Modifying the Anti-inflammatory Effects of High-density Lipoprotein

Benjamin J. Ansell, MD, Gregg C. Fonarow, MD, Mohamad Navab, PhD, and Alan M. Fogelman, MD

Corresponding author
Benjamin J. Ansell, MD
Department of Medicine, David Geffen School of Medicine at UCLA, 100 UCLA Medical Plaza, Suite 525, Los Angeles, CA 90095, USA.
E-mail: bansell@mednet.ucla.edu

Current Atherosclerosis Reports 2007, **9:**57–63
Current Medicine Group LLC ISSN 1523-3804

The anti-inflammatory effects of high-density lipoproteins (HDL) are well documented and include inhibition of low-density lipoprotein (LDL) oxidation, reduction of inflammatory cytokines and vascular leukocyte adhesion molecules, and participation in innate immunity. However, certain conditions, including coronary disease, diabetes mellitus, systemic inflammation, and a diet high in saturated fat, are associated with modification of HDL such that it paradoxically enhances LDL oxidation and/or vascular inflammation. Treatment with statins and/or apolipoprotein A1 mimetic peptides improves HDL's anti-inflammatory functions, and these as well as other medications may represent a novel pathway through which to target atherosclerosis.

Introduction

The inverse relationship between high-density lipoprotein (HDL) cholesterol and coronary heart disease (CHD) reflects many of the atheroprotective effects of HDL [1]. In the absence of systemic inflammation, HDL enhances reverse cholesterol transport, inhibits thrombosis, slows oxidation of low-density lipoproteins (LDL), and decreases production of inflammatory cytokines and adhesion molecules within the vascular wall [2–5]. HDL can also moderate acute inflammation as well, as evidenced by the attenuation of an inflammatory response to either acute ischemia or septic shock following infusion of HDL [6–8].

In response to systemic inflammation, however, HDL can itself be modified to become either less functional or frankly proinflammatory. This conversion can occur in response to an acute inflammatory process, such as influenza infection or elective surgery, or it can reflect conditions that are characterized by a chronic acute phase response such as coronary disease, diabetes mellitus, infections, and some rheumatologic diseases [9]. Previously in this journal, we reviewed the varying functions of HDL and their relationship to systemic inflammation [10••]. In this review, we address proven and emerging means by which HDL's anti-inflammatory functional capacity can be modified.

Dysfunctional and Proinflammatory HDL

Proinflammatory HDL

A systemic inflammatory stimulus can generate HDL that paradoxically promotes, rather than inhibits, vascular inflammation (ie, proinflammatory HDL). This is even grossly visible in the setting of Group A streptococcal infection. A streptococcal virulence determinant known as serum opacity factor binds apolipoprotein A-I (apoA-I) and apoA-II in HDL, inducing a disruption of the usual helical structure of these apolipoproteins that causes release of tiny lipid droplets, leading to serum opacification [11]. Courtney et al. [11] proposed that this conformational change and lipid depletion contributes to the development of proinflammatory HDL.

In addition, the acute phase response generated by an infection leads to increased concentrations of the prooxidant enzyme ceruloplasmin, along with decreased activity of the iron-binding antioxidant transferrin, both contributing to increased oxidative stress within HDL [12]. Van Lenten et al. [13] reported that intranasal administration of influenza virus in mice was associated with decreased levels of the HDL-associated antioxidants paraoxonase and platelet-activating factor acetylhydrolase, along with increased concentrations of ceruloplasmin and apoJ. These changes peaked 3 days after inoculation and were associated with impaired abilities to inhibit monocyte chemotaxis in an artery wall cell co-culture model [13].

A noninfectious acute phase response is associated with similar changes within HDL. Kumon et al. [14] reported that levels of paraoxonase and lecithin-cholesterol acyl transferase fell by 76% and 56%, respectively, in the days following elective laparoscopic cholecystectomy in 12 study patients. These changes coincided with increased serum levels of the acute phase reactants C-reactive protein (CRP) and serum amyloid A (SAA) [14].

Navab et al. [15] have suggested that a chronic form of the acute phase response described by Gabay and Kushner [16] can develop into atherosclerosis and other forms of chronic inflammation, which lead to proinflammatory changes in HDL. Ansell et al. [17•] reported that HDL isolated from most patients with CHD and/or its risk equivalents promoted vascular inflammation. In a group of patients with clinical CHD despite very high HDL cholesterol (HDL-C) levels (≥ 84 mg/dL), the addition of their HDL uniformly increased LDL-mediated monocyte chemotaxis in the co-culture model and enhanced phospholipid oxidation in a cell-free HDL functional assay. Such proinflammatory HDL was seen in 77% and 96% of a different group of 26 subjects with CHD risk equivalents with average HDL-C levels using the monocyte chemotaxis and cell free assays, respectively [17•]. None of the healthy controls that were tested showed evidence for proinflammatory HDL, as their HDL consistently inhibited monocyte recruitment and phospholipid oxidation [17•].

In addition to coronary disease, other chronic inflammatory states are often characterized by abnormally functioning HDL. Impaired antioxidant capacity has been described in several studies of HDL in diabetes mellitus and metabolic syndrome [18–20].

Morena et al. [21] found that HDL isolated from hemodialysis patients showed impaired ability to inhibit LDL oxidation compared with HDL from healthy controls. McMahon et al. [22] recently reported that 45% and 20% of patients with systemic lupus erythematosus and rheumatoid arthritis have frankly proinflammatory HDL, respectively. In contrast, only 4% of healthy controls exhibited proinflammatory HDL ($P < 0.001$ in comparison to both rheumatologic conditions) [22]. Furthermore, the presence of proinflammatory HDL correlated with the amount of oxidized LDL ($r = 0.37$; $P < 0.001$) [22].

The presence of oxidized lipids within the subendothelial space leads to expression of monocyte chemoattractant protein-1 (MCP-1), which upregulates cellular adhesion molecules that enhance monocyte entry. Injection of leumedin (an anti-inflammatory compound) into rabbits generated HDL that was much more effective than HDL from control rabbits in slowing LDL oxidation in vitro [23]. Furthermore, HDL's ability to retard monocyte chemotaxis, and thus limit cellular inflammation, is highly correlated with its ability to slow phospholipid oxidation [17•]. Similarly, HDL's inhibitory effect on monocyte chemotaxis is highly correlated with HDL's facilitation of cholesterol efflux [15]. These observations suggest that these seemingly disparate functions of HDL are linked.

Structure/function changes

Ferretti et al. [24•] recently reviewed the range of chemical modifications to HDL and their associated alterations in HDL functions. Chemical alterations in HDL, its apolipoproteins, and phospholipids can adversely impact HDL's ability to mediate cholesterol efflux and prevent LDL oxidation and its antioxidant enzymatic activities [24•]. ApoA-I can form dimers, trimers, and heterodimers with itself and other apolipoproteins in response to some of these alterations, particularly oxidation (Fig. 1) [24•].

Within the artery wall, macrophage phagocytic enzymes such as myeloperoxidase generate hydrogen peroxide (H_2O_2) and superoxide (O_2-), which can form HDL oxidants such as tyrosyl (from tyrosine), hypochlorite (from chloride ion), and peroxynitrate (from nitric oxide via lipoxygenase) [24•]. Zheng et al. [25] also reported direct oxidation of apoA-I by myeloperoxidase. Transitional metal ions are also capable of peroxidation of polyunsaturated fats within phospholipids in HDL. Oxidized HDL (oxHDL) shows decreased paraoxanase activity, impaired ability to promote cholesterol efflux, and adverse effects on vascular inflammation [24•,26,27]. For example, Girona et al. [26] demonstrated that, in contrast to native HDL, oxHDL actually behaves like oxidized LDL, inhibiting THP-1 human monocyte–derived macrophage production of tumor necrosis factor-α in vitro. Nitrotyrosine and chlorotyrosine modification of HDL following these oxidation steps leads to further impairment in reverse cholesterol transport [28].

Nonenzymatic glycation of HDL, which often accompanies hyperglycemic conditions, is associated with decreased paraoxonase levels and renders HDL more susceptible to oxidation [29,30]. Oxidized glycated HDL is itself less effective as an antioxidant and in facilitating reverse cholesterol transport. These findings may explain the strong correlation between the degree of glycemic control and both HDL's antioxidant capacity [18] and its ability to inhibit monocyte chemotaxis (Navab, Unpublished data).

Ahmed et al. [31] reported that the hydrolysis of HDL by endothelial lipase appears to be important in determining the expression of endothelial adhesion molecules. The hydrolyzed HDL is a potent activator of the peroxisomal proliferator-activator receptor, which in turn upregulates adhesion molecules on the endothelial cell surface [31].

In contrast to these chemical modifications, some alterations of HDL appear to render it more atheroprotective. In apoE-deficient mice, Macdonald et al. [32] demonstrated that intraperitoneal administration of HDL that had undergone tyrosyl oxidation enhanced reverse cholesterol transport and inhibited aortic atherosclerosis better than either unmodified HDL or saline. Wang et al. [33] have shown that tyrosyl oxidation of HDL allows for dimerization of apoA-I, with enhanced reverse cholesterol transport seen, especially with heterodimers of apoA-I and apoA-II.

Dietary Effects

Although a multivariate regression analysis [34] of a population-based study of lifestyle, genetic, and nutritional factors failed to find any relationship between diet and paraoxonase levels, a recent study indicates that

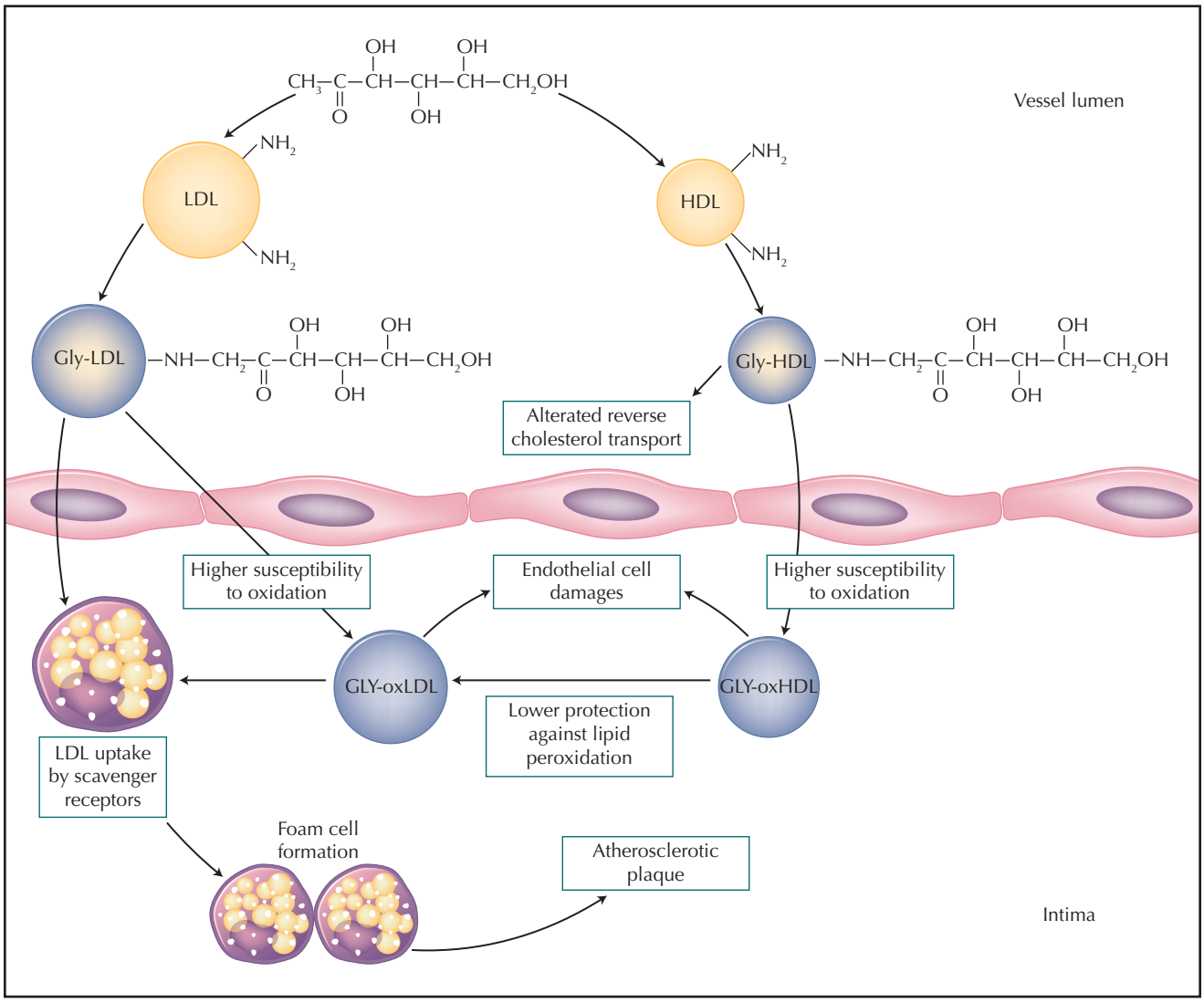

Figure 1. Atherogenic effects of glycation of high-density lipoprotein (HDL), including impaired reverse cholesterol transport, increased susceptibility to oxidation, and decreased ability to slow oxidation of low-density lipoprotein (LDL), which itself is rendered more susceptible to oxidation by glycation. Gly—glycation; ox—oxidized (*Adapted from* Ferretti et al. [24•].)

dietary composition can affect HDL's anti-inflammatory capacity. In a crossover design, Nicholls et al. [35•] compared the effects of two high-fat meals that contained either predominantly polyunsaturated fat (safflower oil) versus saturated fat (coconut oil) sources in 14 healthy volunteers who were approximately 30 years of age. The high-fat meals were administered in a random order, separated by 1 month, and (in the case of female subjects) timed at 7 days following menstruation. HDL was isolated from the subjects in a fasting state, then at 3 and 6 hours following each high-fat meal. Brachial artery reactivity was also assessed at the same time points.

Nicholls's group then assessed the effect of the HDL on expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1) on the surface of human umbilical vein endothelial cells that were activated by tumor necrosis factor-α. The expression of these inflammatory cellular adhesion molecules was significantly lower at both time points following the polyunsaturated fat meal compared with the fasting state. In contrast, the saturated fat meal increased the expression of ICAM-1 and VCAM-1 at 6 hours compared with fasting, as depicted in Figure 2. Importantly, the protein, cholesterol, and phospholipid content; particle size; and electophoretic pattern of HDL were not significantly changed from the fasting state by either meal. The researchers also reported a modest decrease in post-hyperemic flow-mediated dilatation in the subjects after a saturated but not a polyunsaturated fat meal [35•].

Pharmacologic Effects

Statins

In addition to their well-characterized effects on LDL, statins have significant effects on HDL and its function. Although statin therapy is typically associated with only

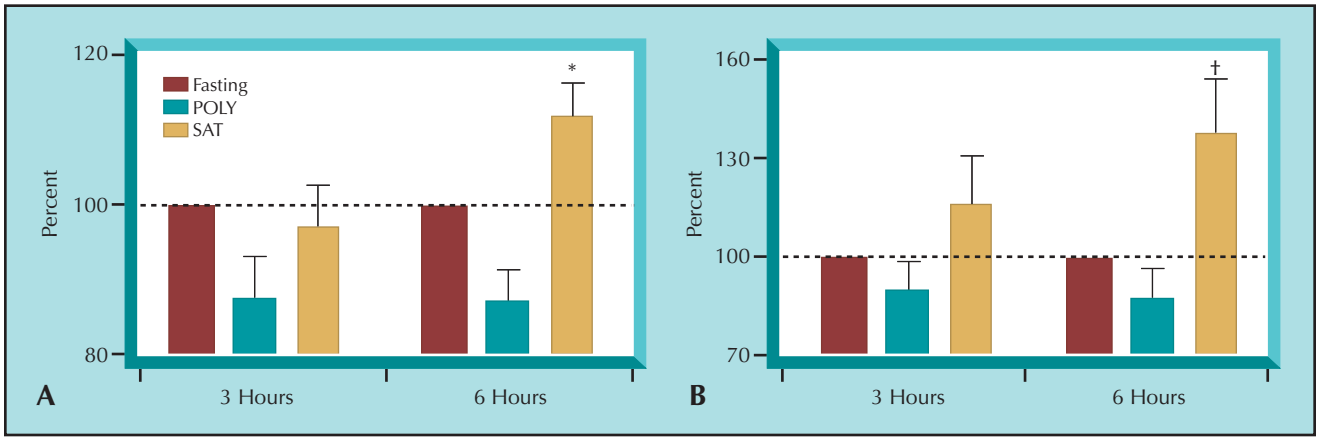

Figure 2. Intercellular adhesion molecule-1 (ICAM-1) (*panel A*) and vascular cell adhesion molecule-1 (VCAM-1) (*panel B*) expression by cultured human endothelial cells after incubation with high-density lipoprotein isolated from subjects following ingestion of meals containing predominantly polyunsaturated (POLY) or saturated (SAT) fat. Results are compared with the ICAM-1/VCAM-1 expression in the presence of high-density lipoprotein isolated from fasting blood (mean ± standard error of mean). *Asterisk* indicates difference between the meals of $P = 0.005$. *Dagger* indicates difference between the meals of $P = 0.007$. (*Adapted from* Nicholls et al. [35•].)

Table 1. Pro- and anti-inflammatory effects of simvastatin on HDL-C

	Inflammatory index*				Anti-inflammatory HDL	
Subjects	**HDL-C, *mg/dL***	**CFA**	**MCA**	***P* value**	**CFA**	**MCA**
Controls	63.5 ± 6.1	0.53 ± 0.15	0.38 ± 0.14	NA	100%	100%
Patients before simvastatin†	57 ± 13	1.19 ± 0.19	1.38 ± 0.91	< 0.001	4%	23%
Patients after simvastatin†	61 ± 14	0.91 ± 0.28	1.08 ± 0.71	0.002	58%	46%

*Inflammatory index is defined as the assay results using the subject's HDL divided by the results of the assay when performed with no HDL present. The fraction of anti-inflammatory HDL in each group is shown using both assays.
†Patients were statin-naive in the setting of coronary heart disease or a risk equivalent.
CFA—cell-free assay; HDL-C—high-density lipoprotein cholesterol; MCA—monocyte chemotaxis assay.
(*Data from* Ansell et al. [17•].)

modest (2%–9%) increases in HDL cholesterol [36], effects on HDL anti-inflammatory function appear to be more substantial. Girona et al. [37] reported that simvastatin treatment in 15 normolipidemic subjects reduced oxHDL formation by 64% ($P < 0.05$) in vivo. Some of this antioxidant activity may reflect the significant inhibition of gene expression of myeloperoxidase that has been seen with statin treatment [38]. Preliminary data also support the role of statin therapy compared with placebo in improving the anti-inflammatory characteristics of HDL treatment in patients with active rheumatoid arthritis [39].

Ansell et al. [17•] analyzed the effects of a 6-week course of simvastatin, 40 mg/d in the 26 aforementioned subjects with CHD or risk equivalents (Table 1). Findings were normalized using an "inflammatory index," which was the results of the assay using the subject's HDL divided by the results of the assay in the absence of HDL. An inflammatory index greater than 1.0 indicates proinflammatory HDL, whereas an index less than 1.0 defines anti-inflammatory HDL. Based upon the cell-free assay of phospholipid oxidation, the statin-naive patients' HDL was proinflammatory (inflammatory index of 1.19 ± 0.19) relative to HDL from healthy age/sex-matched controls (0.53 ± 0.19; $P < 0.001$) [17•]. After 6 weeks of treatment with simvastatin, 40 mg/d, HDL isolated from the same patients showed a reduction in the inflammatory index to 0.91 ± 0.28 (ie, it was mildly anti-inflammatory), but not as much as the control HDL [17•]. The results of the monocyte chemotaxis assay were similar in that the patients' HDL was proinflammatory with an inflammatory index of 1.38 ± 0.91 compared with the anti-inflammatory control HDL (0.38 ± 0.14; $P < 001$) [17•]. After simvastatin treatment, the patient HDL inflammatory index improved to 1.08, but still indicated proinflammatory HDL [17•]. Although only 4% and 23% of patients exhibited anti-inflammatory HDL at baseline using the cell-free and monocyte chemotaxis assays, respectively, approximately half of the patients converted to anti-inflammatory HDL following statin treatment, as shown in Table 1 [17•].

HDL and mimetics

Infusions of HDL and apoA-I have been associated with enhanced reverse cholesterol transport, decreased proinflammatory characteristics of HDL, and atherosclerotic regression, mostly in animal models [40–42]. Five weekly infusions of recombinant apoA-I Milano, the natural form of which is protective against coronary disease, were associated with short-term atherosclerotic regression

(4.2% from baseline), in contrast to an ineffective placebo ($P < 0.001$), in a clinical trial reported by Nissen et al. [43]. The study assessed the change in atheroma volume as assessed by intravascular ultrasound in 47 subjects with acute coronary syndromes. The characteristics of the individuals' HDL have not been reported, but the study provides proof-of-concept that HDL-mediated therapies can have remarkable impact on atherosclerosis.

ApoA-I contains 243 amino acids and must be administered by intravenous infusion, presenting practical barriers to its use as a major therapeutic intervention. However, several different orally active peptides with characteristics similar to native HDL are currently in preclinical investigation. One of these is D-4F, an 18-amino acid peptide that forms an amphipathic helix similar to apoA-I but whose dextro positioning prevents its degradation by human digestive enzymes. The sequence of amino acids is critical to D-4F's activity [44].

Navab et al. [45] reported that D-4F ingested daily by LDL-receptor–null and apoE-null mice diminished atherosclerotic lesion size by 79% and 75%, respectively, without changing cholesterol or HDL cholesterol levels. Analysis of the plasma of treated apoE-null mice 20 minutes after D-4F administration revealed small micelle-like cholesterol-containing particles that migrated in a pre-β chromatographic pattern and were enriched in apoA-I and paraoxonase activity [46]. HDL and these small particles were both very anti-inflammatory and enhanced cholesterol efflux following D-4F treatment, in contrast to the corresponding liquid chromatography fractions prior to treatment [46]. Furthermore, the anti-inflammatory effects of D-4F on HDL are independent of HDL-C level [44].

D-4F also appears to offer protection against the inflammatory response to viral infections. Control LDL-receptor–null mice that were infected with influenza A virus developed severe pneumonia that was associated with proinflammatory HDL and decreased paraoxonase levels [44]. In contrast, when LDL-receptor–knockout mice were pretreated with oral D-4F prior to influenza exposure, the pulmonary inflammatory response was minimal, HDL remained anti-inflammatory, and paraoxonase levels increased [44]. Increased macrophage entry into the control mice's aortae was also seen in the controls following influenza exposure, but not in D-4F–treated mice [44].

A synthetic 10-D amino acid sequence from apoJ known as D-[113-122]apoJ has shown anti-inflammatory and antiatherosclerotic effects similar to D-4F [47]. Like D-4F it associates with HDL, converts it to a more anti-inflammatory particle, and reduces atherosclerotic lesions in apo-E–null mice [47]. However, unlike D-4F, it does not increase pre-β HDL levels. Remarkably, synthesis of the 4-amino acid sequence KRES, which in contrast to D-4F and D-[113-122]apoJ is too small to generate an amphipathic helix, resulted in many of the same properties as the larger peptides [47]. KRES increased HDL cholesterol levels, HDL anti-inflammatory activity, and paraoxonase levels while reducing atherosclerotic lesion size in apo-E–null mice. The peptide also rendered HDL anti-inflammatory in cynomolgus monkeys [47]. Interestingly, altering the amino acid sequence to KERS eliminated all apparent biologic activity [47]. Navab et al. [47 have suggested that the ability of these small amphipathic peptides to enhance HDL anti-inflammatory properties results from the ability of the peptide to associate with HDL and activate antioxidant enzymes within HDL [47].

Table 2. Clinical situations and therapeutic interventions that are associated with proinflammatory and anti-inflammatory changes within high-density lipoprotein

Proinflammatory effect	Proven anti-inflammatory effect
Coronary atherosclerosis	Apolipoprotein A-1 mimetics
Diabetes mellitus	Statins
Systemic lupus erythematosus	Diet high in polyunsaturated fat
Rheumatoid arthritis	**Possible anti-inflammatory effect**
Hemodialysis	Apolipoprotein A-I Milano
Infection	Cholesteryl ester transferase protein inhibitors
Surgery	Delipidated high-density lipoprotein
Diet high in saturated fat	Antirheumatic biologics

A combined therapeutic approach using both statin and D-4F treatment may offer synergy in generating anti-inflammatory HDL and retarding atherosclerosis. In a murine model, separately administered low doses of pravastatin and D-4F had no measurable impact on HDL or its function, but simultaneous treatment with both drugs led to increased levels of HDL-C (22%), apoA-I (19%), and paraoxonase (33%) [48]. Treatment with a combination of both agents compared with placebo was associated with a 79% reduction ($P < 0.0001$) in aortic lesion formation, regression of existing plaque, and decreased lesion macrophage content in mice [48]. In monkeys on the combination therapy but not those taking the agents separately at low dose, HDL became more anti-inflammatory, as indicated by the monocyte chemotaxis assay [48]. Table 2 lists clinical conditions that are associated with proinflammatory and anti-inflammatory changes in HDL, as well as potential and proven anti-inflammatory HDL-based strategies.

Future Directions

Emerging HDL-based therapies

Although there is certainly great therapeutic potential offered by compounds that are being developed to raise HDL-C levels, the functionality of the resultant HDL needs to be considered. Quantitative and qualitative improvements in HDL may provide complementary strategies to slow, if not reverse atherosclerosis. However,

several questions will need to be answered before that promise might be realized. How will emerging therapies targeting HDL-C levels impact cholesterol efflux and the anti-inflammatory capacity of HDL? Will it be necessary to characterize functional changes in HDL resulting from pharmacotherapies such as HDL mimetics that appear to modestly increase the amount of HDL cholesterol? If so, what function(s) will need to be assessed?

Anti-inflammatory therapies

Given the evidence of increased cardiovascular risk in the setting of systemic inflammation and its association with proinflammatory HDL, it is worth considering whether modification of systemic nonvascular inflammation might improve coronary risk. Among important still unanswered questions are the following: Do emerging disease-modifying biologic agents for chronic inflammatory diseases such as rheumatoid arthritis also potentially impact HDL anti-inflammatory function? Conversely, do nonsteroidal anti-inflammatory drugs, which have been associated with increased cardiovascular risk, adversely impact HDL's anti-inflammatory function? Indeed, further insight into the mechanisms of HDL dysfunction and how it can be altered will be crucial to evaluating the roles of emerging HDL-based and non–HDL-based anti-inflammatory therapies.

Conclusions

In acute and chronic inflammatory states, HDL often loses its atheroprotective characteristics and can become frankly proinflammatory. Exercise and diets that emphasize polyunsaturated fats may mitigate this transformation. In addition, statins and HDL mimetics have shown the ability to enhance HDL's antioxidative and anti-inflammatory properties while also improving HDL-mediated cholesterol efflux. Augmenting our current predominantly LDL-based therapeutic approach by improving HDL's anti-inflammatory function may allow for more effective treatment of atherosclerotic risk.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Castelli WP, Garrison RJ, Wilson PW, et al.: **Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study.** *JAMA* 1986, **256:**2835–2838.
2. Rader DJ: **Regulation of reverse cholesterol transport and clinical implications.** *Am J Cardiol* 2003, **92:**42J–49J.
3. Cockerill GW, Rye KA, Gamble JR, et al.: **High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules.** *Arterioscler Thromb Vasc Biol* 1995, **15:**1987–1994.
4. Mineo C, Deguchi H, Griffin JH, Shaul PW: **Endothelial and Antithrombotic Actions of HDL.** *Circ Res* 2006, **98:**1352–1364.
5. Nofer JR, Kehrel B, Fobker M, et al.: **HDL and arteriosclerosis: beyond reverse cholesterol transport.** *Atherosclerosis* 2002, **161:**1–16.
6. McDonald MC, Dhadly P, Cockerill GW, et al.: **Reconstituted high-density lipoprotein attenuates organ injury and adhesion molecule expression in a rodent model of endotoxic shock.** *Shock* 2003, **20:**551–557.
7. Cuzzocrea S, Dugo L, Patel NS, et al.: **High-density lipoproteins reduce the intestinal damage associated with ischemia/reperfusion and colitis.** *Shock* 2004, **21:**342–351.
8. Barter P: **The inflammation: lipoprotein cycle.** *Atheroscler Suppl* 2005; **6:**15–20.
9. Ansell BJ, Watson KE, Fogelman AM, et al.: **High-density lipoprotein function: recent advances.** *J Am Coll Cardiol* 2005, **46:**1792–1798.
10. •• Ansell BJ, Fonarow GC, Fogelman AM: **High-density Lipoprotein: is it always atheroprotective?** *Curr Atheroscler Rep* 2006, **8:**405–411.

This paper summarizes recent research in defining the functional capacity of HDL.

11. Courtney HS, Zhang YM, Frank MW, Rock CO: **Serum opacity factor, a streptococcal virulence factor that binds to apolipoproteins A-I and A-II and disrupts high density lipoprotein structure.** *J Biol Chem* 2006, **281:**5515–5521.
12. Khovidhunkit W, Kim MS, Memon RA, et al.: **Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host.** *J Lipid Res* 2004, **45:**1169–1196.
13. Van Lenten BJ, Wagner AC, Nayak DP, et al.: **High-density lipoprotein loses its anti-inflammatory properties during acute influenza A infection.** *Circulation* 2001, **103:**2283–2288.
14. Kumon Y, Nakauchi Y, Kidawara K, et al.: **A longitudinal analysis of alteration in lecithin-cholesterol acyltransferase and paraoxonase activities following laparoscopic cholecystectomy relative to other parameters of HDL function and the acute phase response.** *Scand J Immunol* 1998, **48:**419–424.
15. Navab M, Anantharamaiah GM, Reddy ST, et al.: **The double jeopardy of HDL.** *Ann Med* 2005, **37:**1–6.
16. Gabay C, Kushner I: **Acute-phase proteins and other systemic responses to inflammation.** *N Engl J Med* 1999, **340:**448–454.
17. • Ansell BJ, Navab M, Hama S, et al.: **Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment.** *Circulation* 2003, **108:**2751–2756.

This paper was the first to demonstrate improvement in anti-inflammatory properties of HDL with statin treatment.

18. Nobecourt E, Jacqueminet S, Hansel B, et al.: **Defective antioxidative activity of small dense HDL3 particles in type 2 diabetes: relationship to elevated oxidative stress and hyperglycaemia.** *Diabetologia* 2005, **48:**529.
19. Gowri MS, Van der Westhuyzen DR, Bridges SR, Anderson JW: **Decreased protection by HDL from poorly controlled type 2 diabetic subjects against LDL oxidation may Be due to the abnormal composition of HDL.** *Arterioscler Thromb Vasc Biol* 1999, **19:**2226–2233.
20. Watson KE, Hama S, Fonarow GC, et al.: **Metabolic syndrome patients have higher plasma lipid hydroperoxides and more pro-inflammatory HDL than dyslipidemic control subjects, even with comparable levels of HDL, hs-CRP and paraoxonase activity.** *Circulation* 2004, **110:**III-52.
21. Morena M, Cristol JP, Dantoine T, et al.: **Protective effects of high-density lipoprotein against oxidative stress are impaired in haemodialysis patients.** *Nephrol Dial Transplant* 2000, **15:**389–395.
22. McMahon M, Grossman J, Fitzgerald J, et al.: **Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis.** *Arthritis Rheum* 2006, **54:**2541–2549.

23. Navab M, Hama SY, Van Lenten BJ, et al.: **A new antiinflammatory compound, leumedin, inhibits modification of low density lipoprotein and the resulting monocyte transmigration into the subendothelial space of cocultures of human aortic wall cells.** *J Clin Invest* 1993, **91:**1225–1230.

24.• Ferretti G, Bacchetti T, Negre-Salvayre A, et al.: **Structural modifications of HDL and functional consequences.** *Atherosclerosis* 2006, **184:**1.

This is an excellent review of chemical modifications of HDL and their impact on HDL function.

25. Zheng L, Nukuna B, Brennan ML, et al.: **Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease.** *J Clin Invest* 2004, **114:**529–541.

26. Girona J, La Ville AE, Heras M, et al.: **Oxidized lipoproteins including HDL and their lipid peroxidation products inhibit TNF-alpha secretion by THP-1 human macrophages.** *Free Radic Biol Med* 1997, **23:**658–667.

27. Jaouad L, Milochevitch C, Khalil A: **PON1 paraoxonase activity is reduced during HDL oxidation and is an indicator of HDL antioxidant capacity.** *Free Radic Res* 2003, **37:**77–83.

28. Zheng L, Settle M, Brubaker G, et al.: **Localization of nitration and chlorination sites on apolipoprotein A-I catalyzed by myeloperoxidase in human atheroma and associated oxidative impairment in ABCA1-dependent cholesterol efflux from macrophages.** *J Biol Chem* 2005, **280:**38–47.

29. Hedrick CC, Thorpe SR, Fu MX, et al.: **Glycation impairs high-density lipoprotein function.** *Diabetologia* 2000, **43:**312.

30. Ferretti G, Bacchetti T, Marchionni C, et al.: **Effect of glycation of high density lipoproteins on their physicochemical properties and on paraoxonase activity.** *Acta Diabetol* 2001, **38:**163–169.

31. Ahmed W, Orasanu G, Nehra V, et al.: **High-density lipoprotein hydrolysis by endothelial lipase activates PPARalpha: a candidate mechanism for high-density lipoprotein-mediated repression of leukocyte adhesion.** *Circ Res* 2006, **98:**490–498.

32. Macdonald DL, Terry TL, Agellon LB, et al.: **Administration of tyrosyl radical-oxidized hdl inhibits the development of atherosclerosis in apolipoprotein E-deficient mice.** *Arterioscler Thromb Vasc Biol* 2003, **23:**1583–1588.

33. Wang WQ, Merriam DL, Moses AS, Francis GA: **Enhanced cholesterol efflux by tyrosyl radical-oxidized high density lipoprotein is mediated by apolipoprotein AI-AII heterodimers.** *J Biol Chem* 1998, **273:**17391–17398.

34. Ferre N, Camps J, Fernandez-Ballart J, et al.: **Regulation of serum paraoxonase activity by genetic, nutritional, and lifestyle factors in the general population.** *Clin Chem* 2003, **49:**1491–1497.

35.• Nicholls SJ, Lundman P, Harmer JA, et al.: **Consumption of saturated fat impairs the anti-inflammatory properties of high-density lipoproteins and endothelial function.** *J Am Coll Cardiol* 2006, **48:**715–720.

This is an elegant study of the effect of dietary fat composition on endothelial cell adhesion molecule expression.

36. Jones PH, Davidson MH, Stein EA, et al.: **Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial).** *Am J Cardiol* 2003, **92:**152–160.

37. Girona J, La Ville AE, Sola R, et al.: **Simvastatin decreases aldehyde production derived from lipoprotein oxidation.** *Am J Cardiol* 1999, **83:**846–851.

38. Kumar AP, Reynolds WF: **Statins downregulate myeloperoxidase gene expression in macrophages.** *Biochem Biophys Res Commun* 2005, **331:**442–451.

39. Charles C, Khanna D, Navab M, et al.: **Effects of high-dose atorvastatin on HDL anti-inflammatory/pro-inflammatory function and disease activity in patients with rheumatoid arthritis. Results of the Statin Anti-Rheumatic Activity (SARA) pilot study.** Paper presented at 2006 Scientific Sessions, American College of Cardiology. Atlanta, GA, 2006.

40. Angelin B, Parini P, Eriksson M: **Reverse cholesterol transport in man: promotion of fecal steroid excretion by infusion of reconstituted HDL.** *Atheroscler Suppl* 2002, **3:**23–30.

41. Badimon JJ, Badimon L, Fuster V: **Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit.** *J Clin Invest* 1990, **85:**1234.

42. Navab M, Hama SY, Cooke CJ, et al.: **Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1.** *J Lipid Res* 2000, **41:**1481–1494.

43. Nissen SE, Tsunoda T, Tuzcu EM, et al.: **Effect of recombinant apoa-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial.** *JAMA* 2003, **290:**2292–2300.

44. Navab M, Anantharamaiah GM, Reddy S, Fogelman AM: **Apolipoprotein A-I mimetic peptides and their role in atherosclerosis prevention.** *Nat Clin Pract Cardiovasc Med* 2006, In press.

45. Navab M, Anantharamaiah GM, Hama S, et al.: **Oral administration of an Apo A-I mimetic peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol.** *Circulation* 2002, **105:**290–292.

46. Navab M, Anantharamaiah GM, Reddy ST, et al.: **Oral D-4F causes formation of pre-beta high-density lipoprotein and improves high-density lipoprotein-mediated cholesterol efflux and reverse cholesterol transport from macrophages in apolipoprotein E-null mice.** *Circulation* 2004, **109:**3215–3220.

47. Navab M, Anantharamaiah GM, Reddy ST, et al.: **Oral small peptides render HDL antiinflammatory in mice and monkeys and reduce atherosclerosis in ApoE null mice.** *Circ Res* 2005, **97:**524–532.

48. Navab M, Anantharamaiah GM, Hama S, et al.: **D-4F and statins synergize to render HDL antiinflammatory in mice and monkeys and cause lesion regression in old apolipoprotein E-null mice.** *Arterioscler Thromb Vasc Biol* 2005, **25:**1426–1432.