Molecular Interactions Leading to Lipoprotein Retention and the Initiation of Atherosclerosis

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Abstract—Atherosclerosis is distinguished by the accumulation of lipoprotein lipid within the arterial wall. An ionic interaction of positively charged regions of apolipoprotein (apo) B with matrix proteins, including proteoglycans, collagen, and fibronectin, is thought to initiate this process. Proteoglycans are complex glycoproteins containing highly negatively charged carbohydrate chains. These proteins are abundant in atherosclerosis lesions, and they associate with apoB-containing lipoproteins. Several specific regions of apoB may mediate this process. Other lipoprotein-associated proteins, including apoE and lipases, might also participate in this process. In addition, retention may occur via lipoprotein association with other matrix molecules or as a consequence of intra-arterial lipoprotein aggregation.

Key Words: atherosclerosis ■ lipoproteins ■ apolipoproteins ■ proteoglycans

Cholesterol was recognized as the lipid present in atheromatous plaques in the 19th century. The epidemiological association between plasma cholesterol or, more precisely, low-density lipoprotein (LDL) and coronary heart disease was well-established by the 1960s. Subsequently, studies of patients with familial hypercholesterolemia demonstrated that increased plasma concentrations of LDL, without other coronary heart disease risk factors, could cause accelerated atherosclerosis. Cholesterol in atheromatous lesions is derived from LDL cholesterol. Raised serum triglyceride levels, a reflection of very-low-density lipoprotein (VLDL) concentrations, are also thought to be risk factors for coronary heart disease. Zilversmit proposed that postprandial lipoproteins, especially chylomicron remnants, are also harmful. High plasma concentrations of remnant lipoproteins created using diet or genetic manipulation in animals are atherogenic in animals. Moreover, remnant lipoproteins have been identified within arteries. The commonality between these atherogenic lipoproteins is the presence of apolipoprotein (apo) B. For this reason, a quest to understand atherogenesis has focused on the biochemistry of apoB interaction with vascular wall molecules.

The theories of how lipoproteins enter and accumulate within the artery have remained rather consistent for decades. Nearly 50 years ago, Page summarized the extant theories in his Connor lecture: lipoproteins infiltrate the artery wall, the lipid is altered to a toxic form, and this promotes an inflammatory response (Figure 1). Increased infiltration could result from alterations in the wall of the vessel caused by endothelial denudation. However, developing atheroma are usually covered by an intact endothelial layer throughout most stages of lesion progression: lipoprotein retention, fatty streak formation, and formation of advanced lesions. Endothelial injuries that are insufficient to cause gross denudation may cause functional modifications leading to reduced barrier functions, ie, increased permeability. This could lead not only to greater amounts of arterial lipoproteins but also to localized concentration of the lipid in very circumscribed areas.

Normal, healthy endothelium transports or “leaks” many molecules, including lipoproteins. Depending on their size, lipoprotein particles are able to penetrate arterial tissue via transcytosis. Human LDLs of normal size (25 to 30 nm) transverse the endothelium efficiently, whereas lipoproteins larger than 70 nm cannot do so because of the size limitation of transcytotic vesicles. Thus, smaller remnant lipoproteins, but not very large, lipid-rich particles appear to be atherogenic.

Which is the more important determinant of lipoprotein accumulation, increased entry or greater retention (that is, decreased egress)? In addition, why do some lipoproteins remain within the lesions and incite pathological processes? Studies by Schwenke et al using labeled lipoproteins have implicated retention, rather than increased influx, as a major determinant of atherosclerosis susceptibility. Thus, the biochemistry of lipoprotein retention, rather than endothelial dysfunction, might be the primary determinant of atherosclerosis. Williams and Tabas coined the phrase “response to retention” to describe the process that initiates lesion formation. The molecular interactions determining how lipoproteins are retained within the vessel wall is the focus of this study.

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Arterial Proteoglycans

Proteoglycans are macromolecules composed of a core protein and complex, linear, long-chain carbohydrates, called glycosaminoglycans (GAGs). GAGs consist of repeating disaccharide units bearing negatively charged sulfate and carboxy groups. GAGs are covalently bound to the core protein via a tetrasaccharide linkage [GlcA-Gal-Gal-Xyl]. There are distinct types of GAGs: chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin, keratan sulfate, and hyaluronan. Several classes of CS and DS containing proteoglycans are found in the artery. These include versican, a large CS containing proteoglycan, and biglycan and decorin, 2 small leucine-rich proteoglycans containing DS and CS/DS, respectively. HS containing proteoglycans, the syndecans and glypicans, are associated with the cell membrane of smooth muscle cells (SMCs) and endothelial cells. Perlecan, a >500-kDa molecule that is the major HS in subendothelial matrix, is also an important component of the artery wall.

The extracellular matrix—comprising collagens, elastin, complex proteoglycans, hyaluronan, and proteins like fibronectin, laminin and tenascin—extends from the basement membrane of endothelial cells to the internal elastic lamina and forms a continuous space in contact with the pericellular region of SMCs and macrophages. Within the extracellular matrix, proteoglycans are distributed in 2 different locations. The proteoglycans of the pericellular environment are anchored to the cell plasma membrane, whereas those secreted by the cells are part of the extracellular space and usually do not physically connect with cells. The proteoglycans of the extracellular space are versican, decorin, and biglycan. The pericellular environment includes the subendothelial matrix perlecan and the cell surface proteoglycans, syndecan and glypican. Plasma lipoproteins, therefore, encounter a variety of types of proteoglycans throughout the artery wall.

The normal human intima is rich in versican and the content increases with lesion progression. In lesions, CS chains are longer than those in proteoglycans from normal regions. In contrast to those of humans, lesions from mice have greater contents of perlecan and relatively less CS/DS.

What factors regulate the production of proteoglycans within the artery? Vascular SMCs, endothelial cells, and macrophages are responsible for the synthesis of arterial wall proteoglycans. Pathological states such as atherosclerosis, hypertension, and diabetes are characterized by altered patterns of proteoglycan synthesis by these cells. The composition of proteoglycans synthesized by vascular cells fluctuates with the physiological state of the cells. Numerous factors, including extracellular matrix components, transforming growth factor (TGF)-β1, platelet-derived growth factor (PDGF), oxidized LDL (Ox-LDL), and nonesterified fatty acids modulate SMC proteoglycan synthesis. TGF-β1 increases the proliferation of SMCs and also enhances binding to LDL and also stimulates SMC proliferation and increases synthesis of versican, but not biglycan and decorin. The combination TGF-β and PDGF has an additive effect on increasing proteoglycan synthesis, primarily versican. However, combining both TGF-β and PDGF resulted in the loss of PDGFs effect on cell proliferation. Thus, the stimulation of proteoglycan synthesis in SMC is not coupled to cell proliferation.

Ox-LDL nonselectively increases the overall molecular sizes of versican, biglycan, and decorin in cultured monkey arterial SMC by increasing GAG chain length. Proteoglycans synthesized in the presence of Ox-LDL had greater affinity for native LDL. Ox-LDL enhances the expression of mRNA for biglycan, but not versican or decorin. Chang et al found that 7-ketocholesterol, lysophosphatidylcholine, and lysosphatidic acid, major products formed during LDL oxidation, regulate proteoglycan synthesis. 7-ketocholesterol, lysophosphatidylcholine, and lysosphatidic acid caused nonspecific chain elongation on each of the major secreted proteoglycan species. Enhanced LDL binding was a property of these modified proteoglycans. These results suggest that lipoprotein oxidation might aggravate atherosclerosis not only by regulating the synthesis of biglycan but also by enhancing the lipoprotein binding properties of all of the major proteoglycans synthesized and secreted by SMCs.

Fatty acids induce alterations in proteoglycans produced by endothelial cells and SMC, and this effect has been postulated to lead to greater atherogenesis in diabetes. Camejo et al reported that exposure of human arterial SMCs to 100 to 300 μmol/L albumin-bound linoleic acid reduced cell proliferation rate, altered cell morphology, and increased the amount of core protein mRNA of versican, decorin, and syndecan 4. GAG chains of decorin increased in size and bound [125I]-LDL with greater affinity compared with control cells. Hypothetically, a similar effect of chronic arterial exposure to high levels of fatty acids could increase SMC production of matrix proteoglycans with a higher capacity to retain apoB lipoproteins.
Proteoglycan production by macrophages has been studied through the use of cultured macrophages and macrophage-like cell lines. Both LDL and Ox-LDL may associate with macrophage cell surface CS and HS.77 Macrophage proteoglycans are not stored within the cell but are released extracellularly or maintained as a part of the cell surface. Owens and Wagner38 studied proteoglycans in control and cholesterol-loaded pigeon macrophages. A high-molecular-weight pericellular CS proteoglycan that was predominantly 6-sulfated, and thus similar to versican, was found only in the cholesterol-loaded cells. In addition, a pool of rapidly turning-over pericellular CS–proteoglycan was found in cholesterol-enriched cells. CS–proteoglycans produced by cholesterol-enriched macrophages also bound plasma LDL.29 This binding was competitively inhibited by heparin, suggesting that heparin and the proteoglycan produced by macrophages bind to the same sites on LDL. These studies collectively indicate that proteoglycan synthesis by macrophages is enhanced when macrophages become cholesterol-enriched and that increased turnover of the proteoglycans and proteoglycan interaction with lipoproteins can be a plausible explanation for enhanced accumulation of LDL in the extracellular space and subsequent uptake by the cells.

Differentiation of THP-1 cells into macrophages results in an increase in the amount, chain length, and sulfation of GAGs.30 Human monocyte-derived macrophages secrete a proteoglycan form of the cytokine macrophage colony-stimulating factor containing a CS chain (proteoglycan–macrophage colony-stimulating factor).31,32 Proteoglycan–macrophage colony-stimulating factor binds LDL in vitro31,32 and could participate in lipoprotein retention.

**Lipoprotein Interaction With Proteoglycans**

**LDL ApoB–Proteoglycan Interaction**

How do lipoproteins associate with proteoglycans? LDL association with proteoglycans was studied in the early 1960s by Gero and Bihara-Varga80–82 when it was recognized that mucopolysaccharides of arteries bound to beta lipoprotein and that complex association might also involve other macromolecules such as fibrinogen.83 Specific GAGs were later studied using gel columns with covalently linked hyaluronan and sulfated GAGs.33 When chromatographed on a column of granulated hyaluronan at physiological pH and ionic strength, VLDL, LDL, and high-density lipoprotein (HDL) lipoproteins emerged with the void volume, indicating that no significant binding had occurred. Equilibration of the same lipoproteins with GAG-substituted agarose gels, at pH 7.4 and in low ionic strength buffers, led to VLDL and LDL binding to gels containing heparin, DS, HS, and CS. In contrast, HDL and acetylated specimens of VLDL and LDL did not bind to the gels at any ionic strength. The observed interactions were interpreted as an ionic binding of positively charged amino groups on apoB to negatively charged groups on the GAGs.

LDL showed affinities in the 10⁻⁷ M range for versican-like proteoglycans secreted by human arterial SMCs34 and affinities in the 10⁻⁸ to 10⁻⁹ M range for decorin from fibroblasts at physiological ionic strength and calcium concentrations.34,35 The stoichiometry of these interactions is difficult to study because of the size heterogeneity of different proteoglycan preparations. It is likely that, at least in vitro, several lipoprotein particles are bound to each proteoglycan. When affinities of LDL for pericellular proteoglycans of fibroblasts and those for the LDL receptor in the same cells were compared, LDL affinity for the receptor was at least 2 orders of magnitude higher than that for the cell surface proteoglycans. However, the capacity of the proteoglycans for LDL binding was much higher.56 A similar observation was made by assessing LDL association with the cell surface; LDL interaction with proteoglycans was a lower-affinity but higher-capacity process.37 These results indicate that proteoglycans by themselves are sufficient for the association of relatively large amounts of LDL in the extracellular intima.

What regions of apoB are responsible for its interaction with proteoglycans? The interaction between LDL and proteoglycans could involve positively charged amino acids in apoB100. A search for multiple positive amino acid-rich regions of apoB led to the description of LDL-derived peptides that associated with heparin affinity gel. Studies by Weisgraber, Camejo, and Hirose, between 1987 to 1988, identified 8 clusters of positively charged amino acids in apoB100.38–40 However, these clusters were identified in delipidated fragments of apoB100 in the presence of urea and binding studies were performed in low-ionic-strength buffers. So, these results might not illustrate the GAG-binding sites that are functional when apoB is associated with lipids or in physiological conditions.

Studies comparing GAG association of whole lipoproteins and genetically modified apoB have clarified the required molecular determinants of apoB binding. Boren et al41 in 1998 identified site B (residues 3359 to 3369) as the heparin-binding region, a reduction in early atherosclerosis initiation proteoglycan preparations. It is likely that, at least in vitro, several lipoprotein particles are bound to each proteoglycan. When affinities of LDL for pericellular proteoglycans of fibroblasts and those for the LDL receptor in the same cells were compared, LDL affinity for the receptor was at least 2 orders of magnitude higher than that for the cell surface proteoglycans. However, the capacity of the proteoglycans for LDL binding was much higher.56 A similar observation was made by assessing LDL association with the cell surface; LDL interaction with proteoglycans was a lower-affinity but higher-capacity process.37 These results indicate that proteoglycans by themselves are sufficient for the association of relatively large amounts of LDL in the extracellular intima.
was observed when mice with similar plasma cholesterol were studied. Later lesions were, however, not different. Similarly, decreased perlecans also reduced early, but not later, lesion size.46 Therefore, different stages of mouse lesion formation might reflect different pathophysiological processes. Processes other than apoB–proteoglycan interaction must promote atherogenesis.

**Effects of LDL Size**

Does the size and composition of LDL affect its interaction with proteoglycans? Using pigeons fed cholesterol-containing diets, an inverse correlation between LDL size and number of LDL particles bound per proteoglycan have been demonstrated.47 This was postulated to be caused by steric hindrance. In contrast, studies in cholesterol-fed monkeys correlated reduced LDL size with less proteoglycan binding.48 In humans, Anber et al50 reported that small, dense LDL from patients undergoing coronary angiography with moderate hypertriglyceridemia and low HDL had greater affinity for human arterial CS proteoglycans. Using a gel mobility shift assay, Olin-Lewis et al50 found that the smaller, denser LDL bound biglycan better than large, buoyant subfractions. In addition, these smaller, denser LDL subfractions had a greater content of apoC-III.

Size and density of lipoproteins could affect the accessibility and conformation of apoB and apoE on lipoproteins. Two studies suggested the presence of buried GAG-binding sites on apoB100 that are unmasked when the protein and lipid composition of LDL are altered. Paananen et al51 showed that proteolysis of apoB100 strengthened the binding of LDL to proteoglycans. LDL treatment with phospholipase A2 increased its binding.52 A neighboring region to site B, site A (residues 3148 to 3158), may become functional when LDL is modified.53

**Ox-LDL**

Does oxidation of LDL increase its propensity for retention? There is some controversy in the literature regarding the interactions between proteoglycans and Ox-LDL, as well as the type of the proteoglycans involved in this interaction. Perhaps this is because of investigation of LDL with different degrees of oxidation. Some studies showed that oxidation of LDL particles decreased their ability to bind to aortic proteoglycans.54 Other studies, however, suggested that mild oxidation of LDL increased its binding to CS proteoglycans32 and to HS proteoglycans,55 whereas heavy oxidation reduced LDL binding to CS to levels lower than that of native LDL binding to CS.53 These processes might be important because the association of LDL with CS proteoglycans increases its ability to be oxidized56,57.

**Lipoprotein(a)**

If the major proteoglycan-binding site in apoB is masked by lipoprotein a [Lp(a)], then how are these particles atherogenic? The apo(a) moiety on Lp(a) might increase its association with SMC CS,58 perhaps via an interaction with the core protein of decorin.59 Alternatively, arterial Lp(a) accumulation might depend on the presence of lipase60 or other matrix components. A third option is that the apoB–apo(a) interaction unmasks the amino terminal apoB–GAG binding site.

**Lipases, Bridging Molecules That Increase Lipoprotein Binding to Proteoglycans**

Are other matrix components required for efficient arterial retention of lipoproteins? In vitro, LDL and other apob-containing lipoproteins bind weakly to heparin and other proteoglycans in physiological ionic-strength environments.61 It is therefore possible that molecules such as lipoprotein lipase (LPL), which enhance the interaction of LDL with proteoglycans, could play an important role in atherogenesis in vivo. In addition to its lipolytic activity, LPL acts as a potent bridge between lipoproteins and cell-surface proteoglycans, components of the extracellular matrix.62,63 The presence of LPL markedly increases the binding of lipoproteins to cultured cells, perfused livers, and matrix components deposited on culture dishes by fibroblasts and endothelial cells. LDL increased LDL and Ox-LDL binding to decorin-coated collagen.54 This bridging ability of LPL is not dependent on its catalytic activity.65 The LPL interaction with lipoproteins has been studied with microtiter plate assays, gel filtration, and Biocore assays. These different systems have yielded disparate data regarding the importance of LPL interaction with LDL lipids versus apoB.66–68

LPL is produced by macrophages and SMCs and has been found in atherosclerotic lesions.69,70 It has been suggested that LPL contributes to the LDL retention in the vessel wall by bridging LDLs to either macrophages or the extracellular matrix.62 Endothelial lipase and hepatic lipase71,72 are also effective in facilitating lipoprotein bridging and have been proposed to have important roles in the pathogenesis of atherosclerosis.

Several experiments with genetically engineered mice have attempted to elucidate the role of vessel wall LPL in atherogenesis. By transplanting either Lpl+/− or Lpl−/− fetal liver cells as the source of hematopoietic cells and, ultimately, of monocytes/macrophages infiltrating the arterial wall, Babaev et al73 showed that LDL receptor knockout mice with reduced macrophage LPL had less fatty streak lesions. In their experiment, development of advanced atherosclerotic lesions was not affected by macrophage LPL production, which is consistent with the observed scarcity of macrophages in this type of lesion. In other studies, bone marrow transplantation altered atherosclerosis and plasma lipid levels.74 Collectively, these experiments suggest that macrophage-derived LPL exerts an atherogenic effect.

**Apolipoproteins**

Despite the fact that apoC-III does not bind biglycan directly, recent data showed that the apoC-III content of apoB-containing lipoproteins was associated with increased binding to the vascular proteoglycan biglycan.50 The hypothesis used to explain this is that apoC-III causes a conformational change by which apoB and/or apoE become more accessible to proteoglycans.

The actions of apoE in atherogenesis are still incompletely understood. By increasing lipoprotein association with HS proteoglycans,75 apoE should increase lipoprotein retention and atherogenesis. Areas in human atherosclerotic plaques enriched in biglycan contain apolipoproteins E, A-I, and B.76 However, apoE overexpression in macrophages leads to
reduced atherosclerosis, suggesting a local role for this protein exclusive of its effects on lipoprotein–proteoglycan interaction.

**Interaction of Lipoproteins and the Nonproteoglycan Extracellular Matrix**

Could matrix molecules other than proteoglycans be critical mediators of lipoprotein retention?

**Collagen**

Aside from proteoglycans, other components of the extracellular matrix interact with lipoproteins. Collagen types I and III, the predominant vascular collagens, bind to native LDL in vitro, and the extent of this binding is related to the net negative charge on LDL. Ox-LDL exhibited even greater collagen binding than did native LDL. By comparing native LDL and Ox-LDL binding to the extracellular matrix of cultured SMC, Chang et al. found that Ox-LDL binding to SMC extracellular matrices involves a nonproteoglycan component of the matrix. More recently, Fukuchi et al. studied native and Ox-LDL within the human coronary using apoB100 as a marker for native LDL and 8-iso-prostaglandin 

F2\[alpha\] as a marker for Ox-LDL. They found that an increase in 8-iso-proteoglycan F2\[alpha\] staining relative to apoB100 staining coincided with increased type I and type III collagen and elastic fibers but rarely with sulfated proteoglycans including decorin.

**Elastin**

An association between elastin and lipid also has been demonstrated. ApoB-containing lipoproteins can be released from atherosclerotic tissue by elastase treatment. In vitro, elastin and LDL have a high-affinity interaction, with increased binding of LDL to elastin isolated from atherosclerotic versus normal arteries. Fibronectin

Greilberger et al. investigated the binding of Ox-LDL to microtiter plates coated with collagens, laminin, fibronectin, or poly-D-lysine. Comparing various collagen types, the binding of Ox-LDL followed this order: type I > type V and type III > type IV > type II. An F(ab')2 fragment from a monoclonal antibody to Ox-LDL inhibited the binding of highly Ox-LDL to type II collagen, laminin, type I collagen, and poly-D-lysine, but not fibronectin.

Matrix proteins such as fibronectin and collagens are masked by the overlying HS proteoglycan. Removal of subendothelial HS, but not CS and DS, by enzymatic treatment increased the binding of Lp(a). When cultured endothelial cells were exposed to Ox-LDL, matrix HS was reduced whereas Lp(a) binding to the HS-depleted matrix was increased. This binding was inhibited when antifibronectin antibodies were used. It was suggested that loss of HS exposed additional Lp(a) binding sites.

**Aggregation of Lipoproteins**

Can lipoprotein retention within the artery occur because of aggregation, a process that is dependent on neither apoB nor proteoglycans? Extracellular lipid visible by electron microscopy, including that in lipid cores of atherosclerotic lesions, may be derived from coalescence of plasma-derived lipoproteins in the extracellular matrix. In fact, after injection of LDL, clusters of LDL compatible with aggregates have been found within the artery. A number of lipoprotein modifications can lead to aggregation. The modifications include lipolysis by sphingomyelinases or phospholipases, or complexation to proteoglycans. In addition, oxidation will accelerate the aggregation of lipoproteins.

In macrophages, it has been proposed that aggregated LDL can be delivered into a labyrinth of surface-connected compartments by a type of phagocytic process termed patocytosis. This represents a unique endocytic pathway operating in macrophages. Aggregated LDL taken-up by macrophages is poorly processed because of an impairment in lysosomal degradation. It has been suggested that intracellular lipoprotein in macrophages may also leave the macrophage without being degraded. Macrophages can disaggregate and release sequestered aggregated LDL by changing plasminogen to plasm, which degrades apoB sufficiently to release it from macrophage surface-connected compartments. Theoretically, this can contribute to the extracellular accumulation of lipoprotein in atherosclerotic lesions.

**Conclusion**

Although the biochemistry of lipoprotein–proteoglycan interactions has been explored in detail, the pathological and therapeutic implications of this knowledge are not yet clear. Proteoglycan composition and synthesis tend to become more LDL-retentive with atherosclerosis. A substantial body of biochemical data shows in vitro interaction between apoB and proteoglycan. However, aside from the information on alterations in early lesions with mutations of apoB, most in vivo information is correlative. Ongoing experiments using mice with genetic deletions of perlecain, biglycan, and decorin should be completed in the near future and will assess the importance of LDL interaction with each of these proteoglycans. In addition, it is likely that additional lipoprotein-binding molecules are important, and perhaps other molecules that mediate retention will be discovered.
There are at least 3 processes (shown in Figure 2) that could allow lipoprotein retention: lipoprotein binding to proteoglycans, lipoprotein association with other matrix proteins, and lipoprotein aggregation. Each of these processes could mediate some lipoprotein retention, and elimination of one pathway may only partially alter atherogenesis. Thus, the importance of each pathway and its complicated interaction will need to be studied.

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