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Review

Altered lipoprotein metabolism in chronic inflammatory states: proinflammatory high-density lipoprotein and accelerated atherosclerosis in systemic lupus erythematosus and rheumatoid arthritis

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Abstract

In this review, the authors discuss the formation and structure of high-density lipoproteins (HDLs) and how those particles are altered in inflammatory or stress states to lose their capacity for reverse cholesterol transport and for antioxidant activity. In addition, abnormal HDLs can become proinflammatory (piHDLs) and actually contribute to oxidative damage. The assay by which piHDLs are identified involves studying the ability of test HDLs to prevent oxidation of low-density lipoproteins. Finally, the authors discuss the potential role of piHDLs (found in some 45% of patients with systemic lupus erythematosus and 20% of patients with rheumatoid arthritis) in the accelerated atherosclerosis associated with some chronic rheumatic diseases.

Overview of the pathogenesis of atherosclerosis

Multiple factors play a role in the development of clinical atherosclerosis, including lipids, inflammation, physical sheer forces, and aging. This review is concerned with the role of high-density lipoproteins (HDLs) in both protecting and promoting atherosclerosis. In quick review then, low-density lipoproteins (LDLs) shuttle in and out of artery walls; when they are minimally or moderately oxidized within the wall (oxLDLs), they become proinflammatory. Endothelial cells are activated, monocytes are attracted into the artery wall, and monocyte/macrophages engulf oxLDLs, forming foam cells. Foam cells are the nidus of atherosclerotic plaque, and their formation is associated with the release of growth factors and

proteinases that cause hypertrophy of arterial smooth muscle and destruction of normal tissue in the artery wall. Monocyte ingress into arterial walls attracts lymphocytes that recognize antigens released by damaged cells, such as heat shock proteins, and contributes to inflammation with release of cytokines. The endothelial cells can also be damaged by products of inflammation and immunity independently of pro-atherogenic lipids, including cytokines (particularly tumor necrosis factor-alpha [TNF-α], interleukin-1 [IL-1], and interferon-gamma), chemokines, pro-oxidants, circulating immune complexes (ICs), and antiendothelial antibodies. Finally, shear stress, hypertension, and aging contribute to points of increased pressure which favor plaque formation and gradual loss of elasticity, resulting in the gradual stiffening of major arteries. Recent reviews of these processes are available [1-5]. In the remainder of this review, we will focus on the interactions between LDLs, oxLDLs, and proinflammatory HDLs (piHDLs).

Overview of the role of apolipoprotein B- and apolipoprotein A-containing lipids in atherosclerosis

Some experts consider that the simplest way to classify the role of various lipids in promoting atherosclerosis is to compare levels of those carrying apolipoprotein B with those carrying apolipoprotein A (apoB and apoA, respectively). High levels of the proatherogenic apoB or low levels of

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ABCA1 = ATP-binding cassette transporter AI; apo = apolipoprotein; b2-GPI = beta2-glycoprotein I; CAD = coronary artery disease; CETP = cholesterol ester transfer protein; DCFH = dichlorofluorescein; HDL = high-density lipoprotein; IC = immune complex; IDL = intermediate-density lipoprotein; IL = interleukin; LCAT = lecithin cholesterol acyltransferase; LDL = low-density lipoprotein; MCP-1 = monocyte chemotactic protein-1; NOS = nitric oxide synthase; oxLDL = oxidized low-density lipoprotein; ox-PAPC = oxidized 1-palmitoyl-2-arachidonyl-sn-3-glycero-phosphorylcholine; PAF-AH = platelet-activating acyl hydrolase; PEIPC = 1-palmitoyl-2-5,6 epoxyisoprostanoyl)-sn-glycero-e-phosphocholine; piHDL = proinflammatory high-density lipoprotein; PLTP = phospholipid transfer protein; PON = paraoxonase; PPAR = peroxisome proliferator-activated receptor; RA = rheumatoid arthritis; SAA = serum amyloid A; SLE = systemic lupus erythematosus; TNF-α = tumor necrosis factor-alpha; VLDL = very-low-density lipoprotein.

antiatherogenic apoA predict accelerated atherosclerosis, manifested as coronary artery disease (CAD) or stroke [5-7]. The following lipids are rich in apoB: low-density lipoproteins (LDLs), very-low-density lipoproteins (VLDLs) (which are also rich in triglycerides), and intermediate-density lipoproteins (IDLs). In contrast, apoA-1 is carried primarily in high-density lipoproteins (HDLs). Thus, there is substantial evidence that high levels of LDLs in plasma are associated with increased risk for atherosclerosis whereas subnormal levels of HDLs are an independent risk factor for the same disease [7,8].

Recently, it has become clear that simple quantitative analysis of HDL lipid/lipoproteins and their subfractions may be inadequate to estimate the role of HDLs in protecting against atherosclerosis. For example, in a controlled prospective trial of the HDL-raising CETP (cholesterol ester transfer protein) inhibitor torcetrapib added to a statin, compared with placebo plus statin, quantitative HDL levels increased 72.1% in 12 months in the torcetrapib/statin group, but athero-sclerotic events were significantly more frequent [9]. The qualitative character of the increased HDLs was not measured in that study. In fact, in states of acute and chronic inflammation, the contents and functions of HDLs can change drastically, converting atheroprotective HDLs to atherogenic HDLs. The focus of this review is to discuss that change and to review data suggesting that altered atherogenic piHDLs may be products of inflammation in patients with rheumatic diseases which play an important role in their predisposition to accelerated atherosclerosis.

Low-density lipoproteins: mechanisms by which oxidized low-density lipoproteins predispose to atherosclerosis

LDLs are the major transporters of cholesterol in the body. They shuttle in and out of arterial walls, where they are major substrates for oxidation. In the artery wall, numerous oxidative molecules are available, including xanthine oxidase, myelo-peroxidase, nitric oxide synthase (NOS), NAD(P)H, lipoxy-genases, and mitochondrial electron transport chains. LDLs are altered by these oxidants to contain reactive oxygen, nitrogen, and chlorine species as well as lipid-derived free radicals [5]. These are oxidized LDLs (oxLDLs), which are potent mediators of endothelial dysfunction and oxidative stress. The result of deposition of oxLDLs is inflammation and the formation of plaque in the artery. oxLDLs activate chemokine and cytokine receptors (such as monocyte chemotactic protein-1 [MCP-1]) on endothelial cells, and monocytes are trapped as they flow past; they enter the artery wall [10]. oxLDLs, in contrast to unmodified LDLs, are recognized by scavenger receptors on monocytes (thus triggering innate immunity). This results in phagocytosis of oxLDLs and formation of the lipid-rich foam cells that are the nidus of plaque. These activated macrophages release pro-inflammatory cytokines and chemokines, causing local tissue damage and stimulating hypertrophy of smooth muscle cells in the artery wall. Inflammation is also expanded by the influx

of lymphocytes. As plaque matures, there is central inflam-mation around lipids, release of proteases and other pro-inflammatory molecules from the inflammatory cells, hyper-trophy of smooth muscle, damage to endothelial cells, bulging of plaque into the lumen of the artery, and formation of a friable fibrous cap over the plaque. Exposure of circulating clotting factors and platelets to plaque is thrombogenic. Thus, the stage is set for impairment and even total blockage of blood flow in the area of plaque, leading ultimately to myocardial infarction, stroke, and tissue death.

High-density lipoproteins: characteristics, synthesis, degradation, and mechanisms by which normal high-density lipoproteins protect from atherosclerosis

Description of high-density lipoproteins and subsets

Plasma HDLs can also be viewed as part of the innate immune system – designed to prevent inflammation in baseline healthy situations and to enhance it when in danger [11]. As shown in Figures 1 and 2, HDLs are a collection of spherical or discoidal particles with high protein content (in the range of 30% by weight) that includes apolipoprotein A1 (apoA1) (approximately 70% of the total proteins) [5]. Their outer portion is a lipid monolayer of phospholipids and free cholesterol; larger HDLs have, in addition, a hydrophobic core consisting of cholesterol esters with small amounts of triglycerides. Proteins in HDLs in addition to apoA1 include apoE, apoA-IV, apoA-V, apoJ, apoC-I, apoC-II, and apoC-III [12,13]. HDL particles also contain antioxidant enzymes paraoxonase (PON), lecithin cholesterol acyltransferase (LCAT), and platelet-activating acyl hydrolase (PAF-AH). Characteristics of a classical HDL molecule are shown in Figure 2a.

Depending on the method used to separate HDLs, there are as many as 10 subsets: some particles contain only apoA1 and others both apoA-I and apoA-II [14]. In general, small dense HDLs are lipid-poor and protein-rich discs, but the majority of HDL particles are spherical and rich in both lipid and protein. There has been dispute as to which of the HDL subsets are most important in protecting from athero-sclerosis, with general agreement that high plasma levels of alpha1-HDLs and apoA-I are protective [13,14]. The HDLs that are measured in routine service laboratories include primarily large, cholesterol-rich HDL particles [5].

Synthesis and degradation of high-density lipoproteins

As shown in Figure 1, small HDL precursors (lipid-free apoA-I or lipid-poor pre-beta-HDLs referred to as immature HDLs in Figure 1) are synthesized in liver and intestine through the action of the enzyme ATP-binding cassette transporter A1 (ABCA1) on precursor protein, then modified in the circulation by acquisition of lipids. Initial lipid acquisition occurs at cellular membranes (listed as macrophages and peripheral tissues in Figure 1) via the ABCA1-mediated efflux of cholesterol and phospholipids from cells onto HDLs

Figure 1

Overview of synthesis, maturation, and disposal of high-density lipoproteins (HDLs). Apolipoprotein A1 (apoA1) is synthesized by the action of ATP-binding cassette transporter AI (ABCA1) in the liver and small intestine and is secreted as immature HDL (imm HDL) particles with large amounts of protein and small amounts of free cholesterol. Macrophages and peripheral tissues also donate free cholesterol and phospholipids to apoA1 to form more immature HDL particles. The action of lecithin cholesterol acyltransferase (LCAT) adds esterified cholesterol to the core of HDLs, leading to mature HDL particles composed of lipoproteins (apoA1 being the most abundant), phospholipids, and cholesterol esters. Cholesterol esters are shuttled to apoB-rich low-density lipoproteins (LDLs) and very-low-density lipoproteins (VLDLs) by the actions of cholesterol ester transfer protein (CETP). Conversely, phospholipids are transferred from LDLs/VLDLs to HDLs by the action of phospholipid transfer protein (PLTP). HDLs, as they break down, donate phospholipids and cholesterol/cholesterol esters, which are bound by SR-B1 receptor on liver cells. LDLs are bound by LDL receptor (LDLR) on hepatocytes. ApoA1 can be reused or secreted by the liver. Cholesterol can be reused or secreted into the bile for disposal. Triangles = apoA1; diamond = apoB. CE, cholesterol esters; FC, free cholesterol; PL, phospholipids; TG, triglycerides. The figure is based, in part, on figures and data in [102] and [103].

Figure 2

Comparison of normal protective anti-inflammatory high-density lipoproteins (HDLs) **(a)** to proinflammatory HDLs **(b)**. Normal HDLs are rich in apolipoproteins (yellow ovals) and antioxidant enzymes (white squares). After exposure to pro-oxidants, oxidized lipids, and proteases, proinflammatory HDLs have less lipoprotein and some, such as the major transporter apolipoprotein A-1 (A-1 in the figure), are disabled by the addition of chlorine, nitrogen, and oxygen to protein moieties: A-1 can no longer stabilize paraoxonase-1 (PON1) so PON1 cannot exert its antioxidant enzyme activity. In addition, pro-oxidant acute-phase proteins are added to the particle (serum amyloid A [SAA] and ceruloplasmin) as are oxidized lipids. The figure is based on information in [2] and [41]. apoJ, apolipoprotein J; CE, cholesterol ester; CE-OOH, cholesteryl linoleate hydroperoxide; GSH, glutathione; HPETE, hydroxyeicosatetraenoic acid; HPODE, hydroperoxy-octadecadienoic acid; LCAT, lecithin cholesterol acyltransferase; PAF-AH, platelet-activating acyl hydrolase.

binding to SR-B1 is activation of endothelial NOS and nitric oxide production.

Mechanisms by which high-density lipoproteins prevent atherosclerosis

Numerous actions of normal anti-inflammatory HDLs contribute to their ability to protect against atherosclerosis (Table 1 and Figure 2a). The first major mechanism for this protection is that normal HDLs participate in reverse cholesterol transport. Reverse cholesterol transport is the shuttling of cholesterol out of cell membranes and cytoplasm (including tissue macrophages, foam cells, and artery walls; Figure 1) into the circulation and then to the liver. The cholesterol efflux is mediated by the interactions of apoA-I, apoA-II, and apoE in HDLs with ABCA1, ABCG1, or ABCG4 transporters and/or SR-BI receptor on cell membranes. The process is rapid, unidirectional, and LCAT-independent, removing both cholesterol and phospholipids from membranes [19]. The cholesterol is transferred to HDL particles in the circulation and from there is transported to the liver [20]. ApoA-I is probably the most important protein in promoting reverse cholesterol transport [21]; treatment with recombinant apoA-I (Milano) variant mobilized tissue cholesterol and reduced plaque lipid and macrophage content in aortas of apoE$^{-/-}$ mice [22]. In addition to reverse cholesterol transport mediated by HDLs, oxLDLs are removed from artery walls by engulfment by

[15,16]. Genetic defects in ABCA1, as in Tangier disease, result in low HDL levels and premature atherosclerosis [1,5,16]. LCAT-mediated esterification of cholesterol then generates large spherical HDL particles with a lipid core of cholesterol esters and triglycerides [5]. These particles are remodeled and fused with other particles. Surface remnant transfer onto HDLs from LDLs and VLDLs is mediated by phospholipid transfer protein (PLTP) [17]. Smaller particles can be generated by the action of CETP, which transfers cholesterol esters from HDLs to apoB-containing lipoproteins (LDLs and VLDLs) [18]. This generates triglyceride-rich HDLs with little cholesterol ester, forming smaller particles of HDLs. The important protein apoA-I can be shed from these small HDLs and form new HDL particles via new interactions with ABCA1 in macrophages, cell membranes of other tissues, or liver. HDL lipids are degraded (a) by selective uptake into other particles, (b) via CETP transfer to LDLs/VLDLs, or (c) as holoparticles taken up by SR-B1 receptors on hepatocytes, primarily via apoE-containing HDLs, after which they are secreted into bile [5]. Another consequence of

Table 1

Proposed mechanisms by which high-density lipoproteins (HDLs) influence atherosclerosis

Normal protective HDLs	Proinflammatory HDLs

Reverse cholesterol transport
    ApoAI and other lipoproteins in HDLs transport cholesterol from
    artery walls and macrophages to other lipids and to the liver for
    recycling or disposal
	Impaired reverse cholesterol transport
	    ApoAI and apoJ are disabled after the addition of chlorine,
	    nitrogen, and/or oxygen
	    Lipoprotein synthesis is reduced by inflammation

Antioxidant activities
    Due primarily to enzymes PON1, lecithin cholesterol acyltransferase,
    platelet-activating acyl hydrolase, and glutathione peroxidase
	Pro-oxidant activities
	    PON1 is disabled by association with altered apoAI
	    Synthesis of enzymes is decreased by inflammation
	    Pro-oxidants serum amyloid A and ceruloplasmin are added to
	    HDLs

Anti-inflammatory activities
    Prevent generation of oxidized LDLs and oxidation of other
    proinflammatory lipids
    Prevent endothelial cells from expressing monocyte chemotactic
    protein-1 and other chemoattractants
    Diminish interactions between T cells and monocytes
	Proinflammatory activities
	    Primarily promote oxidation of LDLs

apo, apolipoprotein; LDL, low-density lipoprotein; PON, paraoxonase.

macrophages using scavenger receptors such as CD36 [23-26].

The second major mechanism for protective capacity of normal HDLs is their antioxidative function. Both proteins and lipids in LDLs are protected from accumulation of oxidation products *in vivo* in the presence of normal HDLs [27,28]. The antioxidative capacity depends on several antioxidative enzymes and several apolipoproteins. Again, apoA-I plays a major role by removing oxidized phospholipids of many types from LDLs and from arterial wall cells [29] and by stabilizing PON – a major antioxidant enzyme in HDLs. ApoE also has antioxidant properties [30] and can promote regression of atherosclerosis [31]. ApoJ at low levels is also antioxidant via its hydrophobic-binding domains [32]. On the other hand, apoA-II may be proatherogenic in that it can displace apoA-I and PON from HDL particles [33]. The major HDL antioxidative enzymes are PON1, platelet-activating factor acyl-hydrolase (PAF-AH), lecithin/cholesterol acyltransferase (LCAT), and glutathione peroxidase [27,29]. PON1 hydrolyzes LDL-derived short-chain oxidized phospholipids. PON1 can destroy biologically active oxLDLs and can protect LDLs from oxidation that is metal-ion-dependent. The association of HDLs with PON1 is required to maintain normal serum activity of the enzyme, possibly by stabilizing the secreted peptide [34,35]. PAF-AH and LCAT can also hydrolyze LDL-derived short-chain oxidized phospholipids [36]. Local arterial expression of PAF-AH (separate from HDLs) also reduces accumulation of oxLDLs and inhibits inflammation, thrombosis, and neointima formation in rabbits [37]. The characteristics of normal HDL particles are illustrated in Figure 2a.

A third protective mechanism relates to HDL interactions with lipids in human arterial endothelial cells. Oxidized 1-palmitoyl-2-arachidonyl-sn-3-glycero-phosphorylcholine (ox-PAPC) and its component phospholipid, 1-palmitoyl-2-5,6 epoxyisopros-tanoyl)-sn-glycero-e-phosphocholine (PEIPC), present in atherosclerotic lesions activate endothelial cells to induce inflammatory and pro-oxidant responses that involve induction of genes regulating chemotaxis, sterol biosynthesis, the unfolded protein response, and redox homeostasis. The addition of normal HDLs to the arterial endothelial cells *in vitro* reduced the induction of the proinflammatory responses, resulting in the reduction of chemotactic activity and monocyte binding. However, the antioxidant activities induced by ox-PAPC and PEIPC were preserved [38].

A fourth mechanism by which normal HDLs protect from atherosclerosis is by downregulating immune responses. This has several components. First, the oxidation of lipids is proinflammatory, as discussed above, and normal HDLs prevent that oxidation. Second, activation of endothelial cells, influx and activation of monocytes/macrophages, and damage to smooth muscle cells resulting from oxLDL deposition in artery walls are all suppressed, as discussed above. Third, cellular contact between stimulated T cells and monocytes is inhibited by HDL-associated apoA-I. This results in decreased activation of monocytes and decreased release of the highly proinflammatory cytokines IL-1β and TNF-α [39].

Transformation of normal, protective high-density lipoproteins to proinflammatory high-density lipoproteins
During acute or chronic inflammation, several changes occur in HDLs, as summarized in Table 1. As part of the acute-phase response, several plasma proteins carried in HDLs are decreased, including PON, LCAT, CETP, PLTP, hepatic lipase, and apoA-I. Acute-phase HDLs are depleted in

cholesterol ester but enriched in free cholesterol, triglyceride, and free fatty acids – none of which can participate in reverse cholesterol transport or antioxidation [40,41]. In these HDLs, levels of the pro-oxidant serum amyloid A (SAA) increase several-fold, as do levels of apoJ (also called clusterin) [42]. In fact, apoA-I is displaced from HDLs by SAA, which is associated not only with disabling HDLs as anti-inflammatory mediators, but with creating piHDLs. These HDLs can be defined as proinflammatory because they actually enhance the oxidation of LDLs and therefore attract monocytes to engulf those oxLDLs [42]. In fact, regulation of SAA, apoA-I, and PON1 is coordinated in murine hepatocytes; as SAA increases, the other two molecules decrease. These changes are promoted by nuclear factor-kappa-B and suppressed by the nuclear receptor peroxisome proliferator-activated receptor-alpha (PPAR-α) [43]. Acute-phase HDLs (including piHDLs) are much less effective than normal HDLs in removing cholesterol from macrophages [44] and delivering cholesterol esters to hepatocytes [45]. Lipids in the altered HDLs are themselves oxidized [46].

We can thus envision the piHDLs as pictured in Figure 2b. In the spherical particles, apoA-I and antioxidative enzymes are partially replaced by the products of oxidation, including oxidized lipids and serum amyloid protein. Such changes have been shown to occur in acute infection, in acute 'trauma' of surgical interventions, and in chronic inflammation. If one measures total HDLs by standard service clinical laboratory methods, they are usually low during periods of acute infection as well as in chronic inflammatory states such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [47-50]. A population study of monocytes from individuals from the general population with low plasma concentrations of HDLs showed increased expression of a cluster of inflammatory genes *(IL-1β*, *IL-8*, and *TNF-α*) and decreased *PPAR-γ* and antioxidant metallothionein genes compared with controls [51]. It seems likely that there are at least two major factors determining whether an individual at any given time point has normal anti-inflammatory HDLs or nonprotective piHDLs, whether inflammation is present, and genetic background. Furthermore, it is likely that the measurement of HDL function shows a 'majority' activity. That is, HDLs consist of numerous particles of different sizes, contents, and activities. In assays for anti-inflammatory versus proinflammatory function of HDLs obtained from test serum, one detects a dominant activity that does not describe the exact distribution of these HDLs. These data would predict that the ratio of normal to proinflammatory HDLs would vary over time. In fact, as discussed below, in our data in patients with SLE, that was not true. piHDL activity in an individual was stable over time without relation to disease activity; normal HDLs were also found repeatedly in some individuals with SLE even during periods of marked disease activity. It is our idea that HDL functions are rooted in genetic susceptibility and influenced by the presence of chronic inflammation in rheumatic diseases.

What are the processes that account for modification of normal HDLs into piHDLs? These are probably complex and include (a) oxidation of lipids and lipoproteins in the HDL particle (by increased activities of peroxidases that occur during inflammation, for example), (b) decreased synthesis of the proteins that populate HDL particles (for example, apoA-I), (c) addition of proteins that may participate in inflammation, and (d) replacement of cholesterol-transporting proteins and antioxidant enzymes by pro-oxidants SAA and ceruloplasmin. This is probably a dynamic situation in which lipids and proteins interact with other lipids and transfer from one particle or lipid-containing membrane to another. Thus, chronic autoimmune inflammation, even if low-grade, in a permissive genetic background may determine a chronic composition of HDLs which is proinflammatory. A study of the protein content of HDLs from patients with CAD compared with HDLs from healthy individuals showed enrichment of CAD HDLs in complement regulatory proteins, serpins, and apoE [52]. It is not clear how this work relates to the piHDLs that are discussed in this review.

Measurement of proinflammatory versus normal high-density lipoproteins
The measurement of the qualitative function of HDLs relies on the ability of normal HDLs to prevent oxidation of LDLs [53-55]. Patient HDLs are isolated from cryopreserved plasma and added to a fluorochrome-releasing substrate, dichlorofluorescein (DCFH), following the addition of LDLs from a normal donor. In the absence of HDLs, the LDLs oxidize *in vitro* and in turn oxidize DCFH, which then gives off a fluorescent signal. In the presence of normal protective HDLs (isolated from a normal donor), oxidation of LDLs is reduced and fluorescence is quenched. Fluorescence released by normal HDLs plus normal LDLs is set as '1.0'. Protective HDLs give a reading of 1 or less and piHDLs give a reading of greater than 1 [55]. Another approach to measuring the inflammatory potential of HDLs is to measure monocyte migration in coculture with aortic or smooth muscle cells in the presence of LDLs and test HDLs [42], although our laboratory has experienced better reliability and reproducibility with the procedurally easier DCFH cell-free assay.

Lipid abnormalities and rheumatic diseases: overview
The prevalence of atherosclerosis is increased in several rheumatic diseases (Table 2), with the highest prevalence being in SLE, followed by RA. The usual lipid profiles (done in routine service laboratories) for SLE and RA, as well as other rheumatic diseases, are shown in Table 2 [47-50,55-59]. With regard to HDLs, the usual profile is for HDL cholesterol to be low in rheumatic diseases associated with systemic inflammation (and triglycerides to be high), although there is variation from study to study in this regard. Quantitative measures of HDLs have not been predictive of subclinical or clinical atherosclerosis in any studies of patients with rheumatic diseases, with major predictors being age and

Table 2

Lipid levels and carotid plaque in patients with rheumatic diseases [47-50,55-59]

	Increase in risk for atherosclerosis	Total cholesterol	LDL-C	HDL-C	Triglycerides	OxLDL	Anti-oxLDL	PiHDL	Plaque/IMT on carotid ultrasound
SLE	OR 7 (general), 50 (females 35 to 44 years old)	Normal	Normal	↓ or normal	Normal	↑	↑	↑↑	↑ plaque all ages Decade 3: 6% Decade 4: 13% Decade 5: 33% Decade 6: 73%
Rheumatoid arthritis	3	↓ or normal	↓ or normal	↓	↑	↑	↑	↑	↑ plaque all ages Decade 3: 7% Decade 4: 52%, Decade 5: 52%
Psoriatic arthritis	1.6	↑ or normal	↓ or ↑	↓	Not done	Not done	Not done	Not done	↑ IMT overall
Ankylosing spondylitis	1.6	↑ or normal	↑	↓	Not done	Not done	Not done	Not done	↑ IMT in patients with high BASMI score
Vasculitis	Not found	Normal	Normal	Normal	Normal	Not done	↑	Not done	↑ plaque: 58%

↑, increased; ↑↑, significantly increased; ↓, decreased; BASMI, Bath Ankylosing Spondylitis Metrology Index; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; oxLDL, oxidized low-density lipoprotein; piHDL, proinflammatory high-density lipoprotein; SLE, systemic lupus erythematosus.

duration of disease with weaker correlations with smoking, high levels of homocysteine, hypertension, antibodies to phospholipids, and diabetes. The role of treatment with glucocorticoids has been variable [2,47-50,55-59]; most studies show a correlation with atherosclerosis but some show either no correlation or a protective effect. In our work, prednisone doses of greater than 7.5 mg daily were significantly associated with piHDLs [55].

Genetic factors predisposing to arterial thrombosis in SLE include homozygosity for variant alleles of mannose-binding lectin, as shown in a Danish cohort [60]. For dysfunctional HDLs in the general population, a polymorphism in apoA-1 (apoA-1 Milano) is associated with reduced clinical events [55,61,62]. Genetic variants of ABCA1 influence cholesterol efflux. Polymorphisms in LCAT, apoA-II, and apoE are all likely to alter the function of HDLs [63,64]. Some genetic variants of PON1 influence levels of that enzyme and are also likely to alter HDL function; at least one also predisposes to SLE [65,66].

Proinflammatory high-density lipoproteins and systemic lupus erythematosus

When qualitative rather than quantitative properties of HDLs are measured, the importance of HDLs to atherosclerosis in SLE and RA becomes apparent. In our studies [55], the presence of piHDLs was common in SLE and a strong predictor of subclinical atherosclerosis. A study of 154 women with SLE compared with 48 women with RA and 72 healthy women showed that piHDLs were present in 45% of

patients with SLE, 20% of patients with RA, and 4% of healthy controls. Differences between each group were statistically significant at a *P* value of less than 0.006. The mean inflammatory indices (<1.0 is normal) were 1.02 ± 0.57 in SLE compared with 0.68 ± 0.28 in healthy controls ($P<0.001$). Since piHDLs can arise and persist for approximately 2 weeks after surgeries, we originally proposed that piHDLs developed from peroxidation of HDLs caused by inflammation associated with active SLE. This hypothesis was supported by a positive correlation between piHDLs and Westergren erythrocyte sedimentation rate levels on multi-variate analysis. However, the presence of piHDLs did not correlate with SLE disease activity measured by Selena-SLEDAI, and the presence of piHDLs or normal HDLs in any given patient was stable over time, regardless of disease activity. Therefore, it seems likely that genetic predisposition also contributes to whether a given individual produces persistent piHDLs. Genetic predisposition is also suggested by the observation that low activity of PON1 in SLE patients compared with a healthy population did not correlate with measures of disease activity/inflammation, although it did correlate with clinical atherosclerosis. The BB phenotype that correlates with high activity of PON1 was absent in all of the SLE patients [67].

piHDLs occur in a larger proportion of patients with SLE compared with RA and also are significantly more frequent in SLE patients who had documented CAD. Recent work has shown that piHDLs are also significantly more frequent in SLE patients with carotid artery plaque [68]. In fact, the

presence of piHDLs in an SLE patient increases the risk for carotid plaque several-fold. Thus, it is likely that identification of piHDLs is a valid biomarker for increased risk for atherosclerosis in patients with SLE. More importantly, understanding the biologic basis for maintaining piHDLs should provide important insights into the pathogenesis of accelerated atherosclerosis characteristic of some patients with SLE. The results of our initial study are summarized in Figure 3.

It is also interesting that measurements of some of the lipoproteins and antioxidant enzymes associated with HDLs are also associated with increased risk for atherosclerosis in SLE. For example, plasma levels of PON1 are reduced in SLE patients [67], as one would expect if HDLs were pro-inflammatory instead of protective (Table 1 and Figure 2b). Enhanced lipid peroxidation, including high levels of oxLDLs, is associated with atherosclerosis in patients with SLE [69]. The increase in oxidation is associated, in part, with the presence of piHDLs rather than antioxidant normal HDLs.

Processes in addition to proinflammatory high-density lipoproteins that may accelerate atherosclerosis in systemic lupus erythematosus

Antibodies may also play a role in the pathogenesis of atherosclerosis, particularly in conditions such as SLE. Elevated levels of antibodies against oxLDLs have been described in the general population and in some studies are predictive of myocardial infarction and the progression of atherosclerosis [70,71]. Other studies, however, have not found any such correlations [72]. Similarly, the presence of antibodies to oxLDLs has uncertain significance in subjects with SLE. Anti-oxLDLs have been described in up to 80% of patients with SLE and antiphospholipid antibody syndrome [73-76]. Titers of antibodies to oxLDLs have also been associated with disease activity in SLE [77]. At least one study has demonstrated that autoantibodies to oxLDLs are more common in SLE patients who have a history of cardio-vascular disease than in SLE controls or normal subjects [78], although in two other studies, anti-oxLDLs and arterial disease were not associated [79,80]. There is some speculation that the increased risk of thrombotic and athero-sclerotic events seen in patients with SLE and antiphos-pholipid antibodies may be due, in part, to a crossreactivity between anticardiolipin and oxLDLs [74]. Cardiolipin is a component of LDLs [81], and indeed, a crossreactivity between anticardiolipin and anti-oxLDL antibodies has been demonstrated [74]. Additionally, beta2-glycoprotein I (β2-GPI), the protein recognized by most antibodies to cardio-lipin, binds directly and stably to oxLDLs [82]. These oxLDL–β2-GPI complexes have been found in patients with SLE and antiphospholipid antibody syndrome and are associated with a risk of arterial thrombosis [83]. Interest-ingly, there is enhanced uptake of oxLDL–β2-GPI complexes by macrophages, probably mediated by macrophage Fc-γ

Figure 3
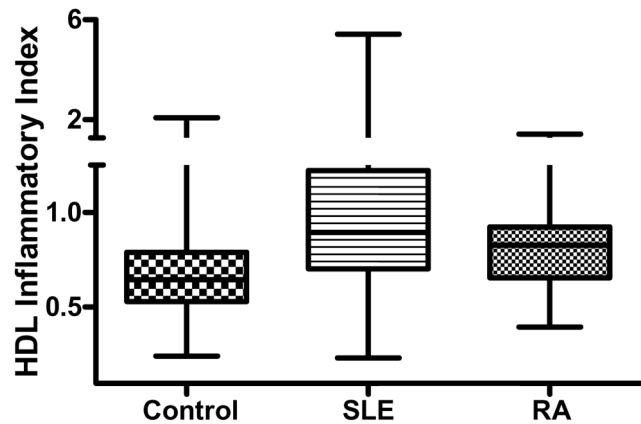
Comparison of the inflammatory indices of high-density lipoproteins (HDLs) isolated from healthy controls (left column) and patients with systemic lupus erythematosus (SLE) (middle column) and rheumatoid arthritis (RA) (right column). Numbers below 1.0 are normal; numbers greater than or equal to 1.0 are proinflammatory. Data are presented as box-and-whisker plots; the ends of each box represent the lowest and highest quartiles, the vertical lines show minimum and maximum values, and horizontal lines in each box indicate median values. Note that inflammatory indices are higher in SLE patients than in healthy controls or RA patients and are higher in RA patients than in healthy controls. Statistical analyses are as follows: SLE versus healthy controls, $P < 0.0001$; RA versus healthy controls, $P = 0.004$; and SLE versus RA, $P = 0.005$. There were 154 individuals in the SLE group, 45 in the RA group, and 74 healthy volunteers. All individuals are female. Data are from [55].

receptors [84]. Thus, oxLDL–β2-GPI complexes may contri-bute to atherosclerosis by increasing formation of foam cells.

ICs have also been described as a risk factor for athero-sclerosis in the general population. In one prospective study of 257 healthy men, the levels of circulating ICs at age 50 correlated with the future development of myocardial infarction [85]. *In vitro* studies have also suggested that LDL-containing ICs may play a role in atherogenesis. Macro-phages that ingest LDL-ICs become activated and release TNF-α, IL-1, oxygen-activated radicals, and matrix metallo-proteinase-1 [86]. LDL-containing ICs have been examined in several studies of SLE subjects, with varying results. In one study of a pediatric SLE population, there was an increase in levels of IgG LDL-ICs in SLE subjects compared with healthy controls, although there was no association with endothelial dysfunction [76]. Another study of an adult SLE population, however, demonstrated no difference from controls in levels of IgG or IgM LDL-containing ICs [69].

In addition to piHDLs, autoantibodies, and ICs, inflammation itself probably contributes to accelerated atherosclerosis in patients with chronic rheumatic diseases. Infiltration of arterial walls with T lymphocytes that recognize various autoantigens and contribute to the release of proinflammatory cytokine and

Figure 4
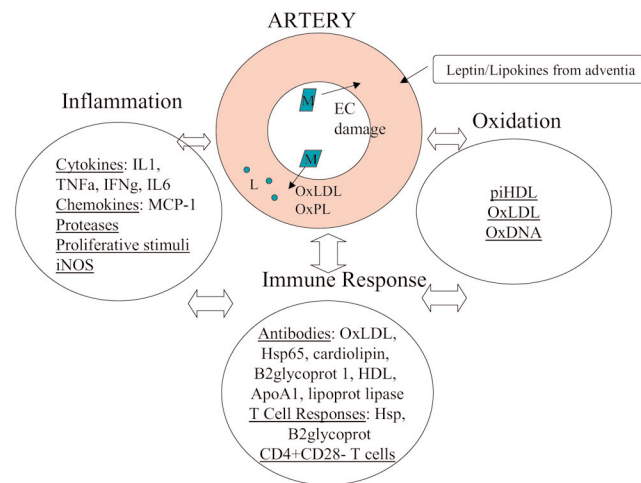
An overview of the pathogenesis of atherosclerosis. The influence of high-density lipoprotein (HDL) and oxidized low-density lipoprotein (oxLDL) on atherosclerosis is one part of the story, as shown in the open circle on the right. However, many other processes impact on arterial health, including additional factors influencing inflammation, oxidation, and the immune response. Proinflammatory HDLs (piHDLs) play a role in each of these processes. EC, endothelial cell; IFNγ, interferon-gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; L, lymphocyte; M, monocyte; MCP-1, monocyte chemotactic protein-1; OxPL, oxidized phospholipid; TNFα, tumor necrosis factor-alpha.

chemokines, and to the pro-oxidative molecules that arise, also accelerates clinical disease [87]. Furthermore, at the adventitial side of the artery, lipokines, cytokines, and chemokines promote inflammation in arteries, particularly the neurtrophil-attractant IL-8 and the monocyte-attractant MCP-1 [88]. Discussion of these risk factors is beyond the scope of this article: they are reviewed elsewhere in more detail [2,89] and their interplay is illustrated in Figure 4.

Proinflammatory high-density lipoproteins and nonrheumatic diseases

Other diseases in which dysfunctional, presumably pro-inflammatory, HDLs have been found include metabolic syndrome [90], poorly controlled diabetes mellitus [91], solid organ transplantation [92], and chronic kidney disease [93]. All of these disorders are characterized by accelerated atherosclerosis, and all have many abnormalities promoting arterial damage – similar to the situation in SLE and RA.

Therapeutic options to restore proinflammatory high-density lipoproteins to normal protective high-density lipoproteins

Several ideas and preliminary studies have been advanced for methods to alter piHDLs and render them more protective against atherosclerosis. It would be ideal in the therapy of rheumatic diseases (a) to be able to identify patients at high risk for accelerated atherosclerosis and (b) to have available

effective, safe therapies. With this in mind, a few trials of statins have been undertaken in an attempt to affect piHDLs. Statins decrease plasma levels of apoB-containing lipo-proteins, particularly LDLs, IDLs, VLDLs, and VLDL remnants. HDL levels rise a small amount, as does apoA-I production. Statins increase the activity of PON1 and reduce LDLs. Recombinant HDL administered intravenously enhances cholesterol efflux and reducs oxidative damage in dys-lipidemic subjects. This has been effective in a small trial to stabilize vulnerable unstable atherosclerotic plaque [94]. In the Ansell series, patients with CAD and piHDLs were treated with simvastatin 40 mg/day for 6 weeks. The mean decrease in the inflammatory index of their piHDLs was 38%, but this was not enough to restore piHDLs to normal range in most patients [95]. In RA, Charles-Schoeman and colleagues [96] treated 30 patients with RA with atorvastatin 80 mg or placebo for 12 weeks. The inflammatory index of patient HDLs fell 15% in statin-treated patients and rose 7% in those on placebo (P <0.026) [96]. Diet and exercise in patients with metabolic syndrome dropped piHDL levels toward normal as the patients lost weight [97].

Amphipathic peptides based on the structures of apoA-1 or apoJ can be administered orally in their D forms. In animal studies, an 18-amino-acid peptide, D-4F, removed lipid oxidation products from HDLs and promoted cholesterol efflux [98]. In monkeys with piHDLs, the inflammatory index of 1.2 fell to 0.5 two hours after administration of D-4F [28], the best studied of these peptides to date. Levels of lipid hydroperoxides fell in both LDLs and HDLs. Preliminary data in patients with coronary disease showed improvement in HDL inflammatory index after administration of D-4F, without any lowering of total HDLs [99]. D-(113-122)apoJ is a nine-amino-acid sequence mimetic that also improves HDL function and inhibits atherosclerosis in animals [100].

Other potential therapies that might alter piHDLs toward more protective particles include decreasing plasma tri-glyceride levels to increase cholesterol esters in HDL cores or decreasing oxidative stress and inflammation hoping to replace SAA with functional apoA-1. Although a recent CETP inhibitor study failed to prevent cardiovascular events (and actually increased them) even though quantities of HDLs rose [9], other CETP inhibitors are under study. It may be that they should be combined with niacin or statins or both. Niacin functions to reduce triglycerides, with a concomitant increase in quantities of HDLs and apoA-1. Fibrate therapy increases HDLs by a small amount and also increases levels of apo-AI and apo-AII [5].

For now, in 2008, physicians caring for patients predisposed to atherosclerosis by SLE or RA or other rheumatic disease, especially with accompanying risk factors like metabolic syndrome, hypertension, diabetes, and older age, should follow standard guidelines for preventing atherosclerosis. This would include statin therapies for high LDLs, niacin for

hypertriglyceridemia, control of hyperglycemia and hypertension, and cessation of smoking. Furthermore, it is likely that the better we control inflammation from the rheumatic disease, the less the patient is predisposed to atherosclerosis and to piHDLs. For example, treatment of RA with methotrexate reduced mortality overall, particularly mortality from cardiovascular disease [101]. Since that was not true of other disease-modifying antirheumatic drugs used for RA in the same study population, the situation is probably more complex than simply reducing the inflammatory 'load' in a given patient. Hopefully, in the next few years, measurement of piHDLs will be established as a routine biomarker of patients at high risk; therapies that correct HDLs from dysfunctional to normal will be improved by new biologics, and currently available therapies that partially correct HDL dysfunction will be more widely used.

Competing interests
The authors declare that they have no competing interests.

Acknowledgments

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