Editorial

Lipoprotein Retention—and Clues for Atheroma Regression

Kevin Jon Williams, Ira Tabas

Abstract—Subendothelial retention of apoB-lipoproteins is the key initiating event in atherosclerosis, provoking a cascade of pathogenic responses. Dissection of the molecular participants provides fresh insight into how this major killer might be reversed. Efflux of harmful lipids derived from retained lipoproteins may be crucial in promoting beneficial remodeling of lesions.

The pathogenic mechanisms responsible for atherosclerosis have been the subject of considerable debate over the decades (reviewed in references 1–5). It is more than a theoretical concern. Despite the clinical successes of current plasma lipid-lowering strategies, such strategies fail to stop most cardiovascular events.[6–8] Pathogenic understanding is needed to guide the development of new strategies to fill this therapeutic void.

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A central role for cholesterol-rich apoB lipoproteins is now clear, but there has been a long controversy regarding triglyceride-rich particles (TRPs) and atherogenesis. Epidemiologic studies have shown a link, although the direct importance of this link has been clouded by the common association of hypertriglyceridemia with additional atherogenic factors, particularly insulin resistance, hypertension, low plasma high-density lipoprotein (HDL) concentrations, and the presence of small dense low-density lipoproteins (LDL). Moreton[9] and Zilversmit[10] advocated a direct role for post-prandial TRPs, which can carry a substantial amount of cholesterol.[11] Moreover, despite their size, TRPs penetrate into the arterial wall and become retained there.[12,13] Extracellular matrix is key to this retention, chiefly involving arterial proteoglycans,[14–16] and proteoglycan-binding sequences were identified in $apoB_{100}$ and $apoB_{48}$ and shown to be pathogenically essential in the initiation of atherogenesis in vivo, though mainly for LDL.[17]

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Öörni et al[18] provide fresh insight into the mechanisms by which TRPs could be retained in the arterial wall and thereby contribute to atherogenesis. Although the affinity of small very low–density lipoproteins (sVLDL) and intermediate-density lipoproteins (IDL) for arterial proteoglycans is lower than that of LDL, sphingomyelinase, an enzyme secreted by endothelial cells into the extracellular mileu of the arterial wall,[19] causes these particles to aggregate and fuse.[18] Once this occurs, their affinity for matrix increases. If similar events occur during atherogenesis, the movement of these particles would be sterically hindered as well, and their egress out of the wall would become unlikely. Moreover, sphingomyelinase-induced aggregation of TRPs increases their ability to load macrophages with cholesterol.[20] Sphingomyelinase-induced aggregation of LDL and Lp(a) also increases their affinity for proteoglycans and their ability to load macrophages with cholesterol.[21,22] Although LDL retention can be increased by several arterial-wall enzymes, Öörni et al found that sphingomyelinase is particularly important for these TRPs.[18]

Can anything helpful be done with this and other discoveries related to the pathogenesis of atherosclerosis? Large, prospective, randomized trials of antibacterials[23–25] and of vitamin E alone or in combination with other antioxidants[26,27] failed to show benefit in human atherosclerotic vascular disease, although it is possible that future studies with other types of these agents could be successful. A shared etiology with cancer was implied by the monoclonal theory of atherosclerosis,[1,28–31] but antineoplastic agents would be impractical in this setting, and the apparent monoclonality of atheromata may arise simply because of unexpectedly large X-inactivation patches within normal human arteries.[32]

This leaves the response-to-retention theory (reviewed in references 3 and 17), which is the focus of the article by Öörni et al, and the inflammation hypothesis,[33,34] which was put forth as a descendant of the response-to-injury model[35] but also has roots in Virchow's earlier concept of "endarteritis deformans."[2,4] The response-to-retention and inflammation theories are intellectually compatible, in that retained and modified lipoproteins within the arterial wall are now generally regarded as the essential stimulus for the activation of endothelium and the recruitment of macrophages, T-cells, and mast cells, as well as a number of important responses that are not inflammatory, such as in-migration of smooth muscle cells.[33,34,36] Hyperbetalipoproteinemia, which quickly leads to lipoprotein retention at atherosclerosis-prone sites,[37–40] provides a powerful stimulus that acts synergistically with local, preexisting turbulent flow to turn on endothelial NF-κB and induce cell-adhesion molecule display, before macrophage infiltration.[41,42] Local enzymatic digestion of these retained lipoproteins, in addition to causing the

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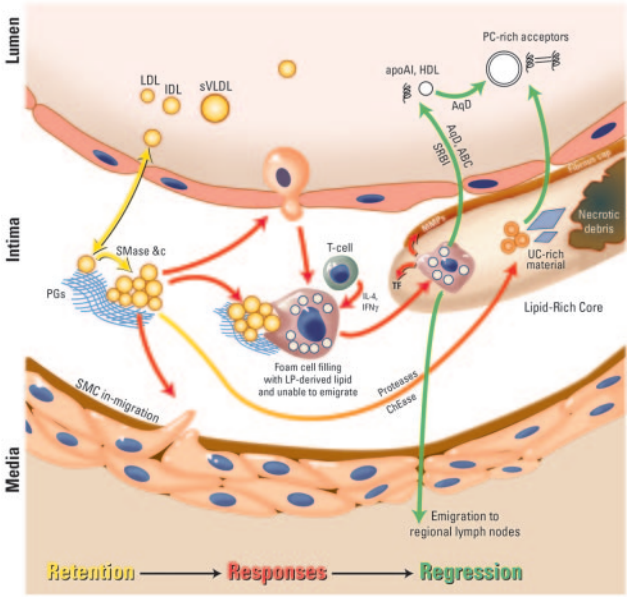
physical changes documented by Öörni et al, triggers the release of proinflammatory products,[43] and it is tempting to speculate that the set of molecules released from retained TRPs might differ from the ones generated from LDL. Macrophages subsequently recruited to the developing lesion secrete molecules, including lipoprotein lipase[44] and additional sphingomyelinase,[45] that promote further lipoprotein retention, modification, and release of biologically active byproducts. Nonenzymatic modifications are likely to contribute to these processes as well.[46]

Of particular note is the recent discovery of lipoprotein-derived lipids that block macrophage emigration, thereby forcing these cells into an abnormal state of persistence within the lesion.[47] Moreover, intracellular accumulation of lipoprotein-derived unesterified cholesterol can lead to macrophage apoptosis, which, in the absence of phagocytic clearance, can contribute to necrotic core formation.[48,49] Thus, retained and modified lipoproteins cause significant derangements of macrophage function, and hence of the inflammatory response. These derangements may help explain why direct antiinflammatory therapeutic agents have not proven beneficial in human atherosclerosis to date,[33,50] although new approaches are being explored.[34,51]

If arterial retention of apoB-lipoproteins is the essential pathophysiologic process in atherosclerosis, it makes the clinical successes of conventional lipid lowering easy to understand. Nevertheless, as noted above, this approach has had limited benefits using our current set of hypolipidemic agents in pre-established arterial disease. One answer may be to push plasma apoB-lipoprotein levels even lower,[52] and combinations of old and new agents might make this possible while keeping side-effects under control, although this still needs additional testing.[53] Another is to interfere with specific molecular participants in lipoprotein retention and modification. Agents to compete with direct apoB–proteoglycan interactions,[3,54] though ingenious, are unlikely to be practical owing to the stoichiometry, and they may lose efficacy as additional molecular processes that facilitate lipoprotein retention come into play during lesion progression.[3,44,55,56] Another strategy related to the work of Öörni et al[18] would be to interfere with the hydrolysis of lipoproteins by sphingomyelinase. High lipoprotein sphingomyelin content is an independent risk factor for atherosclerosis in humans,[57] and inhibition of sphingomyelin synthesis was recently reported to slow arterial lesion development in apoE-null mice out of proportion to its lipid-lowering effect.[58,59] Similarly, genetic targeting of sphingomyelinase in apoE-null mice is associated with a marked reduction in early lesion development[60] (Devlin C, Leventhal A, Kuriakose G, Williams KJ, Tabas I, unpublished data, July 2003 to March 2005).

If retained and modified lipoproteins are the root cause of this disease, then reaching into the arterial wall to remove the offending material should be beneficial, like pulling out a troublesome splinter.[5,6,61,62] Although much progress has occurred recently in our understanding of how apoA-I, HDL, and HDL-like particles mobilize lipids from loaded cells,[63] little attention has been paid to extracellular lipid deposits, which is exactly what Öörni et al created in vitro. Such deposits have long been recognized in human plaques and consist of a variety of components, including retained and aggregated lipoproteins, cholesterol-rich vesicular structures, cholesterol crystals, and debris from necrotic cells.[64–67] This material could be phagocytozed by macrophages, which under healthy conditions might apoptose safely in situ,[68–70] actively exit the plaque,[47] or release their extra lipid to apoA-I and HDL via passive diffusion, ABC transporters, and SR-



Retention, responses, and regression. **Retention** (yellow arrows): ApoB-lipoproteins (LP) continually enter and exit the arterial wall, but some particles adhere to the local matrix long enough to become modified by enzymes and other factors, resulting in essentially irreversible retention. LDL has a major role in these crucial initiating steps. Despite their size, IDL and small VLDL (sVLDL) also participate, particularly through the action of sphingomyelinase (SMase), which causes these particles to aggregate, thereby increasing their affinity for proteoglycans (PG) in the matrix. **Responses** (red arrows): Retained and modified lipoproteins release biologically active byproducts that activate the endothelium and attract monocyte/macrophages and smooth muscle cells. Aggregated lipoproteins in particular are avidly phagocytozed by macrophages, which become foam cells. Other lipoprotein-derived molecules block the normal emigration of foam cells from the developing lesion, so that the lipid that has accumulated cannot be removed through cellular trafficking. Macrophages that persist in the lesion secrete a number of important molecules, including matrix metalloproteinases (MMPs), which weaken the fibrous cap, and tissue factor (TF), a potent procoagulant that is released on plaque rupture. Many of these macrophages eventually accumulate large amounts of lipoprotein-derived unesterified cholesterol (UC) and die by apoptosis. In the absence of effective phagocytosis, the cells become secondarily necrotic. This cellular debris, together with extracellular lipids, leads to necrotic core formation. Lipoproteins and lipoprotein-loaded cells also contribute to the appearance of UC-rich vesicles and crystals. **Regression** (green arrows): Naturally occurring acceptors, apoA-I and HDL, extract unesterified cholesterol and other molecules from lesional cells via aqueous diffusion (AqD), ABC transporters (ABC), and the scavenger receptor-BI (SR-BI). Artificial acceptors rich in phosphatidylcholine (PC), such as liposomes or apoA-I/PC complexes, mobilize material from extracellular deposits, as well as from apoA-I and HDL. Under the proper circumstances, substantial remodeling of the plaque can occur, including efflux of harmful or superfluous lipids, phagocytosis and digestion of debris, and the normal emigration of macrophages from the arterial wall.

BI[63] (see the Figure). In fact, several strategies designed to enhance cellular lipid efflux are currently in human clinical trials,[71–73] and other approaches are being developed.[6,63,74–76] Under the right circumstances, even non-lipid components can improve, including calcification, fibrosis, and tissue factor expression.[77–82]

But in the human disease, much of the retained and modified material remains extracellular. One recent study using insoluble extracts from human plaques indicated that apoA-I and HDL are surprisingly inert at mobilizing non-cellular lipid; only acceptor particles particularly rich in phosphatidylcholine (PC) were effective.[83] The relevance in vivo of this provocative observation remains to be seen, although it suggests that the participation of active phagocytic cells or the administration of exogenous PC-rich acceptors might be helpful, especially given that phagocytes[63] and artificial acceptors[61,83,84] can cooperate with endogenous HDL and its components in the mobilization of lipids for disposal. Consistent with this idea, artificial PC-rich particles have been shown in vivo to mediate "reverse" lipid transport from the periphery to the liver,[85–89] suppress inflammatory responses in dysfunctional vessels during hyperbetalipoproteinemia,[90,91] and shrink arterial lesions both in animals[61,92–94] and, in one small trial, in humans.[72] These results are of current interest, given our recognition of the role of lipid-rich vulnerable plaques in acute coronary events.[95,96]

Everything that we identify, from general theories of pathogenesis to specific molecular participants, must eventually pass muster in the authentic human disease. Regrettably, as shown by the recently completed clinical trials,[23–27] many scientific discoveries are not readily put to use. By this standard, the coming few years will be a crucial test of the idea that lipoprotein retention and its consequences can be reversed. As atherosclerosis continues to grow as a worldwide killer, we must apply knowledge about its pathogenesis toward improving the lives of our patients and their families, despite the nearly insurmountable obstacles of translating discoveries into practice.[97,98]

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