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EDITORIAL

Enhancing Atherosclerosis Regression in Diabetic Mice Through Apo AI (Apolipoprotein AI)

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Regression of atherosclerotic plaque has long been a holy grail of treatment of atherosclerotic cardiovascular disease. The most powerful approach to atherosclerosis regression in multiple species is substantial reduction in atherogenic lipoproteins.[1] The data for atherosclerosis regression in humans are more limited, but studies with pharmacologic reduction in low-density lipoprotein (LDL) have shown modest regression in atherosclerotic coronary artery disease.[2] The contribution of plaque regression, per se, to the reduction in cardiovascular events associated with LDL reduction remains uncertain. However, there remains a substantial residual risk of recurrent cardiovascular events even in the setting of marked reduction in LDL. Objective regression of plaque would be one of the most straightforward findings in phase II that would encourage further clinical development of a novel orthogonal intervention aimed at atherosclerosis.

The concept that targeting high-density lipoprotein (HDL) metabolism could be an approach to atherosclerosis regression has been of interest for decades.[3] Indeed, studies in several preclinical models have indicated that infusion or overexpression of apo AI (apolipoprotein AI), the major HDL protein, can promote regression. In mice, viral vector–mediated gene transfer and overexpression of apo AI was shown to induce atherosclerosis regression,[4] as was transplantation of atherosclerotic aortas into mice transgenically overexpressing apo AI.[5] This concept led directly to the idea of infusion of apo AI-containing recombinant particles in humans with atherosclerotic cardiovascular disease,[6] an approach that is playing out in a phase III cardiovascular outcomes trial (NCT03473223).

A number of groups have shown that diabetic mice experience quantitatively less atherosclerosis regression despite comparable cholesterol-lowering effects.[7,8] Comparable data for the effect of diabetes mellitus on regression in humans are not abundant; however, it is well-established that people with diabetes mellitus have increased incidence of atherosclerotic cardiovascular disease and worse cardiovascular outcomes. Individuals with diabetes mellitus not only have lower levels of HDL cholesterol and apo AI, but clear evidence of reduced HDL function including reduced cholesterol efflux capacity and anti-inflammatory function.[9] Thus, the hypothesis that diabetes mellitus increases cardiovascular risk (and possibly blunts the impact of antiatherosclerotic therapies) by reducing HDL function is of interest.

In this context, Barrett et al in this issue of *Circulation*[10] demonstrate that apo AI overexpression promotes atherosclerosis regression in diabetic mice and elucidate some of the mechanisms by which it may do so. They utilized a model of atherosclerosis regression popularized by the Fisher lab, namely the transplantation of atherosclerotic aortic arches from hypercholesterolemic mice (cholesterol > 1000 mg/dL) into the abdominal aortas of normocholesterolemic wild-type mice

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(cholesterol < 100 mg/dL). Under these conditions of immediate dramatic reduction in exposure to atherogenic lipoproteins, the atherosclerotic plaque in the transplanted donor aortic arch regresses rapidly within 1 to 2 weeks. While the majority of publications using this model have employed *Apoe*$^{-/-}$ mice as donors, the current report used as donors *Ldlr*$^{-/-}$ mice fed a Western diet for 16 weeks. The authors have previously shown that transgenic overexpression of apo AI promotes regression, even in hypercholesterolemic *Apoe*$^{-/-}$ recipient mice, without reduction in cholesterol levels.[6] Here, using transplantation of atherosclerotic aortic arches from Western diet–fed *Ldlr*$^{-/-}$ mice into normocholesterolemic mice, they find that streptozotocin–induced diabetic mice have much less regression after 2 weeks compared with nondiabetic mice. Most importantly, they show that when the diabetic mice were also overexpressing human apo AI, the magnitude of regression was significantly greater despite comparable hyperglycemia and was similar to the regression observed in nondiabetic mice. A group of nondiabetic mice overexpressing apo AI was not included, so it is impossible to assess whether apo AI overexpression was even *more* effective in the presence of diabetes mellitus. Another important question is whether apo AI overexpression would promote regression in diabetic mice without accompanying cholesterol reduction.

Barrett et al went on to explore a number of consequences of apo AI overexpression in streptozotocin-diabetic mice that could potentially contribute to enhancing atherosclerosis regression, with a particular focus on monocytes and neutrophils. Overall leukocyte counts, as well as monocyte and neutrophil counts, were increased in the diabetic mice and normalized in the apo AI overexpressing mice. Furthermore, the numbers of bone marrow common myeloid and granulocyte macrophage progenitor cells were increased in the diabetic mice and were significantly reduced in the apo AI overexpressing mice, consistent with the concept that cholesterol efflux negatively regulates hematopoiesis.[11] Surprisingly, using injection of monocytes labeled with fluorescent beads, the authors showed that overexpression of apo AI significantly reduced monocyte recruitment into the transplanted aortas in diabetic mice back to the level of nondiabetic mice. Interestingly, while the authors have previously shown that rapid atherosclerosis regression in this model is driven in large part by macrophage *egress* from plaques,[1] diabetes mellitus and apo AI overexpression had minimal effects on this process.

There is increasing interest in the role of neutrophils in atherosclerosis. Neutrophil activation—which was stimulated by diabetes mellitus—was significantly reduced by apo AI overexpression as assessed by S100a8/a9 expression in neutrophils and circulating plasma levels of S100A8/A9. Furthermore, in the presence of diabetes mellitus, neutrophils are primed to produce excess neutrophil extracellular traps (NETs), which are known to promote inflammation and atherosclerosis progression.[12] NETs were markedly reduced in the context of plaque regression in wild-type nondiabetic mice; diabetes mellitus resulted in less reduction in NETs, but apo AI overexpression in diabetic mice restored NET reduction to the level of nondiabetic mice. While this is provocative, and potentially implicates NETs in atherosclerosis regression and apo AI in reducing NETs, the degree of "NETosis" was strongly associated with the overall amount of plaque regression and it is impossible to determine cause and effect from these observations. However, impaired cholesterol efflux in myeloid cells markedly increased neutrophil accumulation and NET formation in atherosclerotic plaques,[13] making a causal relationship plausible. Indeed, the connections between cellular cholesterol, regulation of inflammasome activation, and atherosclerosis continue to become stronger and point to interesting new directions of investigation.

One cell type not addressed in these studies that is of the vascular smooth muscle cell. Recent reports suggest that a dedifferentiated vascular smooth muscle cell may give rise to a substantial percent of lipid-laden cells within atherosclerotic plaques.[14] It would be particularly interesting to utilize this transplant model to determine the contribution of these cells to plaque composition, their behavior during the regression phase, and the effects of both diabetes mellitus and apo AI overexpression on their contribution to regression dynamics.

How relevant are these interesting observations to humans and specifically the potential impact of apo AI on atherosclerosis regression in the presence (or absence) of diabetes mellitus? The authors made an attempt to address this question by providing some observational correlative data in a limited sample of 299 patients, half of whom had diabetes mellitus, finding a negative correlation between HDL cholesterol and monocyte/neutrophil counts, monocyte activation, and plasma S100A9 levels. It would be of interest to probe these associations in much larger data sets, and include other measures of monocyte/neutrophil activation and HDL function. But ultimately these associative findings will not address the critical question of whether elevating HDL cholesterol or apo AI causes these effects in humans. The experience with inhibitors of cholesteryl ester transfer protein (CETP) inhibitors to raise HDL cholesterol is not favorable: not only did CETP inhibitors largely fail to reduce cardiovascular events, they have also been associated with modestly increased markers of inflammation.[15] But CETP inhibition is a markedly different intervention from the apo AI overexpression used in these mouse studies. The most analogous intervention in humans is apo AI infusion, of which 3 different approaches have been studied. Two of these, MDCO-216 (apo AI Milano) and CER-001, failed to show significant short-term effects on coronary atherosclerosis

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by intravascular ultrasound, though LDL cholesterol levels were already very low in these studies and there are other limitations to the interpretation of these studies.[7] However, a third apo AI product, CSL112, is being tested in a large phase III cardiovascular outcomes trial (NCT03473223). In light of the results reported by Barrett et al, it will be especially interesting to learn whether CSL112 has effects on monocyte/neutrophil counts and markers of their activation, including NET formation. At least 2 other HDL-targeted interventions, endothelial lipase inhibition (NCT03351738) and recombinant LCAT infusion (NCT03004638), both also very different from CETP inhibition in multiple ways, are being studied in humans. Based on the results of Barrett et al, it is of interest to speculate that CSL112 and possibly these other HDL-targeted interventions could be particularly effective in the setting of diabetes mellitus or other conditions associated with impaired HDL function.

Atherosclerosis regression in the setting of LDL reduction may contribute to its beneficial effects in reducing cardiovascular events, but substantial residual risk exists even in the face of extremely low levels of LDL. While human studies have thus far not been encouraging with regard to HDL-targeted interventions (primarily CETP inhibition), the fact remains that apo AI overexpression in particular promotes atherosclerosis regression in preclinical models and has a variety of effects on cholesterol flux and inflammation that may contribute to the regressive environment. Whether these concepts can be extended into apo AI targeted therapeutic interventions in humans—perhaps focused on individuals with diabetes mellitus and other conditions associated with impaired HDL function—remains to be determined.

ARTICLE INFORMATION

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