Abstract

Vascular inflammation is central to the pathogenesis of the atherosclerotic lesion. In the setting of hypercholesterolemia, vascular inflammation accelerates the accumulation of cholesterol within arterial smooth muscle cells, macrophages, and other immune cells. In disorders such as obesity, diabetes, and thrombosis, a myriad of interactions between sterol metabolites and inflammatory mediators exacerbate cholesterol deposition in the vessel wall, leading to the well-known consequences of stroke, transient ischemic attack, myocardial infarction, and peripheral vascular insufficiency. This review highlights emerging concepts in the regulation of cholesterol synthesis, the lipolytic enzymes involved in cholesterol utilization, and the therapies that successfully modulate vascular inflammation. In addition, developments relating to the role of inflammasomes in the management of cholesterol-mediated inflammation are discussed.

Keywords: Atherosclerosis; Cholesterol Metabolism; Inflammation; Lipases; Lipoproteins; Micro RNA; Transcriptional Factors

Abbreviations


Introduction

Regulating Cholesterol Metabolism

For more than 50 years, we have known that sterols are found in cells of the body owing to their essential role in membrane synthesis and function. Whereas all cells express enzymes for cholesterol biosynthesis, there is no biological pathway for its degradation; thus, cholesterol must be excreted intact. Cholesterol dynamics have been studied intensively over many decades and therapeutic advances in the field have been built upon a detailed understanding of how sterols are transported and metabolized, particularly within the arteries of the liver, heart and brain.

Cholesterol is essential to both the biosynthesis of cellular membranes and the synthesis of sterol-based molecules such as vitamin D, bile acids and steroid hormones [2,3]. Cellular pools of cholesterol are maintained by a balance between de novo biosynthesis and dietary intake versus cholesterol utilization and efflux [2]. Cholesterol circulates in the blood in the form of lipoproteins (chylomicrons, VLDL, LDL, IDL and HDL) and is stored in cells in the form of cholesteryl esters (CEs), which also circulate as lipoproteins [2].

As we age, approximately half of our cholesterol is derived from de novo biosynthesis [2]. Acetyl coenzyme A (acyethyl CoA) arises from the oxidation of amino acids, fatty acids, and pyruvic acid is metabolized to hydroxyl methyl glutaryl coenzyme A (HMG-CoA) [2]. In a rate-limiting step inhibited by the statin class of drugs, HMG-CoA is converted to mevalonate, which, in turn, is converted to cholesterol through a series of enzyme-catalyzed reactions [2]. A detailed understanding of this pathway led to the development of statins, which have revolutionized the treatment of hypercholesterolemia.

Healthy adults synthesize cholesterol at a rate of about 1 gram per day, and on a Western diet, consume approximately 0.4 gram of cholesterol per day [2]. Therefore, normal, total serum cholesterol levels (<200 mg/dL) can usually be maintained by controlling cholesterol synthesis, and inhibition of HMG-CoA reductase by statins can reduce deposition of cholesterol in blood vessels. However, because the rate of cholesterol biosynthesis is also influenced by the rate of intake of dietary cholesterol, attention to diet is important. In addition, excessive reduction of serum cholesterol can be deleterious due to its role in multiple biosynthetic reactions. Therefore, maintaining cholesterol homeostasis is paramount.

Cholesterol levels in plasma are maintained through four regulatory mechanisms, namely HMG-CoA reductase activity, sterol o-acetyltransferase activity, LDL receptor-mediated cholesterol uptake, and HDL-mediated reverse cholesterol transport [2]. HMG-CoA reductase activity is controlled, in turn, by four additional mechanisms, which include feedback inhibition by free cholesterol, enzymatic degradation of HMG-CoA reductase, control of HMG-CoA reductase gene expression through its sterol-sensing domain, and covalent modification of HMG-CoA reductase via phosphorylation and de-phosphorylation reactions [2]. It is well documented that phosphorylation of HMG-CoA reductase, which decreases its activity, is controlled by acyclic AMP (cAMP) dependent signaling pathway [2]. Regulation of cholesterol biosynthesis can be controlled by insulin, which decreases cAMP, thus activating...
cholesterol synthesis, and by glucagon and epinephrine, which increase cAMP, thus decreasing cholesterol synthesis [2]. The basic function of these hormones is to control the availability and delivery of energy to cells. Finally, HMG-CoA reductase stability can vary depending on the level of mevalonate. When cholesterol flux is high, HMG-CoA reductase degradation increases; when the flux is low, degradation of HMG reductase decreases [2]. HMG-CoA reductase contains a sterol-sensing domain, which increases HMG-CoA reductase degradation within the proteasome when sterol levels are high [2].

Regulation of Transcription: MicroRNAs Control Cholesterol Metabolism

MicroRNA-122 (miR-122), which accounts for 70% of all hepatic miRNAs, is a major regulator of lipid metabolism in the liver [4]. Inhibition of miR-122 by RNA antisense results in decreased plasma cholesterol [4]. Mice treated with miR-122 antisense oligonucleotides showed 25-35% reductions in total cholesterol and lipoproteins [4-6]. In African green monkeys, efficient silencing of miR-122 in the liver by anti-miR-122, caused as unstained decrease in total plasma cholesterol [4-6]. These findings highlight the regulatory role that microRNAs can play in modulating lipid metabolism, through target gene expression [4-6].

Three independent studies demonstrated recently that miR-33a, an intronic microRNA located within the sterol-regulated element binding protein (SREBP) gene, can target genes involved in cholesterol trafficking, such as ABCA1 and ABCG1 (Figure 1) [6-8]. miR-33a was identified by genome-wide profiling of miRNAs whose expression was altered by cellular cholesterol content [5-9]. In both macrophages and hepatocytes, miR-33a expression rises during states of cholesterol depletion, and falls in states of cholesterol enrichment [5-9]. Interestingly, several investigators have independently identified miR-33a within intron16 of SREBP, and shown that it is co-transcribed during states of cholesterol depletion [6-9]. Target prediction algorithms identify the cholesterol transporter ABCA1 as a top predicted target gene of miR-33a [6]. The untranslated region of ABCA1 contains three highly conserved miR-33a consensus-binding sites, which mutational analysis has shown to contribute cumulatively to miR-33-dependent ABCA1 repression [6]. Through this mechanism, miR-33a limits the efflux of cholesterol to HDL (Figure 1) [6]. Conversely, antagonism of endogenous miR-33 using anti-sense oligonucleotides can increase ABCA1 expression, thus promoting the efflux of cholesterol to apoA1, the first step in reverse cholesterol transport to the liver [6]. A second sterol transporter, ABCG1, which promotes efflux of cholesterol to HDL, is also targeted by miR-33a [6,9]. Hence, miR-33a, co-generated with SREBP transcription, can increase cellular cholesterol levels by limiting cholesterol export through the down-regulation of ABCA1 and ABCG1 (Figure 1) [6,9]. Finally, altering the expression of miR-33a in vivo by viral delivery of sense or antisense oligonucleotides can significantly alter HDL cholesterol levels in mice such that miR-33 can decrease hepatic ABCA1 expression and circulating HDL [6,9].

Another member of the miR-33 family, miR-33b, is found within intron 17 of the human SREBP gene [5-9]. miR-33a and miR-33b differ by only two nucleotides, which appears to explain their overlapping gene target profile, which includes ABCA1. Therefore, under conditions in which SREBP-1 is activated to promote fatty acid synthesis, miR-33b can coordinate down-regulate ABCA1 expression and cholesterol efflux [5-9]. Together, these findings suggest an important and coordinated role for microRNAs in cholesterol homeostasis (Figure 1).

Regulation of Transcription: Utilization of Cholesterol

Transport of blood cholesterol and cholesteryl esters (CE) by lipoproteins is a complex process. Once cholesterol is synthesized in the liver, it is carried by LDL to tissues throughout the body. LDL, along with chylomicrons, transports dietary cholesterol from the small intestine to the peripheral tissues. The liver synthesizes VLDL, which carries triglycerides. VLDL can be degraded to LDL through the action of an endothelial cell-associated lipoprotein lipase. Once deposited in plasma membranes, cholesterol can be removed by HDL and esterified by the HDL associated enzyme, lecithin-cholesterol acyltransferase (LCAT). Cholesterol captured from peripheral tissues by HDL can be transferred to VLDL or LDL through the action of cholesteryl ester transport proteins (CETP). This reverse cholesterol transport process allows peripheral cholesterol to be returned to the liver and excreted through the bile.
Sterol trafficking is regulated by both metabolic enzymes and cell surface LDL receptors [Figure 2] [4-9]. When cells crave cholesterol, they accelerate both synthesis and uptake processes; conversely, when the need is reduced, synthesis and uptake decrease [4-9]. Regulation of these events occurs by sterol-regulated transcription of key rate-limiting enzymes [2], which are regulated through cleavage of the transcription factor, SREBP. Sterol control of transcription, which affects more than 30 genes involved in the biosynthesis of cholesterol, fatty acids, triglycerides, and phospholipids, requires the presence of sterol regulatory element-1 (SRE -1), an octamer sequence that binds SREBP [6]. Humans express two distinct SREBP genes, SREBP-1a, which regulates all SREBP-responsive genes in both the cholesterol and fatty acid biosynthetic pathways, and SREBP-1c, which primarily controls fatty acid synthesis genes in adipocytes [6].

As outlined by Moore, et al. [6] SREBP regulation is complex (Figure 2). Sterol-mediated activation of the SREBP gene can occur through the liver X receptors (LXRs) [3-6]. LXRs are members of the steroid thyroid hormone superfamily of cytosolic receptors that migrate to the nucleus upon ligand binding [3]. In the nucleus, LXRs regulate gene expression by binding to specific target sequences [3,6]. All SREBPs are proteolytically activated, a process controlled by the sterols in the cell. Full length SREBPs have now been found to possess several domains embedded in either the ER membrane or nuclear envelope [6]. The N-terminal domain contains a helix-loop-helix transcription factor motif that is exposed to the cytoplasmic side of the ER. Two transmembrane domains are followed by a large C-terminal domain exposed to the cytosolic side. The C-terminal domain interacts with SREBP cleavage activating protein (SCAP) (Figure 2), a large ER membrane protein that contains at least 8 membrane-spanning domains [6]. The C-terminal region of SCAP contains four WD-40 motifs (beta transducing motifs of 40 amino acids) that are required for interaction of SCAP with SREBP. The regulation of SREBP activity is controlled within the ER by the interaction of SCAP with insulin-induced proteins-1 and -2 (Insig-1 and -2) [Figure 2] [2,6,9]. When cells have sufficient sterol content, SREBP and SCAP are retained in the ER. The N-terminus of SCAP resembles HMG-CoA reductase, which itself is subject to sterol-stimulated degradation. SCAP and HMG-CoA reductase share a sterol-sensing domain, which allows SCAP to function as the cholesterol sensor in the protein complex [2,6,9]. Thus, when cells have sufficient sterol levels, SCAP binds cholesterol, promoting the interaction with Insig 1.2 allowing the entire complex to be maintained in the ER. Insig proteins activate sterol-dependent degradation of HMG-CoA reductase, which is required for cholesterol synthesis from acetyl CoA [6,9]. Cholesterol can then efflux from the cell via ABCA1/ABCG1, thereby allowing its binding to apoA1-HDL for transport back to the liver [6].

Lipolytic Enzymes in Atherosclerosis: Lipases and Phospholipases

Endothelial cell dysfunction can accelerate atherosclerosis [1,10-12]. Plasma lipoproteins, when bound to the endothelium or while in transit through inter-endothelial “gaps”, can accumulate in arteries. During atherogenesis, inflammation occurs where cytokines can contribute to the development of the plaque [11,13]. Oxidative modification of lipoproteins can alter their structure, predisposing to aggregation. Modified LDL particles, which may contain spongomyelins and lysophosphatidyl choline, can trigger pro-inflammatory reactions. Lipolysis of LDL phospholipids by phospholipase A (PLA) and lipolysis of CE by CE lipase can release free fatty acids, such as arachidonate, which are substrates for the synthesis of pro-inflammatory eicosanoids [12,14].

Lipases are widely distributed enzymes involved in the degradation of CE, triglycerides, and phospholipids [12,14]. Lipoprotein lipase (LPL), hepatic lipase, and endothelial lipase are products of the neutral lipase gene family, and require cofactors, such as apolipoprotein C and colipase, for full stability and catalytic activity [12]. Moreover, their broad substrate specificity allows for pleiotropic biological functions [12]. For example, CE lipase has been reported to modify LDL and HDL in human plasma. In addition, LpL is the extrahepatic enzyme responsible for the hydrolysis of triglyceride-rich plasma lipoproteins [12,15]. LpL is produced by monocyte-derived macrophages and vascular smooth muscle cells found in atherosclerotic lesions [16,17]. Increasing evidence indicates that LpL also functions as a lipoprotein ligand that promotes binding of LDL to LDL receptors, as well as proteoglycans, in the extracellular matrix [12]. Hepatic lipase (HL) catalyzes the hydrolysis of triglycerides and phospholipids in LDL, HDL, and LDL molecules, thus maintaining intracellular...
lipid homeostasis. In an apparent paradox, HL activity seems to correlate positively with atherosclerosis in hypertriglyceridemia, but inversely with atherosclerosis in hypercholesterolemia [12]. Endothelial lipase expression suggests that the enzyme may have a distinctive role in lipoprotein degradation, as it has substantial phospholipase activity, and attacks lipoprotein phospholipids as its major substrate [12].

Phospholipase A$_2$ superfamily members share the ability to hydrolyze the sn-2 bond of phospholipid fatty acids, thereby generating multiple classes of bioactive lipids [18,19]. The five families of phospholipases possessing defined physiological roles include secretory PLA$_{2}$, cytosolic PLA$_{2}$, lysosomal PLA$_{2}$, Group V PLA$_{2}$, and Group VII or platelet activating factor (PAF) acetylhydrolase. PAF generates pro-inflammatory lipid mediators, such as eicosanoids and leukotrienes (Figure 3) [19], which modify a wide range of biologic responses, such as inflammation, allergy, cancer and cardiovascular disease [20]. In addition, PLA$_{2}$ activity can render LDL more susceptible to accumulation in the blood, where it can be oxidized, thus becoming more atherogenic [18]. Clinical studies indicate that PLA$_{2}$ activity correlates with the concentration of C-reactive protein (CRP), an acute phase reactant, suggesting a role for PLA$_{2}$ in acute phase responses [18]. This finding may provide the biologic basis for the clinical finding that elevated plasma PLA$_{2}$ is a strong independent risk factor for atherosclerotic heart disease [18].

Finally, many synthetic and natural compounds (such as

![Figure 3: Effects of Phospholipase A$_{2}$ (PLA$_{2}$) on Lipoproteins and Atherogenesis.](image)

PLA$_{2}$ hydrolyzes phospholipids within lipoproteins, producing arachidonic acid, a substrate for inflammation-promoting eicosanoids and leukotrienes. PLA$_{2}$ also produces smaller lipoproteins whose apoproteins undergo conformational changes that reduce their binding to cells and internalization. As a result, normal clearance of LDL-CE and its catabolism are prevented and modified LDL accumulates in the circulation, increasing plasma cholesterol levels. Exposure of LDLs to circulating reactive oxygen species (ROS) causes oxidation of LDL, which increases lipid accretion and atherogenesis.
eicosanoids) that are lipase inhibitors may have a role in the treatment of inflammatory disorders [10,16]. However, although inhibition of PLA₂ may block formation of a wide variety of secondary inflammatory mediators [16], the diverse array of PLA₂ isoforms makes precise pro-inflammatory targeting difficult. Several cell-permeable PLA₂ inhibitors may interfere with phospholipid metabolism, and, thus, have utility in the treatment of inflammatory conditions [16]. Indeed, the development of highly specific lipolytic enzyme inhibitors should be of great interest in the management of athero-thrombotic vascular disease.

**Targeting Cholesterol and Managing Inflammation**

As stated earlier, the major circulating atherogenic lipoprotein, LDL, promotes cholesterol accretion and a subsequent inflammatory response in the blood vessel wall [10,11]. HDL, on the other hand, promotes cholesterol efflux from cells, thus reducing inflammation [10,11]. Once modified by interaction with matrix proteoglycans, oxidation, or aggregation, LDL is more likely to be retained in the vessel wall, with two pathological consequences [11]. First, modified LDL functions as a ligand for Toll-like receptors 2 and 4 (TLR2/4), stimulating NF-κB-mediated synthesis of NLRP3 and pro-interleukin-1β (IL-1β) [21,22]. Second, accumulation of cholesterol crystals within phagolysosomes can alter lysosome acidification and cause release of cathepsins that activate NLRP3 inflammasome assembly [22]. Inflammasome activation leads to caspase-1 activity, and subsequent maturation and secretion of IL-1β. Thus, cholesterol amplifies the innate inflammatory response via a dual-signaling process [21].

Presently, it is thought that HDL oxidation and myeloperoxidase (MPO) activity can reduce the normal activity of HDL [21-23]. MPO is induced by cytokines and chemokines, and although murine macrophages have lower MPO expression levels than human macrophages, overexpression of human MPO in macrophages of LDL receptor-deficient mice promotes atherosclerosis [24]. In addition, human ApoA1 that has been oxidized by MPO is less effective in promoting both cholesterol efflux via ABCA1 in macrophages, and atherosclerosis regression in mice [25]. Individuals with heart disease have higher levels of MPO-modified ApoA1 than control individuals; their HDL activity is also impaired causing a reduction in ABCA1-mediated cholesterol efflux from macrophages [26]. Although MPO-oxidized ApoA1 represents a small proportion of the total ApoA1 in the inflammatory plaque microenvironment, it may be sufficient to impair macrophage cholesterol efflux [21]. Hence, macrophage inflammatory responses that are mediated by MPO can lead to inactivation of ApoA1 in the arterial wall, reduced macrophage cholesterol efflux, and increased atherosclerosis. This process may also predispose to inflammasome activation [21].

ABCA1 and ABCG1 promote macrophage cholesterol efflux and suppress macrophage inflammatory responses via the TLR receptors [21,22,26]. Possibly through decreased formation of cholesterol-enriched plasma membrane lipid rafts, peritoneal macrophages of mice deficient in ABCA1 and ABCG1 accumulate free and esterified cholesterol, and have enhanced inflammatory responses when exposed to atherogenic lipoproteins [21]. Moreover, defective movement of cholesterol within the plasma membrane of ABCA1⁻/⁻/ABCG1⁻/⁻ macrophages appears to support pro-inflammatory changes [19]. Macrophage-specific deficiency of ABCA1 and ABCG1 results in increased atherosclerosis and hyper-expression of the inflammatory chemokines CC-chemokine ligand 2 (CCL2) and CCL3 within plaques [21,22,26].

Cholesterol uptake in macrophages promotes inflammasome activation and atherosclerosis [21]. Inflammasome activation requires both a priming signal typically mediated by TLR activation, and a second signal involving potassium ion efflux, lysosomal damage, or generation of reactive oxygen species (ROS) [21,22,26]. The initial signal may result from pattern recognition receptor activation, whereas the second signal can be mediated by cholesterol, as a result of either phagocytosis of extracellular cholesterol or CD36-mediated uptake of modified LDL [21,22,26]. Activation of the nucleotide-binding domain leucine-rich repeat-pyrim domain-containing receptor-3 (NLRP3) inflammasome usually leads to maturation and secretion of the pro-inflammatory cytokines, interleukin-1β and interleukin-18 [21,22,26].

Moreover, hypercholesterolemia leads to macrophage cholesterol accretion in macrophages and other immune cells, which promotes inflammatory responses, including increased TLR signaling, inflammasome activation, and recruitment of monocytes/neutrophils to the advancing lesion. Activation of TLR signaling can predispose to decreased cholesterol efflux, which results in further cholesterol accumulation and the amplification of inflammatory responses. Thus, cholesterol metabolic changes occur during inflammation and immunity pathways that participate in the formation and progression of atherosclerotic lesions.

LXR activators reduce atherosclerosis, most likely by stimulating cholesterol efflux [20]. Although they may have efficacy in autoimmune disorders, LXR activator-like drugs are limited in efficacy against atherosclerosis due to untoward effects, such as fatty liver disease and increases in plasma cholesterol [20]. Recent studies have defined specific roles for cholesterol derivatives such as 25-OH cholesterol and 7α25-OH cholesterol in the immune response to infection, providing new insights into the intimate links between sterol metabolism and immunity, and opening the possibility of sterol-targeted interventions as a means of immunosuppression [20,21].

Although plasma LDL cholesterol can be lowered by statins, unacceptable side-effects underscore the need for new therapies. Increasing HDL production can reduce atherosclerosis in animal models, and infusion of ApoA1-phosphatidylcholine complexes appears to reduce coronary atherosclerosis in both humans and animals [11,21]. Moreover, the cholesterol efflux capacity of human plasma HDL is inversely correlated with the atherosclerotic burden in the coronary and carotid arteries [21]. These anti-atherogenic effects could reflect the anti-inflammatory effects of HDL, perhaps through disruption of TLR-mediated signaling.

**Lipids Affecting Inflammation**

In this review we have highlighted the complex interactions between inflammation and atherosclerosis (Figure 4). While inflammation can accelerate atherosclerosis, the reverse may also be true. For example, cholesterol-feeding in animals increases circulating markers of inflammation, such as C-reactive protein (CRP) and serum amyloid A [20]. Moreover, obese humans often have markers of low-grade chronic inflammation thought to be due to excessive nutrients, and reflected in increased levels of circulating CRP and cytokines [20,28]. A potential mechanism is the absorption of endotoxin from the gut in the setting of a high fat diet (metabolic endotoxemia). In addition, studies in animals show that diets high in fatty acids and/or cholesterol are accompanied by evidence of inflammation in arteries, adipose tissue, and liver [28,29]. In adipose tissue, resident and infiltrating macrophages secrete inflammatory mediators (e.g. cytokines) in response to saturated fats. It has been demonstrated, further, that a high-cholesterol diet exacerbates hepatic inflammation, as reflected in increases CD68-positive macrophages, MCP-1, pro-inflammatory cytokines, plasma amyloid, and E-selectin levels in the liver [20,29].

Other studies show that chylomicrons and VLDL induce hepatic...
Inflammation through enhanced scavenger receptor-mediated uptake of these lipoproteins, thus triggering an intracellular inflammatory response [30]. Modified forms of LDL, which may contain oxidized lipids such as oxysterols and oxidized phospholipids are recognized by scavenger receptors, and generate inflammatory mediator responses that may accelerate atherogenesis. We and others have shown that modified lipoproteins can induce vascular inflammation (Figure 4) [11,20,31].

Monocytes infiltrating the vessel wall, as in the liver, can endocytose oxLDL, thereby activating or amplifying signaling pathways, such as the NF-κB and JNK pathway [20]. In addition, the uptake of oxLDL by anti-inflammatory macrophages polarizes these cells towards a more pro-inflammatory phenotype. The accumulation of specific oxidized lipids in the plaque may determine the inflammatory response, albeit some oxidized lipids may exert both anti-inflammatory and pro-inflammatory effects. Moreover, the appearance of cholesterol crystals within the vessel wall of hypercholesterolemic animals activates macrophage inflammasome assembly, thereby triggering IL-1β secretion [20,21].

Ex vivo studies have demonstrated that monocytes can be activated by TG-rich lipoprotein remnants, thus contributing to monocyte-endothelial adhesion and endothelial cell activation processes central to early atherogenesis. Vascular inflammation may also be reduced by HDL, through its ability to reduce endothelial cell VCAM-1 and ICAM-1 expression [11,20,22,26]. HDL reduces endotoxin pro-inflammatory signaling through an SR-BI dependent mechanism [20,32]. In addition, HDL may contain specific lipids, such as lysophospholipid sphingosine-1-phosphate (SIP) that acts as anti-inflammatory signaling molecule [20]. Binding of SIP to one of its five receptors inhibits pro-inflammatory pathways in endothelial and smooth muscle cells, reducing the interaction of circulating monocytes with the arterial wall [33]. The SIP analogue, fingolimod, effectively reduces the progression of atherosclerosis in mice, and is a promising immunosuppressant that is currently in clinical trials [34]. Interestingly, improving HDL function has also been suggested as a novel therapy, based on its ability to modulate activity of innate and adaptive immune cells [35]. HDL can reduce the cholesterol content of immune cell plasma membrane lipid rafts, thereby changing their activity. Taken together, these findings provide early insights into the physiologic role of lipoproteins in modulating inflammatory processes.

Other bioactive lipids can promote inflammation. Saturated fatty acids can increase intracellular NF-κB signaling, probably through TLR pathways [36,37]. In addition, ceramides increase the response of macrophages to endotoxin [38]. Alternatively, fatty acids may bind several ubiquitous G-protein coupled receptors (GPR) [39] with affinities for fatty acids based on chain length and the degree of saturation [20]. For example, intestinal fatty acids bind GPR43, which is thought to be involved in leukocyte recruitment to inflammatory sites; medium-chain fatty acids bind GPR84, causing cytokines to be released from macrophages; and, binding of n-3 fatty acids to GPR120 on macrophages can inhibit TLR-mediated inflammatory pathways [39]. Alternatively, n-3 fatty acids may exert anti-inflammatory and anti-atherogenic effects by reducing plasma TG levels and inhibiting NF-κB signaling [40]. Hence, fatty acids can induce either pro-inflammatory (e.g. saturated n-6 fatty acids) or anti-inflammatory signaling (e.g. n-3 unsaturated fatty acids), depending on individual chemical structures.

Finally, lipid-sensing transcription factors may control lipid metabolism [3,6,20,21,41] by directly regulating inflammatory gene expression, and by interacting with additional transcription factors within inflammatory pathways (Figure 2). For example, peroxisome proliferator-activated receptors (PPAR) not only control lipid metabolism, but also inhibit inflammatory responses and macrophage activation [42]. PPAR is expressed in multiple cell types involved in atherogenesis, such as macrophages, smooth muscle cells, and endothelial cells [20]. PPAR activation also reduces VCAM-1 and ICAM-1 expression in endothelial cells, thereby diminishing recruitment and infiltration of inflammatory macrophages into the vessel wall. Moreover, PPARs modulate macrophage phenotypes by controlling lipid metabolism [11,42]. PPAR can induce an anti-inflammatory phenotype in macrophages, while also diminishing the inflammatory response in other leukocytes [20]. PPAR can even regulate the activation of regulatory T-cells in adipose tissue [21].

The farnesoid X receptor (FXR) also influences the regulation of lipid metabolism and inflammation (Figure 2) [3,20,43]. Activation of FXR regulates bile acid, lipid, and glucose metabolism, and, similar to LXRs, can suppress inflammatory pathways by trans-repression of signaling mechanisms [3,20,43]. FXR activation can protect against endotoxin-induced hepatic inflammation and intestinal inflammation [20]. Although there are conflicting data, some reports suggest that FXR agonists can reduce atherogenesis by reducing inflammation [3,20,44-46].

Liver X receptors (LXRs) are induced by oxysterols in response to elevated intracellular cholesterol, and can also alter cholesterol metabolism (Figure 2) [3,20]. In atherogenesis, LXRs protect against macrophage-derived foam cell formation by activation of reverse cholesterol transport [3,20,47]. In addition, LXRs can inhibit the induction of pro-inflammatory gene expression after endotoxin stimulation or bacterial infection [48] by binding to transcriptional co-repressors or silencers (trans-repression). LXR agonists can also inhibit atherogenesis in some animal models, but differences have been reported between humans and mice in the induction of hyper triglyceridemia upon activation of hepatic LXR, owing to variable activation of the lipogenic transcription factors, SREBP and CREBP [3,20,49]. Ideally, LXR modulators would not perturb triglyceride metabolism [50].

In summary, nuclear lipid sensors exert a spectrum of effects...
on the regulation of lipid metabolism and inflammation. In general, their actions seem to be anti-inflammatory and anti-atherogenic (Figure 4) [20]. Clearly, specific lipids can induce inflammatory cascades, while acute inflammatory stimuli, such as endotoxin and cytokines, can induce pro-atherogenic changes in lipoprotein metabolism. An interesting emerging view is that patients using lipid-lowering drugs, such as the statins, derive additional benefit from their secondary anti-inflammatory effects.

**Future Perspectives Regarding Plaque Stabilization**

Based, in part, on the success of current approaches to managing plaque vulnerability, future treatments to promote plaque stabilization remain on the horizon. Statin therapy, particularly with the use of the new generation of statins, has proven to be effective according to studies highlighted and reviewed by Yla-Herttuala, et al. [51]. For example, patients receiving pravastatin before carotid endarterectomy showed significantly less inflammation and higher carotid plaque collagen content, suggesting plaque stabilization [51]. The ATROCAP study found that plaques from carotid endarterectomy patients on atorvastatin showed a trend towards reduced inflammatory cell infiltration [51]. These results are consistent with the pleiotropic, anti-inflammatory and plaque-stabilizing effects of statins. The recent finding of plaque stabilization with ezetimibe, which lacks pleiotropic effects, lends support to the theory that lipid-lowering alone can reduce inflammation.

A variety of other approaches appear to be effective in lowering LDL, or raising HDL, thereby stabilizing the atherosclerotic plaque [51]. Four recent IVUS clinical trials have shown that β-blockers slow the progression of heart disease. Endothelial function can be improved by rennin-angiotensin inhibitors, and HOPE and ONTARGET clinical trials have shown a larger reduction in clinical events that could be predicted from the reduction in blood pressure. ApoA1-Milano and other HDL-like apoA1 complexes have been shown to regress atherosclerosis possibly via several HDL-related protective mechanisms like reverse cholesterol transport, anti-oxidative activity, endothelial vasoprotection, and reduction of platelet activation. HDL also inhibits the coagulation cascade. In two trials in statin-treated patients with low HDL, modified-release of nicotinic acid significantly reduces carotid atherosclerosis, and the use of slow-release niacin significantly reduces carotid intima-media thickness in comparison with statin therapy. Inhibition of lipoprotein-associated phospholipase A2, which can prevent an increase in the necrotic core, is now under investigation in two major trials (STABILITY and SOLID-TIMI trials). Small molecule antagonists of the pro-atherogenic chemokine receptor CCR5 and its ligand CCL5 may also be useful for plaque stabilization. PCSK9, which favors degradation of the LDL receptor, thereby increasing hypercholesterolemia, is an attractive target for lowering plasma LDL and stabilizing the plaque; the ODYSSEY OUTCOMES trial is now testing this hypothesis [51].

siRNAs against apob100 have been used to reduce LDL levels, albeit it remains to be determined whether this technology will prevent plaque rupture. Finally, immunization with LDL or apob100 fragments reduces atherosclerosis hyperlipidemic animals, suggesting that vaccination may become an effective strategy for preventing vascular disease [51].

**Summary**

In this review, we have discussed reciprocal signaling between inflammatory mediators and lipid metabolites in the context of managing the arterial cholesterol burden and atherogenesis. In the past decade, new discoveries have provided novel therapeutic approaches for the treatment of various forms of hypercholesterolemia in order to reduce atherosclerotic lesion formation [20,21]. Current therapy for atherosclerosis has focused on the use of statins to decrease de novo cholesterol biosynthesis, reduce plaque formation particularly arterial intimal development, and inflammation. Presently, second-generation statins with fewer side effects and plaque stabilizing properties are available [51]. However, because statins are only partially effective in some patients, newly emerging drug targets, particularly those relating to inflammatory pathways, are under investigation. For example, lipoprotein-associated PLA2, is an attractive target, but therapy must be specific enough to avoid extraneous effects on other critical pathways [18]. In addition, blocking synthesis of a specific eicosanoid could divert the arachidonate pool to other pathways, potentially exacerbating arterial disease [18]. Thus far, PLA2 inhibitor trials have shown limited success due to side effects [18], which reflect the limited selectivity of anti-lipase agents, notwithstanding extensive efforts to develop these agents for suppression of atherosclerosis [18].

A key role for regulation of lipid metabolism by miRNAs is also emerging [5-9]. Because multiple genes can be targeted by a single miRNA, and because one gene may be targeted by multiple miRNAs, the miRNA regulatory network appears redundant and poorly defined [5-9]. Yet, recent evidence suggests that circulating extracellular miRNAs are biologically active, particularly in controlling lipolytic enzymes in the setting of inflammation [5-9].

Finally, new research has shown that changes in cholesterol homeostasis can predispose to inflammatory responses via enhanced TLR signaling and/or inflammasome activation [21,22,26]. Although this host adaptation becomes dysfunctional in atherosclerosis, the disease can be managed by increasing HDL or activating LXRs [21,22,26]. Inflammatory mediators have emerged as independent risk factors for atherosclerosis, perhaps due to their ability to interact with each other in the exacerbation of heart disease. Unraveling the complex interactions through which inflammation impacts cholesterol metabolism will undoubtedly stimulate many new approaches for future treatment of deleterious cholesterol deposition in blood vessels.

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