Arteriosclerosis, Thrombosis, and Vascular Biology

***ATVB* IN FOCUS:**
The Science of *ATVB* Early Career Committee

Series Editor: Robert A. Hegele

Macrophages in Atherosclerosis Regression

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ABSTRACT: Macrophages play a central role in the development of atherosclerotic cardiovascular disease (ASCVD), which encompasses coronary artery disease, peripheral artery disease, cerebrovascular disease, and aortic atherosclerosis. In each vascular bed, macrophages contribute to the maintenance of the local inflammatory response, propagate plaque development, and promote thrombosis. These central roles, coupled with their plasticity, makes macrophages attractive therapeutic targets in stemming the development of and stabilizing existing atherosclerosis. In the context of ASCVD, classically activated M1 macrophages initiate and sustain inflammation, and alternatively activated M2 macrophages resolve inflammation. However, this classification is now considered an oversimplification, and a greater understanding of plaque macrophage physiology in ASCVD is required to aid in the development of therapeutics to promote ASCVD regression. Reviewed herein are the macrophage phenotypes and molecular regulators characteristic of ASCVD regression, and the current murine models of ASCVD regression.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: atherosclerosis ■ coronary artery disease ■ inflammation ■ macrophages ■ thrombosis

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of morbidity and mortality worldwide.[1] Vascular inflammation, even after robust cholesterol lowering, is considered an important contributor to the risk of recurrent atherothrombotic events, and macrophages represent a likely contributor to residual inflammatory risk. Initiated by the retention of apoB (apolipoprotein B)-containing lipoproteins in the arterial wall, ASCVD represents a failure to resolve the inflammatory response.[2–4] Vascular lipid deposits activate the immune system leading to the local accumulation of both innate and adaptive immune cells, which facilitates the formation of lipid-rich lesions. Plaques may form in any number of vascular beds, with ASCVD most commonly referring to either coronary artery disease, peripheral artery disease, cerebrovascular disease, or aortic atherosclerosis. Atherosclerotic lesions grow slowly over the years, eventually impeding blood flow and leading to the clinical manifestation of stable angina or claudication. However, obstructive and nonobstructive lesions may also erode or abruptly rupture, resulting in the local accumulation of tissue factor and platelet activation, culminating in rapid thrombotic vascular occlusion and life-threatening conditions, such as myocardial infarction, stroke, acute limb ischemia, and cardiovascular death.

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MACROPHAGES IN ASCVD PROGRESSION

Predominantly derived from circulating monocytes and local proliferation,[5–8] macrophage numbers increase up to 20-fold within mouse aortae during atherogenesis.[2,9,10] Additionally, there is evidence that vascular smooth muscle cells can dedifferentiate to a plaque macrophage-like state.[11–14] Recruitment of monocytes into the intimal space is a process that occurs early in life, with the initial stages evident in infants less than a year old,[15,16] and

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Nonstandard Abbreviations and Acronyms

APC	antigen-presenting cell
apoB	apolipoprotein B
ASCVD	atherosclerotic cardiovascular disease
CCR7	C-C chemokine receptor type 7
FOURIER	Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects with Elevated Risk
HDL-C	high-density lipoprotein cholesterol
HSP	heat shock protein
IL	interleukin
KLF4	Krüppel-like factor 4
LDL	low-density lipoprotein
LDL-C	LDL cholesterol
LDLR	LDL receptor
LXR	liver X receptor
MERTK	tyrosine-protein kinase MER
PCSK9	proprotein convertase subtilisin/kexin type 9
STAT	signal transducer and activator of transcription
TNF-α	tumor necrosis factor-α
Tregs	regulatory T cell
TREM	termed triggering receptor expressed on myeloid cells 2

Highlights

- Macrophages in atherosclerotic cardiovascular disease play a central role in the development of plaques.
- Classically activated M1 macrophages are implicated in initiating and sustaining inflammation, and alternatively activated or M2 macrophages are linked to inflammation resolution.
- Macrophage plasticity makes them attractive therapeutic targets to stem the development of and stabilize existing atherosclerosis.
- Understanding the basis of metabolic and epigenetic reprogramming of macrophage polarization is anticipated to translate to new therapeutic opportunities to promote atherosclerotic cardiovascular disease regression to reduce residual inflammatory risk.

atherosclerotic plaques are prevalent in adolescents and young adults.[17–19] ASCVD represents a chronic inflammatory process that continues throughout adulthood, and for many culminates in a major adverse cardiac event.

A key feature of ASCVD is lipoprotein ingestion and accumulation by arterial macrophages, which gives rise to foam cells. Foam cell buildup contributes to plaque lipid storage and sustained plaque growth.[10,20] Macrophages contribute to the maintenance of the local inflammatory response by secreting proinflammatory cytokines, chemokines, and producing reactive oxygen and nitrogen species. Additionally, macrophages engage in crosstalk with vascular smooth muscle cells, amplifying the inflammatory cycle by producing additional proinflammatory cytokines and extracellular matrix components, further promoting the retention of lipoproteins.[2,21] Plaque macrophages have a decreased ability to migrate, impeding inflammation resolution, promoting the progression of lesions into complicated, rupture-prone plaques. Moreover, this persistent inflammation drives macrophage apoptosis, and in the absence of efficient efferocytosis, leads to the accumulation of debris and apoptotic cells, facilitating necrotic core formation in atherosclerotic plaque.[2,10,22]

A defining feature of macrophages is their plasticity, which allows them to produce a tailored response to local microenvironment stimuli.[23–26] During inflammation, macrophages may act to either promote inflammation or resolve it during wound and tissue repair.[24,27] The classical model of macrophage activation defines both pro- and anti-inflammatory macrophages with distinct physiological roles and activators. At the broadest level, macrophages are classified as either M1, classically activated, or M2, alternatively activated.[23,28] In vitro, M1 macrophages polarize in response to toll-like receptor ligands, interferons, pathogen-associated molecular complexes, lipopolysaccharides, and lipoproteins. Fueled primarily by glycolysis,[29] M1 macrophages contribute to tissue destruction and secrete pro-inflammatory factors including high levels of IL (interleukin)-1β, IL-6, and TNF-α (tumor necrosis factor-α).[26,30,31] Consistent with their inflammatory phenotype, they express pro-inflammatory transcription factors, including nuclear factor-κB and STAT (signal transducer and activator of transcription)-1. M2 macrophages are at the other end of the spectrum with a fatty acid oxidation dependent-phenotype and anti-inflammatory properties.[32] M2 macrophages are polarized in response to the cytokines IL-4 and IL-13 and secrete anti-inflammatory factors such as the IL-1 receptor agonist, IL-10 and collagen. M2 macrophages are characterized by their expression of CD163 (cluster of differentiation 163), mannose receptor 1, resistin like-β, and high levels of arginase-1.

In the context of plaques, macrophages adhering to both the classically activated and alternatively activated subsets are present in human and mouse lesions, with M1 as the predominant subtype.[33–37] In human lesions, macrophages expressing proinflammatory markers are in rupture-prone, unstable regions, and M2-like macrophages in stable regions and the adventitia.[38–43] However, recent evidence suggests that macrophages exist on an activation continuum and that the M1/M2 classification system is an oversimplification of macrophage heterogeneity and their diverse functions.[36,43,44]

In the context of murine ASCVD, several alternative macrophage classifications are described.[36,45] These alternate phenotypes include hemorrhage-residing Mhem

macrophages, which phagocytize and use erythrocyte remnants and hemoglobin deposits.[46–48] This subset is atheroprotective and resistant to foam cell formation, attributed to their high expression of the cholesterol transporters ABCA1 (ATP-binding cassette transporter A1) and ABCG1 (ATP-binding cassette transporter G1) and the nuclear receptors, LXR (liver X receptor)-α and LXR-β.[49] Mox macrophages, a proatherogenic subset induced by oxidized phospholipids that protect from oxidative stress through nuclear factor erythroid-derived 2-related factor 2–mediated expression of antioxidant enzymes such as heme oxygenase 1, thioredoxin reductase 1, and sulfiredoxin-1. In hypercholesterolemic mice, Mox macrophages are reported to account for 30% of plaque macrophages, with M1 and M2 subsets making up 40% and 20% of the remaining cohort, respectively.[50] Finally, M4 macrophages are a subset polarized by platelet factor 4.[51] This population is found in human lesions[52] and characterized by high expression of matrix metalloprotease 7 and S100A8.[53] M4 macrophages are defined as atherogenic based on their production of proinflammatory cytokines (IL-6 and TNF-α) and defective phagocytic properties.[51,52]

Macrophage heterogeneity in plaques was first appreciated using immunohistochemistry and, at the molecular level, by laser capture microdissection.[54,55] Technological advances, including mass cytometry time of flight and single-cell RNA sequencing, have further expanded our knowledge of macrophage heterogeneity in progressing plaques.[56,57] These technologies have assisted in characterizing the heterogeneous nature of plaque macrophages, and have identified a new previously unreported subset identified.[58–60] Termed triggering receptor expressed on myeloid cells 2 (TREM)^{hi} macrophages, this subset expresses high levels of the genes *Trem2*, *Cd9*, *Ctsd,* and *Spp1* and low expression of inflammatory cytokines, with ascribed biological functions of lipid metabolism and cholesterol efflux.[58–60] This unique population is proposed to be cholesterol-enriched and represents foamy macrophages.[59] Altogether, TREM^{hi} macrophages provide an alternate hypothesis in which macrophages subsets in plaques are inflammatory.

As described above, there are various modes of macrophage activation. Collectively, they demonstrate that macrophages in plaques may have only a partial resemblance to M1 and M2 macrophage phenotypes. Further research is necessary to identify gene-expression profiles and transcriptional pathways that underlie the identity and diversity of macrophages in ASCVD. Additionally, whether results in mice are translatable to human plaques, which have distinct phenotypic differences (eg, hemorrhage and rupture), is essential to determine for the development of therapies to reduce macrophage-associated residual inflammatory risk.

ATHEROSCLEROSIS REGRESSION

Macrophages are the hallmarks of ASCVD contributing to plaque development, local inflammation, and the promotion of thrombosis. This central role, coupled with their plasticity, makes macrophages attractive therapeutic targets to stem the progression of plaques and stabilize existing atherosclerosis.

Studies in the 1970s undertaken in nonhuman primates and pigs made the initial observations of macrophages contributing to atherosclerosis regression.[61–63] These seminal studies employed atherogenic high-fat, high-cholesterol diets to induce atherosclerosis progression and subsequent low-fat, low-cholesterol diets to reduce hypercholesterolemia. In both models, 4 to 6 months of regression diet feeding decreased aortic lesion macrophage foam cells, reduced necrotic plaque area, and increased the thickness and density of fibrous caps. An ASCVD regression review in 1985 stated, "it is obvious that the role of macrophages in regression may be very complex and a comprehensive study of such is unattainable by a single experiment by one or a small group of investigators".[61] Since then, the generation of hyperlipidemic mouse models,[64–66] extensively used to model human ASCVD,[67,68] which allow for the rapid, reproducible development of plaques, has further increased the field's understanding of the regulators of plaque progression. In 2001, in response to the need for further murine atherosclerosis model development basic research into the mechanisms that govern ASCVD regression or stabilization was stimulated by the establishment of an aortic transplantation approach.[64]

Clinical trials in humans have demonstrated that robust cholesterol reduction prevents major adverse cardiovascular events.[65,66] Imaging studies using intravascular ultrasound and optical coherence tomography suggest that dramatic LDL (low-density lipoprotein) lowering (ie, statins, PCSK9 [proprotein convertase subtilisin/kexin type 9] inhibition) prevents plaque progression and may even induce plaque regression.[67–70] The development of LDL-C (LDL cholesterol)-lowering therapies that facilitate unprecedented reductions in LDL-C, relative to traditional statins, are likely to provide further insight into the role of residual inflammatory risk and plaque progression and regression.[22,71–74] Advances in imaging techniques provide insight into the compositional changes in remodeling plaques.[75] Optical coherence tomography allows detailed visualization of plaques and provides information on plaque composition (eg, lipids and calcification) and thickness of the fibrous cap, a classical marker of plaque inflammation and vulnerability. Given that plaque lipid concentrations are positively associated with macrophage accumulation, this relationship provides indirect evidence for reduced plaque macrophage count during human ASCVD regression.[76]

Evidence for monocyte and macrophage phenotypes associated with plaque vulnerability are derived from plaques taken from subjects with different stages of atherosclerosis.[41–43,77] However, translation of macrophage studies in mice to human ASCVD regression carries the caveat that responses of monocyte-derived macrophages from mice and humans are still needed to be compared side-by-side.[24] Further imaging advancements indicate

that monitoring of plaque macrophage content and phenotype may one day be a possibility in humans as it is in mice.[78] These data are likely to further our understanding of human and murine lesions during ASCVD regression.[79,80]

Reviewed below are the current murine models of ASCVD regression, the well-described contribution of macrophages, and preclinical efforts to develop macrophage-targeted therapeutics to suppress plaque growth and inflammation.

MOUSE MODELS OF ASCVD REGRESSION

Relative to baseline plaques, murine ASCVD regression encompasses one or more of the following, a reduction in plaque (1) size, (2) cholesterol content, or (3) macrophage content. All models begin with a progression phase to establish a baseline plaque in which plasma apoB lipoprotein levels are high (LDL-C >300 mg/dL), followed by a phase of low atherogenic lipoprotein levels (VLDL [very-low–density lipoprotein] and LDL-C) to induce regression. Preclinical models of ASCVD regression are considered analogous to high-intensity statin treatment in humans.[81]

Plaque Transplantation

Representing the first ASCVD model of regression, the plaque transplant model involves transplanting an atherosclerotic thoracic arch[64] or aortic arch segment[82,83] from a hyperlipidemic donor mouse (eg, *ApoE*$^{-/-}$ or *Ldlr*$^{-/-}$) into a normolipidemic recipient mouse (ie, C57Bl/6J). The rapid change in atherogenic apoB lipoproteins induces plaque regression, characterized by decreased lesion, macrophage, and lipid areas over a short period.[34,64,74,84,85] This original model has been instrumental in elucidating mechanisms that contribute to regression and the assessment of ASCVD-reducing therapies.[30,74,86,87]

Elimination of Atherogenic Lipoprotein Production

Reversa mice are LDLR (LDL receptor) deficient and genetically modified via the introduction of a conditional allele of Mttp (microsomal triglyceride transfer protein) in the liver (*Ldlr*$^{-/-}$*Apob*$^{100/100}$*Mttp*$^{fl/fl}$*Mx1-Cre*$^{+/+}$) and can serve as a reversible ASCVD model.[88] Inhibition of microsomal triglyceride transfer protein (IFN-α [interferon-alpha], IFN-β, or synthetic double-stranded RNA [eg, polyinosinic:polycytidylic acid, pIpC]), in conjunction with a switch to a chow diet, results in reduced VLDL and LDL (apoB lipoprotein cholesterol; >1000 mg/dL to <150 mg/dL) and rapid regression of plaques.[89] Similar changes in atherogenic lipoproteins and plaque regression can also be achieved in *Ldlr*$^{-/-}$ mice treated with the microsomal triglyceride transfer protein inhibitor, such as BMS 212122.[90] Additionally, the elimination of apoB production with an *Apob* antisense oligonucleotide promotes ASCVD regression in *Ldlr*$^{-/-}$ mice.[91]

Modulation of LDL Receptor or ApoE Expression

Early lipid lowering approaches in mice used adeno-associated virus therapies to induce hepatic overexpression of *Apoe* in *ApoE*$^{-/-}$ mice and *Ldlr* in *Ldlr*$^{-/-}$ mice.[92–94] Recent studies in wild-type mice have also described the utility of LDLR antisense oligonucleotides in raising apoB lipoprotein cholesterol levels and withdrawal of the antisense oligonucleotide and subsequent antagonism with sense oligonucleotides to accelerate the reduction of lipid levels and facilitate regression.[95] Similarly, increasing PCSK9 levels, a protein that directs hepatic LDLR for degradation,[96] elevates circulating LDL-C and induces atherosclerosis in wild-type mice.[37,97–99] The PCSK9 adeno-associated virus model can be used for regression studies by a diet switch to chow, with lipid lowering further accelerated by the inclusion of an microsomal triglyceride transfer protein inhibitor to the diet.[37] Given that they override the necessity for complicated and time-consuming backcrosses, ASCVD models that are genotype-independent will facilitate the relatively rapid assessment of factors that regulate ASCVD regression. Further, mechanistic work will benefit from the rapid induction of gene-specific phenotypes in adult mice with established lesions to test candidates that may regulate regression.[100]

Reduction in Dietary Cholesterol

A simple switch in diet from an atherogenic high-fat, high-cholesterol diet to a low-fat, low-cholesterol diet in some atherosclerosis-prone mouse models is sufficient to induce ASCVD regression and comparable to the use of statins or beneficial dietary changes in humans. However, this method of regression is slower and, in some mice apoB lipoprotein cholesterol levels (eg, *ApoE*$^{-/-}$ mice) may not normalize to sufficient levels to achieve regression.[37,101,102]

MACROPHAGES IN ASCVD REGRESSION

Plaque macrophage content is determined by monocyte recruitment and macrophage proliferation, emigration, and death.[2] Historically, atherosclerosis studies have placed a significant emphasis on understanding mechanisms of monocyte recruitment into the vascular wall and devising strategies to block their influx into plaques.[2] However, recent studies show that there are also factors that determine macrophage retention within plaques,[10] and it is hypothesized that if these processes are favorably modulated, plaque macrophage content may be reduced and ASCVD regression achieved.

Broad changes in the plaque macrophage transcriptome are characteristic of ASCVD regression, most commonly distinguished by enrichment of M2-associated transcripts.[54,85,86,103–105] The dynamic change in plaque macrophage phenotype raises the possibility that inducing of macrophage polarization potential in vivo will represent a viable therapeutic option for ASCVD regression and suppress residual inflammatory risk. Detailed below are known factors that modulate plaque macrophage content and phenotype in the context of ASCVD regression.

FACTORS THAT REGULATE PLAQUE MACROPHAGE REGRESSION

Macrophage Trafficking

Plaque macrophage emigration is characteristic of regressing lesions in several murine models of regression.[86,103,106] Consistent with macrophage motility, cytoskeletal-binding, and Rho GTPase genes are among the top enriched transcripts in plaque macrophages in response to lipid lowering.[107] One mechanism for decreased plaque macrophage content shown in the aortic transplantation model is increased macrophage egress to lymph nodes, mediated via CCR7 (C-C chemokine receptor type 7).[87,106] Macrophage CCR7 transcript expression is, in part, regulated by a sterol response element in its promoter. Thus, it is hypothesized that lipid lowering induces plaque macrophage CCR7 expression and migration.[85,108] In mice deficient in low-density lipoprotein receptor-related protein 1, a separate group independently presented evidence for a role of CCR7 in regression. During regression, LRP-1 (low-density lipoprotein receptor-related protein 1) deficiency increased macrophage cholesterol efflux and CCR7 expression and promoted macrophage emigration to lymph nodes and plaque regression.[109]

Epigenome-guided analysis of the transcriptome of plaque macrophages during ASCVD regression revealed activation of the Wnt signaling pathway.[107] Given that in macrophages, Wnt signaling promotes cell motility through a β-catenin-dependent mechanism,[110] and β-catenin knockdown promotes atherosclerosis progression,[111] this pathway may represent an unexplored regulator of ASCVD regression. Additionally, the macrophage retention factors, netrin 1 and semaphorin 3E, transcripts differentially regulated in progressing and regressing plaques, also hold promise as potential mediators of plaque macrophage content given their ability to modulate macrophage retention and migration in progressing plaques.[2,54,112,113]

Monitoring plaque macrophage flux is an essential component in the assessment of the effectiveness of therapies designed to promote ASCVD regression. The most readily used technique in murine regression studies takes advantage of the ability of monocytes to take up fluorophore-labeled beads (eg, Rahman et al,[74] Nagareddy et al,[86] Distel et al,[103] and Potteaux et al[114]). The monocyte bead labeling technique can be used to monitor plaque monocyte entry and macrophage egress.[89] To monitor entry, mice are injected intravenously with beads before harvest (typically 24–72 hours), and to monitor macrophage egress, mice are injected with beads before the induction of regression (typically 24–96 hours). Quantification of bead-bearing cells in plaques at the time of harvest allows for the assessment of monocyte and macrophage trafficking.

This technique represents a relatively rapid and straightforward labeling procedure and does not alter the phenotype of bead-bearing cells.[115] The predominant drawback of this approach is the relatively low incorporation of beads into circulating monocytes (≈5%–10%) and the selectivity of Ly6C^{lo} monocytes in taking up the beads, the monocyte subset with reduced capacity to enter lesions. Prior injection of mice with clodronate liposomes, to deplete all circulating monocytes, can skew bead labeling to the Ly6C^{hi} subset.[116] However, this comes with the inherent drawback of potential depletion of plaque-residing macrophages given their ability to deplete macrophage populations in the spleen, bone marrow, and liver.[116,117] Alternatively, Ly6C^{hi} monocytes may be labeled with the modified thymidine analog EdU (5-ethynyl-2´-deoxyuridine) that at time points less than 72 hours is selectively incorporated in the Ly6C^{hi} subset.[74,114] Ly6C^{hi} monocytes that enter plaques are determined by staining for EdU. Dual staining with the proliferation marker Ki67 allows for the distinction between recruited monocytes, and macrophages proliferating in situ.

Fluorescent reporter lines (eg, GFP-CD68 [green fluorescent protein CD68], CX3CR1-GFP [CX3C chemokine receptor 1 green fluorescent protein]) and congenic mice (eg, CD45.1, CD45.2) also allow for monitoring plaque monocyte/macrophage trafficking.[104,116] The utility of these models is best suited to regression studies undertaken in the transplant model, as this allows for differential labeling of either donor plaque cells or recipient circulating cells to assess regression-mediated changes. The utilization of inducible reporter lines (eg, CreloxP or FLP-FRT [flippase/flippase recognition target] system) that could be triggered at the time of regression also represents a viable method to track myeloid cell trafficking in transplant-free regression models. Fluorescent reporter lines represent a useful model to monitor monocyte/macrophage flux via intravital imaging. However, the relatively short monitoring window and the need to expose the site of interest to detect signals limit this approach.

Macrophage Polarization

Macrophage polarization is considered a dynamic process,[23,26] and editing of this potential is an emerging therapeutic area for a variety of inflammation-based disorders.[23,118] M1 macrophages characterize progression lesions while regressing plaques are enriched in M2 macrophages.[69] M2 macrophage enrichment in plaque regression is consistent with the view that M1 macrophages are pro-atherogenic and promote an unstable plaque, while

M2 macrophages promote tissue repair and plaque stability. In vitro, the phenotypes of M1 and M2 macrophages are reversible, and there is evidence that this may occur in mice in vivo.[119–121] However, whether macrophage interconversion occurs in the context of ASCVD regression, in humans or mice, is largely unknown and is an area of active research. Alternate possibilities for plaque M2 macrophage enrichment during regression are (1) egress of M1 macrophages from plaques, (2) entry of monocytes and their polarization to M2 macrophages, and (3) proliferation of resident yolk-sac-derived M2 macrophages.[6,122]

In atherogenic mice, the deletion of NR4A1, a transcription factor that regulates the Ly6C^{lo} monocyte phenotype and favors M2 macrophage differentiation,[123] results in plaque M1 macrophage enrichment and accelerates atherosclerosis.[124,125] Similarly, the deletion of the transcription factor KLF4 (Krüppel-like factor 4), which promotes M2 and inhibits M1 macrophage polarization,[126] enhances pro-inflammatory M1 macrophage activation, foam cell formation, and accelerates atherosclerosis in *ApoE*$^{-/-}$ mice.[127] Likewise, stimulation of the PPAR-γ (peroxisome proliferator-activated receptor-gamma) pathway, which promotes M2 macrophage polarization,[128] decreases atherosclerosis development in the *ApoE*$^{-/-}$ and Reversa mice.[89,129] Similarly, loss of Akt2 enhances the ability of macrophages to polarize to the M2 state and suppresses atherogenesis.[130] Treatment of *Ldlr*$^{-/-}$ mice with the M2-polarizing cytokine IL-13 promotes a plaque M2 macrophage phenotype, along with an increase in plaque collagen content, and a reduction in monocyte recruitment in lesions and macrophage content.[131] However, it remains to be established whether the mechanisms that promote or hinder macrophage M2 polarization under conditions of hypercholesterolemia can be applied to regressing plaques.

A recent study reported that after reversal of hypercholesterolemia, the recruitment of Ly6C^{hi} monocytes and their STAT6-dependent conversion to M2 macrophages is essential for reducing plaque macrophage content and suppression of ACSVD inflammation during regression.[74] To date, the factors that regulate STAT6-signaling to mediate this change are unknown, but given IL-4 and IL-13 facilitate M2 polarization through a STAT6-dependent pathway, it is hypothesized that local production of these cytokines (eg, by basophils, eosinophils) during regression may mediate this process. Important to note, however, are findings in an alternate model of plaque regression, which found that suppression of monocyte recruitment is essential for plaque macrophage regression.[114] This is consistent with studies in diabetic mice showing impaired regression after lipid lowering because of increased monocyte recruitment.[103] These findings in different murine models of regression reveal that many different pathways positively affect the regression of established plaques. They also highlight that despite robust apoB lipoprotein lowering, plaques did not regress completely, providing evidence for additional unidentified mechanisms that contribute to residual inflammatory risk during regression exist.

A defined function of macrophages is their efferocytotic capacity, an essential process for the resolution of inflammation and plaque stabilization by reducing necrotic core area.[132,133] Macrophage efferocytosis, referring to their ability to clear apoptotic cells and debris, is mediated through receptors including MERTK (tyrosine-protein kinase MER), LRP-1 and CD47 and is regarded as a protective anti-inflammatory function of M2 macrophages.[134,135] Thus, enrichment of M2 macrophages, or enhancement of macrophage efferocytotic capacity (eg, via PPAR-γ activation[136]), may represent a viable strategy to promote ASCVD regression and plaque stabilization.

Recent studies demonstrate that macrophage inflammatory responses and their metabolism are codependent.[137] Classically activated M1 macrophages shift to anabolic metabolism by upregulating either glycolysis or the pentose-phosphate pathway, while M2 macrophages are fueled by oxidative phosphorylation and fatty acid oxidation.[138] In the context of ASCVD, environmental signals including hyperlipidemia, hypoxia, and hyperglycemia skew macrophage polarization toward a glycolytic inflammatory M1-like phenotype, a macrophage phenotype of both unstable murine and human plaques.[77,86,103,139–142] How metabolic shifts in macrophages contribute to lesion progression and stability and the changes that occur after LDL-C lowering are currently unknown. However, preclinical studies provide insight into how reprogramming of macrophages to an anti-inflammatory M2-like phenotype suppresses plaque progression. Antagonism of miR-33, a microRNA elevated in macrophages in progressing lesions, promotes regression[105] and skews macrophages towards an M2 state, as evidenced by increased mRNA expression of genes encoding AMP kinase and fatty acid oxidation, elevated mitochondrial respiration, and decreased glycolysis.[102] Additionally, the treatment of *Ldlr*$^{-/-}$ mice with the M2-polarizing cytokine IL-13 inhibits atherosclerosis progression, in part by its ability to skew plaque macrophage phenotype towards an M2 state.[131]

Cholesterol Efflux

During ASCVD plaque regression, reductions in atherogenic apoB lipoproteins result in an improved HDL-C (high-density lipoprotein cholesterol) to LDL-C ratio. Increased concentrations of functional HDL particles are likely to represent a significant contributor to ASCVD regression, given their ability to mediate cholesterol efflux, facilitate foam cell migration, and induce M2 polarization.[85,120,143] In vitro, treatment of macrophages with HDL enhances the expression of M2 macrophage markers (*arginase 1* and *retnlb*) in a STAT6-dependent process[120] and induces the transcriptional regulator ATF3, a repressor of inflammation.[144] Given that ATF3 activates Wnt/β-catenin signaling in macrophages to mediate migration,[145] a process that is

promoted by HDL in cholesterol-loaded macrophages,[146] these data may provide further mechanistic insight into the beneficial effects of HDL during regression.

In vivo studies further support a role of raising levels of functional HDL particles during the regression phase, as demonstrated by studies where HDL was raised in transplant recipients (by transgenic overexpression of human apoA-I), or by infusion of HDL, and accelerated plaque regression.[85,147] Further, in *ApoE*$^{-/-}$ mice with established lesions, intervention with injections of apoA-I or statin-containing HDL particles suppressed plaque progression.[148–150] Antagonism of miR-33, a negative regulator of circulating HDL levels, as noted above, also represents another option to promote plaque M2 macrophage enrichment and plaque regression.[102,103,105]

The beneficial role of cholesterol efflux in ASCVD regression is supported by preclinical LXR-focused studies. LXR is a transcription factor that induces the expression of genes involved in cholesterol transport and efflux[151,152] and is essential for retarding atherosclerosis progression and promoting atherosclerosis regression.[84,153] Additionally, the nonspecific efflux molecule, cyclodextrin, promotes atherosclerosis regression via LXR-mediated macrophage reprogramming to improve cholesterol efflux and exert anti-inflammatory effects.[154]

The lymphatic vasculature, localized in the adventitia, may also represent an underappreciated pathway contributing to ASCVD regression.[155] In murine models, the lymphatic system was shown to be a critical component of reverse cholesterol transport, facilitating the effective removal of cholesterol effluxed from plaque macrophages.[155,156] In the aortic transplant model, lymphatic-mediated reverse cholesterol transport accounted for 50% of cholesterol delivery from cholesterol-loaded macrophages into the plasma compartment and was essential for ASCVD regression.[156] In mice, hypercholesterolemia is proposed to induce lymphatic dysfunction and drive atherosclerosis.[157,158] While it remains to be established whether lymphatic function is restored after cholesterol-lowering in mice, experimental evidence indicates that during the regression phase, plaque cholesterol content is reduced and monocyte-derived cells exit plaques with some reaching lymph nodes.[156] However, a recent report indicates that the proximity of a macrophage to the lymphatic vasculature before lipid lowering determines its egress capacity, rather than functional changes to the cell.[115] Whether lymphatic-mediated cholesterol mobilization represents a significant plaque stabilizing or regression-inducing pathway in humans is unknown.[159]

DIABETES MELLITUS IMPAIRS PLAQUE MACROPHAGE INFLAMMATION RESOLUTION

Despite advances in therapies to reduce CVD risk, patients with diabetes mellitus have a 2- to 4-fold higher risk of ASCVD and associated morbidity and mortality.[66,160,161] Notably, diabetes mellitus not only increases CVD events but also impairs the resolution or regression of ASCVD.[161] Consistent with the clinical data, diabetic mice have impaired atherosclerosis regression, as measured by the quantity and inflammatory state of plaque macrophages after aggressive lipid lowering.[103,104,162,163] Diabetes mellitus in mice also increases monocytosis by activation of myelopoiesis, enhancing monocyte infiltration and plaque macrophage content, and impairs the polarization of plaque macrophages to the M2 state despite lipid lowering.[86,104,164] Recently, we considered the outcome of raising functional HDL in diabetic mice and established that HDL can overcome diabetes mellitus–mediated impairments to regression by promoting a plaque M2 macrophage phenotype and suppressing aberrant myelopoiesis on lipid lowering.[103,143] In the context of regression, we ascribe the beneficial effects of raising functional HDL to its cholesterol efflux capacity and anti-inflammatory functions—pathways dysregulated in both diabetic patients and mice.[143]

THERAPEUTIC TARGETING OF PLAQUE MACROPHAGES

Even with potent cholesterol reduction, many patients experience a major adverse cardiac event. This is highlighted by data from the FOURIER trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects with Elevated Risk), which demonstrate that patients with ASCVD randomized to a PCSK9 inhibitor (in conjunction with statin therapy) reached a median LDL-C of 30 mg/dL; 10% of all patients still experienced a cardiovascular event during a median follow-up of 26 months.[165] These data, along with other trials, of potent LDL-C lowering, demonstrate that lowering cholesterol alone is not sufficient to completely reduce ASCVD-associated morbidity and mortality.[70,166–170] While robust LDL-C lowering is maintained with PCSK9 inhibitors and may induce ASCVD regression, the degree of regression is limited.[70] Residual inflammatory risk has emerged as a mechanism predisposing individuals to cardiovascular events, which remains even after aggressive LDL-C lowering therapies in humans.[22,72,73] Additionally, even in mice, aggressive LDL-C lowering does not, in most cases, lead to the complete regression of lesions (eg, [74,143]). Indeed, maximal plaque regression did not occur if a decrease in macrophage inflammation was prevented at the same time lipid levels were lowered.[74] In combination with lipid lowering therapies, specific targeting of plaque macrophage-mediated inflammation may represent a viable option to reduce residual inflammatory CVD risk in humans and accelerate plaque regression in both humans and mice. Namely, therapies that reduce plaque macrophage content by promoting macrophage efferocytosis, emigration, or polarization to a pro-resolving phenotype are likely to have beneficial clinical outcomes when coupled with optimal medical therapies.

Current preclinical efforts have included the targeted delivery of LXR agonists to reduce plaque macrophage inflammation and promote cholesterol efflux (eg, Guo et al[171] and Yu et al[172]). While the benefits of LXR-pathway activation are appreciated, preclinical studies have not translated well clinically, as synthetic LXR ligands strongly activate sterol regulatory element-binding protein 1c, inducing hypertriglyceridemia.[173] Recently, however, desmosterol and synthetic desmosterol mimetics were shown in vivo to specifically target LXR pathways in macrophages and have minimal effects on hepatocytes, providing a potential new therapeutic strategy.[174] In addition to reducing plaque lipid content, increased cholesterol efflux would be expected to favorably affect the inflammatory state of macrophages and enhance their ability to emigrate.[89,146]

Preclinical and clinical observations indicate that the reprogramming of plaque macrophages to an anti-inflammatory M2 phenotype will promote ASCVD regression and plaque stabilization. Treatment of *Ldlr*$^{-/-}$ mice with helminth-derived antigens, a eukaryotic parasitic worm that strongly induces anti-inflammatory, immune responses, was found to suppress myeloid cell activation, intraplaque inflammation (TNF-α, MCP-1) and reduce the recruitment of macrophages to lesions.[175] These studies raise the interesting hypothesis that helminth-derived components or alternate strategies to induce M2 polarization may provide novel opportunities to mediate ASCVD regression and reduce systemic inflammation.[176]

During ASCVD regression, the balance of M1 and M2 macrophages switches, with M2-like macrophages more

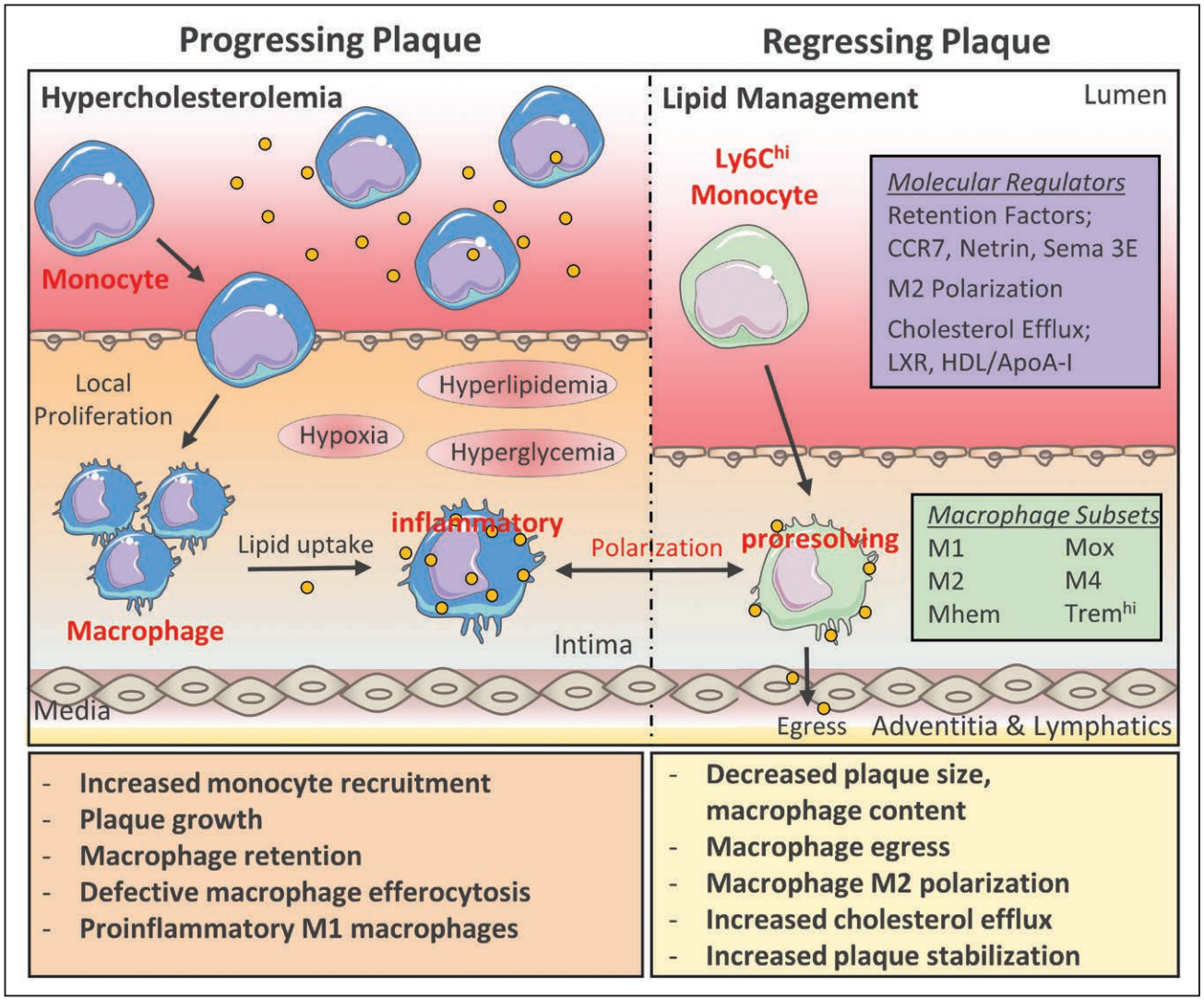

Figure. Dynamics of macrophage plasticity and trafficking in atherosclerosis.
Atherosclerotic lesions are characterized by proinflammatory macrophages which sustain lesion growth by contributing to local and systemic plaque inflammation. Atherosclerosis development and macrophage dysfunction is accelerated during hypercholesterolemia (high LDL-C [low-density lipoprotein-cholesterol), and hyperglycemia. Atherogenic lipid-lowering remodels lesions towards a stable phenotype, a process driven mainly by macrophages. Broad changes in the plaque macrophage transcriptome are characteristic of atherosclerosis regression, most commonly distinguished by enrichment of M2-associated transcripts. The dynamic change in plaque macrophage phenotype raises the possibility that inducing of macrophage polarization potential in vivo will represent a viable therapeutic option for atherosclerotic cardiovascular disease regression and suppress residual inflammatory risk.

predominant. The role of metabolic shifts in determining the phenotype of macrophages in lesions and how this alters in response to cholesterol-lowering is an area of active research. Further research to decipher what induces metabolic and epigenetic reprogramming in plaque macrophages, and factors that promote or inhibit macrophage polarization in vivo (ie, mitochondrial function, inducible glycolysis inhibitors[177,178]) will likely translate to new therapeutic opportunities to promote ASCVD regression.

VACCINE-MEDIATED SUPPRESSION OF PROINFLAMMATORY IMMUNE RESPONSES

Plaque macrophages and dendritic cells can function as APC (antigen-presenting cells) to activate members of the adaptive immune system including T and B cells, and evidence of APC-T cell interaction suggests antigen-specific immune activation through immune synapses in the plaque.[179] The interaction of plaque macrophages with T cells is increasingly recognized to alter macrophage and plaque inflammation, as factors secreted by T helper type 1, Th2, and regulatory T cells (Tregs) can differentially skew macrophage phenotype.[180] Tregs can dampen effector T cell responses by secretion of anti-inflammatory cytokines,[181–183] promote the polarization of M1 macrophages to M2 macrophages by secretion of IL-10, and reduce macrophage lipid accumulation.[184,185]

The beneficial effects of Tregs to influence macrophage phenotype indicate that therapies promoting endogenous and antigen-specific Treg activity may alleviate plaque inflammation. In the context of atherosclerosis, the primary antigens identified to be responsible for triggering T cell activation are epitopes of oxLDL (oxidized low-density lipoprotein),[186] apoB-100,[187] and HSP (heat shock protein) 60/65.[188] Research into immunization with antigenic proteins and peptides for the resolution of ASCVD by balancing pro- and anti-atherogenic T cell responses is currently confined to preclinical studies.[189,190] This approach, however, holds promise as atherosclerosis-relevant antigens have been shown to induce antigen-specific Tregs and be atheroprotective in mice.[191] These studies provide optimism that a therapy that restores tolerance to autoantigens may represent a viable strategy to reduce residual inflammatory risk in humans with ASCVD and promote ASCVD regression by polarizing macrophages to a tissue reparative state.

CONCLUSIONS

We increasingly recognize that on lipid lowering, macrophages can modulate plaque progression, regression, rupture, erosion, or stabilization. Detailed knowledge of how macrophage physiology contributes to clinical outcomes is essential to understand processes that promote macrophage pro-resolving characteristics. Further research is necessary to identify gene-expression profiles and transcriptional regulators of macrophage phenotype and function and reconcile how divergent plaque macrophage phenotypes (ie, M1, M2, Mhem, Mox, M4, and Trem^{hi}) contribute to ASCVD stability. Currently, pro-resolving macrophages that participate in efficient efferocytosis, tissue remodeling, migration, and suppression of inflammatory processes are considered optimal for atherosclerotic regression (Figure). Thus, the development of therapeutics to enrich this phenotype may reduce cardiovascular morbidity and mortality by resolution of residual inflammatory risk.

Additionally, despite preclinical models playing a crucial role in expanding our understanding of the heterogeneous nature of plaque macrophages, it is essential to ascertain how these findings translate to human pathophysiology. Translation to humans is essential given that the events that precipitate myocardial infarction, stroke, and acute limb ischemia (eg, plaque erosion and rupture), do not occur in current murine models. Recent advancements in lipid lowering therapies, which facilitate robust and sustained LDL-C reductions, are likely to increase our understanding of changes to plaque composition during regression. Additionally, an increased understanding of the role of macrophages in human ASCVD through omics studies and characterization of human tissue are anticipated to reconcile murine and human ASCVD and facilitate the development of strategies to promote ASCVD regression.

ARTICLE INFORMATION

Received December 24, 2018; accepted October 31, 2019.

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Acknowledgments

Sincere thanks to Edward A. Fisher and Jeffrey S. Berger and for their support and guidance and critical reading of this work. Additional thanks to the Reviewers of this paper and my fellow past and present lab members who have contributed to my knowledge and understanding of macrophages.

Sources of Funding

T.J. Barrett is supported by an American Heart Association Career Development Award (18CDA34110203AHA) and an American Society of Hematology Award (18-A0-00-1001884).

Disclosures

None.

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