Formation of Dysfunctional High-Density Lipoprotein by Myeloperoxidase

Stephen J. Nicholls, Lemin Zheng, and Stanley L. Hazen*

Recent studies identify the presence of high-density lipoprotein (HDL) particles in patients with cardiovascular disease, which are "dysfunctional," lacking in typical atheroprotective properties, and promoting proinflammatory effects. The mechanisms for generating dysfunctional HDL have been unclear. New evidence points to a role for myeloperoxidase (MPO)-generated oxidants as participants in rendering HDL dysfunctional within human atherosclerotic plaque. Myeloperoxidase was recently shown to bind to HDL within human atherosclerotic lesions, and biophysical studies reveal MPO binding occurs via specific interactions with apolipoprotein (apo) A-I, the predominant protein of HDL. This likely facilitates the observed selective targeting of apoA-I for site-specific chlorination and nitration by MPO-generated reactive oxidants in vivo. One apparent consequence of MPO-catalyzed apoA-I oxidation includes the functional impairment of the ability of HDL to promote cellular cholesterol efflux via the adenosine triphosphate binding cassette-1 transport system. Myeloperoxidase-mediated loss of the atheroprotective functional properties of HDL may thus provide a novel mechanism linking inflammation and oxidative stress to the pathogenesis of atherosclerosis. (Trends Cardiovasc Med 2005;15:212–219) © 2005, Elsevier Inc.

Stephen J. Nicholls, Lemin Zheng and Stanley L. Hazen are at Center for Cardiovascular Diagnostics and Prevention, Cleveland Clinic Foundation, Cleveland, Ohio.

* Address correspondence to: Stanley L. Hazen, MD, PhD, Cleveland Clinic Foundation, Lerner Research Institute, Center for Cardiovascular Diagnostics and Prevention, 9500 Euclid Avenue, NE-10, Cleveland, OH 44195, USA. Tel.: (+1) 216-445-9763; fax: (+1) 216-636-0392; e-mail: hazens@ccf.org.

Since the seminal observation in the Framingham study that levels of plasma high-density lipoprotein (HDL) cholesterol serve as the strongest lipoprotein predictor of clinical risk (Gordon et al. 1977), numerous population (Gordon et al. 1989) and animal (Badimon et al. 1989, Plump et al. 1994) studies have established the atheroprotective properties of HDL. In addition to the well-characterized ability to promote reverse cholesterol transport (Fielding et al. 1995), HDL has reported antioxidant, antiinflammatory, and antithrombotic activities (Nofer et al. 2002, Bielicki and Oda 2002, Barter et al. 2004). Direct infusion of apolipoprotein (apo) A-I in animal models inhibits progression of atherosclerotic plaque (Miyazaki et al. 1995), and in recent studies, reconstituted forms of HDL in humans demonstrate both dramatic benefit on endothelial function (Spieker et al. 2002, Bisoendial et al. 2003) and regression of atherosclerotic burden (Nissen et al. 2003).

Despite the numerous demonstrated atheroprotective effects of HDL, high levels of the lipoprotein are not always protective in subjects, suggesting that not all HDLs function to prevent atherosclerosis. There remains a lack of consensus on how to manage patients with low levels of HDL. Raising HDL is a secondary goal in the current lipid management guidelines of the National Cholesterol Education Program (Grundy et al. 2004). Moreover, clinically available therapeutic agents have only limited ability to substantially elevate plasma HDL. As a result, there is no consensus supporting a target level for HDL elevation. This discrepancy results, in part, from the recognition that the quality of HDL may be as important as its quantity.

A growing body of evidence supports the notion that some HDL is "dysfunctional" or "proinflammatory", facilitating leukocyte recruitment and cellular activation phenotypes. Oxidative processes have been linked to the presence of proinflammatory HDL, though the precise pathways responsible for its formation are not established (Barter et al. 2004). Navab et al. (2000, 2001a, 2001b, 2001c) have shown that detection of proinflammatory HDL may serve as a useful marker for gauging susceptibility for atherosclerosis in subjects (Ansell et al. 2003). These investigators found that HDL isolated from patients with coronary artery disease and plasma HDL levels in the normal or high range promoted rather than inhibited monocyte chemotaxis in response to oxidized low-density lipoprotein (LDL), suggesting a possible mechanism for why normal levels of HDL are not always protective.

This paper focuses on studies characterizing dysfunctional or proinflammatory forms of HDL, with particular emphasis on recent insights into pathways contributing to the oxidative modification of HDL in vivo, and their apparent influence on the functional properties of the lipoprotein. A series of recent reports identify novel in vivo modifications of apoA-I within human atherosclerotic plaque that are catalyzed by the enzyme myeloperoxidase

(MPO). These modifications appear to have a detrimental impact on the beneficial properties of the lipoprotein, inhibiting both lipid binding and adenosine triphosphate binding cassette-1 (ABCA-1)-dependent cholesterol efflux activities. The relationships between MPO-generated reactive oxidant species and impaired atheroprotection of HDL provide additional mechanistic links between inflammation, oxidative stress and atherogenesis.

- **Dysfunctional Forms of High-Density Lipoprotein**

Despite the consistent demonstration that a low plasma HDL is a strong predictor of clinical risk, it is apparent that many patients with "normal" or even "elevated" plasma HDL experience clinical events. In fact, nearly half of the clinical events in the Framingham cohort occurred in subjects with plasma HDL concentrations ≥40 mg/dL (Kwiterovich 1998). It has been proposed that HDL with impaired functional properties within subjects of this cohort may lead to either a loss of protective benefit or even an actual promotion of atherogenic events (Ansell et al. 2003).

It is therefore of interest that HDL recovered from different subjects often demonstrates marked heterogeneity in its in vitro functional properties. For example, Ashby et al. (2001) demonstrated that HDL isolated from distinct subjects differed markedly in their ability to inhibit cytokine-induced expression of the adhesion molecule vascular cell adhesion molecule-1 by endothelial cells. Several investigators have similarly reported that HDL isolated from diabetic subjects has impaired ability both to promote cellular cholesterol efflux and to prevent the oxidation of LDL (Gowri et al. 1999, Syvanne et al. 1996).

Another intriguing observation is that the antiinflammatory properties of HDL reportedly decline in the setting of the acute phase response. Van Lenten et al. (1995) compared the functional properties of HDL isolated from subjects before versus after elective surgery. High-density lipoprotein isolated preoperatively demonstrated antiinflammatory properties, such as the ability to inhibit LDL oxidation and subsequent monocyte

chemotaxis. In contrast, HDL isolated postoperatively promoted both LDL oxidation and monocyte chemotaxis. Functional properties of HDL during acute phase responses have been further studied in humans, rabbits, and mice (Ashby et al. 2001, Van Lenten et al. 1995, 2001). The transition to a proinflammatory form of HDL is reportedly associated with alterations in the composition of circulating HDL-associated proteins. Both reductions in the HDL contents of paraoxonase and platelet-activating factor acetylhydrolase, as well as parallel elevations in HDL content of serum amyloid A and ceruloplasmin, are reported (Van Lenten et al. 1995). These alterations in composition may underscore the observed effects of such proinflammatory HDL on monocyte chemotaxis (Ansell et al. 2003). Of interest, 6 weeks of treatment with simvastatin reportedly reduced the extent of monocyte chemotaxis induced by HDL preparations isolated from subjects with prior proinflammatory HDL (Ansell et al. 2003). These results further support the notion that the quality, rather than the

quantity, of circulating HDL may serve as the more important determinant of overall cardiovascular risk.

Whereas HDL with apparent proinflammatory properties has been widely reported, the underlying mechanism(s) responsible for generating these functionally heterogeneous HDLs, and the chemical components responsible, remain largely unexplored. A leading hypothesis suggests that the nature and degree of oxidative modification of HDL may be responsible for the functional and biologic heterogeneity.

- **High-Density Lipoprotein as a Selective Target of Oxidation in the Artery Wall**

Low-density lipoprotein oxidation is widely believed to play an important role in the development of atherosclerotic plaque. Native LDL has little effect on cells of the arterial wall, whereas oxidatively modified forms of LDL induce numerous proatherosclerotic effects, including promotion of cholesterol deposition and foam cell formation (Partha-

Table 1. Preferential nitration and chlorination of apoA-I in serum of patients with established cardiovascular disease and in atherosclerotic lesions

	NO_2Tyr		$ClTyr$	
	Median (IQR) (μmol oxTyr/molTyr)	P value	Median (IQR) (μmol oxTyr/molTyr)	P value
Serum				
Total protein				
Control	6.1 (3.9–7.8)		1.6 (0.6–2.4)	
CVD	9.0 (5.7–12.9)	<.001	1.9 (1.3–3.1)	.07
apoA-I				
Control	438 (335–598)		186 (114–339)	
CVD	629 (431–876)	.005	500 (335–650)	<.001
Normal artery				
Total protein	55 (24–143)		63 (25–128)	
apoA-I	401 (185–637)	<.001	678 (299–1311)	<.001
Atheroma				
Total protein	108 (51–346)		232 (111–431)	
apoA-I	2340 (1665–5050)	<.001	3930 (1679–7005)	<.01

Results are shown for median and interquartile (IQR) ranges of NO_2Tyr and $ClTyr$ contents of (**A**) total protein and apoA-I circulating in serum of patients with and without cardiovascular disease (CVD) and (**B**) total protein and apoA-I isolated from a normal arterial wall and atherosclerotic lesions, expressed as the mole ratio of oxidized to parent amino acid tyrosine. The *P* values shown are for comparisons of NO_2Tyr or $ClTyr$ content (**A**) in serum between control and CVD groups and (**B**) between normal arterial wall and atherosclerotic lesions. Adapted with permission from J Clin Invest 2004;114:529.