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Visions & Reflections

Retention of atherogenic lipoproteins in atherogenesis

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Abstract. Atherosclerosis is a multifactorial disease whose pathogenesis is still unclear. Mounting evidence, however, supports the concept that subendothelial retention of apoB100-containing lipoproteins is the initiating event in atherogenesis. Subsequently, a series of biological responses to this retained material leads to specific molecular and cellular processes that promote lesion formation.

Key words. Apolipoprotein B; atherosclerosis; proteoglycan; retention; glycos aminoglycan; transgenic mice.

The causal relationship between blood cholesterol and atherosclerosis is no longer in doubt and numerous epidemiological studies have established that elevated levels of low-density lipoprotein (LDL) and other apolipoprotein (apo)B-containing lipoproteins are linked to the incidence of cardiovascular disease. Other risk factors for cardiovascular disease can accelerate the atherosclerotic process, but in the absence of dyslipidemias contribute little to atherogenesis [1].

However, the mechanism(s) by which hypercholesterolemia induces the pathobiological changes that lead to the disease remain unclear, and several hypotheses have been articulated to explain the events that initiate atherogenesis. The 'response-to-injury' hypothesis states that endothelial injuries that are insufficient to cause gross denudation but severe enough to cause functional modifications are key to atherogenesis. A major hypothesized change in endothelial function was increased permeability particularly to atherogenic lipoproteins [2]. The 'oxidation' hypothesis highlights the importance of oxidized lipid as an important source of pathogenic substances [3]. Finally, the 'response-to-retention' hypothesis invokes a critical role in atherogenesis for the retention of atherogenic lipoproteins by extracellular matrix molecules [1]. While these hypotheses are not mutually exclusive, and may even be considered mutually compatible with differences in emphasis, recent evidence strongly supports the response-to-retention hypothesis.

The response-to-retention hypothesis

The response-to-retention hypothesis emphasizes the causative role of dyslipoproteinemia in the development of atherosclerosis and is based on pioneering work carried out in the 1970s and 1980s [4–6]. The basis for the hypothesis is that atherogenic lipoproteins that gain entry to the subendothelial space are bound and retained through ionic interactions between positively charged residues on the atherogenic lipoproteins, and negatively charged residues in the extracellular matrix molecules. Of these extracellular matrix components, proteoglycans in particular appear to play an important role [7]. Proteoglycans are macromolecules composed of a core protein and complex, long-side-chain carbohydrates, called glycosaminoglycans (GAGs), which consist of repeating disaccharide units, all bearing negatively charged, usually sulfate or carboxylate, groups [7]. In vitro, LDLs bind with high affinity to many proteoglycans found in the artery wall, especially the chondroitin sulfate proteogly-

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can versican and the two small leucine-rich proteoglycans decorin and biglycan, which are produced by smooth muscle cells. Several growth factors as platelet-derived growth factor (PDGF) and transforming-growth factor (TGF) β increase both the net synthesis of proteoglycans by smooth muscle cells, and the GAG chain length of the proteoglycans [8, 9].

The consequence of the retention of atherogenic lipoproteins is not only a net accumulation of lipid, but also prolonged exposure to local oxidants and other non-oxidative enzymes in the vessel wall. A growing body of evidence supports an important effector role for variously modified lipoproteins and their constituents in triggering an inflammatory reaction that accelerates lesion development [1].

Direct interactions between apoB and GAGs

The interaction between LDL and proteoglycans involves positively charged amino acids in apoB100, the protein moiety of LDL, that bind ionically with the negatively charged GAGs on the proteoglycans [1]. Several laboratories have contributed to the identification of eight clusters of positively charged amino acids in apoB100 [10–12]. However, these clusters were identified in delipidated fragments of apoB100 in the presence of urea or with short synthetic apoB peptides. Thus, which of the GAG-binding sites are functional when apoB is associated with lipids (e.g., when it is incorporated into LDL) was not clear. To identify the main proteoglycan-binding site in apoB100, specific mutations were introduced into the human apoB100 gene and transgenic mice expressing mutant forms of human recombinant LDL were generated. The recombinant LDLs were isolated, characterized, and tested for their ability to bind to proteoglycans [13]. These studies identified site B (i.e., residues 3359–3369) as the proteoglycan-binding site of apoB100, and showed that positively charged arginine and lysine residues of site B are critical for binding to proteoglycans. Interestingly, site B coincides with the LDL-receptor-binding site of LDL [14]. However, the proteoglycan-binding activity and the LDL receptor binding can be discriminated and a single lysine to glutamic acid substitution at residue 3363 impairs the ability of apoB100 to bind to extracellular vascular proteoglycans, but not the LDL receptor [13].

The conformation of apoB100 on the surface of the LDL particle is dependent on the composition of the core lipids, the surface phospholipid content, and the diameter of the lipoprotein particle. Thus, other binding sites than site B may become functional in modified LDL. Paananen and Kovanen [15] noted that proteolysis of apoB100 strengthened the binding of LDL to proteoglycans, suggesting the exposure of buried GAG-binding sites. Likewise, LDLs modified by treatment with secretory group IIA phospholipase A_2 ($sPLA_2$), a strong independent risk factor for coronary heart disease [16], bind more avidly than unmodified LDLs to proteoglycans [17]. Camejo and coworkers originally suggested that site A (residues 3148–3158) may become functional in modified forms of LDL [18]. Recent results have confirmed this and shown that site A acts cooperatively with site B in the association with proteoglycans in LDL modified with $sPLA_2$ [C. Flood, M. Gustafsson and J. Borén, unpublished observation]. The results also showed that the triglyceride content of LDL influences the conformation of apoB and decreases the affinity for GAGs. This mechanism is likely mediated by a conformational change of site B and is, in contrast to $sPLA_2$-modified LDL, independent of site A.

ApoB exists in two forms, apoB100 and apoB48. ApoB100 consists of 4536 amino acids, while apoB48 corresponds to the amino-terminal 48% of apoB100. The editing process that converts apoB100 mRNA to apoB48 mRNA and the expression patterns of the two proteins are well established [19]. In humans, apoB100 is expressed in the liver, forming very low density lipoprotein (VLDL), while apoB48 is synthesized in the intestine, forming chylomicrons. Several species (e.g., rodents) express apoB48 also in the liver where it assembles VLDL.

The finding that site B lies in the carboxyl-terminal half of apoB100 and is absent in apoB48 presented a paradox because elegant studies using gene-targeted mice expressing only apoB48 or only apoB100 have shown that apoB100- and apoB48-containing lipoproteins are equally atherogenic [20]. Furthermore, apoE-deficient mice, which are the most widely used experimental model of atherosclerosis because of their ability to spontaneously produce atherosclerotic lesions [21], contain mainly apoB48-containing lipoproteins. This paradox was recently solved by showing that the proteoglycan-binding site of apoB48 (i.e., site B-Ib at residues 84–94) is located in the amino terminus of apoB, and that it is masked by the carboxyl terminus of apoB100 in apoB100-containing LDL (fig. 1) [22]. Thus, it is exposed and functional in apoB48 but masked and nonfunctional in apoB100-containing LDL. The presence of a proteoglycan-binding site in the amino-terminal region of apoB is consistent with the response-to-retention hypothesis and provides a possible explanation for the dual atherogenicity of apoB48- and apoB100-containing lipoproteins.

The size of lipoprotein particles is linked to their ability to penetrate arterial tissue via transcytosis. Human LDLs of normal size (i.e., 25–30 nm) transverse the endothelium efficiently, whereas lipoproteins greater than 70 nm cannot do so because of the size limitation of transcytotic vesicles [23]. Thus, fewer apoB48-containing chylomicron remnants are retained within the intima, than the

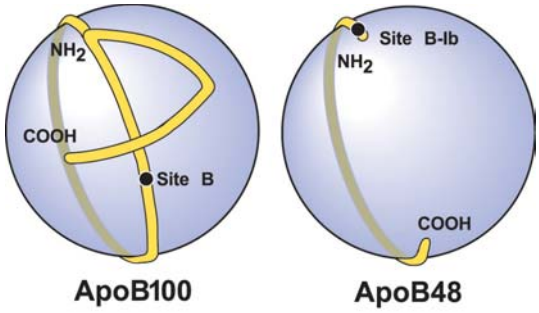

Figure 1. Schematic of the proteoglycan-binding sites of apoB100 and apoB48. The first 89% of apoB100 enwraps the LDL particle like a belt, and the carboxyl-terminal 11% constitutes a 'bow' that crosses over the belt. The proteoglycan-binding site in apoB100 is located in the carboxyl-terminal half of apoB100 (site B), and is absent in apoB48. The proteoglycan-binding site in apoB48 (site B-Ib) is located in the amino-terminus of apoB, and is 'masked' by the carboxyl terminus of apoB100. The projection of apoB on the 'back' of the lipoprotein particles has been simplified.

smaller apoB100-containing LDLs. However, a greater number of retained particles does not necessarily translate to greater cholesterol mass, since chylomicron remnants contain approximately 40 times more cholesterol per particle than do apoB100-containing LDL particles [23].

Indirect interactions of LDL to the extracellular matrix

In addition to apoB, other protein components of lipoproteins are also implicated in binding to proteoglycans. ApoE, a component of VLDL remnants and lower-density fractions of high-density lipoproteins (HDLs), can bind to the negatively charged GAG chains of several proteoglycans [24]. In particular, apoE has been shown to colocalize with biglycan in human atherosclerotic lesions [25]. The HDL protein, apoA-I, is also present in human [25] and mouse [26] lesions. ApoA-I has been shown to modulate lipoprotein binding to proteoglycans and recent results indicate that it regulates hepatic lipase activity by binding to sites on the proteoglycans and dislodging the bound hepatic lipase [27]. High circulating apoC-III levels are associated with increased atherosclerosis risk, and very recent data also show that the apoC-III content of apoB-containing lipoproteins is associated with increased binding to the vascular proteoglycan biglycan despite the fact that apoC-III does not bind biglycan directly. The enhanced biglycan binding may result from a conformational change associated with increased apo C-III content by which apoB and/or apoE become more accessible to proteoglycans [28]. A high plasma lipoprotein (a) [Lp(a)] level is closely related to coronary heart disease and Lp(a) is avidly retained in the artery wall because it can bind to the extracellular matrix through either its apo(a) component or its apoB100 component [29]. In vitro studies have shown that Lp(a) binds to several extracellular matrix proteins, including fibrin, collagen, laminin, fibronectin, and proteoglycans [30]. Even though fibrin interacts with high affinity with the apo(a) moiety of Lp(a), proteoglycans seem to be the most important extracellular matrix component for binding of Lp(a) [30].

In addition to apolipoprotein, several lipases have been shown to mediate 'bridging' between lipoproteins and heparin sulfate proteoglycans (HSPGs) on the cell surface, which results in increased cellular uptake and degradation of lipoproteins. Lipoprotein lipase (LPL) which is secreted by smooth muscle cells and macrophages in atherosclerotic lesions [31], has been shown to act as a bridge between GAG and extensively oxidized LDL, which are sufficiently depleted of positive charges to inhibit direct binding to GAG [32, 33]. The bridging function of LPL does not require a catalytically active enzyme [34]. Hepatic lipase which is synthesized by mouse and human macrophages [35], and endothelial lipase which is synthesized by endothelial cells [36] are also effective in facilitating lipoprotein bridging and both have been proposed to have important roles in the pathogenesis of atherosclerosis. The retention of atherogenic lipoproteins may well be initially governed by binding of apoB100 and/or apoE to GAG, but shifting to lipase-mediated binding when macrophages infiltrate the intima and secrete LPL [33].

In vivo evidence supporting the response-to-retention hypothesis

Atherogenesis is a complex process, thus presenting difficulties for experimentally dissecting the chronology of the processes involved and generating in vivo evidence supporting the response-to-retention hypothesis. Sequential studies by several laboratories provided the first insights into the relative association of lipoproteins with arterial tissue [37–41]. Of particular importance are the landmark studies by Schwenke and Carew that indicated that trapping and retention of lipoproteins is the initiating event in atherosclerosis [42, 43]. These studies showed in vivo that the early prelesional accumulation of atherogenic lipoproteins within the arterial wall is focally concentrated in sites that are known to be prone to the later development of atherosclerotic lesions. However, the rates of lipoprotein entry into prelesional susceptible versus resistant sites were not different. This finding indicated that retention, not enhanced endothelial permeability to lipoprotein influx, is the key pathological event in atherogenesis.

The technological advances during the past decade and the power of mouse genetics recently enabled directly

testing of the response-to-retention hypothesis and investigation of the biological significance of the interaction between apoB100 and artery wall proteoglycans in atherogenesis. The key for these studies was the generation of transgenic mice expressing recombinant human wild-type LDL with normal proteoglycan binding, or genetically engineered LDL that fails to bind proteoglycans (i.e., proteoglycan-binding-defective LDL). The mice were fed a Western diet for 20 weeks and the extent of the vessel wall covered by atherosclerotic lesions was quantitated (fig. 2). The results showed convincingly that mice expressing LDL that is defective in proteoglycan binding had greatly reduced atherogenesis, and that the decreased atherosclerotic potential of this LDL was indeed due to its ability to interact with artery wall proteoglycans. This study provides the first direct experimental evidence that binding of LDL to artery wall proteoglycans is an early step in atherogenesis [44].

Why do humans develop coronary heart disease?

Humans have higher plasma LDL cholesterol than most other mammalian species, and a propensity of spontaneously developing atherosclerosis and coronary heart disease [45]. The mean LDL level in lower animals averages 32 mg/dl (0.8 mmol/l) [46] and atherosclerosis does not develop when serum cholesterol concentrations are below 80 mg/dl (2.1 mmol/l) [1]. A feasible explanation for this difference is that humans during evolution were exposed to wide fluctuations in the food supply, promoting the selection of DNA sequences that enhance the utilization of dietary nutrients. Our genome has had insufficient time to adapt to the dietary abundance and physical inactivity of the modern era. Consequently, diseases of dietary excess such as atherosclerosis and diabetes have become the major cause of death and disability in the Western world.

A tentative hypothesis is that an enhanced recruitment of LDL by endothelial cell surface HSPGs during evolution enhanced the utilization of dietary nutrients by facilitating the interaction of LDL with other molecules, such as LPLs or apoE, which could then mediate uptake or degradation of LDL via the lipoprotein-receptor-related protein or other pathways. However, with high plasma levels of atherogenic lipoproteins, the same mechanism leads to the initiation of atherosclerosis.

Therapies for reducing arterial retention of atherogenic lipoproteins

In addition to reducing the concentration of proatherogenic lipoproteins in plasma, interventions that reduce the interaction between atherogenic lipoproteins and the extracellular matrix may be beneficial in attenuating arterial cholesterol accumulation. Two suggested possibilities have recently been proposed. These are the blocking/prevention of GAG elongation on proteoglycans [47, 48] and the potential of small oligosaccharides to prevent interaction between LDL and proteoglycans [49], as potential therapeutic targets for intervention in the pathological process of binding and retention of lipoproteins within the vascular wall.

Summary

Atherosclerosis is a multifactorial disease whose pathogenesis is still unclear. Mounting evidence, however, supports the concept that subendothelial retention of apoB100-containing lipoproteins is the initiating event in atherogenesis. Retained lipoproteins can directly or indirectly provoke all known features of early lesions and, by stimulating local synthesis of proteoglycans, can further accelerate retention and aggregation. Thus, atherosclerosis is initiated by subendothelial retention of atherogenic lipoproteins.

Figure 2. Sudan IV-stained aorta from a mouse expressing human wild-type recombinant LDL (upper) or LDL that fails to bind proteoglycans (e.g. proteoglycan-binding defective LDL) (lower).

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