HDL and the Inflammatory Response Induced by LDL-Derived Oxidized Phospholipids

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Abstract—Oxidation of low density lipoprotein (LDL) phospholipids containing arachidonic acid at the sn-2 position occurs when a critical concentration of “seeding molecules” derived from the lipoxygenase pathway is reached in LDL. When this critical concentration is reached, the nonenzymatic oxidation of LDL phospholipids produces a series of biologically active, oxidized phospholipids that mediate the cellular events seen in the developing fatty streak. Normal high density lipoprotein (HDL) contains at least 4 enzymes as well as apolipoproteins that can prevent the formation of the LDL-derived oxidized phospholipids or inactivate them after they are formed. In the sense that normal HDL can prevent the formation of or inactivate these inflammatory LDL-derived oxidized phospholipids, normal HDL is anti-inflammatory. HDL from mice that are genetically predisposed to diet-induced atherosclerosis became proinflammatory when the mice are fed an atherogenic diet, injected with LDL-derived oxidized phospholipids, or infected with influenza A virus. Mice that were genetically engineered to be hyperlipidemic on a chow diet and patients with coronary atherosclerosis, despite normal lipid levels, also had proinflammatory HDL. It is proposed that LDL-derived oxidized phospholipids and HDL may be part of a system of nonspecific innate immunity and that the detection of proinflammatory HDL may be a useful marker of susceptibility to atherosclerosis. (Arterioscler Thromb Vasc Biol. 2001;21:481-488.)

Key Words: HDL ■ LDL ■ atherosclerosis ■ oxidized phospholipids

The events involved in fatty streak formation resemble those elicited by mycobacteria. Over the past decade, there has been increasing evidence that this inflammatory response may, in part, be elicited by the oxidation of phospholipids contained in LDL. Several oxidized phospholipids that are able to induce the genes and proteins necessary for the cellular response seen in the fatty streak have been identified in mildly oxidized LDL and in lesions of animal models of atherosclerosis. Two of these oxidized phospholipids, 1-palmitoyl-2(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine (POVPC) and 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine (PGPC), both induced monocytes to bind to endothelial cells. However, PGPC but not POVPC also induced neutrophils to bind to endothelial cells. Indeed, POVPC strongly inhibited lipopolysaccharide-mediated induction of neutrophil binding and expression of E-selectin protein and mRNA. This inhibition by POVPC was mediated by a protein kinase A–dependent pathway that resulted in downregulation of nuclear factor-κB–dependent transcription. PGPC, on the other hand, induced both E-selectin and vascular cell adhesion molecule-1 (VCAM-1) expression on endothelial cells. On the basis of studies in Xenopus laevis oocytes, Leitinger et al concluded that POVPC and PGPC bound to different receptors. Furthermore, they demonstrated that at concentrations equal to those present in mildly oxidized LDL, POVPC prevented the induction of neutrophil binding and E-selectin expression in endothelial cells despite the presence of PGPC. Thus, we hypothesized that the relative concentrations of POVPC and PGPC will determine whether an acute (neutrophilic) or chronic (monocytic) inflammation would result in any given tissue. A third group of oxidized phospholipids that also induce monocye binding to endothelial cells was identified as 1-palmitoyl-2(5,6-epoxyisoprostane E2)-sn-glycero-3-phosphorylcholine (PEIPC). Autoantibodies specific for products of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (Ox-PAPC), including POVPC, have been identified in apoE-deficient mice and have been shown to inhibit macrophage uptake of oxidized LDL. These antibodies have also been shown to bind to apoptotic cells and inhibit their phagocytosis by macrophages, indicating that these oxidation-specific epitopes mediate macrophage recognition of apoptotic cells. These antibodies, which have been found in atherosclerotic...
lesions, were found to be structurally and functionally identical to classic “natural” T15 anti-phosphorylcholine antibodies that are of B-1 cell origin and that have been reported to provide protection against virulent pneumococcal infection.14

The inflammatory response elicited by the oxidized phospholipids (eg, Ox-PAPC) found in mildly oxidized LDL is mediated in part by the induction in endothelial and smooth muscle cells of monocyte chemotractant protein-1 (MCP-1)15; macrophage colony stimulating factor (M-CSF),16 a member of the GRO family of chemokines17; P-selectin18; and interleukin 8 (IL-8).19 Additionally, these oxidized phospholipids induce the accumulation of connecting segment-1 of fibronectin on the apical surface of endothelial cells by activating endothelial β1 integrins, particularly those that associate with α5 integrins.20 Connecting segment-1 serves as the endothelial ligand that binds to αβ1 (very late antigen-4, VLA4) on monocytes, thus promoting adhesion of the monocytes to activated endothelial cells.20 The induction of monocyte binding to endothelial cells exposed to mildly oxidized LDL also involves lipoygenase (LO) metabolites.21 The mechanism for the induction of MCP-1 and IL-8 in endothelial cells exposed to mildly oxidized LDL, Ox-PAPC, POVP, or PGPC appears to involve the lipid-dependent transcription factor peroxisome proliferator-activated receptor-α.19

The response of endothelial cells to these oxidized phospholipids appears to be genetically determined.22-25 Using a novel explant technique, Shi et al22 isolated endothelial cells from the aortas of inbred mouse strains with different susceptibilities to diet-induced atherosclerosis. The response of these endothelial cells to mildly oxidized LDL was determined by measuring levels of mRNA for inflammatory genes, including MCP-1, M-CSF, and the oxidative stress susceptibility to diet-induced atherosclerosis. The response from the aortas of inbred mouse strains with different allele (apoE–/–).24 Although the C3H.apoE –/– mice had higher susceptibilities to atherosclerosis in other mouse models.

Formation of LDL-Derived Oxidized Phospholipids That Induce an Inflammatory Response

LDL is usually thought of as the major source of extracellular cholesterol. However, LDL is also a major source of extracellular phospholipid. As noted above, some of these phospholipids can yield oxidized phospholipids that induce an inflammatory response. Subbanagounder and colleagues11 found that the major structural determinant of the biological activity of oxidized phospholipids was at the sn-2 position. Substituting stearoyl for palmitoyl at the sn-1 position or ethanalamine for choline at the sn-3 position did not alter bioactivity.11 All oxovaleroyl phospholipids studied stimulated monocyte binding and inhibited lipopolysaccharide-induced expression of E-selectin.11 All oxovaleroyl phospholipids but not the glucaroyl phospholipids induced monocyte binding without increasing VCAM-1.11 Glutaroyl phospholipids but not oxovaleroyl phospholipids stimulated E-selectin and VCAM-1.11 However, intact phospholipid molecules were required for bioactivity, since activity was destroyed after treatment of the phospholipids with phospholipase (PL) A2, PLAs, or PLC.11 The levels of POVP, PGPC, and PEIPC were increased 3- to 6-fold in rabbit atherosclerotic lesions and corresponded to ∼116, 62, and 85 μg/mL POVP, PGPC, and PEIPC, respectively.11 These levels were ∼10 to 20 times higher than those needed to activate endothelial cells in culture.11

The concept that LDL must be “primed” for oxidation has emerged from the work of many laboratories. Sevanian and colleagues26 described a subpopulation of freshly isolated LDL that was enriched in lipid hydroperoxides, which they named LDL+. Parhasarathy,27,28 Witzius and Steinberg,29 Witzius,30 Chisolm,31 Thomas and Jackson,32 Frei and colleagues (Shwarey et al33 and Polidori et al34), and Thomas et al35 all studied LDL oxidation by metal ions in vitro and, on the basis of their findings, concluded that LDL must be “seeded” with reactive oxygen species before it can be oxidized. Thomas and Jackson32 and Parhasarathy28 suggested that LOs might play a role in this seeding of LDL.

Watson et al4 used defatted albumin to remove the inflammatory lipids from mildly oxidized LDL. Because the lipid-binding properties of apoA-I36-38 are greater than those of defatted albumin, Navab et al39 reasoned that if freshly isolated LDL contained seeding molecules, incubating the LDL with apoA-I and then separating the apoA-I from the LDL might result in a transfer of the seeding molecules from LDL to apoA-I. They hypothesized that this simple strategy could result in the concentration of seeding molecules on apoA-I, from which they could be extracted, identified, and characterized. When freshly isolated LDL was incubated with apoA-I and then separated from apoA-I, the resulting LDL could not be oxidized by human artery wall cells, nor could it induce human artery wall cells to produce monocyte chemo-tactic activity. However, when the lipids that were transferred from LDL to apoA-I were extracted from the apoA-I and subsequently added back to the treated LDL, the reconstituted LDL was readily oxidized and induced monocyte chemotactic activity.40 Similar results were obtained with an apoA-I mimetic peptide.40 Analysis revealed that the apoA-I–associated seeding molecules removed from freshly isolated
HDL and LDL-Derived Oxidized Phospholipids

Figure 1. Formation of LDL-derived oxidized phospholipids. LDL contains PAPC that has a mass to ion ratio (m/z) of 782.4. Arachidonic acid is shown in the diagram at the sn-2 position of PAPC. The 12-LO pathway generates HPETE and HPODE, which directly associate with LDL or interact with cholesteryl linoleate (Chol.18:2) to form cholesteryl linoleate hydroperoxide (CE-OOH), which then associates with LDL. Although the diagram depicts CE-OOH as being formed and then associating with LDL, the CE-OOH could also be formed within LDL after HPODE and HPETE are associated with LDL. When a critical concentration of HPETE, HPODE, and CE-OOH is reached in LDL, PAPC is oxidized forming the pro-inflammatory oxidized phospholipids found in mildly (MM) oxidized LDL. The 3 oxidized phospholipids depicted in MM-LDL are POVPC (m/z 594.3), PGPC (m/z 610.2), and PEIPC (m/z 828.6). See text for explanation of abbreviations.

second step, the seeded LDL is trapped in the artery wall and receives further seeding molecules derived from the LO pathway(s) of nearby artery wall cells. In the third step, a critical level of seeding molecules relative to phospholipids is reached in the LDL, and a nonenzymatic oxidation process generates POVPC, PGPC, PEIPC, and other similar molecules. Many factors likely determine the critical level of seeding molecules needed relative to the phospholipids in LDL to generate the inflammatory oxidized phospholipids. These include the concentration of antioxidants in LDL, the concentration of phospholipids in LDL with arachidonic acid at the sn-2 position, and the content of platelet-activating factor acetylated hydrolase (PAF-AH) in LDL.

The Role of HDL in Modulating the Inflammatory Response Induced by LDL-Derived Oxidized Phospholipids

As noted above, the major apolipoprotein of HDL, apoA-I (but not apoA-II), prevented the formation of LDL-derived oxidized phospholipids by removing seeding molecules from LDL and/or from artery wall cells. However, apoA-I was active only in a preincubation step: adding apoA-I in a coincubation together with LDL did not prevent LDL oxidation or LDL-induced monocyte chemotactic activity. In contrast to apoA-I, apol was effective in preventing both LDL oxidation and LDL-induced monocyte chemotactic activity during coincubation with LDL.

Paraoxonase (PON) is a component of HDL that has been demonstrated both to prevent the formation of mildly oxidized LDL and to inactivate LDL-derived oxidized phospholipids once they are formed. Mackness et al described the role of PON in preventing metal ion oxidation
of LDL. Aviram and colleagues\textsuperscript{57–59} reported that PON has a peroxidase activity that may explain its ability to render freshly isolated LDL resistant to oxidation by human artery wall cells.\textsuperscript{60} PAF-AH, another enzyme associated with some HDL particles, has also been shown to be unable to inactivate LDL-derived oxidized phospholipids.\textsuperscript{61} A third enzyme associated with HDL that may play a role in preventing the formation of and inactivating LDL-derived oxidized phospholipids is lecithin:cholesterol acyltransferase.\textsuperscript{50–54} A fourth HDL-associated enzyme that reduces organic hydroperoxides and is inhibited by physiological concentrations of homocysteine is plasma reduced glutathione selenoperoxidase.\textsuperscript{55} Thus, normal HDL contains several enzymes that can potentially prevent the formation of and inactivate the inflammatory LDL-derived oxidized phospholipids. Except for PAF-AH, the other 3 enzymes are associated exclusively with HDL. Whereas PAF-AH is associated with both LDL and HDL in human plasma, Stafforini and colleagues\textsuperscript{56} have suggested that for the prevention of LDL oxidation, PAF-AH transfers to HDL where it functions more efficiently.

Direct proof of a role for 1 of these HDL-associated enzymes in the development of atherosclerosis has been provided in mouse models. Shih et al\textsuperscript{57} demonstrated that mice lacking the serum PON gene were susceptible to organophosphate toxicity and diet-induced atherosclerosis. In other studies, these authors demonstrated that combined serum PON-knockout/apoE-knockout mice exhibited increased lipoprotein oxidation, with higher levels of POVPC, PGPC, and PEIPC in their IDL and LDL fractions and that the double-knockout mice had significantly more atherosclerosis compared with apoE-knockout mice.\textsuperscript{58} This finding is especially interesting because the apoE-knockout mice at baseline had low levels of PON,\textsuperscript{45} yet removal of their residual PON activity clearly added to lipoprotein oxidation and the development of atherosclerosis,\textsuperscript{58} indicating the importance of PON to these processes.

**LDL-Derived Oxidized Phospholipids and HDL as Components of a System of Nonspecific Innate Immunity**

Sepsis is a major cause of fetal wastage and infant death, especially during the first year of life when the infant’s specific immune system is immature. It is likely that a system of nonspecific innate immunity resulted, in part, in response to this evolutionary pressure. We propose here that LDL-derived oxidized phospholipids and HDL may be part of a system of nonspecific innate immunity. As noted above, natural antibodies to LDL-derived oxidized phospholipids have been identified.\textsuperscript{14} Napoli and colleagues\textsuperscript{59} reported the presence of fatty streaks in human fetal aortas. In that study,\textsuperscript{59} serial sections of the arch, thoracic, and abdominal aortas were immunostained for recognized markers of atherosclerosis, including macrophages, apoB, and the oxidation-specific epitopes malondialdehyde and 4-hydroxynonenal-lysine. The authors concluded that because LDL and oxidized LDL were frequently found in the absence of monocyte-macrophages, while the opposite was rare, suggested that intimal LDL accumulation and oxidation contributed to monocyte recruitment in vivo.\textsuperscript{59} Naturally occurring antibodies to the LDL-derived oxidized phospholipids have been viewed as a component of the innate immune system.\textsuperscript{60} We hypothesize that the oxidative environment created by the inflammatory response to LDL-derived oxidized phospholipids may also be part of a system of innate immunity that evolved to protect the fetus and infant against sepsis. Napoli and colleagues\textsuperscript{59} found that maternal hypercholesterolemia enhanced fatty streak formation in fetal aortas. If our hypothesis is correct, there may also have been evolutionary pressure to select for maternal hypercholesterolemia and this may, in part, explain why hypercholesterolemia is common among many human populations.

Further evidence to support a role for LDL-derived oxidized phospholipids as part of a system of nonspecific innate immunity comes from studies of HDL and LDL during an acute-phase response. Van Lenten et al\textsuperscript{61} reported that HDL, during an acute-phase response in humans (induced by surgery) or in rabbits (induced by injection of croton oil), lost PON and PAF-AH activities and gained the pro-oxidants ceruloplasmin and serum amyloid A. As a result of these changes, HDL was converted from an anti-inflammatory to a proinflammatory particle, as judged by its ability to protect against or enhance LDL oxidation by artery wall cells or to protect against or enhance LDL-induced monocyte chemotactic activity.\textsuperscript{61}

More recently, Memon et al\textsuperscript{62} demonstrated that LDL taken from Syrian hamsters after they had been injected with bacterial lipopolysaccharide, zymosan, or turpentine contained increased amounts of conjugated dienes and lipid hydroperoxides as well as lysophosphatidylcholine, and the acute-phase LDL had a shorter lag phase when oxidized with metal ions in vitro. On the basis of these studies, Hajjar\textsuperscript{63} raised the question as to whether oxidized lipoproteins and infectious agents are in collusion to accelerate atherosclerosis. Although the answer to Hajjar’s question\textsuperscript{63} may well be yes, the reason that these systems evolved could not have been to accelerate atherosclerosis. More likely they evolved as part of a system of nonspecific innate immunity.

Van Lenten et al\textsuperscript{64} sacrificed B6 mice either before or 2, 3, 5, 7, or 9 days after intranasal infection with 10\textsuperscript{5} plaque-forming units of influenza A. Peak infectivity in the lung was reached by 72 hours and returned to baseline by 9 days.\textsuperscript{64} No viremia was observed at any time. PON and PAF-AH activities in HDL decreased after infection, reaching their lowest levels 7 days after inoculation.\textsuperscript{64} The ability of HDL from infected mice to inhibit LDL oxidation and LDL-induced monocyte chemotactic activity in human artery wall cell cocultures decreased with time after inoculation.\textsuperscript{64} As the infection progressed, LDL more readily induced monocyte chemotaxis. Peak IL-6 and serum amyloid A plasma levels were observed 2 and 7 days after inoculation. HDL apoA-I levels did not change, but apoJ and ceruloplasmin levels in HDL peaked 3 days after infection. Ceruloplasmin markedly increased and remained elevated throughout the time course, whereas apoJ levels decreased toward baseline after the third day. It was concluded that alterations in the relative levels of PON, PAF-AH, ceruloplasmin, and apoJ in HDL occurred during acute influenza infection and caused HDL to lose its anti-inflammatory properties.\textsuperscript{64}

In other studies, Van Lenten et al\textsuperscript{65} found that a key cytokine in the acute-phase response, IL-6, was required for short-term regulation of PON but not of MCP-1 and was not
required for the long-term downregulation of PON by an atherogenic diet in susceptible B6 mice. In short-term feeding experiments (1 to 7 days), Hedrick et al.\textsuperscript{66} found that there was a dramatic decrease in HDL cholesterol, apoA-I, and PON in susceptible B6 LDL receptor–knockout mice that was associated with a rapid increase in HDL lipid hydroperoxides and formation of high-molecular-weight forms of apoA-I that contained an epitope recognized by a monoclonal antibody that recognizes POVPC. Measurement of the levels of apoA-I complexes associated with immunoglobulins, together with the time course of events, suggested that preformed antibodies to oxidized lipid–apoA-I complexes were present before the atherogenic diet was administered.\textsuperscript{66} It was concluded that on feeding the atherogenic diet, the number of epitopes increased to a critical threshold, and this resulted in the clearance of the immune complexes.\textsuperscript{66} Ox-PAPC induced IL-6, a potent acute-phase response mediator, when injected into B6 LDL receptor–knockout mice.\textsuperscript{65} HDL from B6 mice on a chow diet inhibited LDL oxidation, whereas HDL from the same mice on an atherogenic diet promoted oxidation.\textsuperscript{67} The latter was enriched in apoJ, which is a marker of the acute-phase response.\textsuperscript{44} In contrast, HDL from C3H mice that were resistant to diet-induced atherosclerosis protected LDL from oxidation, whether the mice were maintained on a chow or an atherogenic diet,\textsuperscript{67} and HDL from C3H mice on the atherogenic diet did not have increased levels of the acute-phase reactant apoJ.\textsuperscript{45} These studies suggest a link between proinflammatory HDL and decreased PON activity.\textsuperscript{8,45,67–69} Leitinger et al.\textsuperscript{8} found that an atherogenic diet resulted in the formation of proinflammatory HDL particles (AP-HDL). B, In the basal state, HDL contains apoA-I and apoJ as well as 4 enzymes, PON, PAF-AH, lecithin:cholesterol acyltransferase (LCAT), and plasma reduced glutathione selenoperoxidase (GSH peroxidase) that can prevent the formation of or inactivate the inflammatory LDL-derived oxidized phospholipids found in mildly oxidized LDL. As a result, in the basal state, HDL may be considered anti-inflammatory. During the acute-phase reaction, A-I may be displaced by the pro-oxidant acute-phase reactant SAA. Another pro-oxidant acute-phase reactant, ceruloplasmin, associates with HDL as does the anti-oxidant acute phase reactant apoJ. PON, PAF-AH, and LCAT decrease in HDL during the acute-phase reaction, and the lipid hydroperoxides HPETE, HPODE, and cholesteryl linoleate hydroperoxide (CE-OOH) increase in HDL. A-II and GSH peroxidase are shown as unchanged during the acute-phase reaction although there are no data on the latter. The net effect of the changes in HDL during the acute-phase reaction is the production of pro-oxidant, proinflammatory HDL particles (AP-HDL). B, In the basal state, HDL prevents the formation of and inactivates the LDL-derived oxidized phospholipids shown in Figure 1. As a result, HDL favors the maintenance of noninflammatory LDL and the conversion of the proinflammatory, mildly oxidized LDL (MM-LDL) to a noninflammatory state. In contrast, during an acute-phase reaction, HDL favors the conversion of LDL to the proinflammatory MM-LDL. As discussed in the text, the acute-phase reaction can be truly acute, as in the case of a viral infection, or it may become chronic, as in mice that are genetically susceptible to diet-induced atherosclerosis when they are fed an atherogenic diet or in some patients with normal blood lipids and atherosclerosis.

![Figure 2](image)

Figure 2. The acute-phase (AP) reaction favors the formation of proinflammatory HDL and mildly oxidized LDL. A, In the basal state, HDL contains apoA-I and apoJ as well as 4 enzymes, PON, PAF-AH, lecithin:cholesterol acyltransferase (LCAT), and plasma reduced glutathione selenoperoxidase (GSH peroxidase) that can prevent the formation of or inactivate the inflammatory LDL-derived oxidized phospholipids found in mildly oxidized LDL. As a result, in the basal state, HDL may be considered anti-inflammatory. During the acute-phase reaction, A-I may be displaced by the pro-oxidant acute-phase reactant SAA. Another pro-oxidant acute-phase reactant, ceruloplasmin, associates with HDL as does the anti-oxidant acute phase reactant apoJ. PON, PAF-AH, and LCAT decrease in HDL during the acute-phase reaction, and the lipid hydroperoxides HPETE, HPODE, and cholesteryl linoleate hydroperoxide (CE-OOH) increase in HDL. A-II and GSH peroxidase are shown as unchanged during the acute-phase reaction although there are no data on the latter. The net effect of the changes in HDL during the acute-phase reaction is the production of pro-oxidant, proinflammatory HDL particles (AP-HDL). B, In the basal state, HDL prevents the formation of and inactivates the LDL-derived oxidized phospholipids shown in Figure 1. As a result, HDL favors the maintenance of noninflammatory LDL and the conversion of the proinflammatory, mildly oxidized LDL (MM-LDL) to a noninflammatory state. In contrast, during an acute-phase reaction, AP-HDL favors the conversion of LDL to the proinflammatory MM-LDL. As discussed in the text, the acute-phase reaction can be truly acute, as in the case of a viral infection, or it may become chronic, as in mice that are genetically susceptible to diet-induced atherosclerosis when they are fed an atherogenic diet or in some patients with normal blood lipids and atherosclerosis.

in this model the PON activity was responsible for the proinflammatory HDL.\textsuperscript{69} PON has also been associated with atherosclerosis in humans. James and colleagues\textsuperscript{70} reported that smoking was independently associated with significant decreases in serum PON activities and concentrations in patients with coronary artery disease and that cessation of smoking led to an increase

Proinflammatory HDL as a Potential Marker of Susceptibility to Atherosclerosis

HDL has been previously described as a “chameleon-like” lipoprotein,\textsuperscript{3} being anti-inflammatory in the basal state and proinflammatory during an acute-phase response. As noted above, LDL-derivated oxidized phospholipids were found to induce IL-6 in hepatocytes and to repress PON mRNA levels.\textsuperscript{65} A number of mouse models that exhibit susceptibility to atherosclerosis have been found to have proinflammatory HDL and decreased PON activity.\textsuperscript{5,45,67–68} Leitinger et al.\textsuperscript{8} found that an atherogenic diet resulted in the formation of oxidized phospholipids in the livers of mice that were genetically susceptible to diet-induced atherosclerosis, and these oxidized phospholipids were increased further in mice transgenic for secretory PLA\textsubscript{2}.\textsuperscript{8} Presumably, these oxidized phospholipids induced an acute-phase response, which resulted in proinflammatory HDL in mice susceptible to diet-induced atherosclerosis.\textsuperscript{8,45,67} ApoE\textsuperscript{−/−} mice also had evidence of proinflammatory HDL and low PON activity.\textsuperscript{45} HDL from transgenic mice overexpressing apoA-II had proinflammatory HDL and developed atherosclerosis on a chow diet.\textsuperscript{68,69} Total HDL concentrations in the transgenic mice overexpressing apoA-II were elevated but the PON activity was not, resulting in a concentration of PON in HDL of approximately half normal.\textsuperscript{69} Addition of exogenous PON to the HDL of the transgenic mice overexpressing apoA-II converted the HDL from proinflammatory to anti-inflammatory, suggesting that
in serum PON within months. This group of patients had mildly elevated LDL cholesterol levels on average and normal HDL cholesterol levels that were lowest in the smokers. The relationship of PON to coronary heart disease is complicated by the polymorphisms present in humans. However, the concentration of PON and its activity were significantly lower in patients immediately after myocardial infarction compared with those in age- and sex-matched controls. Forty-two days after infarction, PON activity had increased but was still lower than in controls. Analysis of PON1 genotypes did not discriminate between patients and controls.

Navab and colleagues found that PON activity in HDL was significantly lower in 24 patients with angiographically documented coronary artery disease who were normolipidemic and who were neither diabetic nor taking hypolipidemic medications. However, there was an overlap between patients and 29 age- and sex-matched controls. As noted above, PON is just 1 of at least 4 enzymes and 2 apolipoproteins associated with HDL that can potentially modulate the formation of or inactivate LDL-derived oxidized phospholipids. Therefore, it was not surprising that there was overlap in PON activities between patients and controls. To focus on whole HDL rather than on PON exclusively, Navab et al studied a subset of the patients to determine whether their HDL was anti-inflammatory or proinflammatory. HDL from 10 of 10 of the patients not only failed to inhibit LDL oxidation by artery wall cells and the biological activity of Ox-PAPC but, on average, also actually increased LDL oxidation and enhanced the biological activity of Ox-PAPC. HDL from 10 of 10 age- and sex-matched controls inhibited both LDL oxidation and the biological activity of Ox-PAPC. These data suggest that some patients with coronary artery disease and normal HDL cholesterol levels have proinflammatory HDL. These data also suggest that proinflammatory HDL may be a marker of susceptibility to atherosclerosis in humans as it appears to be in mice. However, large-scale studies will be required to determine the true predictive value of such tests. Such studies would have to take into consideration the age of the subject. At birth, human HDL cholesterol levels are equal to or greater than adult levels. However, PON activity at term is approximately half of what it is at age 2 years. Low PON activity relative to HDL cholesterol levels at birth may be part of a nonspecific innate immune system that protects the infant against sepsis as discussed above. However, the persistence of proinflammatory HDL into adulthood may predispose and predict susceptibility to atherosclerosis.

There is increasing evidence that markers of inflammation and the acute-phase response, including induction of C-reactive protein, predict susceptibility to risk for coronary syndromes. It has been suggested that the acute-phase response can become chronic and that a state of low-grade systemic inflammation is a consequence of being overweight. It may well be that the changes seen in HDL in mice with diet-induced atherosclerosis and in some patients with normal blood lipid levels represent a chronic acute-phase response. This chronic acute-phase response could be perpetuated, in part, by LDL-derived oxidized phospholipids and may be exacerbated by the infections and stresses (e.g., surgeries) that humans endure in modern society. Figure 2A summarizes the changes that occur in HDL during an acute-phase response, and Figure 2B indicates what the impact of these changes in HDL would be on the balance between LDL and mildly oxidized LDL in the artery wall. If the concentration of LDL-derived oxidized phospholipids determines the intensity of the inflammatory response in the artery wall, then the balance between noninflammatory LDL (i.e., LDL that does not induce artery wall cells to make proinflammatory molecules such as MCP-1) and mildly oxidized LDL, which is proinflammatory, would in part determine plaque vulnerability and hence, susceptibility to heart attack and stroke. As indicated in Figure 2, HDL likely plays a key role in modulating these processes and hence, in preventing or promoting heart attack and stroke.

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