

The role of non-resolving inflammation in atherosclerosis

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Non-resolving inflammation drives the development of clinically dangerous atherosclerotic lesions by promoting sustained plaque inflammation, large necrotic cores, thin fibrous caps, and thrombosis. Resolution of inflammation is not merely a passive return to homeostasis, but rather an active process mediated by specific molecules, including fatty acid-derived specialized pro-resolving mediators (SPMs). In advanced atherosclerosis, there is an imbalance between levels of SPMs and proinflammatory lipid mediators, which results in sustained leukocyte influx into lesions, inflammatory macrophage polarization, and impaired efferocytosis. In animal models of advanced atherosclerosis, restoration of SPMs limits plaque progression by suppressing inflammation, enhancing efferocytosis, and promoting an increase in collagen cap thickness. This Review discusses the roles of non-resolving inflammation in atherosclerosis and highlights the unique therapeutic potential of SPMs in blocking the progression of clinically dangerous plaques.

Introduction

Atherosclerosis is a lipoprotein-driven, non-resolving chronic inflammatory disease of the arterial subendothelial space (intima) and involves activation of both the innate and adaptive branches of the host immune system. This disease process is the underlying cause of fatal heart attacks and strokes, which are responsible for the majority of deaths worldwide (1). Despite the availability of safe and effective LDL-lowering therapy, the incidence of cardiovascular disease has been increasing in the United States and other developed countries (2). A deeper understanding of the cellular mechanisms that promote atherosclerotic lesions, particularly the unique subset of advanced lesions that trigger vascular disease, may identify novel therapeutic targets to help stem this tide.

Atherosclerosis begins with the retention of apolipoprotein B-containing lipoproteins within the subendothelial intima of arteries, which leads to the activation of endothelial cells; recruitment of leukocytes, particularly Ly6c^{hi} monocytes; and accumulation of cells, extracellular matrix, and lipids (3). Monocytes differentiate into macrophages, the major leukocyte subset in the subendothelial region, and these macrophages then become foam cells through their ingestion of retained lipoproteins. The interaction of macrophages with retained apolipoprotein B-containing lipoproteins, some of which become modified by oxidation and other processes, triggers proinflammatory responses (4). Eventually, myofibroblasts derived from medial smooth muscle cells and possibly other sources enter the intima and lead to the formation of a collagenous fibrous cap that overlies the lipid-rich plaque core (1). Cells found in the atherosclerotic lesion, including macrophages, T cells, and dendritic cells, promote expression of proinflammatory cytokines and eicosanoids that sustain proinflammatory responses (5, 6). Plaque necrosis develops in the setting of contin-

ued inflammatory stimuli, defective clearance of dead cells, and primary necroptosis, and then plaque necrosis further amplifies the inflammatory response (7–9). Over time, the integrity of the fibrous cap may break down, leading to plaque rupture, which can trigger acute occlusive thrombosis and the associated clinical sequelae associated with tissue infarction (9–11). While the role of persistent inflammation has long been recognized in the progression of atherosclerosis, recent work by a number of groups has also identified defects in inflammation resolution as a key causal factor (12–15). Here, we present an overview of how impaired inflammation resolution drives atherosclerosis and discuss the therapeutic implications of this concept.

Resolution of inflammation and specialized pro-resolving mediators

Acute inflammation evolved as a physiological host defense response against both microbial and sterile pathogens (16). When acute inflammation cannot be controlled or resolved, chronic inflammation develops, which can result in organ dysfunction (17). Examples include autoimmune disease, asthma, Alzheimer's disease, diabetes, obesity, and cardiovascular disease (12, 18–22). The acute inflammatory response is divided into two phases: initiation and resolution. During the initiation phase, chemical messengers including cytokines, chemokines, and lipid-derived mediators, e.g., prostaglandins and leukotrienes, recruit immune cells to the site of injury (17). Polymorphonuclear neutrophils (PMNs) arrive first and begin to phagocytose and neutralize pathogens. Simultaneously, monocytes are recruited to the area of inflammation and differentiate into macrophages that help to further clear pathogens as well as begin to orchestrate the next stage, the resolution of inflammation (23). This stage is characterized by quelling of the inflammatory response but with the continued clearance of pathogens; phagocytic removal of apoptotic cells in a process known as efferocytosis; and repair of damaged tissue. Resolution is characterized by a switch in the production of lipid mediators from proinflammatory

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mediators, e.g., leukotrienes, toward specialized pro-resolving mediators (SPMs), e.g., resolvins and lipoxins (24–26).

Proinflammatory lipid mediators, consisting of prostaglandins and leukotrienes, are formed from the *n*-6 fatty acid precursor arachidonic acid (AA). These mediators increase blood flow, endothelial cell permeability, and extravasation of PMNs (27). Lipid mediators that participate in resolution were identified using liquid chromatography–tandem mass spectrometry–based analysis of resolving inflammatory exudates from *in vivo* animal models and human cell culture systems (28). SPMs consist of at least four distinct families: lipoxins, resolvins, protectins, and maresins (25, 26, 29, 30). Like prostaglandins and leukotrienes, lipoxins are produced from *n*-6 fatty acid–derived AA (26). The other families of SPMs, including resolvins, protectins, and maresins, are generated from *n*-3 fatty acid–derived eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or docosapentaenoic acid (DPA) (25, 29). Interestingly proinflammatory lipid mediators are able to trigger the synthesis of the SPMs, suggesting a direct cell biological link between the inflammatory and resolving phases of the inflammatory response (24).

In response to an inflammatory stimulus, polyunsaturated fatty acids including AA, EPA, DHA, and DPA are released from phospholipids by phospholipase enzymes and then catalyzed by a series of lipoxygenases, namely, 5-lipoxygenase (5-LOX), 12-lipoxygenase (12-LOX), and 15-lipoxygenase (15-LOX), to produce lipid mediators of inflammation and resolution (27). Proinflammatory leukotrienes such as leukotriene B₄ (LTB₄) and leukotriene C₄ (LTC₄) are derived from AA by 5-LOX and act on GPCRs such as leukotriene B₄ receptor 1/2 (BLT1/2) and cysteinyl leukotriene receptor 1/2 (CysLT1/2). Activation of these receptors leads to increased expression of inflammatory pathway enzymes, e.g., 5-LOX and cyclooxygenase-2 (COX-2), and inflammatory cytokines, e.g., IL-6 and IL-8, which then promote the recruitment of neutrophils. Similarly to leukotrienes, SPMs also bind and act on their cognate GPCRs, which leads to the induction of pro-resolving cellular processes (31). These processes include apoptosis and decreased recruitment of leukocytes; efferocytosis; macrophage phenotypic shift from proinflammatory to pro-resolving; and tissue repair processes, including reconstitution of cells, matrix, and blood and lymph vessels (32, 33).

Imbalance of inflammatory and resolving mediators drives atherosclerosis progression

An imbalance of pro-resolving to proinflammatory lipid mediators has recently been identified as a key feature of advanced atherosclerosis in human and murine plaques (14, 15). Recent work has shown that regions of human atherosclerotic plaques characterized by large necrotic cores and thin fibrous caps had low levels of the pro-resolving mediator resolvin D1 (RvD1) and high levels of the proinflammatory mediator LTB₄ (14). Conversely, plaque regions containing less necrosis and thicker fibrous caps demonstrated high levels of RvD1 and relatively lower levels of LTB₄ (14). Thus, the ratio of RvD1 to LTB₄ correlated strongly with plaque severity. Consistent with these findings, advanced aortic root plaques of *Ldlr*^{-/-} mice fed a high-fat diet for 17 weeks showed a lower ratio of lesional RvD1 to LTB₄ than did early atherosclerotic lesions from *Ldlr*^{-/-} mice fed a high-fat diet for 8 weeks (14). Another study

showed that the ratio of the SPMs maresin-1 (MaR1) and RvD2 to the proinflammatory mediators LTB₄, prostaglandin A₁, prostaglandin D₂, and prostaglandin E₂ was lower in *Apoe*^{-/-} mice fed a high-fat diet for 16 weeks as compared with mice fed the diet for 4 or 8 weeks (15). An important area of future research will be understanding how the plaque environment affects mediator synthesis and/or degradation. Nonetheless, as reviewed in the following sections, evidence is now accumulating that this imbalance is causal in the development of features of clinically significant plaques, including continuous Ly6c^{hi} monocyte influx; inflammatory macrophage polarization; defective efferocytosis; and thinning of the stabilizing collagenous fibrous cap overlying lesions (Figure 1).

Lipoxygenases play a critical role in the biosynthesis of both proinflammatory and pro-resolving mediators and thus can be critical regulators of their balance. Two key enzymes, 5-LOX and 12/15-LOX, have been studied in this context. Notably, humans express three related forms of this enzyme, 12-LOX, 15-LOX type 1, and 15-LOX type 2, while mice express only one form, which is usually referred to as 12/15-LOX because it catalyzes both 12- and 15-LOX reactions (34). 5-LOX plays a role in the formation of both proinflammatory leukotrienes and pro-resolving lipoxins, and the determination of which product is generated in a given setting is dependent on 5-LOX subcellular localization (34). Nuclear 5-LOX is located near leukotriene A₄ (LTA₄) hydrolase, which converts the product of 5-LOX, LTA₄, to LTB₄. In contrast, when 5-LOX is cytoplasmic, LTA₄ is converted to lipoxin A₄ (LXA₄) by cytoplasmic 12/15-LOX. In macrophages, the nuclear localization of 5-LOX is mediated by MAPKAPK2-mediated (MK2-mediated) 5-LOX phosphorylation, and thus when MK2 is activated in macrophages, the inflammation/resolution balance is shifted toward inflammation (35–37).

Expression analysis of lipoxygenases has demonstrated that 5-LOX is significantly higher in human carotid atherosclerotic lesions compared with healthy arteries, where it has been found to have a predominantly nuclear localization (38, 39). Moreover, 5-LOX expression in atherosclerotic plaques correlated with patients' clinical symptoms, including transient ischemic attack, stroke, and amaurosis fugax (38). The concept that 5-LOX is involved in atherosclerosis is also supported by human genetic findings. In a cohort of patients from the Los Angeles Atherosclerosis Study, Dwyer and colleagues identified a variant allele of *ALOX5* that was associated with increased carotid intima-media thickness. Further, they observed that increased dietary intake of AA enhanced the atherogenic effect of the variant genotype, whereas increased dietary intake of *n*-3 fatty acids diminished its effect (40). These data are consistent with the concept that an increase in the ratio of AA-derived LTB₄ to *n*-3 fatty acid–derived LXA₄ promotes plaque progression. Supportive of a proinflammatory role for 5-LOX in atherogenesis, murine studies have demonstrated that the absence of 5-LOX in *Apoe*^{-/-} mice dramatically decreased atherosclerotic lesion size (41). In contrast to 5-LOX, human studies have suggested that 15-LOX may be atheroprotective. In a case-control study, a polymorphism in *ALOX15* that was associated with higher enzyme activity was enriched in a population of control subjects without coronary artery disease (CAD) versus patients with CAD (42). Consistent with this finding, the Coronary Artery Risk Development in Young Adults (CARDIA) study

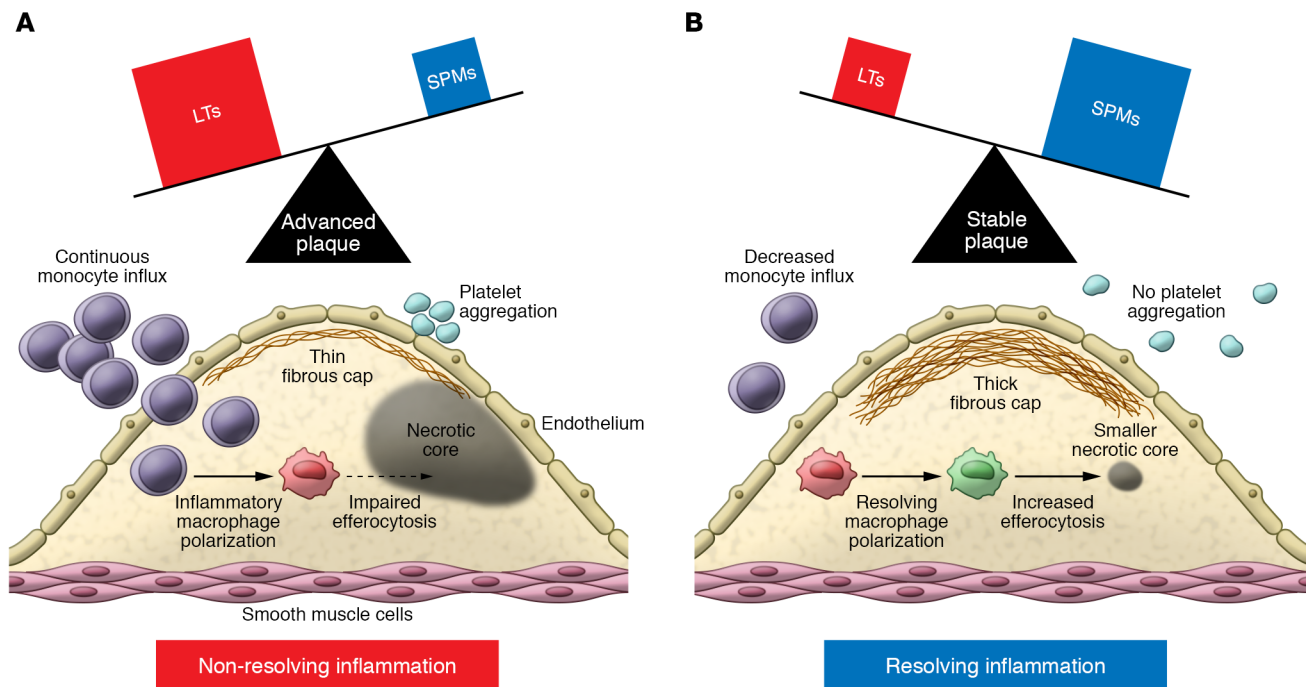


Figure 1. Imbalance of SPMs and leukotrienes (LTs) promotes instability of atherosclerotic plaques. When the SPM/LT ratio is low (**A**), resolution of inflammation is impaired, leading to sustained inflammatory Ly6^{Ch} monocyte influx, platelet aggregation, proinflammatory macrophage polarization, impaired efferocytosis, large necrotic cores, and thin fibrous caps. Conversely, an increase in the SPM/LT ratio (**B**) promotes resolution, characterized by attenuated Ly6^{Ch} monocyte influx, pro-resolving macrophage polarization, enhanced efferocytosis, decreased necrotic core formation, and thicker fibrous caps.

found that a SNP in the coding region of *ALOX15* was associated with both a 20-fold reduction in 15-LOX activity in vitro and a 1.6-fold increased risk of CAD (43). In a hypercholesterolemic rabbit model, overexpression of 15-LOX in macrophages was protective against early atherosclerosis (44). Similarly, transgenic mice overexpressing 12/15-LOX on the *ApoE*^{-/-} background and fed a chow diet showed attenuated atherosclerosis progression, which was thought to be due to increased 12/15-LOX-mediated synthesis of LXA₄ (12). However, Merched and colleagues demonstrated that the role of 12/15-LOX in atherosclerosis is not straightforward. These authors found that although 12/15-LOX-transgenic *ApoE*^{-/-} mice on a chow diet had reduced atherosclerotic lesion area compared with control *ApoE*^{-/-} mice, lesion size was similar between 12/15-LOX-transgenic *ApoE*^{-/-} and control *ApoE*^{-/-} mice when Western diet feeding was used (12, 45). Consistent with these results, they showed that hyperlipidemia decreased 12/15-LOX-derived LXA₄ in the plasma of Western diet-fed 12/15-LOX-transgenic *ApoE*^{-/-} mice and that LDL-supplemented medium blocked LXA₄-induced efferocytosis in cultured macrophages. The mechanisms linking high-fat diet or LDL to decreased SPM plasma level and impaired SPM-mediated functions remain to be elucidated (45).

To identify a potential direct effect of SPMs on the progression of atherosclerosis, recent studies examined the effect of treating established lesions in atherosclerosis-prone mice with lipid mediators, including RvE1, RvD1, RvD2, RvD5_{n-3 DPA}, MaR1, or aspirin-triggered LXA₄ (ATL) (Table 1). Administration of RvE1, an E-series resolvins derived from EPA, to *ApoE*^{-/-} Leiden transgenic mice fed a high-fat/high-cholesterol diet showed a significant reduction in lesion progression, accompanied by

decreased expression of genes involved in atherosclerosis, including those encoding ADAM17, TNF, and IFN- γ (46). The ADAM17 effect is particularly interesting given that Mer proto-oncogene tyrosine kinase (MerTK), a key macrophage efferocytosis receptor in atherosclerotic lesions, is cleaved by this protease, resulting in defective lesional efferocytosis (47). Additionally, because TNF- α and IFN- γ modulate macrophage polarization toward a more proinflammatory phenotype, a decrease in the expression of these cytokines may promote a pro-resolving macrophage phenotype and thereby favorably affect the resolution process (48). In cholesterol-fed rabbits with periodontitis, administration of RvE1 to the gums led to decreases in periodontal inflammation, atherosclerotic lesional inflammation, and necrotic core area (49). The D-series resolvins RvD1 led to smaller necrotic cores, enhanced rates of lesional efferocytosis, and thicker collagen caps when administered to high-fat-fed *Ldlr*^{-/-} mice (14). Similarly, administration of RvD5_{n-3 DPA} (derived from *n*-3 docosapentaenoic acid) to high-fat-fed *ApoE*^{-/-} mice decreased atherosclerotic lesion size and reduced leukocyte and platelet activation (50). Treatment of *ApoE*^{-/-} mice with RvD2 and MaR1 also promoted plaque stability by decreasing lesional macrophage numbers, halting expansion of necrotic cores, and thickening collagen caps (15). ATL treatment was shown to block atherosclerosis progression in high-fat-fed *ApoE*^{-/-} mice by reducing lesional macrophage infiltration and apoptotic cell accumulation, and this protective effect required its cognate receptor, formyl peptide receptor 2 (FPR2/ALX) (51). In addition to SPMs, the proteins annexin A1 and IL-10 have shown pro-resolving effects in the setting of experimental inflammation (52–56). Drechsler et

Table 1. The effect of lipoxygenase and SPM receptor genetic engineering and SPM and pro-resolving protein administration on atherosclerosis in animal models of atherosclerosis

	Animal model	Diet	Outcome	References
Lipoxygenases				
5-LOX-null mutation	<i>Alox5^{-/-} Ldlr^{-/-}</i> mice	Western	Decrease in aortic lesion size	41
12/15-LOX overexpression	12/15-LOX-transgenic <i>ApoE^{-/-}</i> mice	Chow	Decrease in aortic lesion size, increase in LXA ₄	12, 45
12/15-LOX overexpression	12/15-LOX-transgenic <i>ApoE^{-/-}</i> mice	Western	No change in aortic lesion size	45
15-LOX Mφ overexpression	Human 15-LOX-transgenic rabbit	Western	Decrease in aortic lesion size	44
SPMs				
ATL administration	<i>ApoE^{-/-}</i> mice	Western	Decrease in aortic lesion size	51
RvD1 administration	<i>Ldlr^{-/-}</i> mice	Western	Decrease in aortic lesional necrosis and ROS, increase in fibrous cap thickness and efferocytosis	14
RvD2 and MaR1 administration	<i>ApoE^{-/-}</i> mice	Western	Decrease in aortic lesional necrosis and Mφ, increase in fibrous cap thickness	15
RvD5 _{n-3 DPA}	<i>ApoE^{-/-}</i> mice	Western	Decrease in aortic lesion size, decrease in platelet-leukocyte aggregates	50
RvE1 administration (combination with atorvastatin)	<i>ApoE*3</i> Leiden transgenic mice	Western	Decrease in aortic lesion size and expression of proatherogenic genes	46
RvE1 administration (oral-topical)	Rabbit	Western	Decrease in aortic lesion size and inflammatory cell infiltration to lesion	49
Receptors				
FPR2/ALX (murine Fpr2)	<i>Fpr2^{-/-} Ldlr^{-/-}</i> mice	Western	Decrease in aortic lesion size and plaque stability	65
BLT1	<i>Blt1^{-/-} ApoE^{-/-}</i> mice	Western	Decrease in aortic lesion size at 4–8 weeks, no change in lesion size at 19 weeks	70
ChemR23/ERV1	<i>Chemr23/Ervt^{-/-} ApoE^{-/-}</i> mice	Western	Increase in aortic lesion size, increase in necrotic core size	72
	<i>Chemr23/Ervt^{-/-}</i> marrow transplanted to <i>Ldlr^{-/-}</i> mice	Western	Increase in aortic lesion size, increase in necrotic core size	72
Pro-resolving protein				
Ac2-26 (annexin A1 peptide) administration (i.v.)	<i>ApoE^{-/-}</i> mice	Western	Decrease in aortic lesion size and lesional Mφ accumulation	57
Ac2-26 administration (nanoparticles)	<i>Ldlr^{-/-}</i> mice	Western	Decrease in aortic lesion size, lesional necrotic core, and ROS, increase in fibrous cap thickness	14

al. demonstrated that the absence of annexin A1 in high-fat-fed *ApoE^{-/-}* mice led to increased arterial myeloid cell recruitment and atherosclerotic lesion size. Further, the intravenous delivery of the annexin A1-derived peptide fragment Ac2-26 to *ApoE^{-/-}* mice led to a reduction in arterial macrophage accumulation and plaque size (57). Moreover, when Western diet-fed *Ldlr^{-/-}* mice with pre-established atherosclerotic lesions were treated with atherosclerosis-targeting nanoparticles containing Ac2-26, the plaques showed decreases in overall plaque area, necrotic core size, and lesional oxidative stress and increases in fibrous cap thickness and IL-10 expression (58). In another study, treatment of *Ldlr^{-/-}* mice with nanoparticles containing IL-10 led to similar improvements in advanced plaques (59). Together, these studies demonstrate that atherosclerosis progression and vulnerable plaque features can be ameliorated by treatment with exogenous SPMs or pro-resolving proteins. Although these pro-resolving mediators act on distinct receptors, they appear to induce similar atheroprotective mechanisms, including enhanced efferocytosis, pro-resolving macrophage polarization, decreased necrotic core formation, and increased collagen synthesis with thickened fibrous cap development. Despite these important

studies, the precise mechanisms of the atheroprotective effects of SPMs remain incompletely understood.

Recent studies have also shown that the biosynthesis of SPMs is regulated by circadian rhythm. Using lipid mediator profiling of healthy volunteers, Colas et al. demonstrated that plasma concentrations of the DPA-derived D-series resolvins (RvD_{n-3 DPA}) are diurnally regulated. Further, the pattern of regulation was significantly impaired in patients with cardiovascular disease (50). These patients were also found to have increased leukocyte activation and higher levels of circulating platelet-leukocyte aggregates, which have been shown to be a marker of inflammation (50). These findings are particularly interesting given previous work showing that proinflammatory Ly6c^{hi} monocytes and the proinflammatory cytokines IL-6 and TNF-α are also regulated by circadian rhythm (60, 61). These mechanisms may contribute to the development of increased atherosclerosis in mice with disruptions in components of the circadian clock (62, 63). Together, these findings suggest that diurnal regulation of SPM production may be important in their cardioprotective effects.

Along with the lipid mediators themselves, the role of their receptors in atherosclerosis has also been examined, though prog-

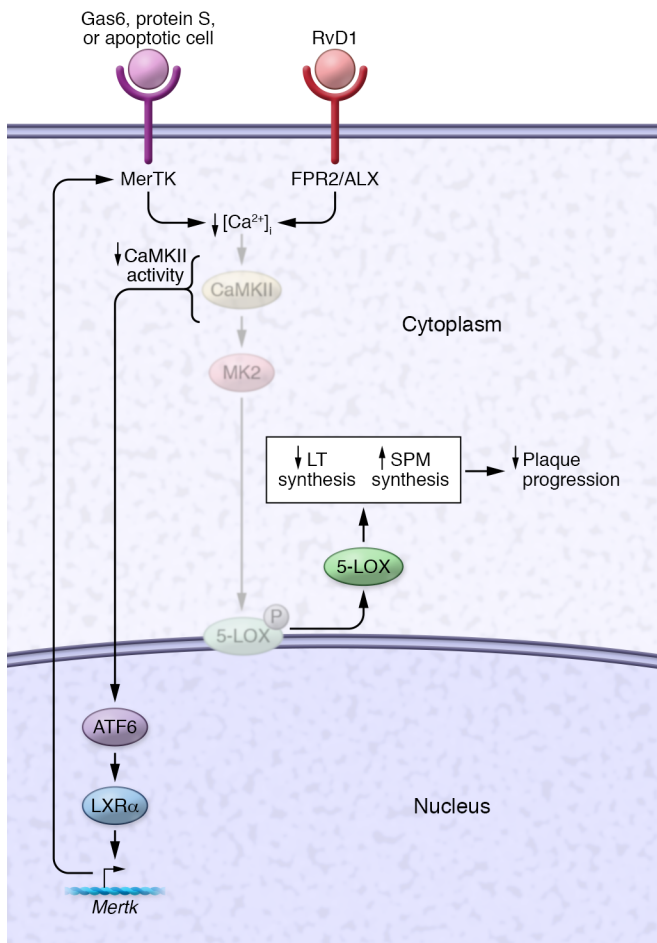
Table 2. SPM receptors and their atherorelevant downstream effects

Receptor	Ligands	Atherorelevant downstream effects	References
ALX/FPR2	AT-LXA ₄	↑ Efferocytosis	33
	LXA ₄	↑ Efferocytosis, ↓ PMN infiltration to tissue, ↓ proinflammatory cytokine production, ↑ antiinflammatory cytokine production	82, 121–123
	RvD1	↑ Efferocytosis, ↓ PMN infiltration to tissue, ↓ proinflammatory cytokine production, ↑ antiinflammatory cytokine production	25, 64, 68, 124
	Annexin A1/Ac2-26	↑ Efferocytosis, ↓ PMN infiltration to tissue, ↑ neutrophil apoptosis	52, 57, 58, 69, 95, 96, 125
BLT1	LTB ₄ (agonist)	↑ Leukocyte chemotaxis, ↑ foam cell formation	70
	RvE1 (antagonist)	↓ PMN infiltration to tissue, ↓ NF-κB activation, ↓ platelet aggregation, ↓ LTB ₄ -induced calcium mobilization	126–128
	RvE2 (antagonist)	↓ PMN chemotaxis/infiltration to tissue, ↑ phagocytosis	126, 129
ChemR23/ERV	RvE1	↓ PMN chemotaxis/infiltration to tissue, ↓ phagocytosis, ↓ proinflammatory cytokine production	84, 126, 127, 130
	RvE2 (partial agonist)	↓ PMN chemotaxis/infiltration to tissue, ↑ phagocytosis	128, 129
FPR1	Annexin A1/Ac2-26	↓ PMN chemotaxis/infiltration to tissue, ↓ leukocyte–endothelial cell interaction	69, 97, 131
GPR18/DVR2	RvD2	↑ Efferocytosis, ↓ PMN infiltration to tissue, ↓ leukocyte–endothelial cell interaction, ↓ monocyte, PMN, platelet activation	50, 83, 108
GPR32/DRV1	LXA ₄	↑ Efferocytosis, ↓ PMN infiltration to tissue, ↓ proinflammatory cytokine production, ↑ antiinflammatory cytokine production	82, 121–123
	RvD1	↑ Efferocytosis, ↓ PMN infiltration to tissue, ↓ proinflammatory cytokine production, ↑ antiinflammatory cytokine production	25, 64, 68, 124
	RvD3	↑ Efferocytosis	132
	RvD5	↑ Phagocytosis, ↓ proinflammatory cytokine production	109

ress has been hindered by the complex and sometimes nonspecific relationship between resolving mediators and their receptors (Table 2). In addition, mechanistic studies have been limited in some cases by the lack of a murine homolog for human receptors. For example, human GPR32, an RvD1 receptor, does not exist in mice, which instead appear to largely utilize the FPR2/ALX receptor for RvD1 as well as for LXA₄ (64). Interestingly, although genetic targeting of the FPR2/ALX receptor in mice prevented atherosclerosis progression as predicted, it actually decreased plaque stability in high-fat-fed *Ldlr*^{-/-} mice, which may be due to non-SPM ligands for FPR2/ALX, such as serum amyloid A, that are proinflammatory (65). Notably, the targeting strategy used by these authors led to the deletion of both *Fpr2* and *Fpr3*, which are the murine homologs of human FPR2/ALX (66, 67). The findings from this study are consistent with those of prior studies showing that the FPR2/ALX receptor induces both proinflammatory and pro-resolution actions depending on the activating ligand. For example, activation of FPR2/ALX by prokaryotic peptides induces leukocyte chemotaxis, while annexin 1 and its related N-terminal peptides cause a decrease in neutrophil migration (67–69). Deletion of BLT1, the receptor for LTB₄, decreased formation of early lesions in *ApoE*^{-/-} mice fed the Western diet for 4–8 weeks, but it did not lead to any change in lesion size after 19 weeks of high-fat-diet feeding (70). In a related study, treatment of short-term Western diet-fed *Ldlr*^{-/-} mice or chow diet-fed *ApoE*^{-/-} mice with a BLT1 antagonist led to reduced lesion area and decreased lesion-associated CD11b expression (71). These combined data suggest that BLT1 signaling is important in early atherogenesis but that other proath-

erogenic processes become dominant in more advanced atherosclerosis. Finally, a recent study demonstrated that deficiency of the receptor for RvE1, ChemR23/ERV1, in *ApoE*^{-/-} mice or in the hematopoietic cells of *Ldlr*^{-/-} mice leads to increased lesion size and larger necrotic cores (72). Further, the loss of ChemR23/ERV1 was associated with decreased phagocytosis in vitro, decreased expression of genes involved in phagocytosis, and increased expression of genes relevant to macrophage lipoprotein uptake. Together, these data suggest an atheroprotective role for ChemR23/ERV1.

Macrophages show a high degree of plasticity and can perform either proatherogenic or antiatherogenic activities in response to environmental stimuli (73). As mentioned above, proinflammatory macrophages are prominent in unstable regions of plaques, whereas abundant pro-resolving macrophages are present in stable regions of plaques (74). Further, atherosclerotic plaques undergoing regression demonstrate increased numbers of pro-resolving macrophages, which presumably promote tissue repair. In this context, SPMs are capable of promoting phenotypic skewing in macrophages. For example, the administration of RvD2 and MaR1 to atheroprone mice promotes polarization of macrophages toward a pro-resolving phenotype (15). Interestingly, pro-resolving macrophages have been shown to synthesize higher levels of SPMs than proinflammatory macrophages (75), suggesting a resolution-amplification cycle. Pro-resolving macrophages have also been shown to have a higher efferocytic capacity than proinflammatory macrophages. Moreover, the pro-resolving protein IL-10 also induces polarization of macrophages toward a pro-resolving phenotype with enhanced efferocytosis (76, 77).



Role of SPMs in atherosclerotic lesional efferocytosis

Efferocytosis, the receptor-mediated clearance of apoptotic cells, has a critically important role in successful resolution responses, and its impairment has been highlighted in many non-resolving chronic inflammatory diseases, including atherosclerosis. Failure to clear apoptotic cells from atherosclerotic plaques in a timely fashion leads to secondary necrosis, which generates heightened inflammation owing to the release of inflammatory damage-associated molecular patterns (DAMPs) from the necrotic cells (4, 78–80). Therefore, efferocytosis is a crucial step in the resolution of lesional inflammation in chronic non-resolving inflammatory diseases. Interestingly, SPMs are able to increase the efferocytic capacity of macrophages (25, 81–83), and efferocytosis, in turn, promotes SPM production by macrophages, including LXA₄, RvE1, and PD1 (75, 81, 84). SPMs, in particular the lipoxins, also act as chemoattractants that recruit monocytes that, after differentiating into macrophages, enhance the overall efferocytic capacity of resolving lesions (82, 85, 86). Thus, similar to the situation mentioned above with resolving macrophages, there is a positive resolution feedback cycle involving SPMs and efferocytosis.

Specific phagocytic receptors binding to phosphatidylserine on apoptotic cells have been implicated in the regulation of efferocytosis in atherosclerotic lesions. For example, genetic targeting of *Mertk* in fat-fed *Apoe*^{-/-} or *Ldlr*^{-/-} mice led to impaired efferocytosis, accumulation of apoptotic cells, and enlarged necrotic cores

Figure 2. RvD1 and MerTK signaling promote pro-resolving pathways in macrophages. Activation of signaling through the FPR2/ALX receptor by RvD1 or through the MerTK receptor by Gas6, protein S, or apoptotic cells (with Gas6 or protein S on the surface of the apoptotic cells) lowers cytoplasmic calcium and thereby decreases the activity of CaMKII. The decrease in CaMKII activity triggers at least two pro-resolving processes. First, it lowers MK2 activity, which increases nonphosphorylated 5-LOX and promotes its nuclear-to-cytoplasmic translocation. In the cytoplasm, 5-LOX mediates the synthesis of SPMs, e.g., LTB₄ in the presence of AA and RvD1 in the presence of DHA, and in the nucleus, 5-LOX causes a concomitant decrease in LT synthesis. The resulting increase in the SPM/LT ratio suppresses plaque progression. Second, suppression of CaMKII activity induces an ATF6-LXR α -MerTK pathway that increases the synthesis of MerTK and thus amplifies the pro-resolving program. Not shown here is that ADAM17-mediated MerTK cleavage, which occurs in advanced lesional macrophages, suppresses both efferocytosis and resolution in macrophages in vitro and in advanced atherosclerotic lesions.

(87, 88). MerTK deficiency also leads to impaired SPM production by macrophages, exemplifying the aforementioned efferocytosis/resolution–amplification loop that, when disrupted, can cause devastating consequences (89). As mentioned above, the MerTK receptor can be inactivated by the metalloproteinase ADAM17, which cleaves the receptor and causes it to shed its soluble extracellular domain (47). Interestingly, high levels of soluble MerTK have been detected in both human and murine atherosclerotic lesions, suggesting that in advanced atherosclerosis, inactivation of the MerTK receptor in this fashion is one mechanism by which efferocytosis is impaired (89). In further support of this idea, analysis of human carotid plaques revealed that expression of ADAM17 is increased while that of MerTK is decreased in macrophages surrounding the necrotic cores as compared with macrophages in peripheral regions of the plaques (90). In addition, extracts obtained from the regions around necrotic cores induced cleavage of MerTK and upregulated expression of ADAM17 in cultured THP-1 macrophages (90).

Our group has demonstrated recently that intact MerTK controls the subcellular localization of 5-LOX, which, as mentioned in the previous section, favors production of SPMs (91). Under inflammatory conditions, 5-LOX is phosphorylated by MK2 and translocated to the nucleus, where it promotes the generation of LTB₄ (36, 37). MerTK signaling inhibits MK2 and thereby leads to cytosolic localization of 5-LOX and the generation of LXA₄ in macrophages. When MerTK is cleaved or genetically deleted, 5-LOX localizes to the nuclear membrane, and there is an increase in leukotrienes (91). Moreover, the atherosclerotic lesions of mice engineered to express a cleavage-resistant version of MerTK (MerTK^{CR}) and crossed onto the *Ldlr*^{-/-} background demonstrate attenuated nuclear localization of 5-LOX in macrophages, enhanced SPMs, increased rates of efferocytosis, smaller necrotic cores, and thicker fibrous caps compared with the lesions of *Ldlr*^{-/-} mice with wild-type MerTK (89, 91). SPMs themselves are able to augment and therefore amplify this pathway. For example, RvD1, which is also induced by successful efferocytosis, can promote cytosolic localization of 5-LOX to favor the generation of LXA₄ (37).

The mechanism by which MerTK signaling and RvD1 promote cytoplasmic 5-LOX and subsequent SPM generation in

macrophages has recently been revealed (Figure 2). RvD1, by activating its receptor FPR2/ALX, lowers cytosolic calcium, which suppresses activity of the calcium-sensitive kinase calcium/calmodulin-dependent protein kinase II- γ (CaMKII γ). CaMKII, which is activated in macrophages of advanced, but not early, mouse and human atherosclerotic lesions (92), activates MK2. Thus, the RvD1-mediated decrease in CaMKII activity lowers MK2 activity, which in turn leads to decreased phosphorylation and increased cytoplasmic localization of 5-LOX (68). Activation of the MerTK receptor by its ligands Gas6 and protein S or by apoptotic cells that have Gas6 or protein S on their surface also suppresses MK2 activity; decreases nuclear phospho-5-LOX, which is the form that leads to LTB₄ synthesis; and increases SPMs (91). Recent evidence suggests that suppression of CaMKII activity is upstream of this effect, as it is with the RvD1-FPR2/ALX pathway. Finally, as yet another example of feedback and amplification in the resolution response, suppression of CaMKII activity has another pro-resolving role in macrophages, i.e., in addition to increasing SPM biosynthesis: its inactivation leads to an increase in *Mertk* transcription and MerTK-mediated efferocytosis via a pathway involving the transcription factors ATF6 and LXR α (92). In vivo, genetic deletion of CaMKII γ from myeloid cells in *Ldlr*^{-/-} mice enhances the efferocytic capacity of macrophages in atherosclerotic plaques and decreases plaque necrosis and inflammation (92). Thus, CaMKII activity in lesional macrophages represents an important hub orchestrating defective efferocytosis and impaired resolution. For this reason, its therapeutic inhibition may be considered as a new strategy to block advanced plaque progression.

In addition to efferocytosis, SPMs play a role in the apoptosis of neutrophils after they have engulfed pathogens, which is a critical step in resolution. For example, in a model of acute lung injury, RvE1, through its receptor BLT1, promoted apoptosis of neutrophils after their ingestion of opsonized *E. coli* by increasing caspase-3 and caspase-8 activation and by suppressing ERK- and Akt-dependent prosurvival signaling pathways (93). Similarly, aspirin-triggered lipoxins signal via FPR2/ALX, ERK, and Akt to block prosurvival signaling in neutrophils (94). Further, annexin A1 has also been shown to promote neutrophil apoptosis by a pathway involving L-selectin shedding from neutrophils, inhibition of PMN adhesion to endothelial cells, and activation of caspase-3. This pathway also leads to the externalization of annexin A1 on the PMNs, which promotes efficient efferocytosis of the cells (95–97). Finally, SPMs, including 15-epi-LXA₄ and RvD1, promote autophagy and survival pathways in macrophages, which enhances efferocytosis and tissue resolution (98).

Antiinflammation versus resolution therapy in atherosclerosis

In view of the evidence suggesting that chronic inflammation plays an important role in plaque progression, therapeutic targeting of inflammation has been considered as a complementary strategy to LDL reduction for lowering the risk of atherosclerotic vascular disease. This idea recently made a quantum leap in plausibility with the publication of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) trial, which examined approximately 10,000 post-myocardial infarction patients to determine whether inhibition of IL-1 β with canakinumab, a monoclonal anti-

body directed against the proinflammatory cytokine IL-1 β , could decrease cardiovascular events in patients with a history of myocardial infarction and elevated C-reactive protein (CRP) levels (99). More than 90% of the subjects were on concomitant statin therapy. In animal and preclinical models, IL-1 β has been shown to promote inflammation in atherosclerosis by inducing the expression of leukocyte adhesion proteins, e.g., ICAM-1, E-selectin, and VCAM-1, that contribute to the accumulation of leukocytes in lesions (100, 101). The CANTOS investigators demonstrated that 150 mg of canakinumab administered every 3 months led to a statistically significant reduction in the primary composite endpoint of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death (hazard ratio 0.85 vs. placebo, $P = 0.021$) (102). This effect was associated with a decrease in CRP but no significant changes in LDL or HDL in the plasma. Thus, the CANTOS trial showed a beneficial effect of blocking inflammation independently of the role of lipids, highlighting the promise of therapy directed toward inflammation in cardiovascular disease. A second trial exploring the potential benefit of low-dose methotrexate for cardiovascular disease is the Cardiovascular Inflammation Reduction Trial, whose results are expected in 2020 (103). Methotrexate, which has long been a mainstay of therapy for autoimmune disease, including rheumatoid arthritis, psoriasis, and psoriatic arthritis, has been shown to attenuate levels of the proinflammatory cytokines IL-6 and TNF- α as well as CRP in the plasma of these patients (104).

A concern with the use of antiinflammatory drug therapy is the potential to compromise of host defense (105–107), and in CANTOS there was a significant increase in the number of fatal infections associated with canakinumab therapy (incidence rate 0.31 vs. 0.18 events per 100 person-years, combined dose groups vs. placebo, $P = 0.02$) (102). In this context, translational cardiovascular researchers are beginning to consider the alternative strategy of boosting resolution to lower risk of cardiovascular disease, because this strategy would attack one of the root causes of advanced plaque progression while having the capacity to actually boost host defense rather than suppress it (108, 109). One possibility would be to increase the precursors of SPMs through dietary supplementation of *n*-3 fatty acids. This idea would be valid only if these precursors were rate-limiting for SPM biosynthesis in a manner that could increase the level of lesional SPMs. Conversely, if a major cause of low SPMs in lesions were a defect in a biosynthetic pathway *distal* to the step where *n*-3 fatty acids enter the pathway, e.g., excessive phosphorylation of 5-LOX (above), this strategy would not be expected to be effective. Indeed, when the outcomes of a number of *n*-3 fatty acid trials are considered together, the results have been rather disappointing. While observational population-based studies have suggested that a *n*-3 fatty acid-rich diet is associated with lower incidence of cardiovascular disease (110, 111), subsequent randomized clinical trials of *n*-3 fatty acid supplementation yielded mixed results, with only some suggesting a beneficial effect in preventing cardiovascular events (112–115). There have been questions related to the dose and types of *n*-3 fatty acids used in these trials, and two new cardiovascular outcome studies comparing statin therapy alone with statins plus either high-dose EPA or combined DHA/EPA are currently under way.

In view of the imbalance in SPMs versus leukotrienes in advanced atherosclerotic plaques (116, 117), investigators have

tested the effect of atreleuton, a 5-LOX inhibitor, to lower leukotrienes. However, this enzyme is also involved in the synthesis of lipoxins and resolvins (above). Phase II studies conducted in patients with acute coronary syndromes demonstrated that while leukotriene production was decreased in patients given atreleuton, vascular inflammation within the ascending aorta and carotids as measured by fludeoxyglucose (FDG) PET did not show any significant change (118, 119). This lack of efficacy in this early attempt at resolution-targeting therapy may have been due to the dual role of this enzyme in SPM synthesis.

The most direct way to implement resolution mediator therapy is to administer exogenous mediators. Examples summarized in Table 1 and discussed above illustrate the concept that exogenous administration of SPMs provides a protective effect in animal models of atherosclerosis. These preclinical studies, together with the theoretical advantages of pro-resolution versus antiinflammatory therapy outlined above; the fact that SPMs are already being tested in clinical trials for other chronic inflammatory diseases (ClinicalTrials.gov, NCT00799552 and NCT00941018); and the new opportunities for plaque therapy in humans in the wake of CANTOS support further research into using resolution mediators to block plaque progression in humans.

Conclusions and future directions

Despite years of steady progress in the treatment and prevention of cardiovascular disease, death rates attributed to heart attack and stroke are again rising in the United States, presumably owing to the epidemic of obesity and diabetes (2). Aggressive lipid lowering remains the mainstay of therapy for atherosclerosis, but there is room for the development of complementary therapeutics. Although the recent CANTOS trial has demonstrated that targeting inflammation can successfully reduce cardiac mortality in high-risk populations, it has also confirmed that antiinflammatory therapies run the risk of compromising host defense. Our understanding of the etiology of atherosclerosis has recently evolved to include failed resolution as a prominent causal feature for the most clinically relevant advanced plaques, opening up the exciting

possibility that clinical plaque progression could be targeted in a manner that would lessen immunosuppression. Moreover, in the context that obesity and type 2 diabetes are major risk factors for atherosclerotic cardiovascular disease — and a major reason why cardiovascular disease remains the leading cause of death (2) — a role for chronic inflammation and failed resolution in metabolic diseases is also emerging (120). Thus, resolution therapy may represent a novel avenue for the integrated treatment of cardiometabolic disease. Although we know that there is an imbalance of proinflammatory versus pro-resolving mediators in advanced atherosclerosis, we do not yet fully understand why the resolution process fails in this setting. On the one hand, we can consider the direct approach of administering exogenous SPMs, as discussed above. However, more mechanistic strategies must also be considered, and this will require a deeper understanding of how SPMs are synthesized and catabolized and how they work on effector cells to carry out various resolution processes. Finally, with the increasing availability of large genetic data sets, incorporating a functional genomic approach to the identification of gene signatures associated with defective inflammation resolution may help identify new targets as well as provide a strategy for the tailoring of therapy to specific individuals.

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