Apolipoprotein E (apoE) has long been recognized as an important regulator of plasma lipoprotein metabolism and cholesterol homeostasis. However, despite decades of research into the biology of apoE, new aspects of its function continue to emerge. In this issue, Li et al demonstrate that apoE regulates the expression of an anti-inflammatory microRNA, miR-146a, in monocytes/macrophages. The authors reveal that miR-146a represses nuclear factor-κB (NF-κB) signaling in these cells and that intravascular delivery of miR-146a mimetics can inhibit atherogenesis in mouse models. These findings establish that enhancing miR-146a expression can antagonize atherogenesis and provide an impetus to pursue microRNA-based therapies for vascular inflammatory diseases.

ApoE Attenuates Atherosclerosis via miR-146a

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Initial work that characterized apoE function in the cardiovascular system demonstrated that apoE mediates the uptake of chylomicron remnants and triglyceride-rich very low and intermediate density lipoproteins in the liver. In arteries, together with apoA-I, apoE facilitates cellular cholesterol efflux from macrophage foam cells. Genetically engineered deficiency of Apoe in mice results in elevated plasma very low and intermediate density lipoproteins and spontaneous atherogenesis. Over the past 2 decades, this model has served as the mainstay of atherosclerosis research. Global Apoe deficiency can be rescued by transplantation of wild-type bone marrow, which normalizes plasma cholesterol levels and dramatically reduces atherosclerosis. Attenuated atherosclerosis is also observed in Apoe-null mice bred with transgenic mice expressing human apoE specifically in macrophages. These mice have plasma cholesterol levels comparable with control littermates. Interestingly, a recent study revealed that global hypomorphic expression of apoE has no effect on plasma cholesterol, yet atherosclerosis is suppressed and the expression of monocyte and endothelial cell inflammatory genes are reduced relative to complete Apoe deficiency in the low-density lipoprotein receptor–deficient (Ldlr−/−) background. These studies highlight the importance of apoE in cholesterol efflux and other antiatherogenic functions.

Independent of its critical functions in lipoprotein metabolism, early studies also revealed immunomodulatory properties of apoE, and an ability to regulate cell proliferation and differentiation. More recently, it was shown that apoE suppresses toll-like receptor 4– and toll-like receptor 3–induced interleukin-12 and other T helper-1-type proinflammatory cytokines in mice. These effects may be mediated by very low density lipoprotein receptor and apoE receptor-2 signaling, since transfection of mouse RAW264.7 macrophages with these receptors downregulates the expression of proinflammatory M1 markers and upregulates anti-inflammatory M2 markers. However, the endogenous receptors for apoE, and additional receptor-independent pathways in macrophages, remain poorly understood. Three major APOE polymorphic alleles are present in humans (APOE2, APOE3, and APOE4). The rare APOE4 isoform is strongly associated with several inflammatory diseases, including cerebrovascular, cardiovascular, and Alzheimer’s diseases. Interestingly, macrophages transfected with human APOE4 expression constructs have enhanced NF-κB induction and inflammatory gene expression compared with cells expressing human APOE3. APOE4 transgenic mice also have an elevation in innate immune signaling in macrophages compared with APOE3 transgenic mice and have reduced survival in sepsis models. Administration of APOE mimetic peptides (which do not affect lipoprotein metabolism) can reduce inflammatory markers in mouse models of sepsis and enhance survival, and human subjects with a single APOE4 allele have elevated inflammatory responses after exposure to lipopolysaccharide and have more severe complications of sepsis. Taken together, these studies support an anti-inflammatory function of APOE, especially APOE3, and implicate a regulatory role in NF-κB signaling.

The NF-κB/Rel family of transcription factors mediate the expression of many agonist-induced genes implicated in inflammation, survival, and proliferation and can protect cells from cytokine-triggered apoptosis. Exposure to various stimuli, including cytokines and toll-like receptor (TLR) ligands, induces NF-κB transcriptional activity via the activation of intracellular signaling pathways that promote the proteasome-mediated degradation of inhibitor of NF-κB (IκB), which acts to sequester NF-κB in the cytoplasm of quiescent cells. NF-κB is subsequently imported into the nucleus where it transactivates gene expression through interactions with various cofactors and other transcription factors. Considering the critical role that NF-κB plays in orchestrating the inflammatory response, fine-tuning of this pathway is essential. Indeed several negative feedback regulators are direct targets of this

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transcriptional pathway and their induction acts to limit the intensity and the duration of the inflammatory response. These include proteins that antagonize the NF-κB signaling pathway, such as IκBα and A20, as well as several microRNAs, most prominently, miR-146a. MicroRNAs bind primarily to the 3′ untranslated regions of target mRNAs and suppress protein production. In the case of miR-146a, multiple components of proinflammatory signaling pathways (including TRAF6, IRAK1, IRAK2, MyD88, RelB, STAT1, CARD10, and TLR4) have been identified as bona fide targets.18 Deletion of miR-146a in mice has revealed that this microRNA dampens and extinguishes inflammatory signaling cascades. Mice deficient in miR-146a have prolonged T-cell responses, excessive and lethal responses to endotoxin and develop autoimmunity and tumors.18 These phenotypes are invariably associated with enhanced and prolonged NF-κB-dependent responses.

Cellular levels of miR-146a may affect the susceptibility to inflammation-related diseases. Intriguingly, a single-nucleotide polymorphism in the transcript that is processed into mature miR-146a affects the amount of miR-146a made and is associated with risk of coronary artery disease in certain populations.19 Although miR-146a is assumed to be protective against atherosclerosis because of its ability to suppress NF-κB signaling and endothelial cell activation,20 levels of miR-146a are paradoxically elevated in human atherosclerotic plaques,21 as well as in the circulation22 and plaques23 of atherosclerotic mice (ie, Apoe−/− mice). This may be because of the strong activation of NF-κB signaling in plaque, implying that miR-146a is induced as part of a negative feedback loop. Understanding the mechanisms that control the cellular levels of miR-146a will yield insight into pathways that might be involved in regulating atherogenesis. Although transcriptional mechanisms are undoubtedly involved, recent studies have shown that miR-146a is present in the circulation24 and transfer to recipient cells may also modulate the steady state levels of this microRNA. In addition, it is noteworthy that a network of microRNAs have been uncovered that impinge on inflammatory signaling pathways,18 and this includes miR-2125 and miR-14726 (identified as apoE-regulated microRNAs by Li et al). It is therefore likely that miR-146a is not the only microRNA that affects vascular inflammation. Indeed, mouse models of atherosclerosis have revealed an antiatherogenic role for miR-181b, which suppresses the nuclear import of NF-κB proteins in the endothelium,22 and a proatherogenic role for miR-155, which represses Bcl6, a negative regulator of NF-κB in macrophages.23

Li et al now provide compelling evidence that apoE, which is highly expressed by monocytes and macrophages, can positively regulate miR-146a transcription and expression in these cells, and thereby suppress NF-κB activation and inflammatory gene expression (see Figure). The extent to which augmented miR-146a expression contributes to the constellation of beneficial, lipid-independent effects of apoE remains to be fully elucidated, and it would be informative to assess the anti-inflammatory effect of apoE on miR-146a null monocytes/macrophages. It is possible that the regulation of additional microRNAs (such as miR-21 and miR-147a) or NF-κB–independent pathways may contribute to apoE’s effects.

The authors show that apoE induces the expression of PU.1, a transcription factor that has been previously implicated in miR-146a transcription.27 Exploring whether additional PU.1 target genes (including microRNAs and mRNAs) contribute to the apoE anti-inflammatory phenotype seems warranted. A major unanswered question is how apoE induces PU.1. Is this a receptor-dependent or -independent effect, and what signaling pathway(s) are involved?

An important and exciting finding in the article is that intravascular injection of a miR-146a mimic can recapitulate the protective effects of apoE in both Ldlr−/− and Apoe−/−;Ldlr−/− mouse models of atherosclerosis. This represents a novel potential therapeutic strategy. However, the mechanisms by which the miR-146a mimic achieves atheroprotection remain to be fully explored. Is inhibition of NF-κB signaling the only mechanism? Is the mimic primarily delivered to myeloid cells? Presumably elevated miR-146a in peritoneal macrophages reflects that the mimic was also delivered efficiently to macrophages in atherosclerotic lesions. Of note, the same intravascular injection technique was used...
to efficiently deliver a miR-181b mimic to arterial endothelium, where this microRNA suppressed NF-κB activity through antagonism of nuclear import of NF-κB subunits.22 Thus, it will be important to determine whether miR-146a mimic is also delivered to vascular endothelium, where miR-146a is capable of efficiently suppressing endothelial cell activation.20 This is an important consideration in light of previous atherosclerosis studies in which NF-κB signaling was inhibited specifically in either endothelial cells or macrophages. These studies showed that inhibition of NF-κB activation in endothelial cells reduced atherogenesis,28 whereas inhibition of NF-κB activation in macrophages resulted in enhanced atherosclerosis in Ldlr−/− mice.29

Atherosclerosis is a chronic and complex disease that progresses through distinct stages. Macrophage accumulation in early lesions is dependent on endothelial cell activation and monocyte recruitment. Later stages are characterized by stable or diminishing macrophage burden, macrophage renewal dependent on proliferation rather than monocyte recruitment, increasing myeloid cell apoptosis, secondary necrosis because of defective efferocytosis and formation of a necrotic core. Inhibition of inflammation in the early stages of atherosclerosis reduces the extent of lesions, delays the transition to subsequent stages, and influences the progression and characteristics of mature lesions that include smooth muscle cell infiltration, matrix deposition, fibrous cap formation, and calcification. NF-κB activation in endothelial cells induces the expression of leukocyte adhesion molecules and chemokines required for monocyte recruitment, and genetic deletion of endothelial NF-κB signaling28 or of the resultant proinflammatory genes results in reduced atherogenic lesion formation. However, NF-κB also mediates increased expression of genes that protect cells from apoptosis and potentially contribute to the resolution of inflammation. Inhibition of NF-κB signaling in macrophages may induce increased apoptosis and necrosis in advanced lesions and may decreased the production of interleukin-10, an anti-inflammatory cytokine. Thus, it seems that the role of NF-κB signal transduction in atherosclerosis is complex, and therapeutically targeting a specific cell type at a particular stage of atherogenesis will likely be important. Perhaps through fine-tuning of NF-κB signaling, miR-146a can attenuate macrophage inflammatory gene expression without eliciting detrimental effects on macrophage survival.

MicroRNAs represent an exciting therapeutic target for several human diseases, and preclinical studies have shown utility in modulating atherogenesis through manipulation of microRNA-based pathways.22,30,31 The first human trials of microRNA-based therapeutics are underway and there is reason for optimism. For instance, a promising Phase II clinical trial has revealed that miravirsen, a miR-122 inhibitor, can inhibit hepatitis C virus replication.32 In addition, a first-in-class Phase I clinical trial is assessing liposome-mediated microRNA mimic delivery to replace a tumor suppressive microRNA, miR-34, in liver cancers and hematologic malignancies.33 There are several significant challenges that must be overcome for successful application of microRNA mimetic approaches in the clinic, especially in atherosclerosis, a disease that develops over decades. Defining a prolonged treatment regimen may be difficult, impractical, and costly, and intravascular delivery will be problematic in terms of compliance. Technological advances, such as microRNA mimetic-eluting stents, may be required to make this type of therapeutic approach practical. Delivery to the desired cell type to limit off-target effects is a major hurdle, especially with intravascular administration of liposomes, where numerous cell types are likely targeted. Because NF-κB in macrophages plays a key role in mediating host defense, it will be vital to ensure that delivery of miR-146a mimetics (or other microRNAs that inhibit NF-κB activation) to macrophages will not adversely affect responsiveness to pathogens. Perhaps therapeutically enhancing the regression of established plaques may be more practical than attempting to inhibit plaque formation. One can begin by investigating the role of miR-146a and other microRNAs in atherosclerosis regression models. The findings by Li et al have certainly provided further motivation to pursue miR-146a manipulation in atherosclerosis and other inflammatory diseases.

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