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Inflammatory proteins on HDL: What are we measuring?

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In this issue of *Translational Research*, Deeg and colleagues demonstrate that statin therapy is associated with a significant reduction in plasma levels of glycophosphatidylinositol-specific phospholipase D (GPI-PLD), a protein produced primarily by the liver (1) and carried on a minor subset of circulating high density lipoprotein (HDL) particles (2). However, the circulating form of GPI-PLD is not enzymatically active, and the authors also report that statins do not regulate GPI-PLD expression in an hepatocyte cell line. Therefore, why are these results intriguing? They are of interest in light of previous work implicating GPI-PLD in inflammatory processes (3-8), as well as more recent studies identifying a variety of inflammatory modifications of HDL (9-13). These observations raise the intriguing possibility that the GPI-PLD lowering seen in humans is due to an effect of statins on an extrahepatic source of GPI-PLD production. If so, what might this extrahepatic source be, and what evidence is there that proteins produced at an extrahepatic site might associate with HDL?

GPI-PLD and Extrahepatic Inflammation

While hepatocytes classically have been thought to be the primary source of GPI-PLD, several lines of evidence have implicated GPI-PLD in inflammation. First, several proteins involved in the regulation of inflammation have GPI anchors, including decay accelerating factor (CD55) and CD59 (3, 4), as well as a splice variant of vascular cell adhesion molecule-1 (5). Second, macrophages can produce GPI-PLD (8), and GPIs and GPI-PLD cleavage products have been shown to induce macrophage expression of the inflammatory cytokines interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) (6, 7). GPI-PLD is present in human atherosclerotic plaques, where it localizes predominantly with foam cell macrophages, and to a lesser extent with retained HDL particles (8). Moreover, the subset of macrophages with detectable GPI-PLD also contain lipid oxidation products, implicating macrophage activation in GPI-PLD expression by plaque macrophages (8).

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HDL is readily exposed to the atherosclerotic plaque

A recent body of literature has suggested strongly that plaque constituents may modify HDL. HDL are the smallest of the plasma lipoproteins, and likely have ready entry into atherosclerotic plaques (14). In fact, the primary mechanism by which HDL is presumed to decrease atherosclerosis is the process of reverse cholesterol transport, in which HDL enters the plaque, accumulates excess cholesterol from cholesterol-laden plaque cells, and then exits the plaque to transport this cholesterol back to the liver for metabolism and excretion as bile (15). However, HDL also can be retained on plaque extracellular matrix proteins in human atherosclerosis (14), particularly if it contains additional proteins, such as apolipoprotein E (14), phospholipid transfer protein (16), or serum amyloid A (17), each of which enhances ionic interactions of HDL particles with plaque proteoglycans (18).

Inflammatory modifications of plasma and plaque HDL

Recently, a series of reports have suggested a variety of ways in which HDL may be modified by macrophage-generated oxidants (9-12). Several years ago, Daugherty reported that human atherosclerotic plaque macrophages can produce the enzyme myeloperoxidase (MPO), which generates hypochlorous acid (HOCl) (19). HOCl has been shown to generate 3-chlorotyrosine adducts on apoA-I (9, 11). 3-chlorotyrosine levels are increased in HDL of coronary artery disease patients as compared to controls, and are enriched in atherosclerotic plaques (9, 11). In addition, reactive intermediates derived from nitric oxide (NO) generate specific 3-nitrotyrosine adducts on proteins (20), and 3-nitrotyrosine also is enriched in HDL isolated from CAD patient plasma or atherosclerotic plaques (10, 11).

Even more recently, evidence that plasma HDL may acquire inflammatory proteins has been published (13). Using a shotgun proteomics approach that employed mass-spectrometric techniques and a stringent approach to protein identification, Vaisar and colleagues found 42 proteins that differed substantially in amount between HDL isolated from plasma of CAD patients and controls (13). Several of the proteins unique to CAD patient plasma HDL also were detected on HDL isolated from atherosclerotic plaques. Interestingly, many of the proteins unique to atherosclerotic HDL were involved in complement regulation or had serine-type endopeptidase inhibitor activity (13).

HDL modifications: We can't necessarily assume that these occur solely (or predominantly) in plaques

While these findings demonstrate convincingly that HDL may be oxidatively modified (9-12) and accumulate inflammatory proteins (13), it is premature to assume that these changes have occurred solely, or even predominantly, through exposure of HDL to inflammatory cells in atherosclerotic plaques. Another, and perhaps more likely, site of inflammatory HDL modification is visceral adipose tissue, which is highly prone to develop inflammatory cell infiltration in the setting of genetic insulin-resistance and/or diet-induced obesity (21, 22). Particularly when one considers the large difference in volume of adipose tissue as compared to atherosclerotic plaque, it is hard to ignore the possibility that adipose tissue may serve as a major site for HDL inflammatory modifications. Moreover, as participants in the study of Deeg et al did not have clinical cardiovascular disease, it seems more plausible to posit visceral adipose tissue, as opposed to atherosclerotic plaque, as an extrahepatic site of GPI-PLD accumulation by HDL. Finally, Deeg and colleagues have reported previously that insulin resistance is associated with higher levels of HDL-associated GPI-PLD (23), further implicating visceral adipose tissue as a potential source of GPI-PLD.

Statin-mediated reduction in GPI-PLD: evidence for an anti-inflammatory effect of statins?

The possibility that statins reduce GPI-PLD levels through reducing its expression at one or more extrahepatic sites is supported by the following observations: 1) Deeg et al demonstrate that statins do not reduce GPI-PLD expression in a hepatocyte cell line, 2) GPI-PLD can be produced by activated macrophages (8), and 3) HDL not only can be covalently modified by inflammatory cell-generated oxidants (9-12) but also can accumulate inflammatory proteins (13). It should be noted that GPI-PLD was not among the proteins identified in the Vaisar study (13), probably because the stringent protein-identification criteria excluded detection of low-abundance proteins, such as GPI-PLD. Taken as a whole, these findings provide presumptive evidence that statins may have extrahepatic, anti-inflammatory effects.

Conclusions

Do these findings imply that GPI-PLD could be a biomarker for plaque inflammation? The evidence is insufficient to support this at present, especially given that GPI-PLD could arise from extrahepatic sources other than atherosclerotic plaque. In addition, because the statin-associated decrease in GPI-PLD correlated well with decrease in apolipoprotein B (apoB), it is unlikely that GPI-PLD measurement would provide additional clinical information beyond that provided by LDL or apoB levels, at least in terms of gauging response to statin therapy. Moreover, participants in the study of Deeg et al. did not have clinical CVD, so it would be necessary to confirm whether GPI-PLD levels are increased in those with CVD. Finally, it has been difficult to demonstrate that any of the inflammatory biomarkers currently available adds significant, additional prognostic information beyond that available from traditional CV risk factors (24, 25). Nonetheless, the study by Deeg et al., as well as others (9, 10, 13), add to a growing body of literature suggesting that inflammatory modifications of HDL may provide unique insights into the intertwined pathophysiologies of obesity, insulin resistance and atherosclerosis.

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