggested. Also, recent data from the same laboratory support the hypothesis that complement activation occurs in the extracellular matrix of atherosclerotic plaques in humans. In their in vitro system Hakala et al explored the release of LAL and CathD with opsonized zymosan. In vivo however, the situation may be more complex and exposure of macrophages to immunocomplexes can release additional lysosomal and intracellular enzymes that can also contribute to LDL modification. A critical point of the atherogenic mechanism proposed is that, once released in the extracellular intima, LAL and CathD are immobilized by yet uncharacterized structures and that the enzymes remain active toward LDL. No evidence that this is the case is available. However, this is possible since lipoprotein lipase, phospholipase A2, and myeloperoxidase, which can also act on LDL and are most likely secreted by macrophages and smooth muscle cells, are active in lesions. Similarly, all three of these enzymes also have strong affinities for proteoglycans of the extracellular intima and are detected there. This colocalization with proteoglycan-bound LDL may facilitate their effects, as may be the case for LAL and CathD. The potential atherogenic role of released lysosomal acidic lipase and CathD will be strengthened if the enzymes present in the extracellular intima of arterial are shown to be active.

Hakala et al explored the modifications induced in LDL by conditioned media obtained after stimulation with opsonized zymosan. The media contained appreciable amounts of LAL and CathD and led to apoB-100 fragmentation and fatty acid release from LDL lipids. The authors also provide convincing verification that these two enzymes were responsible for the observed modifications, including induction of particle aggregation. The authors discussed in detail the point whether lysosomal enzymes designed to have maximal activ-
ities at acidic pH in the organelles could be active in the more neutral milieu of the extracellular media. Accordingly, they cite the possibility that macrophages proton pumps and hypoxic conditions could acidify the immediacy of macrophase-rich regions. However this discussion, interesting as it is, was not really necessary since they found that, in macrophase conditioned media, with neutral phosphate buffer, the enzymes quite efficiently hydrolyzed apoB-100, cholesteryl esters, and triglycerides. Logically, the authors set off to explore whether the observed modifications had consequences for the interactions of LDL with macrophages and smooth muscle cells. The results show that the modified and aggregated LDL was efficiently taken up by macrophages and smooth muscle cells by pathways involving specific and nonspecific LDL receptors. This resulted in intracellular lipoprotein deposits and lipid vacuoles, although the nature of the accumulated lipids was not explored.

For many years, extensive evidence has become available indicating that most of the cholesterol and cholesteryl esters found in lesions originate from plasma apoB-lipoproteins. Cholesteryl esters with a fatty acid composition almost identical to plasma LDL and immunoreactive apoB-containing particles can be extracted in appreciable amounts from human atherosclerotic lesions. These complexes and aggregates show apoB-100 extensively fragmented and a reduction in the cholesteryl esters phospholipids and triglycerides that are susceptible to lipases or to oxidation of the unsaturated fatty acids in the sn-2 position. This is not compatible with the sometimes-exaggerated idea that all modified LDL is taken up and ends up in macrophages and smooth muscle cells. The article from Hakala and colleagues supports the hypothesis that extensive potentially atherogenic modifications of LDL retained in the intima take place outside cells. The final suggestion from the authors consequently indicates that the action of the lysosomal enzymes on LDL in the intima may contribute both to formation of extracellular lipid aggregates and accumulation of lipids by cells. I encourage the readers of this original contribution to read the corresponding online supplement to obtain a more complete notion of its careful design and documentation.

Schematic illustration of the potential atherogenic effects of LAL and CathD secreted by macrophages as proposed by Hakala et al. The cells secrete the enzymes into the surrounding extracellular matrix under the stimuli of immune complexes and complement activation. These enzymes could further modify LDL retained in the extracellular matrix contributing to generation of bioactive lipids and neo-epitopes from fragmented apoB-100. The action of the enzymes on LDL induces aggregation of the particles that may initiate the formation of lipid deposits in the extracellular intima.

References
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