Visions & Reflections

Retention of atherogenic lipoproteins in atherogenesis

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Received 3 July 2003; received after revision 13 August 2003; accepted 15 August 2003

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-
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 A2 (sPLA₂), 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 sPLA2 [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 sPLA₂-modified LDL, independent of site A.

**ApLB 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 non-functional 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 chylomicle remnants are retained within the intima, than the