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Crosstalk between Macrophages and Vascular Smooth Muscle Cells in Atherosclerotic Plaque Stability

Arif Yurdagul Jr.[1,*]

[1]Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences, Shreveport, LA, 71130, USA

Abstract

Most acute cardiovascular events are due to plaque rupture, with atheromas containing large necrotic cores and thin fibrous caps being more susceptible to rupture and lesions with small necrotic cores and thick fibrous caps being more protected from rupture. Atherosclerotic plaques are comprised of various extracellular matrix proteins, modified lipoprotein particles, and cells of different origins, i.e. vascular cells and leukocytes. Although much has been revealed about the mechanisms that lead to plaque instability, several key areas remain incompletely understood. This In-Focus Review highlights processes related to cellular crosstalk and the role of the tissue microenvironment in determining cell function and plaque stability. Recent advances highlight critical underpinnings of atherosclerotic plaque vulnerability, particularly impairments in the ability of macrophages to clear dead cells and phenotypic switching of vascular smooth muscle cells. However, these processes do not occur in isolation, as crosstalk between macrophages and vascular smooth muscle cells and interactions with their surrounding microenvironment play a significant role in determining plaque stability. Understanding these aspects of cellular crosstalk within an atherosclerotic plaque may shed light on how to modify cell behavior and identify novel approaches to transform rupture-prone atheromas into stable lesions.

Graphical Abstract.

[*]**Correspondence to:** Arif Yurdagul Jr., Louisiana State University Health Sciences, Shreveport, LA, 71130, arif.yurdagul@lsuhs.edu.

DISCLOSURES

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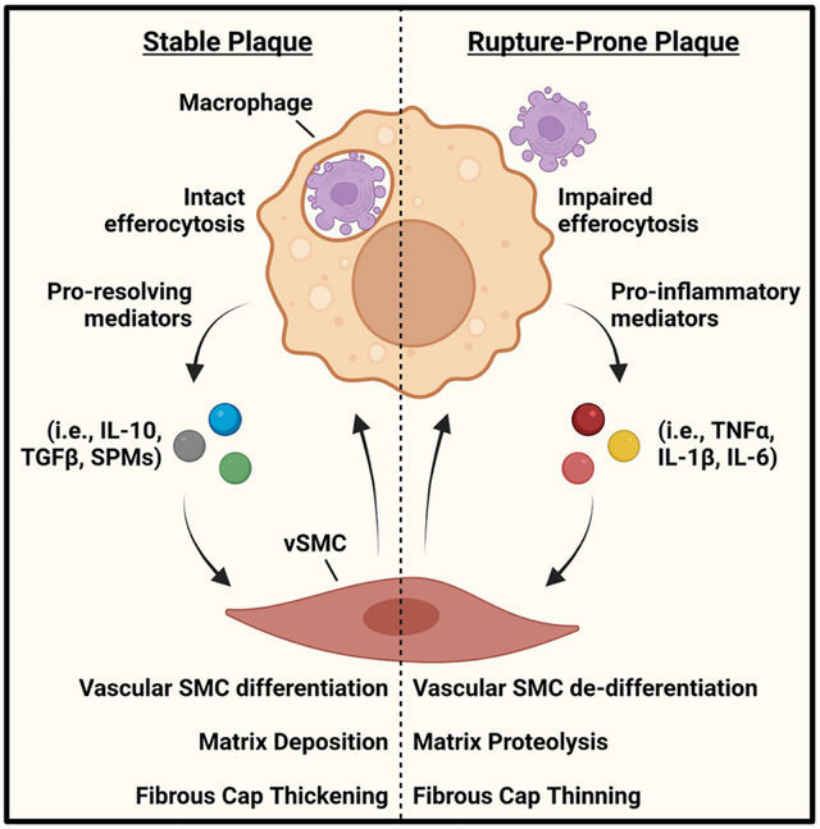

Features of Stable and Unstable Atherosclerosis. Stable plaques are characterized by a thick fibrous cap and small necrotic core. These plaques also show features of vSMC quiescence and have low MMP activity. Macrophages in stable plaques also show intact efferocytosis. Rupture-prone plaques contain large necrotic cores and thin fibrous caps. These atheromas also show features associated with vSMC de-differentiation and contain high MMP activity and ECM proteolysis. Macrophages from rupture-prone atheromas also display impaired efferocytosis. The stability of an atherosclerotic plaque is owed to a balance between resolving mediators and inflammatory factors such that an increase in resolution mediators drive a more stable plaque, whereas an increase in inflammatory factors promote a more vulnerable plaque.

Keywords

macrophages; vascular smooth muscle cells; atherosclerosis; ECM remodeling; efferocytosis

Background

Exponential advancements in molecular and cellular technologies have significantly expanded our understandings of atherosclerotic cardiovascular disease (ASCVD)[1]. It is now evident that the interplay between soluble factors, cell-cell interactions, and the tissue microenvironment governs atherosclerosis progression and plaque stability[2]. Subendothelial retention of plasma-derived apolipoprotein B-containing lipoproteins in the intima of medium-to-large-sized arteries drives a low-grade inflammatory response that,

if sustained, initiates atherosclerosis[3]. These retained lipoproteins undergo extracellular modification that activate endothelial and vascular smooth muscle cells and promote the secretion of pro-inflammatory chemokines and cytokines that drive the recruitment of leukocytes[4]. Monocyte-derived macrophages also secrete factors that modify lipoproteins further, promoting a feed-forward loop of lipid modification and chronic inflammation[5]. Furthermore, impairments in the phagocytic clearance of apoptotic cells (ACs) by macrophages also sustain inflammation, and together with the continued accumulation of modified lipoproteins, rampant cell death, and pooling of extracellular lipids, a necrotic core is formed. These necrotic cores are clinically dangerous as they often are the site of intraplaque hemorrhaging, contain highly thrombogenic material, and act as the nucleating site for calcium deposition[6]. These intimal inflammatory responses also cause vascular smooth muscle cells (vSMCs) in the tunica media to de-differentiate towards a synthetic phenotype, characterized by high rates of proliferation and migration, and drive plaque expansion that occludes the lumen through fibroproliferative remodeling[7].

Powerful therapies exist to lower plasma LDL cholesterol and impede atherosclerosis progression. However, despite aggressive lipid-lowering strategies, ASCVD remains the leading cause of morbidity and mortality worldwide. The recent success of the CANTOS and COLCOT trials demonstrate that targeting aspects of inflammation will complement our current approaches that lower circulating cholesterol in treating ASCVD[8, 9]. However, individuals that are undergoing treatments to lower inflammation experience a heightened rate of infection-related adverse events. Therefore, strategies aimed specifically at transforming rupture-prone atheromas into stable plaques may be a more attractive approach (Fig. 1). In this review, we summarize recent work that is beginning to elucidate the molecular and cellular mechanisms that determine plaque stability and highlight the major questions facing the field.

The Role of Vascular Smooth Muscle Cells in Fibroproliferative Remodeling as a Determinant in Fibrous Cap Formation

During homeostasis, vascular smooth muscle cells (vSMCs) regulate blood pressure and distribution and provide structural integrity for blood vessels. However, vSMCs are remarkably plastic and can significantly contribute to vascular remodeling under physiological and pathological settings. This is owed to their ability to undergo dynamic switching between a differentiated, termed “quiescent”, and de-differentiated, termed “synthetic”, phenotype[10]. Normally, vSMCs proliferate and migrate at an extremely low rate and express an exclusive repertoire of vSMC-specific genes, which include αSMA, γSMA, Calponin, SM22α, smoothelin, and SM-MHC[11]. This is dominantly controlled by myocardin-serum response factor (SRF) and kruppel-like factor 4 (KLF4)[12, 13]. Vascular SMC-specific genes contain the conserved DNA recognition element $CC(A/T\text{-rich})_6GG$ (CArG), which requires the transcription factor SRF bound directly to the coactivator Myocardin to drive gene transcription. However, this mechanism can be antagonized by KLF4, which suppresses myocardin expression and prevents SRF from associating with CArG boxes by binding to G/C repressor elements[14]. Importantly, deletion of KLF4 in vSMCs mitigates the loss in vSMC-specific genes during injury, limits proliferation, and

significantly lowers vascular remodeling during atherosclerosis[15, 16]. KLF4-dependent de-differentiation of vSMCs also promotes their conversion towards a macrophage-like state[17].

While positive remodeling during atherosclerosis progression preserves vessel patency by expanding outwards, negative remodeling, which occurs once the vessel has expanded by more than 40%, occludes lumen diameter as atherosclerosis advances[18]. However, myocardial infarctions and strokes are caused by the sudden rupture of atherosclerotic plaques, which may not be overly stenotic, and subsequent luminal thrombosis[19]. Central to plaque stability is the composition and size of the fibrous cap overlying a necrotic core (Fig. 1). Because vSMCs are the major producer of ECM proteins in an atheroma, their population within the fibrous cap directly correlates with plaque stability[20 21]. Interestingly, these vSMCs that dominate the protective fibrous cap re-express genes associated with vSMC differentiation, such as αSMA and SM22. Therefore, the prevailing theory has been that while vSMC de-differentiation directly contributes to plaque size, phenotypic switching also plays a beneficial role in plaque stabilization by forming the protective ECM-dense fibrous cap. However, these beneficial functions may be negatively regulated by the inflammatory components within the fibrous cap.

Extracellular matrix (ECM) composition and organization govern cell function, and reciprocally, cells actively remodel their surrounding ECM[22]. During development, the ECM undergoes controlled matrix remodeling whereby organ morphogenesis occurs uninterrupted. However, uncontrolled remodeling disrupts tissue function, causes tissue fibrosis, and propagates non-resolving inflammatory diseases[23]. Laminins, polymerized collagen I, collagen III, collagen IV, small amounts of fibronectin, and elastin fibers comprise the ECM during the early stages of atherosclerosis. In contrast, the ECM at later stages of atherosclerosis is comprised of monomeric collagen I, fibronectin, fibrinogen, vitronectin, and osteopontin[2, 24, 25]. Interactions with the ECM are largely mediated by the integrin family of cell-matrix adhesion receptors. Encompassing 18 α subunits and 8 β subunits, integrins heterodimerize into 24 distinct integrin pairs and regulate a panoply of signaling pathways[26]. Vascular SMCs interact with the surrounding ECM through the laminin-binding integrins α3β1 and α7β1 and the collagen-binding integrins α1β1 and α2β1[27]. However, the ECM proteins assembled during atherosclerosis bind to the integrins α5β1, αvβ3, and αvβ5[28]. Vascular SMC function is influenced by their interactions with the ECM. For instance, the integrin α1β1, which binds to collagen I, is highly expressed in contractile SMCs, and phenotypic switching of vSMCs towards a de-differentiated state decreases integrin α1 gene expression[29]. Interestingly, collagen I can exist either in a polymeric form, rich in healthy tissue niches, and in a monomeric form, thought to be abundant in advanced stages of atherosclerosis due to high MMP activity[30]. In its polymeric form, collagen I promotes vSMC quiescence, whereas in its monomeric form, collagen I drives vSMC de-differentiation[30]. As another example, ligation of integrin α7β1 to laminin in vSMCs lowers ERK activation, reduces proliferation, and prevents vascular remodeling[31]. Consistently, inhibiting vSMC interactions with matrix proteins that are abundant in advanced stages of atherosclerosis using αvβ3-blocking peptides or function-blocking antibodies lowers vSMC proliferation and reduces atherosclerosis formation[32, 33].

In addition to directly governing signaling cascades, the microenvironment also plays a role in retaining modified LDLs, pro-inflammatory cytokines, and many growth factors that perpetuate fibroproliferative remodeling and inflammation within the atheroma. Glycosaminoglycans (GAGs), polysaccharides containing repeating disaccharide units on proteoglycans, directly interact with oxidized LDL, leading to its uptake by macrophages[34]. Consistently, enhancing LDL interactions with proteoglycans by deleting APRIL, a cytokine known to bind proteoglycans, drives LDL accumulation, enhances macrophage recruitment to atherosclerotic lesions, and worsens necrotic core formation[35]. As another example, TNFα interacts with laminin and fibronectin, which sustains inflammation[36, 37]. Interactions between most chemokines with heparan sulfate proteoglycans drive oligomerization and favor their presentation to receptors on leukocytes and promote directed migration towards an ECM-rich chemokine gradient[38]. GAGs also interact and retain a wide range of growth factors, including PDGF-BB, TGFβ, HGF, FGF, and IGF[39]. Similar to other matrix-bound soluble factors, growth factors show disparate signaling pathways compared to their soluble-only versions. For instance, soluble growth factors are rapidly internalized and degraded upon binding to their cognate receptor. However, growth factors become immobilized once bound to the ECM and are then resistant to endocytosis and degradation, which sustains growth factor signaling. Also, growth factor receptors can associate with cell-matrix adhesion proteins that potentiate growth factor-dependent proliferation, migration, and angiogenesis[40].

PDGF-BB is a dominant growth factor that drives vSMC de-differentiation, proliferation, and migration. Inhibiting PDGF-BB signaling by blocking PDGF receptors lowers fibrous cap formation in ApoE knockout mice fed a high-fat diet[41]. Interestingly, PDGF-BB-dependent vascular SMC migration and expansion of the neointima can be antagonized by IL-1β[42]. This is consistent with mouse models demonstrating that administration of an IL-1β neutralizing antibody decreased overall plaque burden and is supported by the success of the CANTOS trial, where canakinumab was shown to lower mortality from cardiovascular disease[8, 43]. In contrast to these findings, an elegant study demonstrated that when neutralizing antibodies against IL-1β were delivered to mice with pre-established atherosclerosis vSMC incorporation into the fibrous cap was significantly reduced, macrophage migration was enhanced, and beneficial outward remodeling was impaired[44]. The features associated with rupture-prone atheromas can be enhanced by IFNγ, as elevated expression of this cytokine blunts collagen synthesis by vSMC and drives apoptosis[45, 46]. Furthermore, destruction of the ECM by matrix metalloproteinases (MMPs) weaken the protective fibrous cap. For instance, rupture-prone plaques in humans show enhanced MMP activity compared to stable plaques, and MMPs are considered a risk factor for future cardiovascular events[47]. Furthermore, MMP2 and MMP9 knockout mice show reduced plaque size and exhibit features of plaque stability[48, 49]. Consistently, promoting vSMC differentiation by increasing TGFβ signaling or over-expressing insulin growth factor-1 specifically in vSMCs enhances fibrous cap thickening[50, 51]. As another example, deletion of KLF4 specifically in vSMCs enhances fibrous cap thickening and is associated with an increase in αSMA-positive cells in the fibrous cap[13].

The Role of Efferocytosis by Macrophages in Inflammation Resolution as a Determinant in Necrotic Core formation

Phagocytosis of apoptotic cells, termed “efferocytosis”, prevents secondary necrosis, terminates inflammatory responses, and activates pro-resolving pathways[52]. These protective functions are compromised when efferocytosis is impaired, leading to non-resolving inflammatory diseases. Cells that die early during atherosclerosis formation become rapidly cleared, but through a variety of mechanisms, efferocytosis becomes defective as atherosclerosis progresses[53, 54]. Macrophages interact with apoptotic cells through a unique repertoire of direct and indirect receptors, yet many of these are either downregulated or cleaved as atherosclerosis advances. As one example, MerTK expression decreases and what remains on the cell surface of macrophages is cleaved by the disintegrin and metalloproteinase domain-containing protein 17 (ADAM17)[55]. Mice expressing a cleavage-resistant form of MerTK have plaques with smaller necrotic cores that is associated with increased lesional efferocytosis[56]. Importantly, MerTK also stimulates signaling pathways that dampen inflammation and drive the synthesis of specialized pro-resolving lipid mediators (SPMs), which are lipid species that significantly blunt atherosclerosis progression[57–59]. Another mechanism of impaired efferocytosis during atherosclerosis is oxidized LDL-dependent downregulation of LRP1. Treating macrophages *in vitro* with oxidized LDL drives epsin-mediated, ubiquitin-dependent internalization and degradation of LRP1. Consistently, loss of epsin 1 and 2 in myeloid cells lowers plaque size and necrotic core area by enhancing LRP1-mediated efferocytosis[60]. Furthermore, LRP1 in macrophages is necessary for anti-CD47 blocking antibodies to increase efferocytosis, which have been shown to reduce atherosclerosis progression[61]. The bridging molecule MFGE8 ligates ACs to integrin αVβ3 and transglutaminase 2 on macrophages to drive AC internalization, yet MFGE8 expression declines as atherosclerosis advances. Mice with a genetic deletion in MFGE8 in hematopoietic cells show an increase in plaque size and necrotic core areas[62]. Altogether, impairments in efferocytosis unequivocally lead to post-apoptotic necrosis and cause necrotic core expansion (Fig. 1).

The breakdown, metabolism, and response to AC-derived molecules by macrophages, termed “efferotabolism”, is an exciting new area of study as it continues the successive clearance of dead cells and transitions the termination of inflammation into resolution[53]. After an AC has been broken down in the phagolysosome, the digested components burden a macrophage with substances that must either be rapidly metabolized or exported[63–67]. For instance, intracellular cholesterol rises when a macrophage degrades a foamy AC. Phagolysosomal cholesterol is then trafficked to the ER and is acted on by acyl-CoA:cholesterol acyltransferase to esterify free cholesterol to cholesterol fatty acid esters, which restrict the membrane-damaging effects of elevated levels of free cholesterol[68]. Also, AC-derived sterols stimulate PPAR and LXR to drive the expression of ABCA1 and ABCG1 to promote cholesterol efflux[69]. When macrophages are polarized towards an Arg1-positive pro-resolving phenotype, arginine from an AC is converted into ornithine after efferocytosis. This then becomes decarboxylated by the enzyme ODC1 into putrescine, which drives cytoskeletal remodeling to mediate the successive clearance of multiple ACs[66]. Furthermore, putrescine also maintains MerTK expression on the surface

of macrophages, which mediates IL-10 production upon engagement with an AC[70]. An inability to convert AC-derived arginine into putrescine reduces continual efferocytosis, lowers collagen deposition in the fibrous cap, and prevents a reduction in lesion size and necrotic core area during atherosclerosis regression[66]. Interestingly, a non-canonical route of glutaminolysis that is dependent on glutaminase 1 supports efferocytosis by promoting oxidative phosphorylation[65]. Disrupting this unique route of glutaminolysis in myeloid cells using $Gls1^{fl/fl}$ mice showed impairment in AC clearance *in vivo* and worsened atherosclerosis[65].

The Role of vSMC and Macrophage Crosstalk in Plaque Stability

Pro-resolving macrophages, particularly ones actively clearing apoptotic cells, secrete a panoply of molecules that drive features of plaque stability. Factors released by macrophages after efferocytosis that stimulate the waning of inflammation and initiate the active process of resolution include IL-10, TGFβ, and SPMs[3]. In turn, these secreted factors act on surrounding vSMCs to promote specific functions relating to plaque stability (Fig. 2).

TGFβ is one of the more well-known pro-resolving factors secreted by macrophages after efferocytosis. Human clinical studies demonstrate that higher levels of circulating TGFβ are associated with plaque stability[71]. Similarly, immunohistochemistry revealed that TGFβ levels were lower in rupture-prone atheromas[72]. TGFβ enhances vSMC contractile gene expression and also stimulates the expression of ECM genes[73, 74]. Furthermore, TGFβ suppresses inducible nitric oxide synthase and interleukin 6 (IL-6) in vSMCs by lowering SMAD3 signaling[75]. These reports suggest that TGFβ is protective during atherosclerosis by reducing inflammation and enhancing ECM production. Another pro-resolving factor released by efferocytosing macrophages is IL-10. This pro-resolving cytokine has potent protective effects in vSMCs. For instance, IL-10 inhibits LPS-induced IL-6 secretion by inactivating NFκB. Furthermore, IL-10 reduces balloon injury-induced vascular remodeling by inhibiting vSMC proliferation and migration[76]. Through similar mechanisms, IL-10 also inhibits monocrotaline-induced pulmonary arterial hypertension and angiotensin II-induced hypertension[77, 78].

In advanced atherosclerosis, there is an imbalance between specialized pro-resolving lipid mediators (SPMs), such as resolvins and lipoxins, and pro-inflammatory factors. SPMs actively drive inflammation resolution, increase the clearance of dead cells by macrophages, and enhance features of plaque stability during atherosclerosis[79]. Lipoxins, resolvins, protectins, and maresins comprise the family of SPMs and are derived from *n*-3 fatty acid-derived eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), or docosahexaenoic acid (DHA)[79]. EPA, DPA, and DHA are released from phospholipids by phospholipases and then acted on by either 5-lipoxygenase, 12-lipoxygenase, or 15-lipoxygenase to yield SPMs[80]. In addition to the protective effects of SPMs in macrophages, SPMs also positively influence vSMC function. Resolvin D1 and D2 (RvD1 and RvD2) dose-dependently inhibit vSMC proliferation, migration, superoxide production, and proinflammatory gene expression. Furthermore, these D-series resolvins inhibit neointimal hyperplasia following arterial angioplasty[81]. RvE1 and lipoxins inhibit PDGF-induced vSMC migration by inhibiting PDGFR activation[82]. As another example, maresin 1 (MaR1) blunts CCL2

production in vSMCs by inhibiting TNFα-dependent NFκB activation[83]. Furthermore, MaR1 treatment also blocks vSMC migration towards PDGF and attenuates neointimal hyperplasia[84].

Alterations in cell metabolism following efferocytosis also contribute to macrophage-vSMC crosstalk. Internalization of an AC stimulates aerobic glycolysis and expression of the lactate transporter Slc16a1[85]. Upon its release by efferocytosing macrophages, lactate then acts in a paracrine manner in nearby macrophages to upregulate production of the pro-resolving mediators TGFβ and IL-10. Interestingly, lactate is also taken up by vSMCs, and vSMCs take on a more proliferative and matrix-producing phenotype in an environment high in lactate[86]. However, there appears to be a balance in lactate in the microenvironment as uncontrolled uptake of lactate by vSMCs accelerates calcification[87]. Polyamines synthesized from macrophages after efferocytosis may be secreted and act on vSMCs in atheromas to promote plaque stability. Polyamines can promote vSMC migration and proliferation, features that lead to fibrous cap thickening. Interestingly, polyamine uptake by vSMCs requires caveolin-1 (Cav-1), and vSMCs isolated from Cav-1 KO mice are highly migratory and proliferative in a manner that is polyamine-dependent[88]. Altogether, these studies point to a model whereby macrophages produce pro-resolving mediators to restrict necrotic core expansion, and surrounding vSMCs respond by establishing a matrix-dense fibrous cap (Fig. 2).

Substantial advances in the field of bioinformatics, particularly in single-cell RNA sequencing (scRNA-seq), has provided detailed analysis of the transcriptome in individual cells and is beginning to validate the existence of cellular crosstalk within atherosclerotic plaques[89]. For instance, scRNA-seq has been used to profile the cellular landscape of atherosclerotic plaques from both humans and mice, revealing a larger diversity of immune cells in lesions than previously appreciated. In individuals with stroke, macrophages show high expression of CCL5, and interestingly, its interaction with its cognate receptor CCR5 on vSMCs drives proliferation, de-differentiation, and vascular remodeling, demonstrating the presence of cell-cell communication within atherosclerotic plaques[90, 91]. As another example, using “scTalk”, an advanced network-based modeling method using confirmed interactions from StringDB, suggest that vSMCs signal to fibroblasts via C3 complement and MMP2[92]. New insights provided by scRNA-seq have also confirmed that vSMCs execute de-differentiation programs and give rise to a population of SEM cells (stem cell, endothelial cell, monocyte) that may further transform into fibrochondrocyte-like cells, taking on features associated with inflammation and ECM degradation[92, 93].

Concluding Remarks and Future Directions

Cholesterol-lowering therapies are an effective strategy to lower the risk of ASCVD. However, despite the availability of tools that aggressively lower LDL-cholesterol, the residual risk that remains causes ASCVD to continue being the leading cause of morbidity and mortality world-wide. The success of the CANTOS and COLCOT trials demonstrate that lowering inflammation is a key requisite for attenuating this residual risk[8, 9]. However, individuals on anti-inflammatory therapies remain susceptible to infection-related adverse outcomes. Therefore, therapeutic strategies that focus on transitioning a rupture-prone

plaque into a stable plaque may be more desirable. The concept that macrophages and vSMCs crosstalk within atheromas and that their communication can either promote plaque stability or contribute to plaque vulnerability remains understudied. Achieving a deeper mechanistic insight into understanding the crosstalk between vSMCs and macrophages will lead to strategies that limit matrix proteolysis, enhance ECM deposition in the fibrous cap, tune vSMC phenotype, and drive the pro-resolving function of macrophages. Furthermore, defining how the tissue microenvironment contributes to the non-resolving aspect of cardiovascular disease may lead to identifying how soluble and insoluble factors guide macrophage and vSMC communication within atheromas.

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Nonstandard Abbreviations and Acronyms:

AC	apoptotic cell
Arg1	arginase 1
ASCVD	atherosclerotic cardiovascular disease
ECM	extracellular matrix
GAGs	glycosaminoglycans
IL	interleukin
KLF4	kruppel-like factor 4
LRP1	LDL receptor related protein 1
MaR1	maresin 1
MerTK	MER tyrosine-protein kinase
ODC1	ornithine decarboxylase 1
Rv	resolvin
SPMs	specialized pro-resolving mediator
SRF	serum response factor
vSMC	vascular smooth muscle cell

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HIGHLIGHTS

- Atherosclerotic plaque stability is controlled by the balance between resolution mediators and pro-inflammatory factors.
- The tissue microenvironment influences macrophage and vSMC function by tuning responses to soluble and insoluble factors.
- Cellular crosstalk between macrophages and vSMCs control plaque stability.

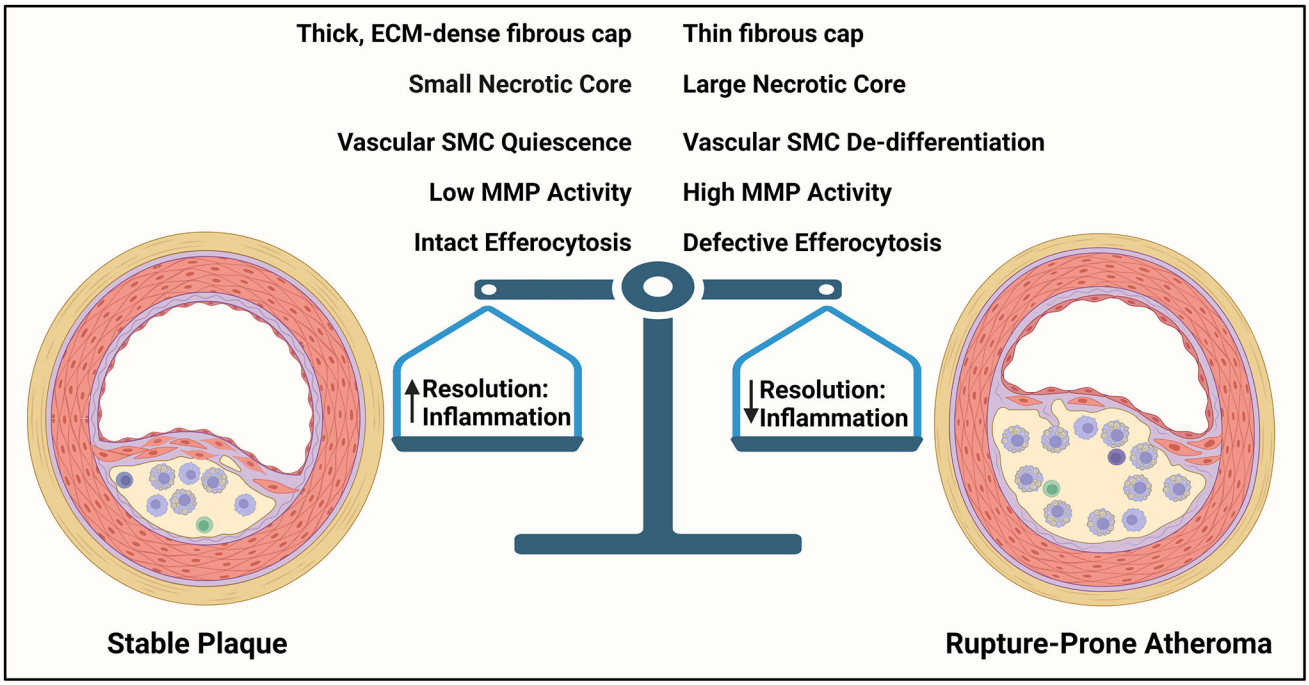

Figure 1. Reciprocal Crosstalk between Macrophages and vSMCs.

Macrophages clearing dead cells produce pro-resolving mediators, such as IL-10, TGFβ, and SPMs. These in turn cause vSMCs to differentiate and deposit ECM, leading to thicker fibrous caps. However, when efferocytosis is impaired, macrophages release pro-inflammatory mediators, such as TNFα, IL-1β, and IL-6 that cause vSMC de-differentiation, ECM degradation, and fibrous cap thinning. These functions are reciprocal and can lead to either plaque stability or rupture-prone atheromas.

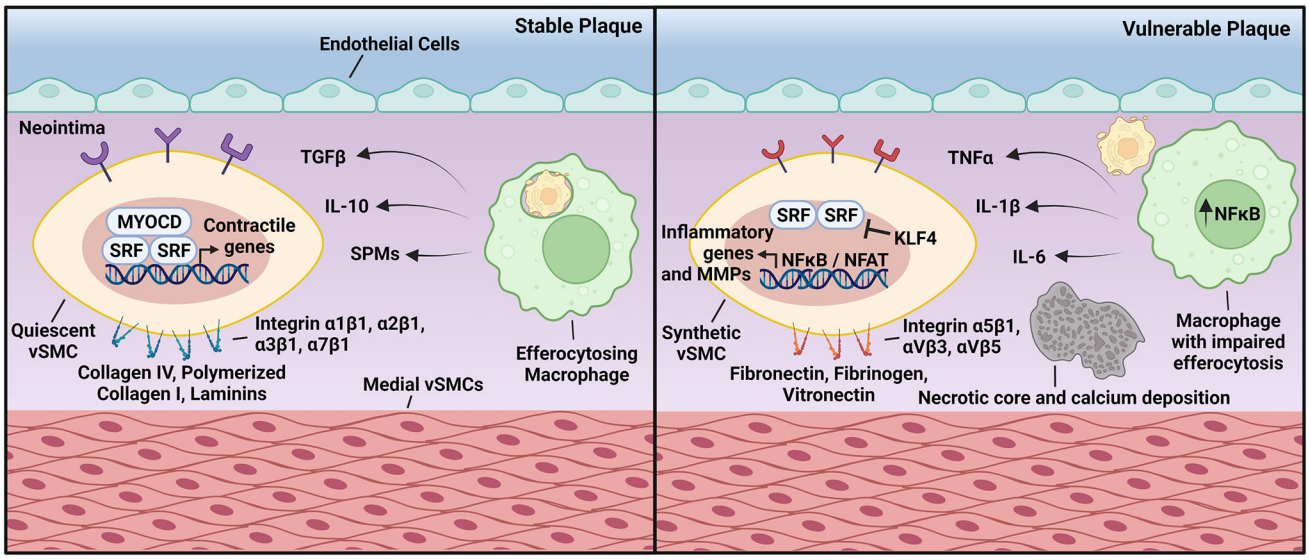

Figure 2. Crosstalk Between Macrophages and vSMCs in Atherosclerosis.

(Left) In stable atherosclerosis, lesional macrophages clearing apoptotic cells produce TGFβ, IL-10, and SPMs. These pro-resolving molecules bind to receptors present on the surface of vSMCs that drive Myocardin/SRF binding to CArG boxes, which promote the expression of genes associated with vSMC quiescence. This can be further enhanced by the interaction between vSMCs and the surrounding ECM through integrins α1β1, α2β1, α3β1, and α7β1. (Right) Plaques vulnerable to rupture contain macrophages with impaired efferocytosis, which leads to the secretion of the proinflammatory cytokines TNFα, IL-1β, and IL-6. These bind to their cognate receptors in vSMCs and downregulate Myocardin expression. Inflammatory cytokines also stimulate the association of KLF4 to G/C elements that prevent SRF binding to CArG boxes. Interactions between vSMCs and the ECM proteins fibronectin, fibrinogen, and vitronectin cause NFκB and NFAT activation and promotes the expression of MMPs and inflammatory genes.