HHS Public Access

Author manuscript

Matrix Biol. Author manuscript; available in PMC 2019 March 25.

Published in final edited form as:

Matrix Biol. 2018 October ; 71-72: 262–282. doi:10.1016/j.matbio.2018.05.010.

THE HEPARAN SULFATE PROTEOGLYCAN GRIP ON HYPERLIPIDEMIA AND ATHEROSCLEROSIS

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Abstract

Heparan sulfate proteoglycans are found at the cell surface and in the extracellular matrix, where they interact with a plethora of proteins involved in lipid homeostasis and inflammation. Over the last decade, new insights have emerged regarding the mechanism and biological significance of these interactions in the context of cardiovascular disease. The majority of cardiovascular disease-related deaths are caused by complications of atherosclerosis, a disease that results in narrowing of the arterial lumen, thereby reducing blood flow to critical levels in vital organs, such as the heart and brain. Here, we discuss novel insights into how heparan sulfate proteoglycans modulate risk factors such as hyperlipidemia and inflammation that drive the initiation and progression of atherosclerotic plaques to their clinical critical endpoint.

INTRODUCTION

Despite significant therapeutic progress made over the last 3 decades cardiovascular disease remains globally the leading cause of death for both men and women [1, 2]. The majority of cardiovascular disease-related deaths are caused by complications of atherosclerosis, a disease characterized by thickening of the arterial wall and narrowing of the arterial lumen. Atherosclerosis is a very complex, maladaptive inflammatory process initiated by accumulation of apolipoprotein (apo) B-lipoprotein remnants, such as chylomicron remnants and low-density lipoproteins (LDL), in the matrix beneath the endothelial cell layer (or intima) of large and medium-sized arteries [3]. Fatal or life-threatening complications occur when the narrowing of arteries reduces blood flow to critical levels in vital organs, such as the heart and brain, causing unstable angina, myocardial infarction, or stroke.

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Abbreviations are listed in Table 1.

Many of the cardiovascular disease risk factors are influenced by the composition and expression of proteoglycans in different tissues. In this review, we summarize recent studies focused on the impact of heparan sulfate proteoglycans (HSPGs) on hypertriglyceridemia and atherosclerosis development and resolution. A primer is provided to describe the process underlying the initiation and progression of atherosclerosis for readers unfamiliar with these subjects. A detailed discussion follows on the role of HSPGs in LDL and triglyceride rich-lipoprotein (TRL) metabolism and their impact on atherosclerosis.

ATHEROSCLEROSIS INITIATION AND PROGRESSION

Atherosclerotic lesions initiate by the trapping of remnant lipoproteins in the underlying extracellular matrix of damaged or activated arterial endothelium [4, 5]. Plaques develop predominantly at sites of altered hemodynamic flow (low shear stress and non-linear or turbulent flow) such as branch points and sites with increased vascular curvature. The exact mechanism behind the increased lipoprotein permeability at these sites remains unclear, but it may be related to the misorientation of endothelial cells and altered barrier function of the endothelial layer [6]. Elevated levels of circulating apoB-lipoprotein levels further promote trapping of remnant lipoproteins in the subendothelial matrix. Trapped remnant apoB-lipoproteins undergo aggregation by vascular proteoglycans and oxidation mediated by lipoxygenases and reactive oxygen species, generating oxidized LDL (oxLDL). Oxidized phospholipids released during the latter process activate the overlying endothelium by stimulating expression of chemokines and adhesion molecules that attract and mediate migration of monocytes, T cells and neutrophils into the intima [3, 7]. Monocytes entering the plaque differentiate into macrophages and internalize aggregated LDL and oxLDL particles. Due to these modifications remnant lipoproteins are no longer recognized by the LDL receptor (LDLR), whose expression is inversely modulated by cellular cholesterol levels. Instead, cholesterol-rich lipoprotein remnants get internalized via phagocytosis or scavenger receptors without constraints and induce massive cholesterol accumulation turning macrophages into lipid droplet-laden cells, called foam cells. These foam cells are retained and progressively accumulate in the subendothelial matrix in the lesions with ensuing chronic low-grade production of cytokines and chemokines. As a result, naïve macrophages entering the plaque to clear the modified remnant lipoproteins and then differentiate into pro-inflammatory macrophages as opposed to the alternatively-activated or resolving macrophages [8]. These proinflammatory macrophages in turn attract more monocytes, T cells and neutrophils, amplifying a chronic inflammatory response [9, 10]. The continuous accumulation of inflammatory macrophages secreting cytokines, such as interleukin (IL)-1β, IL-6 and tumor-necrosis factor, locks the system in a state of non-resolving chronic inflammation [11]. Over time, the aforementioned sterile inflammation intensifies and transitions the lesions into more complex advanced plaques with narrowing of the arterial lumen and deposition of fibrous elements. An adaptive resolution-repair response, including efferocytosis of apoptotic foam cells and formation of a fibrous cap, prevents an overwhelming majority of lesions from causing clinical symptoms [12]. A small subset of lesions progress to produce significant clinical effects and death as a result of plaque rupture, thrombus formation and subsequently arterial occlusion [13].

A decline in cardiovascular disease-associated mortality was initiated by promoting lifestyle changes in combination with LDL cholesterol lowering drugs that either increase hepatic LDLR expression (statins and proprotein convertase subtilisin/kexin type 9 [*PCSK9*] inhibitors) or reduce cholesterol absorption (ezetimibe) [14, 15]. Unfortunately, an expanding variety of at-risk populations and residual risk after LDL lowering therapies are responsible for a recent halt in this downward trend. In part this is driven by the increased prevalence of modifiable risk factors other than LDL cholesterol levels, such as diabetes, a high body-mass index and obesity, hypertension, decreased high-density lipoprotein (HDL) and elevated plasma triglyceride levels or hypertriglyceridemia,.

HYPERTRIGLYCERIDEMIA, AN IMPORTANT CARDIOVASCULAR DISEASE RISK FACTOR

Intense LDL lowering by statins caused a significant decline in deaths caused by cardiovascular disease. Recently this decline in deaths reached a plateau and hypertriglyceridemia is now considered a prevalent risk factor driving this stagnation [15]. With a population incidence of 20–28%, hypertriglyceridemia, is defined as plasma triglyceride levels above 150 mg/dL (1.7 mM). Plasma triglyceride levels are determined by an intricate balance between *de novo* synthesis in the liver (very low-density lipoproteins, VLDL), intestinal absorption of dietary fats (chylomicrons), lipolysis in the peripheral circulation, and hepatic clearance. Chylomicrons and VLDL released into the circulation undergo rapid lipolytic processing primarily by lipoprotein lipase (LPL) immobilized on the capillary endothelial surface in oxidative tissues (heart, skeletal muscle and brown adipose tissue) and storage tissues (white adipose tissue). Lipolysis results in triglyceride hydrolysis, release of free fatty acids and production of triglyceride-rich remnant lipoproteins (TRLs) including smaller VLDL and intermediate-dense lipoproteins (IDLs). Free fatty acids are utilized locally for energy production or stored until fasting conditions liberate them from adipose tissue. The generated TRL remnants undergo rapid clearance in the liver by receptors located on the basal membrane of hepatocytes facing the space of Disse. If the circulation time of TRLs is prolonged, for example in receptor mutants, they can undergo further hydrolysis. Lipid exchange proteins (e.g. cholesterol exchange transfer protein [CETP] and acyltransferases [lecithin cholesterol acyltransferase, LCAT] generate LDL particles that are predominantly cleared by the LDLR in the liver and in peripheral tissues.

LPL AND HSPGs

Proteoglycans are a distinct subset of glycoproteins ubiquitously expressed by all animal cells, as integral components of the glycocalyx and the extracellular matrix [16]. Proteoglycans are distinguished from other glycoproteins by the covalent attachment of one or more long linear glycosaminoglycan chains (Fig. 1). The sulfated glycosaminoglycan chains (heparan sulfate, heparin, chondroitin/dermatan sulfate, and keratan sulfate) are the most negatively charged biopolymers found in nature varying in length from 40–300 sugar residues (~20–150 nm) and charge densities of up to −4/disaccharide. The degree and pattern of sulfation is highly variable and creates binding sites for various proteins, including growth factors, membrane receptors, proteases and their inhibitors, apolipoproteins, lipases, and various extracellular matrix proteins. Proteoglycans regulate cell-cell and cell-

extracellular matrix interactions, serve as co-receptors for growth factor signaling and endocytic clearance receptors, and enable formation of depots for growth factors gradients of morphogens during development. Due to their localization, ubiquitous expression and dense negative charge, proteoglycans are involved in some way in many, if not all, physiological and pathophysiological processes including lipid metabolism and atherosclerosis [16, 17].

Typical HSPGs contain 1–3 HS chains, and additionally can contain chondroitin sulfate/dermatan sulfate (CS/DS) and other types of glycans such as asparagine-linked N-glycans and threonine/serine-linked mucin-type O-linked glycans. In total there are 19 known HSPGs subdivided into four categories based on their cellular localization (Table 2): (i) transmembrane proteoglycans (syndecan 1–4, betaglycan, CD44v3, NRP1, APLP2, CD47), (ii) the cell surface glycophosphatidylinositol (GPI)-anchored proteoglycans (glypicans 1–6), (iii) the secreted extracellular matrix proteoglycans (agrin, collagen XVIII and perlecan) and (iv) the intracellular secretory vesicle proteoglycan serglycin. It is important to note that HSPGs may not contain the full complement of HS chains and can possess chains of different lengths, thus creating great heterogeneity. Recent advances in glycoproteomic tools have allowed identification of previously unknown proteoglycans, confirming the suspicion that the number of HSPGs may be underestimated [18, 19].

HSPGs are important regulators of plasma triglyceride levels because of their impact on LPL-mediated lipolysis and hepatic TRL clearance. LPL is synthesized predominantly by myocytes, adipocytes and macrophages and is presented on the lumenal side of the endothelium in tissue capillaries where it can process VLDL and chylomicrons and release free fatty acids in the local environment (Fig. 2) [20]. For many years it was assumed that endothelial HSPGs were responsible for the retention and presentation of LPL on the lumenal side of capillary beds [21, 22]. The HSPG-LPL concept was supported by observations that binding of LPL to cultured endothelial cells was abrogated by heparin lyases or added heparin and that many of the apolipoproteins (apo) on TRLs, such as ApoE and ApoAV, also bound HS allowing approximation of LPL and TRLs [23]. Furthermore, LPL contains multiple heparin-binding domains and an intravenous injection of heparin releases LPL into the circulation, presumably by liberation of endothelial cell-bound enzyme. However, it was subsequently shown that glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) is the primary receptor for LPL [24]. Initially assumed to be an endothelial-specific HDL binding receptor, it became clear that mice and humans lacking functional GPIHBP1 present with severe hypertriglyceridemia due to absence of LPL presentation on the luminal face of capillary endothelium [25–27]. This important interaction was missed in prior studies because the isolation and cultivation of endothelial cells results in complete loss of GPIHBP1 expression. GPIHBP1 binds to LPL via a negatively charged cysteine-rich Ly6-domain, thus explaining why heparin can displace the enzyme from the complex. GPIHBP1 not only presents and stabilizes the catalytic activity of the LPL homodimers [28], but it also shuttles the enzyme from the basolateral side of the endothelia to the lumenal side [29]. Interestingly, transport and presentation of LPL occurs in regions on the capillary endothelium devoid of a glycocalyx, where margination (i.e. binding) of TRLs can occur (Fig. 2) [30].

An intriguing finding in GPIHBP1-deficient (*Gpihbp*$^{-/-}$) mice was that LPL did not spontaneously diffuse into lymph and circulation [29, 31]. Instead plasma LPL in *Gpihbp*$^{-/-}$ mice is much lower and the enzyme accumulates in endothelial interstitial spaces where it can be released upon heparin injection [29, 32]. This retention was not mediated by endothelial HSPGs based on studies of *Ndst1*$^{fl/fl}$*Tie2Cre*$^{+}$ mice which express endothelial HSPGs with less sulfated HS chains; *Ndst1*$^{fl/fl}$*Tie2Cre*$^{+}$ mice are normolipemic with no detectable differences in plasma LPL mass and activity, endothelial LPL presentation or TRL margination. Bishop et al. provided *in vivo* evidence suggesting that interstitial HSPGs might be responsible for retention of LPL. Collagen XVIII (COL18) is one of the major basement membrane HSPGs produced by endothelial cells [33] and its genetic manipulation resulted in lower circulating LPL mass and activity, fasting hypertriglyceridemia and postprandial dyslipidemia [34]. Humans with Knobloch Syndrome caused by a null mutation in the vascular form of COL18 also present lower than normal plasma LPL mass and activity and exhibit fasting hypertriglyceridemia [34]. Fasting and postprandial dyslipidemia were explained by the fact that loss of COL18 resulted in thickening of the basement membrane, which delayed transport of LPL from its sites of production in the parenchyma to GPIHBP1 on the basolateral side of the endothelium, thus reducing luminal LPL presentation [34].

The observations in COL18-deficient mice suggest that the HS chains attached to the protein core bind and retain LPL in the interstitial space (Fig. 2). Allan et al. showed that treatment with heparin lyase of histological slides of heart and brown adipose tissue from *Gpihbp*$^{-/-}$ mice released the accumulated interstitial bound LPL thereby confirming that the HS chains attached to interstitial HSPGs (most likely COL18) are responsible for this robust retention [31]. These authors found that LPL can transfer from HSPGs on cultured cells to soluble GPIHBP1, GPIHBP1-coated agarose beads, and nearby GPIHBP1-expressing cells, most likely due to weak association of LPL with HS compared to higher affinity for GPIHBP1. The question remains if this dynamic transfer of LPL between HSPGs and GPIHBP1 is affected by metabolic stress or dysfunction.

HEPATIC HEPARAN SULFATE CONTRIBUTES TO TRL CATABOLISM

The HS chains on HSPGs assemble by the copolymerization (catalyzed by a heterodimer complex between EXT1 and EXT2) of alternating N-acetylated glucosamine and glucuronic acid residues on a tetrasaccharide primer (glucuronic acid-galactose-galactose-xylose-) that is covalently bound to a serine residue in the extracellular matrix proteoglycans and on extracellular domain of the core proteins of membrane proteoglycans (Fig 1). The chains undergo various modifications (Fig. 1B) that convert subsets of *N*-acetylated glucosamine residues to *N*-sulfated glucosamine units (catalyzed by members of the NDST family of enzymes), epimerization of nearby glucuronic acid residues to iduronic acid (catalyzed by a C5 epimerase), and additional sulfation reactions at C6 of glucosamine units, C2 of uronic acids, and C3 of *N*-sulfoglucosamine units (catalyzed by HS6ST, HS2ST and HS3ST isozymes, respectively) [35, 36]. These reactions generally occur in the Golgi, using nucleotide sugars and an activated form of sulfate (PAPS). Mature HSPGs at the cell surface or in the extracellular matrix can undergo further modification by extracellular heparanase, which cleaves the chains at a limited number of sites and sulfatases (SULF 1 and 2) that

selectively remove sulfate groups from the C6 position of glucosamine residues. These post-synthetic modifications can release bound proteins and modulate signaling reactions relevant to atherosclerosis [37, 38]. Heparanase activity will also render core proteins (such as syndecans) more susceptible to cleavage by sheddases consequently affecting their residence time on the cell surface [39].

Different cell and animal models are used to study the structure and function of HSPGs, especially in the context of physiological and pathophysiological processes. One approach relies on germ-line or tissue-specific mutation of HSPG core proteins and biosynthetic enzymes in vivo, and siRNA-mediated silencing or CRISPR/Cas9 gene targeting techniques in vitro. Many of the enzymes, including *Ext1, Ext2*, *Ndst1*, *Hs2st*, and *Hs6st1* are critical during development, thus necessitating conditional and/or temporal inactivation using the Cre-LoxP system in mice (Fig. 1) [40–47]. In contrast to loss of function mutations, transgenic overexpression of biosynthetic genes rarely results in noticeable alteration in the content or composition of HSPGs probably due to their presence in catalytic excess. However, overexpression of individual proteoglycan core proteins, heparanase and SULF1 and SULF2 can profoundly affect HSPG structure and function.

It had long been recognized that hepatic HSPGs were important for the clearance of remnant TRLs based on the observation that infusion of bacterial heparin lyase into the portal vein of rats delayed TRL clearance [48–50]. With an average chain length of 40–60 disaccharides carrying ~1.3 sulfates per disaccharide, liver HS is considerably more sulfated and slightly shorter in length compared to most other tissues [51–53]. Structural studies in mice lacking biosynthetic enzymes established the structural features on liver HS required for TRL binding. Hepatocyte-specific inactivation of *Ndst1* (*Ndst1*$^{fl/fl}$*AlbCre*$^{+}$) and *Hs2st* (*Hs2st*$^{fl/fl}$*AlbCre*$^{+}$) expression in the mouse established that both *N*-sulfation and *2-O*-sulfate groups on liver HS are required for binding of the TRL [54, 55]. Mice lacking either of these enzymes presented with fasting and postprandial hypertriglyceridemia as well as delayed clearance of injected human TRLs to the same extent as observed in *Ext1*$^{fl/fl}$*AlbCre*$^{+}$ mice, which fail to make HS chains [56] (Fig. 1).

NDST1 inactivation reduced overall HS sulfation on hepatocytes by 50% due to coupling of the biosynthetic reactions (including 2-*O*- and 6-*O*-sulfation), rendering the normally highly sulfated liver HSPGs incapable of binding apolipoproteins exposed on the TRLs [54, 57, 58]. Similar fasting and post-prandial hypertriglyceridemia was also observed in *Hs2st*$^{fl/fl}$*AlbCre*$^{+}$ mice. For biosynthetic reasons, the reduction of 2-*O*-sulfation results in a nearly stoichiometric increase in the levels of 6-*O*-sulfation and *N*-sulfation [55], suggesting that 6-*O*-sulfation and *N*-sulfation of glucosamine residues might be less important for TRL binding. However, competition experiments using chemically *N*-desulfated, re-*N*-acetylated heparin, 2-*O*-desulfated heparin and 6-*O*-desulfated heparin showed that *N*-sulfate and 2-*O*-sulfate groups were required to block binding, whereas 6-*O*-sulfate groups were not [55]. Consistent with this idea, inactivation of hepatic *6-O*-sulfotransferase *Hs6st1* in mice (*Hs6st1*$^{fl/fl}$*AlbCre*$^{+}$) did not result in hypertriglyceridemia [55]. These observations suggest that *N*- and 2-*O*-sulfation are necessary for TRL binding and uptake. Loss of either one will therefore result in defective HSPG-mediated hepatic TRL clearance.

While initial results in mice suggested that 6-*O*-sulfation was not required for interaction of TRLs with HS, a subsequent study suggested that elevated expression of the HS endo-6-*O*-sulfatase, SULF2, correlated with hypertriglyceridemia in Type 2 diabetic (T2D) mouse models (*db/db* mice) and human obese patients [59–61]. Increased *SULF2* expression was associated with reduced 6-*O*-sulfation of hepatic HS and impaired VLDL binding to hepatocytes isolated from *db/db* mice [59, 62]. Inhibition of hepatic SULF2 expression with an antisense oligonucleotide increased 6-*O*-sulfation as well and corrected the hypertriglyceridemia by normalizing postprandial TRL metabolism [62]. These results suggest a relevant role for *6-O*-sulfation in mediating TRL binding to HS, presumably catalyzed by another member of the *Hs6st* family of sulfotransferases. In obese human patients a SNP in SULF2 was associated with elevated postprandial TRL levels, as well as Hba1c (a marker for hyperglycemia) and plasma glucose levels [60, 61]. These findings suggest that SULF2 may modulate insulin reception and resistance and in this way alter TRL clearance [63–65].

The C5 epimerase, GLCE, converts glucuronic acid to iduronic acid, the more prevalent uronic acid substrate for 2-*O*-sulfation [35] (Fig. 1). An association was noted between heterozygous mutations in *GLCE* (single nucleotide polymorphisms in its coding sequence), reduced plasma HDL cholesterol, and elevated triglyceride levels in a Turkish cohort [66]. However, it remains unclear if the SNPs alter the expression, enzymatic activity or substrate recognition by the enzyme. Heterozygous *Glce* mice ($Glce^{+/-}$) on an $Apoe^{-/-}$ background had a significant moderate elevation in both plasma triglyceride and total cholesterol on a high-fat diet compared to $Apoe^{-/-}$ mice [66]. On a wild-type background $Glce^{+/-}$ mice do not present with hyperlipidemia and so it remains to be determined if the hyperlipidemia in $Apoe^{-/-}Glce^{+/-}$ mice results from impaired TRL clearance, altered LPL activity/localization or altered VLDL or chylomicron production.

Transgenic mice overexpressing the human heparanase gene showed the importance of HS in TRL metabolism [67, 68]. Overexpression of heparanase under control of the chicken β-actin promoter results in a 95% reduction in the HS chains attached to hepatic proteoglycan core proteins without leading to an overall decrease in hepatic HSPG expression [67, 69]. Heparanase transgenic mice have fasting and postprandial hypertriglyceridemia due to delayed hepatic clearance of postprandial TRLs without alterations in post-heparin plasma LPL activity. Despite earlier reports [67], no differences in food consumption and body weight were observed when mice were fed a high-fat diet [67, 68]. Although the data is consistent with the idea that HSPGs act as receptors for remnant clearance, the constitutive overexpression of heparanase in multiple tissues generates a significant amount of HS oligosaccharides in the circulation. It is possible that these HS fragments occupy HS-binding sites on apolipoproteins on TRLs before they enter the space of Disse and thereby competitively prevent interaction of TRLs with HSPGs on the cell surface of hepatocytes. It is also peculiar that the hyperlipidemic phenotype was only observed on a high fat diet and not a chow diet. Under these conditions, the animals likely developed insulin resistance (T2D), which will augment hepatic VLDL output and decrease clearance via both HSPG-dependent and HSPG-independent mechanisms [63–65]. Interestingly administration of the heparanase inhibitor, PG545, to chow-fed $Apoe^{-/-}$ mice reduced plasma glucose levels but

had no significant impact on plasma triglyceride levels [70]. Whether PG545 impacts plasma triglyceride metabolism in animals fed a high-fat diet is unknown.

Heterozygous loss of EXT1 or EXT2 enzyme activity is associated with a moderate reduction in HS chain length and results in the development of a bone disease called Hereditary Multiple Exostoses [40, 41, 71, 72]. Compound inactivation of EXT1 and LDLR in mice resulted in a moderate fasting hyperlipidemia, suggesting again that chain length is important for HS mediated TRL clearance [56]. However no significant impact was seen in mice heterozygous for EXT1 or in patients with Hereditary Multiple Exostoses. These findings suggest that partial deficiency in the LDLR sensitizes TRL clearance to alteration in HS chain length. Taken together, the studies of Ndst1, Hs2st, Ext1 loss-of-function mutants and heparanase overexpression studies provide genetic evidence that HSPGs participate in TRL clearance in vivo.

HEPATIC SYNDECAN-1, AN INDEPENDENT REMNANT CLEARANCE RECEPTOR

Hepatocytes express multiple HSPGs, raising the question as to the identity of the relevant HSPG active in TRL metabolism. Early in vitro studies of CHO cells showed that syndecan-1 (SDC1) will bind and internalize VLDL enriched with LPL. Subsequent studies in mice confirmed that SDC1 is the dominant proteoglycan receptor that facilitates natural remnant particle clearance in vivo under fasting and post-prandial conditions [73–75]. Inactivation of SDC1 expression was associated with elevated plasma triglyceride levels due to impaired hepatic remnant uptake, and the effect was selective based on lack of lipid phenotype in $Sdc3^{-/-}$ and $Sdc4^{-/-}$ mice [73]. Furthermore, induction of a disintegrin and metalloproteinase 17 (ADAM17) causes shedding of hepatic SDC1 (Fig. 3) and reduces TRL clearance, resulting in fasting hypertriglyceridemia in mice [76]. SDC1 is also the primary HSPG receptor responsible for uptake and binding of TRL remnants by human hepatocytes and human hepatoma cells [76]. Thus, there is compelling genetic and biochemical evidence that SDC1 acts as an endocytic receptor.

Syndecan-1 is a type I transmembrane protein bearing up to three HS and two chondroitin/dermatan sulfate chains (Fig. 3). Treatment of human hepatocytes with heparin lyases revealed that HSPG dominate the receptor binding sites, representing at least 90% of the binding capacity, and accounting for ~50% of remnant clearance [76]. Internalization of SDC1 and delivery of bound lipoproteins to lysosomes occurs relatively slowly ($t_{1/2}$ ~30–45 min) through clathrin- and caveolin-independent, raft-dependent endocytosis, and likely involves oligomerization of SDC1 [49, 77, 78]. Compared to the fast internalizing receptors, LDLR ($t_{1/2}$ ~10 min) and LRP1 ($t_{1/2}$ ~0.5 min??), clearance through SDC1 is rather slow. However, the binding capacity of SDC1 for remnants exceeds the capacity of LDLR and LRP1 by at least an order of magnitude, most likely due to the multivalency afforded by the multiple HS chains on SDC1. Under fasting conditions, occupancy of SDC1 is likely less than 10% of its capacity, whereas LDLR and LRP1 may be saturated via TRL and LDL binding. Thus, one function of SDC1 receptors may be clearance of remnants under post-

prandial conditions. As discussed below, SDC1 receptors also remove a unique subset of TRL remnants of characteristic size and apolipoprotein composition (Fig. 4).

The idea that SDC1 is an independent endocytic receptor was originally controversial. Initially it was proposed that hepatic HSPGs capture the incoming remnant TLRs in the liver sinusoid and then handoff the remnants to the cell surface receptor LRP1 [58]. It was also suggested that formation of an HSPG-LRP1 complex is required for internalization of HSPG bound TRLs via its association with this rapid endocytic receptor [79]. These possibilities cannot be fully excluded, but ample evidence strongly infers that SDC1 is an independent endocytic remnant receptor in its own right [80]. An important observation is that hepatocyte-specific ablation of *Lrp1* does not result in accumulation of plasma triglycerides unless *Ldlr* expression is also absent [81–83]. Crossing $Ldlr^{-/-}Lrp1^{fl/fl}AlbCre+$ mice with $Ndst1^{fl/fl}AlbCre+$ aggravated the dyslipidemia, demonstrating unequivocally that the HSPG receptors can act independently [82, 84]. Reciprocal, coordinate regulation of LDLR and LRP1 with SDC1 apparently does not occur, and unlike LDLR and LRP1 expression, SDC1 expression is not affected by alterations in circulating cholesterol levels.

The structural elements of the SDC1 core protein that mediate hepatic remnant internalization remain to be established. *In vitro* alanine scanning mutagenesis of the SDC1 cytoplasmic domain using a hybrid FcR-SDC1 construct revealed the presence of a juxtamembrane endocytic motif, MKKK (Fig. 3) [77]. Upon ligand binding this MKKK motif is responsible for SDC1 dissociation from tubulin, phosphorylation of the cytoplasmic tail and subsequent internalization of the complex. The relevance of the MKKK endocytosis motif for TRL clearance in vivo has not yet been determined. Interestingly the MKKK motif is also present in SDC3 and SDC4 (but not SDC2) suggesting that other regulatory elements, or its localization and/or level of expression differentiate SDC1 as the dominant HSPG clearance receptor. Further studies of SDC1 are needed to understand how its structure relates to its capacity to bind and internalize remnant particles (Fig. 3).

The SDC1-mediated TLR internalization process also requires binding of intracellular adaptor proteins such as Src kinase, cortactin and flotilin-1 (FLOT1) to the SDC1 cytoplasmic domain [77]. Interestingly, hepatic *Flot1* expression is reduced in murine T2D models presenting with hypertriglyceridemia. Rescue of liver *Flot1* expression in these models corrected the hyperlipidemia by improving hepatic TRL clearance [77]. These results suggest that reduced FLOT1 binding to SDC1 is partially responsible for the T2D associated hypertriglyceridemia. However, other studies of streptozocidin-induced T1D in $Ndst1^{fl/fl}AlbCre+$ mice demonstrated that hypertriglyceridemia under these conditions was not related to hepatic HS assembly [85].

SYNDECAN-1 CLEARS REMNANT LIPOPROTEINS ENRICHED IN APOCIII

Remnant TRLs released after LPL mediated lipolysis are not uniform in size, charge, or composition [57, 82, 84]. Evidence suggests that SDC1 preferentially clears TRL remnants with a diameter between 20–40 nm enriched with ApoE and ApoAV (Fig. 4) [57, 82, 84]. In contrast LDLR and LRP1 favor binding of TLRs that are 30–60 nm in diameter and enriched for ApoE [82]. Binding of TRLs to SDC1 requires simultaneous binding to ApoE and

ApoAV to HS, but is independent of ApoB, even though all three apolipoproteins can bind to heparin [57]. Removal of ApoAV from TRLs or antibodies to ApoAV prevented TRL binding to purified HS and SDC1 and blocked SDC1-mediated uptake of TRLs by human hepatoma cells. In mice, the absence of either ApoE or ApoAV results in profound accumulation of small TRLs in the circulation, which correlates with increased atherosclerosis development [57]. Thus, SDC1 is responsible for clearing small, more atherogenic TRLs.

Unexpectedly the TRLs circulating in *Ndst1*$^{fl/fl}$*AlbCre*$^{+}$ mice also contain an abundance of ApoCIII, an apolipoprotein not known to bind HS chains or heparin. ApoCIII is a 8.8 kDa glycoprotein mainly produced in the liver, and to a lesser extent in the intestine, and is present in TRL, LDL and HDL particles [86]. The importance of ApoCIII in triglyceride metabolism became clear when inactivating mutations affecting its expression in humans were shown to correlate with lower plasma triglycerides [87] and protect against cardiovascular disease [88–90]. Similarly, transgenic expression of *Apoc3* in mice results in hypertriglyceridemia [91], whereas a null mutation in *Apoc3* decreases triglyceride levels [92]. Initially, ApoCIII was thought to raise triglyceride levels by inhibiting LPL-dependent lipolysis [93, 94]. Recently, it has become apparent that ApoCIII inhibits clearance of TRL remnants via LDLR and LRP1 and not by inhibiting LPL [84, 95, 96]. These studies also showed that ApoCIII accumulates on plasma TRLs in mice lacking hepatic SDC1, but not in mice lacking LDLR and/or LRP1 (Fig. 4), suggesting that SDC1 specializes in clearance of small, ApoCIII-rich atherogenic TRLs.

Recent studies have shown that administration of *Apoc3* antisense oligonucleotides reduced fasting triglycerides by 35–50% in mice defective in hepatic *Ndst1*, *Lrp1*, or *Ldlr*, and in animals with combined deletions of *Ndst1* and *Lrp1* or *Ldlr* [84]. However, administration of *Apoc3* antisense oligonucleotides to mice lacking both *Lrp1* and *Ldlr* had no effect on plasma TG levels. Furthermore, reduction of ApoCIII production enhanced the rate of clearance of TRLs in mice expressing only LDLR and LRP1 (i.e., lacking SDC1), but had no effect in mice expressing only SDC1 (i.e., lacking LDLR and LRP1). These findings reinforce the notion that ApoCIII prevents clearance through the LDLR/LRP1 axis, and that SDC1 can mediate clearance of TRLs independently of ApoCIII [84]. Thus, SDC1 is the primary receptor responsible for clearance of ApoCIII-rich TRLs, consistent with the observation that mice expressing functional SDC1 have very little ApoCIII on circulating TRLs [84]. Hepatic TRL clearance becomes increasingly more reliant on SDC1 in pathophysiological conditions that dramatically increase ApoCIII expression such as high fat feeding, insulin resistance and T2D [84]. T2D also increases expression and secretion of SULF2 in hepatocytes, reducing 6-*O*-sulfation of HS. The combination of these factors along with increased VLDL production and impaired LRP1 translocation might explain the severe hypertriglyceridemia often manifested by T2D patients.

HEPARAN SULAFTE PROTEOGLYCANS PRESENT PCSK9

In contrast to TRLs, LDL particles are eliminated from the circulation independently of SDC1 receptors and only through ApoB-mediated binding to LDLR. Mutations in *LDLR* or *APOB* genes cause familial hypercholesterolemia, an autosomal dominant genetic disorder

characterized by hypercholesterolemia [97]. SNPs in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) are also associated with familial hypercholesterolemia [97], reflecting the ability of PCSK9 to reduce cell-surface LDLR expression on hepatocytes. Binding of PCSK9 to the EGF-A domain of the LDLR allows formation of a PCSK9-LDLR complex, which undergoes endocytosis and lysosomal degradation [98, 99]. By inducing lysosomal degradation, PCSK9 prevents LDLR recycling back to the plasma membrane, thus reducing LDLR receptor content. When expressed at high levels, PCSK9 diminishes hepatic LDL clearance and raises circulating LDL-cholesterol [99]. Monoclonal antibodies directed against PCSK9 increases LDL clearance and are now prescribed to lower the incidence of cardiovascular disease in patients [100].

There is still a great deal of mystery surrounding the mechanism behind the PCSK9-mediated lowering of hepatic LDLR expression. In fact, many lines of evidence suggest the existence of liver-specific co-receptors or adaptor proteins for PCSK9 [101, 102]. Glypican 3 (GPC3), a member of the family of GPI-anchored HSPGs (Table 2), was identified in a proteomic search as an extracellular interaction partner that could regulate the PCSK9-LDLR complex formation [101]. Reciprocal co-immunoprecipitation experiments in hepatoma cell lines (HepG2 and Huh7) overexpressing a tagged PCSK9 established that GPC3 binds mature secreted PCSK9 as well as intracellular unprocessed pro-PCSK9. Stable knockdown of GPC3 in hepatoma cells resulted in increased LDLR protein expression (without affecting LDLR mRNA expression) and greater LDL uptake [101], whereas GPC3 overexpression had the opposite effect, i.e. less PCSK9 mediated degradation of LDLR. The physiological relevance of some of these findings has been questioned because GPC3 expression in adult liver is almost undetectable [101, 103]. GPC3 expression in embryonic liver suggests that it might play a role in modulating PCSK9 during embryogenesis or liver regeneration [101, 103].

It remains unclear if the interaction of the HS chains on GPC3 mediates the interaction with PCSK9. In order to detect HSPGs on a Western blot one needs to enzymatically remove the HS chains using heparin lyases. In the absence of such a treatment the variable number and length of the attached HS chains render the HSPG heterogeneous in mass, which in Western blots manifests as a ‘smear’ of immunoreactive material at a higher molecular weight than predicted from the amino acid sequence of the core protein. Co-immunoprecipitation of PCSK9, however, identified a distinctly sharp band for GPC3 on Western blot in the absence of heparin lyase treatment suggesting that PCSK9 mostly interacts with unglycosylated GPC3 core protein. This observation might explain why mutant mice lacking HS biosynthetic enzymes do not present with elevated plasma cholesterol levels [48, 54–57, 84].

Gustafsen and colleagues recently established that PCSK9 binds heparin using an affinity column [102]. Addition of heparin to HepG2 cells increased LDLR expression and correlated with increased secretion of PCSK9 in the medium. Mutation of the heparin-binding site on PCSK9 impaired PCSK9-mediated downregulation of LDLR expression in HepG2 cells and in mice [102]. The heparin binding site is opposite to the LDLR binding domain. Thus, exogenous heparin may displace PCSK9 from cell surface HSPGs and prevent its presentation to the LDLR. However, monoclonal antibodies targeting the PCSK9 heparin-binding site do not affect PCSK9-LDLR interactions [102], but these antibodies

have the same therapeutic effects in mice as the PCSK9 monoclonal antibody, Evolocumab, which targets the binding of PCSK9 binding to LDLR [100]. Interestingly, heparanase transgenic mice present with elevated circulating PCSK9 levels and increased hepatic LDLR expression [102]. Possibly, the binding of PCSK9 to cell surface HSPGs may be a mechanism to sequester PCSK9 after its secretion or to facilitate its internalization.

HEPARAN SULFATE, A KEY MODULATOR OF LDL RETENTION

ApoB is present on TLR remnants, IDL, LDL, and Lipoprotein(a) [Lp(a)], an LDL-like lipoprotein in which ApoB is covalently linked to Apo(a). A great body of work supports the idea that the key initiating event in atherogenesis is the retention, or trapping, of cholesterol-rich ApoB-containing lipoproteins. Particles of ≤70 nm in diameter can enter the subendothelial space in large and mid-sized arteries via a poorly understood pathway of transendothelial movement [104]. Once trapped the ApoB-lipoproteins within the arterial wall undergo oxidation and covalent modification and generate a maladaptive local immune response that causes atherosclerosis initiation and progression [5]. According to this ‘response-to-retention’ hypothesis, proteoglycans mediate remnant lipoprotein retention (Fig. 5). The arterial extracellular matrix is rich in chondroitin and dermatan sulfate proteoglycans such as versican and biglycan, as described in great detail by Wight and colleagues [105, 106].

Elevated circulating levels of TRL remnants, LDL, and Lp(a) increases the chance that these ApoB-lipoproteins get trapped in the extracellular matrix of the arterial vessel wall at susceptible sites, often associated with regions of turbulent blood flow [4, 5]. The difference between susceptible and resistant sites in arteries does not reflect the overall transendothelial influx of apoB-lipoproteins, but rather by retention of infiltrating lipoproteins [107]. Evidence supports that HS as well as chondroitin/dermatan sulfate in the arterial extracellular matrix drives retention of remnant lipoproteins. One of the prominent HSPGs in the arterial extracellular matrix is perlecan (HSPG2), a multidomain 450 kDa core protein containing three HS chains [108, 109]. Perlecan is abundantly present in atherosclerotic lesions and increases in content when early lesions progress to advanced plaques [110, 111]. *Apoe*$^{-/-}$ mice heterozygous for perlecan present with greater atherosclerosis burden compared to *Apoe*$^{-/-}$ mice suggesting its importance in cardiovascular disease [112]. Tran-Lundmark *et al.* assessed the importance of HS chains attached to perlecan in mediating LDL retention by studying an HS-deficient perlecan mouse (*Hspg2*$^{\Delta 3/\Delta 3}$) generated by inactivating exon 3 coding for a segment of the protein containing the HS attachment sites (serine residues 65, 71 and 76 in Domain-I) [113, 114]. Crossing *Hspg2*$^{\Delta 3/\Delta 3}$ mice with hypercholesterolemic *Apoe*$^{-/-}$ mice resulted in a dramatic reduction in vascular LDL retention and atherosclerosis formation. Paradoxically, the subendothelial LDL influx in the arteries was greater than in *Apoe*$^{-/-}$ mice. Because perlecan serves both as a structural barrier and retentive molecule, the increased influx was explained by loss of barrier activity. The findings support the notion that the capacity to retain remnant lipoprotein rather than influx rate, determines early (and possibly advanced) development of atherosclerosis. Overall the experiments underscore the importance of HS in the arterial extracellular matrix for LDL retention (Fig. 5). Domain II in the perlecan core protein can also bind and retain LDL in the extracellular matrix [111]. This HS-independent interaction relies upon sialic

acid residues associated with the mucin type O-glycans on Domain II. Interestingly, *Hspg2*$^{+/-}$ and *Hspg2*$^{\Delta 3/\Delta 3}$ mice on a chow diet or on a Western diet did not present with hypertriglyceridemia or hypercholesterolemia even when crossed with hyperlipidemic mouse models (*Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice) [34, 112, 113]. Thus, perlecan seems to play a primary role in arterial biology related to atherosclerosis, rather than lipid metabolism in the circulation, liver or other peripheral tissues.

ENDOTHELIAL HEPARAN SULFATE AND ATHEROSCLEROSIS DEVELOPMENT

Trapped remnant lipoproteins in the subendothelial matrix undergo oxidation and modification by malondialdehyde, and the modified phospholipids activate the overlying endothelium, resulting in expression of cell adhesion molecules and attraction of inflammatory cells, including neutrophils and monocytes ([115–118]. Evidence in mice support that in atherosclerosis monocyte rolling on activated endothelium is mediated by P-selectin [119] and adhesion is driven by vascular cell adhesion molecule-1 (VCAM-1) and integrin $\alpha_4\beta_1$ [120–124]. Once leukocytes bind to the endothelium a set of chemokines and their complementary receptors direct monocyte transmigration into the subendothelium by penetrating endothelial junctions or possibly by transmigration. A number of chemokines play a key role in intimal leukocyte accumulation [116, 125]. CCL2-CCR2 and the CCL5-CCR1/CCR5 promote accumulation of the classical proinflammatory Ly-6C^{high} subset of monocytes [126] [127–129]. Fractalkine (CX_3CL1)-CX_3CR1 in contrast seems to mediate recruitment of Ly6C^{low} monocytes [128–130]. Monocyte recruitment in mouse atherosclerotic lesions persists during lesion progression and is directly proportional to the severity of the lesion [131].

Heparin binds to both P-selectin and L-selectin and block their interaction with their endogenous glycoprotein ligands [132–134]. Endothelial HS is important for leucocyte extravasation via L-selectin as well as for the presentation and signaling of many of the aforementioned cytokines (Fig. 5) [46, 134–137]. Yet, very little is known about the impact of endothelial HS on the initiation and progression of atherosclerotic lesions. Baeyens et al studied the impact of SDC4 on atherosclerosis [6]. SDC4-deficient mice (*Sdc4*$^{-/-}$) crossed onto *ApoB*$^{100/100}$*Ldlr*$^{-/-}$ mice had a massive atherosclerotic plaque burden and developed lesions in regions normally devoid of plaques as a consequence of endothelial misalignment due to the loss of SDC4 [6, 138, 139]. The misalignment amplified endothelial inflammation by promoting NF-κB activity and reducing anti-inflammatory kruppel-like factor-2 and −4 activities [140, 141].

Reduced sulfation of endothelial HS in *Ndst1*$^{fl/fl}$*Tie2Cre*$^{+}$ mice results in increased rolling velocity of neutrophils and reduced firm adhesion to the endothelium in cremaster muscle venules [135, 142]. Inactivation of HS3ST1 in mice (*Hs3st1*$^{-/-}$), an enzyme involved in installation of sulfate groups at C3 of *N*-sulfoglucosamine residues in HS and formation of the antithrombin binding site (Fig. 1), had a similar effect after LPS-induction of leukocyte rolling [142]. These findings suggest that antithrombin binding to HS can compete or prevent proper leukocyte extravasion. Although antithrombin binding sites are typically

abluminal in normal tissues, binding was prominent across the endothelium at atherosclerosis prone sites [142]. Initial attempts to study this interaction in hypercholesterolemic mice failed due to the embryonic lethality of *Hs3st1* deficiency, but this question can now be addressed by backcrossing the strain onto a pure C57Bl/6 background [143] (Esko et al, unpublished). Analysis of the human *HS3ST1* gene showed that SNP rs16881446$^{G/G}$ in the *HS3ST1* gene is associated with reduced *HS3ST1* mRNA expression in human primary endothelial cells and is overrepresented in a population with more severe coronary artery disease (Fig. 5) [142]. These findings are quite provocative and suggest that subtle changes in endothelial HS composition could play a role in atherogenesis [46, 134–137].

Studies of neutrophil depletion and enhancement in hypercholesterolemic mice has established a causal role for neutrophils in atherosclerosis development [144, 145]. Neutrophil infiltration amplifies local lesion inflammation by secretion of cathelicidin and cathepsin G thereby promoting adhesion and recruitment of monocytes by the endothelium [144, 146–148]. In addition to release of proteases and reactive oxygen species (ROS), neutrophils can in response to cholesterol crystals release large web-like structures of DNA and neutrophil-derived proteins by a process known as NETosis (neutrophil extracellular trap (NET) formation) [147][146]. In atherosclerotic plaques NETs stimulate production of cytokines by lesion macrophages and Type-I Interferons (IFNs) by plasmacytoid dendritic cells (Fig. 5) ([147, 149]. On the luminal side of the endothelium NETs are observed in close proximity to proteoglycan-rich areas of erosion-prone human plaques, suggesting that neutrophils induce endothelial cell death and promote plaque erosion and thrombus formation [146, 150]. Many aspects of neutrophil recruitment and neutrophil host-defense mechanism are regulated or influenced by HS on the endothelium or neutrophil [46, 134–137]. Recent observations established that neutrophil HS are an important component of NETs (Fig. 5) [151]. The strong connection between HS and neutrophil biology encourage further studies on the importance of endothelial and neutrophil HS on atherosclerosis disease progression.

MACROPHAGE HEPARAN SULFATE ATTENUATES PLAQUE INFLAMMATION AND PROMOTES RESOLUTION

Once monocytes enter the subendothelial extracellular matrix they differentiate into macrophages, which scavenge the trapped and modified remnant lipoproteins. Oxidative modification of apoB on LDL renders the particles unrecognizable by LDLR, but they can be taken up by scavenger receptors such as scavenger receptor (SR)-A, SR-BI and CD36 [152, 153]. Loss of LDLR-mediated LDL uptake results in diminished negative feedback regulation of LDLR expression and cholesterol biosynthesis. The lack of negative feedback regulation combined with constitutive scavenger receptor activity results in unrestricted cellular cholesterol loading. The continuous uptake of modified ApoB-remnant lipoproteins turns macrophages into sedentary lipid-loaded foam cells.

Boyanovsky discovered in murine macrophage cell lines that SDC4 was a scavenger receptor important for the removal of LDL modified by a secretory phospholipase A_2

(sPLA$_2$) [154]. Loss of macrophage SDC4 reduced uptake of sPLA$_2$-modified LDL (but not oxLDL), and increased SDC4 expression promoted uptake of modified LDL via macropinocytosis (Fig. 5). sPLA$_2$ hydrolysis of LDL is enhanced when bound to arterial chondroitin and HS proteoglycans, further promoting cholesterol loading in a glycosaminoglycan-dependent manner [155–157]. Macrophage SDC4 was able to bind the sPLA$_2$-modified LDL as well, but failed to induce foam cell formation, possibly because it lacks the ability to undergo macropinocytosis [154]. The atheroprotective effect of ω−3 polyunsaturated fatty acids (such as fish oils) is suggested to be in part mediated by its ability to reduce macrophage Sdc4 expression and consequently foam cell formation [158, 159].]

Loss of *Ndst1* in murine macrophages (*Ndst1*$^{fl/fl}$*LysMCre*$^{+}$) resulted in a decrease in overall HS sulfation and was associated with a marked increase in foam cell conversion induced by aggregated LDL (agLDL) [160]. The increased foam cell conversion was a consequence of increased activity of Acyl-CoA:cholesterol acyltransferase (ACAT) 1 and ACAT2, enzymes that convert cholesterol into cholesterol esters using long-chain fatty acyl-coenzyme A [161, 162]. In general, the formation of cholesterol esters positively correlates with foam cell formation [163]. Activation of macrophages in the mutant led to greater atheroma burden when *Ndst1*$^{fl/fl}$*LysMCre*$^{+}$*Ldlr*$^{-/-}$ mice were fed a high-fat diet but did not affect plasma lipid and lipoprotein levels [160].

The mechanism underlying the activation of macrophages in *Ndst1*$^{fl/fl}$*LysMCre*$^{+}$ mice was analyzed in detail. Macrophages secrete constitutively a low level of IFN-β [164, 165], which binds to cell surface HS by way of a patch of positively charged residues on one face of the protein. Thus, cell surface HSPGs control basal activation of macrophages in a cell-autonomous fashion by maintaining IFN-β reception in a quiescent state through sequestration of IFN-β. Consistent with this hypothesis, the reduced sulfation resulting from *Ndst1* inactivation increased baseline signaling through IFN receptors 1 and 2. Reduction of HS sulfation increased the expression of many pro-inflammatory genes in addition to ACAT2, including CCL2 and CCL5, turning most monocytes into activated Ly-6C^{high} macrophages [160]. The increased Type I IFN signaling increased lesion macrophage content and influx of Ly-6C^{high} monocytes into established atherosclerotic lesions, creating a vicious cycle of foam cell conversion and attraction of even more monocytes [129, 166].

It cannot be excluded that the increased macrophage influx in to *Ndst1*$^{fl/fl}$*LysMCre*$^{+}$ mice was to some extent the consequence of decreased macrophage efferocytosis in the lesions [12]. Loss of macrophage SDC1 was shown to delay plaque resolution and clearance of macrophages in a peritonitis model [160, 167]. *Sdc1*$^{-/-}$ macrophages displayed both reduced migration potential and reduced efferocytosis of apoptotic macrophages in a transwell system. Similar to *Ndst1*$^{fl/fl}$*LysMCre*$^{+}$ mice, the loss of SDC1 was associated with increased inflammation and increased chemo-attraction of leukocytes [167, 168]. Of note, activated murine (and human) Ly-6C^{high} macrophages have little to no detectable SDC1 expression whereas Ly-6C^{low} resolving macrophages and macrophages undergoing efferocytosis express the highest levels of SDC1 of any macrophage population subset [167, 168]. The increased inflammation and reduced efferocytosis correlated with a significant increase in atherosclerotic development in *ApoE*$^{-/-}$*Sdc1*$^{-/-}$ mice fed a high-fat diet. Similar to the

Ndst1$^{fl/fl}$*LysMCre*$^{+}$ mice, the lesions in *ApoE*$^{-/-}$*Sdc1*$^{-/-}$ mice fed a high-fat diet were far more advanced and had a greater Ly-6C^{high} macrophage content. No difference in plasma cholesterol levels were noted in wither model, yet one would expect to find a difference in plasma triglyceride levels [57]. The authors did not report if *ApoE*$^{-/-}$*Sdc1*$^{-/-}$ mice accumulated atherogenic TRLs making it difficult to conclude to what extent the atherosclerosis phenotype was driven by the macrophage phenotype [57, 167, 168].

SUMMARY

Taken together, the various studies of HSPGs and the enzymes involved in HS formation demonstrate a central role played by these glycoconjugates in lipid homeostasis and atherogenesis. In some cases, these processes seem to depend on specific HSPGs acting in a cell autonomous manner in endothelial cells, neutrophils, or macrophages. So far the findings indicate that increased HSPG expression and sulfation are associated with reduced cardiovascular disease risk. Further studies are warranted of other cell types in the arterial wall (e.g. smooth muscle cells, dendritic cells and T-cells) and other HSPGs. The overall subtle changes in HS structure afforded by mutations in specific sulfotransferases (e.g. HS3ST1 or NDST1 in the macrophage) raise the possibility that natural variation in HSPG expression or composition could contribute to dyslipidemias, inflammation, and atherogenesis in humans [169–172]. Conditions that result in proteolytic shedding of cell surface HSPGs [173] or desulfation [61] and cleavage of the chains [68] might render some individuals more prone to cardiovascular disease [174]. Genetic association studies provide the first strong evidence for this hypothesis in cardiovascular disease patients [66, 142]. Future investigations will need to address how tissue specific and temporal differences in HSPGs expression and HS structure impact cardiovascular disease outcomes.

ACKNOWLEDGEMENTS

The authors wish to thank generous support fromFondation Leducq and grant HD089067 from the National Institute of Health (to P.L.S.M.G) and grants GM33063 and HL107150 from the National Institutes of Health (to J.D.E.).

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Highlights

- We provide an overview of heparan sulfate proteoglycan structure and assembly
- We describe the role of matrix and membrane heparan sulfate proteoglycans in lipoprotein metabolism.
- We show the evidence that hepatic syndecan-1 mediates triglyceride-rich lipoprotein clearance.
- We describe the importance of neutrophil, endothelial cell and macrophage heparan sulfate proteoglycans in atherogenesis.

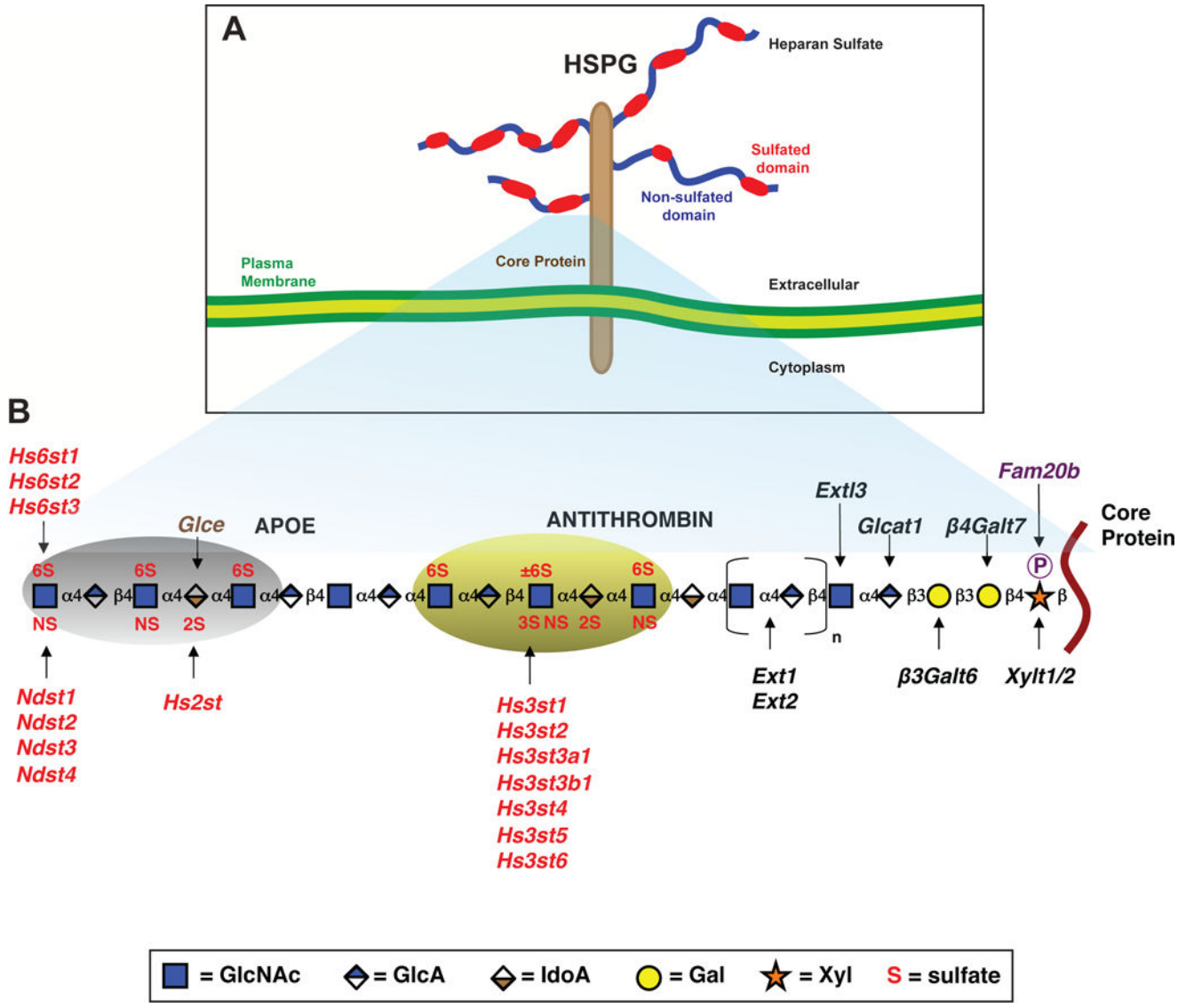

FIGURE 1. Heparan sulfate (HS) structure.

HS biosynthesis commences by the copolymerization of alternating *N*-acetylated glucosamine and glucuronic acid residues on a tetrasaccharide primer (glucuronic acid (GlcA)-galactose (Gal)-galactose-xylose (Xyl)-) that is covalently bound to a serine residue in the extracellular domain of the core proteins of membrane proteoglycans and extracellular matrix proteoglycans. **A,** The chains undergo various modifications as shown in the top of the figure by red shading. The modifications occur in clusters of variable length (sulfated domains), which are interspersed by unmodified domains (non-sulfated domains) indicated in blue. **B,** HS biosynthetic enzymes convert subsets of *N*-acetylated glucosamine (GlcNAc) residues to *N*-sulfoglucosamine units (catalyzed by members of the NDST family of enzymes), epimerization of nearby glucuronic acid residues to iduronic acid (IdoA) (catalyzed by a C5 epimerase [Glce]), and additional sulfation reactions at C6 of glucosamine units, C2 of uronic acids, and C3 of *N*-sulfoglucosamine units (catalyzed by HS6ST, HS2ST and HS3ST isozymes). The modified domains make up binding sites for

protein ligands as depicted for antithrombin and ApoE. The HS chains can be further modified once they arrive at the cell surface or in the extracellular matrix by two endosulfatases (Sulf1 and Sulf2), which remove specific sulfate groups located at C6 of glucosamine units, or by the action of extracellular heparanase (not shown).

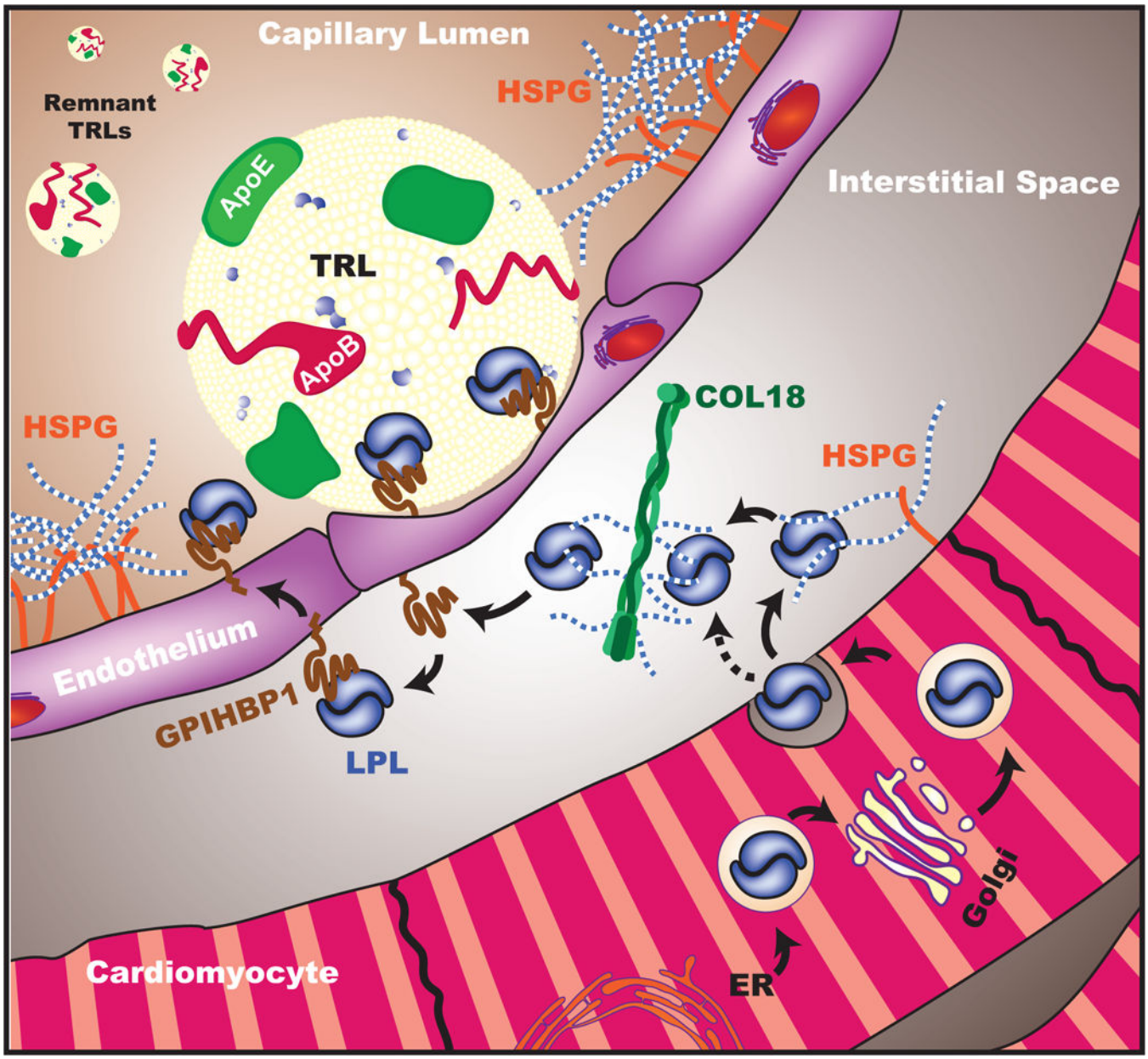

FIGURE 2. The role of HSPGs in LPL metabolism.

Lipoprotein lipase (LPL) is synthesized by parenchymal cells including myocytes, macrophage cardiomyocytes and adipocytes. After being processed in the Golgi mature LPL is secreted into the interstitial spaces. Once secreted, LPL is initially captured by cell surface HSPGs and transferred to the interstitial HSPG, COL18 (dashed arrow). Bound LPL is then transferred to GPIHBP1 on the basolateral surface of capillary endothelial cells. GPIHBP1 binding stabilizes LPL activity and allows the transendothelial transport of the LPL-GPIHBP1 complex to the lumenal side of the endothelium. In the capillary lumen, the LPL-GPIHBP1 complex facilitates TRL margination in regions of the endothelium devoid of HSPGs. The TRL-LPL-GPIHBP1 interaction allows LPL-mediated triglyceride hydrolysis to occur. TRLs reach a critical size thereby limiting the interaction with the LPL-GPIHBP1

complex and resulting in the release of TLR remnants into the circulation. LPL, Lipoprotein Lipase; COL18, Collagen XVIII; GPIHBP1, glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1.

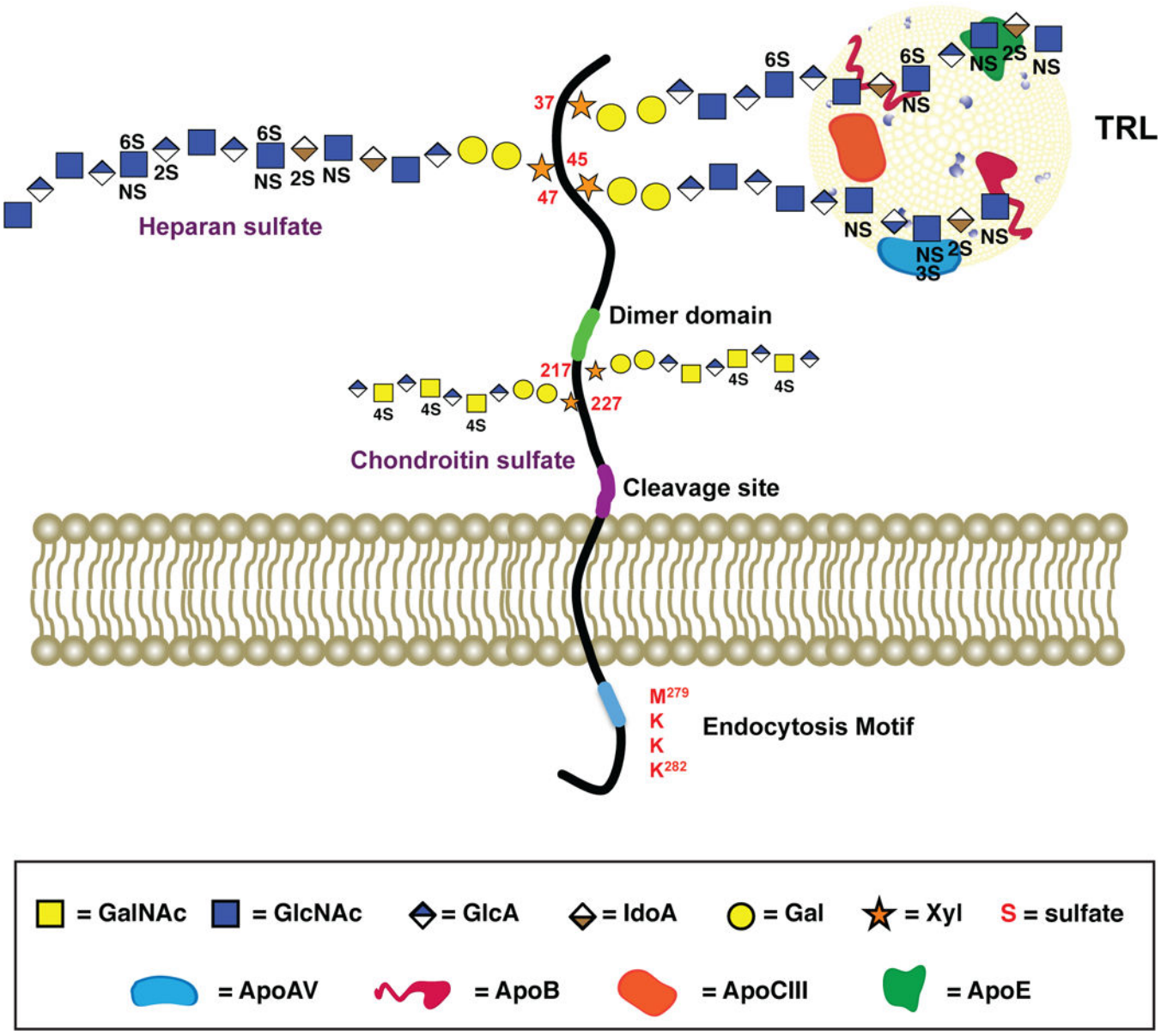

FIGURE 3. Syndecan-1 structure and binding to remnant lipoproteins.

SDC1 is a type I transmembrane protein bearing up to three heparan sulfate and two chondroitin/dermatan sulfate chains. It undergoes homodimerization as well as proteolytic cleavage. The cleavage results in shedding of the large extracellular ectodomain bearing the HS chains. The short cytoplasmic tail contains the MKKK-endocytosis motif and interacts with a number of cytosolic proteins such as FLOT1 that play a role in clathrin- and caveolin-independent, raft-dependent endocytosis. A TRL containing ApoCIII is shown binding to the heparan sulfate chains via the interaction of sulfated domains with ApoE and ApoAV. GalNAc, *N*-acetylgalactosamine

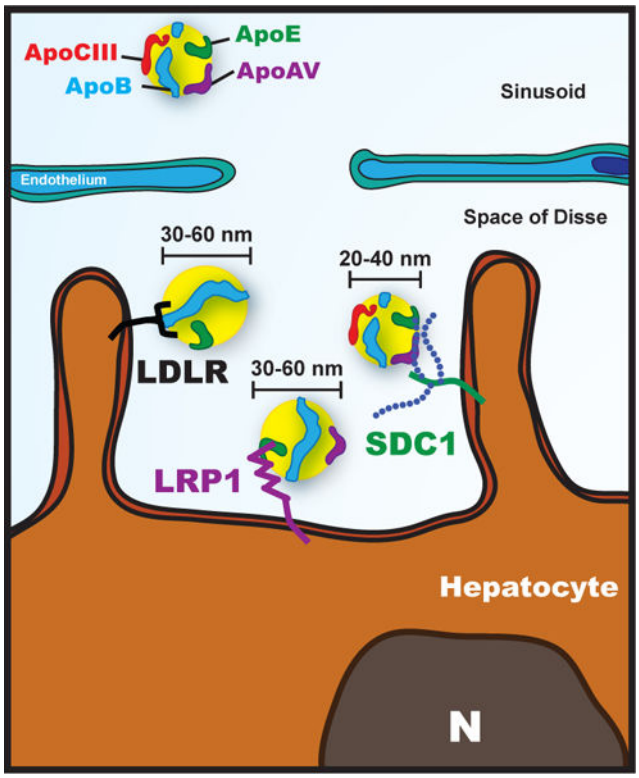

FIGURE 4. Hepatic syndecan-1 and triglyceride-rich lipoprotein clearance.

After lipolytic processing of lipoproteins in the circulation by LPL, remnant TRLs enter the Space of Disse through gaps in the hepatic endothelium. The remnants clear rapidly through three distinct hepatocyte receptors, LDLR, LRP1 and a single HSPG, syndecan-1 (SDC1). SDC1 preferentially clears TRL remnants with a diameter between 20–40 nm enriched with ApoE, ApoAV and ApoCIII. In contrast LDLR and LRP1 favor binding of TLRs that are 30–60 nm in diameter and enriched for ApoE and devoid of ApoCIII. LRP1 will also clear particles via the interaction with apoAV. Binding of TRLs to SDC1 requires simultaneous binding to ApoE and ApoAV to HS, but binding is independent of ApoB. Compared to the fast internalizing receptors LDLR ($t_{1/2}$ ~10 min) and LRP1 ($t_{1/2}$ ~0.5 min), clearance through SDC1 ($t_{1/2}$ ~45 min) is relatively slow. SDC1, Syndecan-1; LDLR, Low-density Lipoprotein Receptor; LRP1,LDLR-Related Protein 1; n; nucleus.

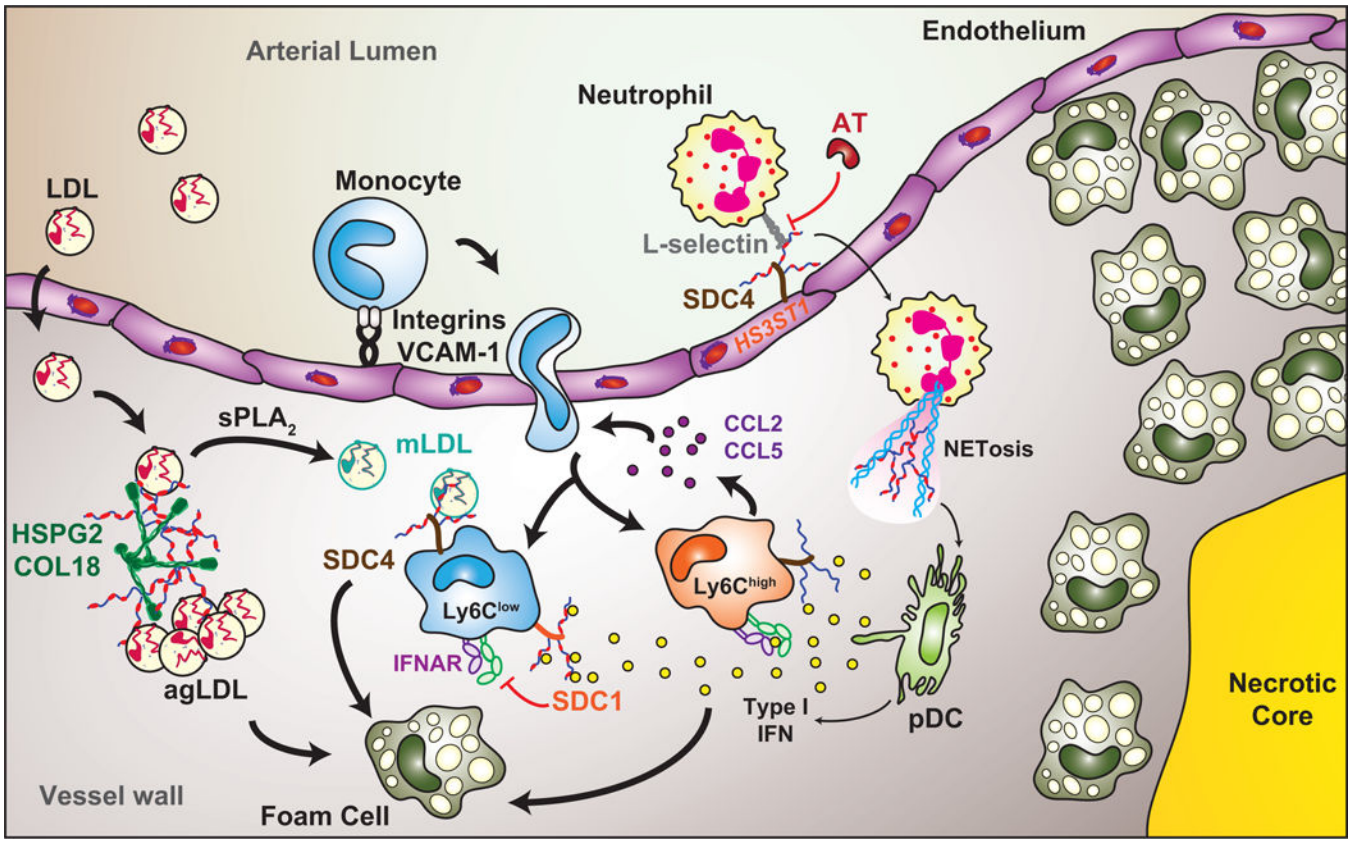

FIGURE 5. The importance of neutrophils, endothelium and macrophages HSPGs in atherogenesis.

Vascular HSPGs such as perlecan (HSPG2) and COL18 are responsible for trapping of LDL entering the subendothelial vessel wall. Trapped LDL aggregates (agLDL) undergo oxidation by reactive oxygen species and partial lipolysis by sPLA2, thereby generating modified LDL (mLDL). The process activates the overlying endothelium to express vascular adhesion molecule-1 (VCAM1) which stimulates infiltration of monocytes. Invested monocytes will differentiate into macrophages and clear mLDL via syndecan 4 (SDC4) and other scavenger receptors (not shown). The macrophages convert into sedentary foams cells that will reside in the intima of the arteries where they perpetuate a chronic inflammatory response that will drive further infiltration of monocytes. Monocytes expressing SDC1 and sulfated HSPGs will differentiate into the resolving Ly6C^{low} macrophages (Ly6C^{low}) due to reduced Type I interferon (IFN) signaling. Monocytes lacking HS sulfation and SDC1 are more susceptible to Type I IFN and differentiate into classical proinflammatory Ly6C^{high} macrophages (Ly6C^{high}) secreting cytokines such as CCL2 and CCL5 that aggravate the chronic inflammation. Increased Type I IFN signaling will also result in increased expression of ACAT1 and ACAT2 and promote foam cell conversion of Ly6C^{high} macrophages. SDC4 can also modulate the infiltration of neutrophils and based on indirect observations (smaller arrows). Evidence suggests that antithrombin (AT) blocks SDC4-mediated infiltration of neutrophils if HS3ST1 is expressed by the endothelial cells. The increased neutrophil influx will further increase the secretion of Type I IFN by plasmacytoid dendritic cells (pDC). The overall process promotes a self-perpetuating cycle, resulting in

excessive foam cell conversion, lipid-induced necrosis, in the formation of a necrotic core and unstable lesions that can rupture and clog arteries.

Table 1.

List of Abbreviations

ACAT	Acyl-CoA:cholesterol acyltransferase
ADAM17	A disintegrin and metalloproteinase 17
APO	Apolipoprotein
COL18	Collagen XVIII
CS/DS	Chondroitin sulfate/dermatan sulfate
FLOT1	Flotilin-1
GLCE	C5 epimerase
GPIHBP1	glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1
HDL	High-density Lipoprotein
HS	Heparan Sulfate
HSPG	Heparan Sulfate Proteoglycan
HSPG2	Perlecan
IDL	Intermediate-dense Lipoprotein
IFN	Interferon
IL	Interleukin
LDL	Low-density Lipoprotein
LDLR	Low-density Lipoprotein Receptor
Lp(a)	Lipoprotein(a)
LPL	Lipoprotein Lipase
LRP1	Low-density Lipoprotein Receptor-related Protein 1
NET	Neutrophil extracellular trap
PCSK9	Proprotein Convertase Subtilisin/Kexin type 9
SDC	Syndecan
$sPLA_2$	Secretory phospholipase A_2
SR	Scavenger receptor
SULF	Sulfatase
T1D/T2D	Type-1 diabetes/Type-2 diabetes
TRL	Triglyceride-rich Remnant Lipoprotein
VLDL	Very Low-density Lipoproteins

Table 2.

The Vertebrate Heparan Sulfate Proteoglycans

Proteoglycan	Core protein (kD)	Number and type of GAG chains	Tissue distribution	Proposed functions in CVD	Human disease associations
Secreted Proteoglycans					
Perlecan	400	1–3 HS, 0–2 CS	Basement membranes, other ECM, cartilage	Lipoprotein retention in the vessel wall	Rare mutations causing severe skeletal malformations
Agrin	200	1–3 HS	Basement membranes, brain and neuromuscular junctions	Unknown	
Collagen type XVIII	147	2–3 HS	Basement membranes, longest isoform more widespread	LPL processing	Mutations give rise to Knobloch syndrome, with multiple ocular, neural tube closure defects and hypertriglyceridemia
Membrane-bound Proteoglycans					
Syndecans 1–4	31–45	1–3 HS, 0–2 CS	Most nucleated cells	Hepatic TRL clearance; uptake of $sPLA_2$ modified LDL in macrophages; attenuation of macrophage and endothelial inflammation	Dysregulation in several cancers, e.g. myeloma, mammary carcinoma
Betaglycan	110	0–1 HS, 0–1 CS	Fibroblasts	Unknown	Tumor suppressor; commonly lost in ovarian cancer
Glypicans 1–6	~60	1–3 HS	Epithelial and mesenchymal cells, brain	Unknown	Simpson-Golabi-Behmel overgrowth syndrome,hepatocellular carcinoma progression (GPC3)
Intracellular Granule Proteoglycans					
Serglycin	10–19	10–15 heparin/CS	Mast cells, other leukocytes, endothelial cells	Unknown	Inflammation, cancer progression

HS, heparan sulfate; CS, chondroitin sulfate; DS, dermatan sulfate; CD44v3, NRP1, APLP2, CD47 are not listed because there is no genetic evidence that supports their role in atherogenesis.