Regression of Atherosclerosis: Insights from Animal and Clinical Studies

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ABSTRACT

Background: Based on studies that date back to the 1920s, regression and stabilization of atherosclerosis in humans has gone from just a dream to one that is achievable. Review of the literature indicates that the successful attempts at regression generally applied robust measures to improve plasma lipoprotein profiles. Examples include extensive lowering of plasma concentrations of atherogenic apolipoprotein B and enhancement of reverse cholesterol transport from atheromata to the liver.

Findings: Possible mechanisms responsible for lesion shrinkage include decreased retention of atherogenic apolipoprotein B within the arterial wall, efflux of cholesterol and other toxic lipids from plaques, emigration of lesional foam cells out of the arterial wall, and influx of healthy phagocytes that remove necrotic debris as well as other components of the plaque. This review will highlight the role key players such as LXR, HDL and CCR7 have in mediating regression.

Conclusion: Although much progress has been made, there are many unanswered questions. There is, therefore, a clear need for preclinical and clinical testing of new agents expected to facilitate atherosclerosis regression with the hope that additional mechanistic insights will allow further progress.

Key Words: atherosclerosis, CCR7, HDL, macrophages, regression

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INTRODUCTION

Atherosclerosis, a chronic inflammatory disease that occurs within the artery wall, is one of the underlying causes of vascular complications such as myocardial infarction, stroke, and peripheral vascular disease. Atherogenesis is a process that occurs over many years with the initiation phase being the subendothelial accumulation of apolipoprotein B-containing lipoproteins (apoB). These particles undergo modifications, including oxidation and hydrolysis, leading to the activation of endothelial cells. These cells secrete chemoattractants called chemokines that interact with specific receptors expressed on monocytes essentially "recruiting" the cells into the lesion. The monocytes then roll along the endothelial cells via interactions of specific selectins (ie, P-selectin glycoprotein ligand-1) with attachment being mediated by monocyte integrins such as very late antigen-4 and lymphocyte function-associated antigen 1 to the respective endothelial ligands vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1. Once attached, a process called diapedesis

occurs by which monocytes enter the subendothelial space. Having accessed the subendothelial space, recruited monocytes differentiate into macrophages, a process driven by interactions with the extracellular matrix and cytokines, including macrophage colony-stimulating factor and members of the tumor necrosis factor family. The uptake of oxidized low-density lipoprotein (LDL) by the macrophages occurs via scavenger receptors, notably the type A scavenger receptor and CD36, a member of the type B family. The cholesteryl esters of the apoB particles that are ingested are hydrolyzed into free cholesterol, which occurs in late endosomes. The free cholesterol is then delivered to the endoplasmic reticulum where it is re-esterified by acyl-coenzyme A:cholesterol ester transferase. It is this process that leads to the macrophages having the "foamy" appearance. It is well-known that macrophages contribute to formation of the necrotic core and fibrous cap thinning that characterizes the vulnerable plaque. How do these macrophages ultimately contribute to the vulnerable plaque? Macrophage-derived matrix metalloproteinases (MMPs) are a family of proteins that can degrade various types of extracellular matrix and hence promote rupture. Moreover, once activated, certain MMPs can activate others. Studies have shown a temporal and spatial correlation between the presence of macrophages in rupture-prone shoulder regions of plaques, thinning of the fibrous cap in these regions,

and local accumulation of activated MMPs. Another potential mechanism of how macrophages may promote plaque thinning and increase vulnerability is via causing smooth muscle cell (SMC) apoptosis. Vulnerable plaques show evidence of SMC death and decreased numbers of SMCs. Even after plaque rupture, the macrophage continues to play a role as it secretes prothrombotic tissue factor thereby accelerating thrombus formation.[1-8]

The idea that human atheromata can regress at all has met considerable resistance over the decades.[1-3] Resistance to the idea of lesion regression has been due to the fact that advanced atheromata in humans and in animal models contain components that give an impression of permanence, such as necrosis, calcification, and fibrosis. Furthermore, numerous theories have been proposed to explain atherogenesis that included processes thought to be difficult, if not impossible, to reverse including injury,[6] oxidation,[7] and cellular transformations resembling carcinogenesis.[8] In this review, data are presented that demonstrate that indeed changes in the plaque environment can stabilize and regress even advanced lesions.

PLAQUE REGRESSION: EVIDENCE FROM ANIMAL STUDIES

Regression of Atherosclerosis: Is It Possible?

In the 1920s, Anichkov and colleagues reported that switching cholesterol-fed rabbits to low-fat chow over 2 to 3 years resulted in arterial lesions becoming more fibrous with a reduced lipid content,[9] which from a modern perspective suggests plaque stabilization.[10,11] To our knowledge, however, the first prospective, interventional study demonstrating substantial shrinkage of atherosclerotic lesions was performed in cholesterol-fed rabbits and was reported in 1957.[12] The dietary regimen raised total plasma cholesterol to ~26 mmol/L (~1000 mg/dL) and induced widespread lesions involving ~90% of the aorta. To mobilize tissue stores of cholesterol, animals received IV bolus injections of phosphatidylcholine (PC). After <10 days of treatment, the remaining plaques were scattered and far less severe than initially, and 75% of arterial cholesterol stores had been removed.

Over the next 20 years, similar arterial benefits from injections of dispersed phospholipids were reported by a number of groups using a variety of atherosclerotic animal models, including primates.[4] Given the heavy reliance of atherosclerosis research on animal models, it is surprising that these impressive, reproducible results were largely ignored, even in numerous historical reviews of regression.[1,3,5,9,13,14]

The concept of regression gained support with a short-term study in squirrel monkeys,[15] and more extensive work reported that advanced arterial lesions in cholesterol-fed Rhesus monkeys underwent shrinkage and remodeling during long-term follow-up when their diet was switched to low-fat or linoleate-rich diets.[13,16] The cholesterol-feeding induction period lasted 17 months, producing widespread coronary lesions, with fibrosis, cellular breakdown, intracellular and extracellular lipid accumulation, and 60% luminal narrowing. The subsequent regression period lasted 40 months, bringing total plasma cholesterol values down to ~3.6 mmol/L (~140 mg/dL) and resulting in the loss of ~66% of coronary artery cholesterol, substantial reduction in necrosis, some improvement in extracellular lipid levels and fibrosis, and substantial lesion shrinkage, so that only 20% luminal narrowing remained.[13,16] Further work confirmed and extended these findings.[9,14] Three decades ago, in an overview of this work, it was concluded that "In the primate the answer is clear: all grades of induced lesions studied to date improve … the primate lesion shows amazing metabolic responsiveness: some extracellular as well as intracellular lipid is depleted, there is resolution of necrotic lesions, crystalline lipid tends to diminish slowly, and fibroplasia is eventually contained."[13]

Regression of advanced lesions in cholesterol-fed swine after reversion to a chow diet demonstrated an important sequence of events.[17] Histological examination of atheromata from these animals immediately after the high-cholesterol induction phase showed hallmarks of complex plaques, including necrosis and calcification. The regression regimen reduced total plasma cholesterol to ~1.8 mmol/L (~70 mg/dL), implying an even lower LDL cholesterol (LDL-C) level. Interestingly, the early phase of regression showed loss of foam cells from the lesions, and an increase in non−foam-cell macrophages around areas of necrosis. Long-term, the necrotic areas virtually disappeared, indicating removal of the material by a flux of functioning, healthy phagocytes.[17]

To revive the long-neglected finding of rapid atherosclerosis regression after injections of dispersed phospholipids, researchers have sought to determine the underlying mechanism of action.[4,18] Aqueous dispersions of PC spontaneously form vesicular structures called liposomes. Initially, cholesterol-free PC liposomes remain intact in the circulation[19] and can mobilize cholesterol from tissues in vivo[19-22] by acting as high-capacity sinks into which endogenous high-density lipoprotein cholesterol (HDL-C) shuttles lipid.[4,23,24] Bolus injections of PC liposomes rapidly restore normal macrovascular and microvascular endothelial function in hyperlipidemic animals,[22] remove lipid from advanced plaques in rabbits in vivo,[25] and rapidly mobilize tissue cholesterol in vivo in humans.[26] Importantly, the optimum liposomal size (~120 nm) has been achieved in animal model studies, which allows these particles to gradually deliver cholesterol to the liver without suppressing hepatic LDL receptor expression or raising plasma concentrations of LDL-C.[21,27]

In 1976, success in atherosclerosis regression also was achieved in rabbits following reversion to normal chow diet in combination with hypolipidemic and other agents.[9] Decades later, a series of studies achieved

shrinkage of atheromata in rabbits with injections of HDL or HDL-like apoA-I and PC disks.[28,29] Interestingly, a lipid-lowering regimen in rabbit, however, was found to diminish local proteolytic and prothrombotic factors in the artery wall, again consistent with remodeling of atheromata into a more stable phenotype.[30]

Unlike humans, mice have naturally high plasma HDL-to-LDL ratios, providing a strong intrinsic resistance to atherosclerosis. Drastic manipulations of plasma lipoproteins are required, therefore, to induce arterial lipoprotein accumulation and sequelae. A revolution in murine atherosclerosis research began in the 1980s when researchers began applying transgenic techniques to create mice that were models of human lipoprotein metabolism.[31,32] With the emerging technique of gene inactivation through homologous recombination (knock out), came the ability to recreate important aspects of human lipid metabolism in mice. Most mouse models of atherosclerosis are derived from two basic models: the apoE-null (apoE-/-) mouse[33,34] and the LDL receptor-null (LDLR-/-) mouse.[35] In these models, the normally low plasma apoB levels are increased to atherogenic levels by eliminating either a ligand (apoE-/-) or a receptor (LDLR-/-) for lipoprotein clearance. Feeding these modified mice with a cholesterol-enriched and fat-enriched diet (Western diet; WD) increased plasma apoB levels to an even greater degree, resulting in accelerated plaque formation in the major arteries.

Using the animal models mentioned previously, atherosclerosis progression and the factors that can modulate it were studied extensively. Using apoE-/- mice expressing human apolipoprotein AI (hAI/apoE-/-), Choudhury and colleagues demonstrated that normalization of HDL levels inhibited plaque progression.[38] However, in terms of studying regression, gene transfer was the first strategy used in mice. For example, injection of LDLR-/- mice that had developed fatty streak lesions after a 5-week WD, with an adenoviral vector containing cDNA encoding human apoA-I caused a significant increase in HDL-cholesterol levels and, importantly, regression of fatty streak lesions at a sampling point 4 weeks later.[36] The ability of HDL-like particles to rapidly remodel plaques in mice was shown by infusion of apoA-I$_{Milano}$/PC complexes, a variant of apoA-I identified in individuals who exhibit very low HDL-C levels. Infusion of this complex reduced foam cell content in arterial lesions in apoE-/- mice within 48 hours.[37] This finding was corroborated by a specific transplantation model reported in 2001,[38] described later. Although another HDL protein, apoM, has been overexpressed in mice to retard plaque progression,[39] evaluation of its role in regression has not yet been reported.

Another major target of gene transfer to achieve regression in mice is hepatic overexpression of apoE, which increases the clearance of plasma atherogenic lipoproteins through receptors in the liver for LDL[35] and for postprandial lipoprotein remnants.[40-43] Following successful transient reduction of atherosclerosis progression in apoE-/- mice with short-term adenoviral-mediated expression of apoE,[44] a number of laboratories capitalized on the greater duration of apoE expression afforded by "second-generation" viral vectors.[45] For example, in LDLR-/- mice fed a WD for 14 weeks to develop plaques rich in foam cells ($\sim$ 50% macrophage content), increased expression of apoE resulted in considerable plaque regression, despite having no discernable effect on fasting plasma lipoprotein levels.[46] These findings were attributed in part to the entry of expressed apoE into the vessel wall, consistent with other studies[47-50]; however, another plausible mechanism is that expressed apoE might also have improved clearance of atherogenic lipoproteins in the postprandial state.

Transplantation Model of Atherosclerosis Regression

To further explore cellular and molecular mechanisms of atherosclerosis regression in murine models, new approaches have been developed to rapidly induce robust improvements in the plaque environment and to trigger lesion remodeling and regression. Our study group developed the technique of transplanting a segment of plaque-containing aorta from a (WD-fed) hyperlipidemic apoE-/- mouse (ie, an extremely pro-atherogenic milieu consisting of high-plasma apoB levels and low HDL cholesterol levels), into a wild-type recipient (ie, rapidly normalizing the lipoprotein environment, which is sustainable indefinitely). This approach allows analysis of plaques of any degree of complexity.[52,53]

It was demonstrated that transplanting early lesions[51,52] or advanced, complicated plaques into wild-type recipients substantially reduced foam cell content and increased the number of SMCs, particularly in the cap, which is consistent with plaque stabilization and regression.[53,54] The loss of foam cells from early lesions was surprisingly rapid, with large decreases evident as early as 3 days post-transplantation (Fig. 1).[51,52] With advanced lesions, all features regressed after 9 weeks, including necrosis, cholesterol clefts, and fibrosis.[53,54]

By using the transplantation model, cellular and molecular features of the regressing plaque were characterized. An early question we sought to answer concerned the fate of the disappearing foam cells—was their disappearance due to apoptosis and phagocytosis by newly recruited macrophages, or emigration? Interestingly, we found that the rapid loss of foam cells was largely accounted for by their emigration into regional and systemic lymph nodes. Furthermore, we found that the wild-type milieu provoked foam cells to display markers characteristic of both macrophages and, surprisingly, dendritic cells, which enabled emigration.[51,52,55-59]

Using laser microdissection to remove foam cells from regressing and non-regressing plaques,[60] analyses revealed the presence of mRNA for CCR7,[52] chemokine
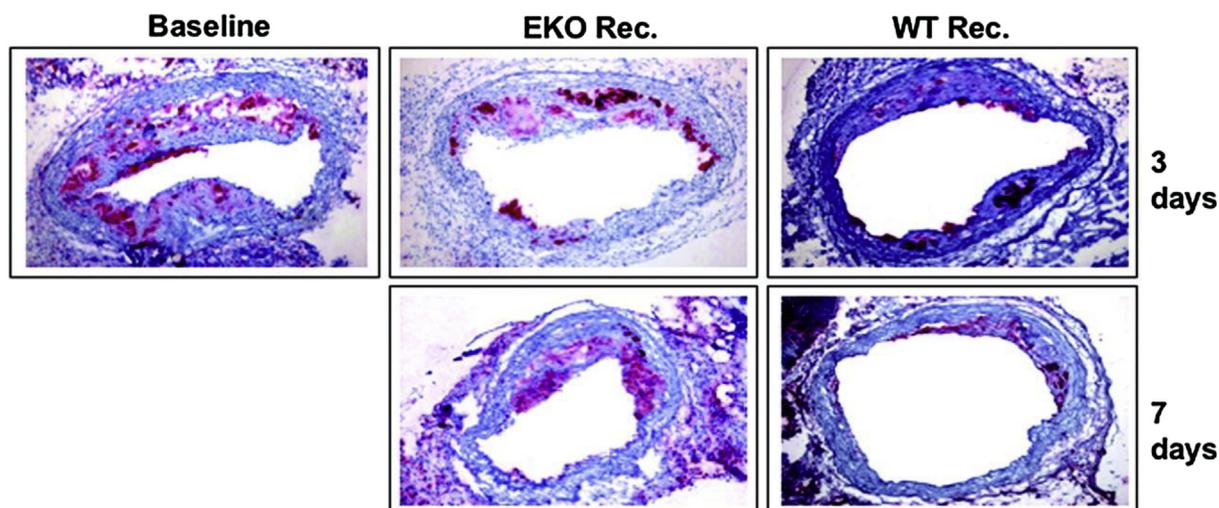
Figure 1. Regression of plaques in the mouse transplantation model. ApoE-/- mice were fed a Western diet for 16 weeks to develop advanced atherosclerosis. Aortic arches from these mice were either harvested and analyzed by histochemical methods, or they were transplanted into apoE-/- ("progression") or wild-type ("regression") recipient mice. Three or 7 days later, the same analyses were performed. Shown are the histochemical results for the foam-cell marker CD68 (red). The pictures show the immunostaining of representative aortic lesions in cross section. The virtual absence of foam cells can be seen in the "regression" group. In contrast with the "regression" results, the "progression" group showed persistence of foam cells. Reproduced from Trogan E. et al.[52]

(C-C motif) receptor 7, which is required for dendritic cell emigration.[61] Interestingly, injection of wild-type recipient animals with antibodies against the 2 CCR7 ligands, CCL19 and CCL21, inhibited the majority of foam cells from emigrating from the aortic transplant lesions—establishing a functional role for CCR7 in regression.[52]

In addition, mRNA concentrations of several well-known proteins implicated in atherothrombosis, such as VCAM-1, monocyte chemotactic protein 1, and tissue factor, are decreased in foam cells during regression. In addition, the level of mRNA for the nuclear oxysterol liver X receptor (LXR) α—known to be induced in vitro by oxidized sterols[62,63]—significantly increased in vivo, as did its anti-atherogenic target ATP-binding cassette 1 (ABCA-1).[52] Intriguingly, systemic administration of an LXR agonist caused lesion regression in LDLR-/- mice,[64] although the concomitant development of fatty liver has dampened enthusiasm for this approach in humans.[65] Interestingly, it has been determined that LXR activation in macrophages promoted regression in vivo and was dependent on CCR7 expression.[66] It is unlikely that regression of atherosclerosis occurs only through a single mechanism. A recent report showed that netrin-1, a neuroimmune guidance cue, was secreted by macrophages in human and mouse atheroma, where it inactivated the migration of macrophages toward chemokines (such as CCL19, ligand for CCR7) linked to their egress from plaques.[67] These findings suggest that inhibition of netrin-1 may be one method of inducing regression of atherosclerosis. Overall, these findings indicate that regression does not simply comprise of the events leading to lesion progression in reverse order; instead it involves specific cellular and molecular pathways that eventually mobilize all pathologic components of the plaque.

HDL and Plaque Regression

At least 3 plasma parameters are changed in the transplantation model when regression was observed: non-HDL levels decreased; HDL levels were restored from ∼33% of normal to wild-type levels; and apoE was now present. For the purpose of this review, we focus on the HDL change. To selectively test this as a regression factor, we adopted the transplant approach by using as recipients human apoAI transgenic/apoE-/- mice (hAI/EKO) or apoAI-/- mice.[68,69] Briefly, plaque-bearing aortic arches from apoE-/- mice (low HDL-C, high non−HDL-C) were transplanted into recipient mice with differing levels of HDL-C and non−HDL-C: C57BL/6 mice (normal HDL-C, low non−HDL-C), apoAI-/- mice (low HDL-C, low non−HDL-C), or hAI/EKO mice (normal HDL-C, high non−HDL-C). Remarkably, despite persistent elevated non−HDL-C in hAI/EKO recipients, plaque CD68+ cell content decreased by >50% by 1 week after transplantation, whereas there was little change in apoAI-/- recipient mice despite hypolipidemia. Interestingly, the decreased content of plaque CD68+ cells was associated with their emigration and induction of their chemokine receptor CCR7.[70] These data are consistent with a recent meta-analysis of clinical studies that demonstrated atherosclerosis regression (assessed by intravascular ultrasound [IVUS]) after LDL lowering was most likely to be achieved when HDL was also significantly increased.[71]

The induction of CCR7 is also likely related to changes in the sterol content of foam cells when they are placed in a regression environment, given that its promoter has a putative sterol regulatory element (SRE). This idea is in agreement with a report that

demonstrated that loading THP-1 human monocytes with oxidized LDL suppresses the expression of this gene.[72] Notably, we have found that statins, potent regulators of SRE-dependent transcription can induce CCR7 expression in vivo and promote regression via emigration of CD68+ cells in a CCR7-dependent manner.[73] Recently, it was reported that both atorvastatin and rosuvastatin can promote regression of atherosclerosis as assessed by IVUS.[74] Our data, therefore, suggest that activation of the CCR7 pathway may be one contributing mechanism.

Another aspect of interest has been the effect of HDL on the inflammatory state of CD68+ cells in plaques. A number of benefits from this can be envisioned such as a reduced production of monocyte-attracting chemokines and plaque "healing" by macrophages prodded to become tissue remodelers (M2 macrophages). There are multiple reasons for HDL to have anti-inflammatory effects on plaques, including the antioxidant properties of its enzymatic and nonenzymatic components, the ability to remove normal and toxic lipid species from cells, and the dampening of toll-like receptor signaling by regulating plasma membrane cholesterol content.[3,75] It is important to note that in CD68+ cells laser-captured from the plaques, normalization of HDL-C led to decreased expression of inflammatory factors and enrichment of markers of the M2 macrophage state.[70,76] Macrophage heterogeneity in human atherosclerotic plaques is widely recognized, with both M1 (activated) and M2 markers being detectable in lesions,[77,78] but little is known about the factors that regulate M2 marker expression in plaques in vivo.

Cholesterol homeostasis recently has been investigated with microRNAs (miRNA), which are small endogenous non−protein-coding RNAs that are post-transcriptional regulators of genes involved in physiological processes. MiR-33, an intronic miRNA located within the gene encoding SRE-binding protein-2, inhibits hepatic expression of both ABCA-1 and ATP-binding cassette subfamily G member 1 (ABCG-1), reducing HDL-C concentrations, as well as ABCA-1 expression in macrophages, thus resulting in decreased cholesterol efflux. Interestingly, enrichment of M2 markers in plaque CD68+ cells was observed in LDLR-/- mice treated with an antagomir of miR-33.[79] The treated mice also exhibited plaque regression (fewer macrophages). The therapeutic potential of miR-33 antagomirs to cause similar benefits in people was suggested by plasma levels of HDL being raised in treated non-human primates.[80] Thus, antagonism of miR-33 may represent a novel approach to enhancing macrophage cholesterol efflux and raising HDL-C levels in the future.

It was recently reported,[81] using Mendelian randomization, that some genetic mechanisms (ie, endothelial lipase polymorphisms) that raise plasma HDL-C do not seem to lower risk for myocardial infarction.

These data potentially challenge the concept that rising of plasma HDL-C will uniformly translate into reductions in risk for myocardial infarction. However, it is important to note that these results should not lead one to abandon the concept that HDL is beneficial but rather may indicate that it is time to alter the HDL hypothesis: It is not the quantity of HDL but rather the quality or functionality that is critical. Clinical trials that have HDL function as an endpoint rather than simply the level are needed.

EVIDENCE FROM CLINICAL STUDIES

Statins, Niacin, HDL, and CETP Inhibitors

The first prospective, interventional study to demonstrate plaque regression in humans was conducted in the mid-1960s, in which approximately 10% of patients (n = 31) treated with niacin showed improved femoral angiograms.[82] Larger trials of lipid lowering have since shown angiographic evidence of regression; however, although statistically significant, the effects were surprisingly small, particularly in light of large reductions in clinical events.[1,3,83] This "angiographic paradox" was resolved with the realization that lipid-rich, vulnerable plaques have a central role in acute coronary syndromes. A vulnerable plaque is characterized by being small, causing less than 50% occlusion, and being full of intracellular and extracellular lipid, rich in macrophages and tissue factor, with low concentrations of SMCs, and with only a thin fibrous cap under an intact endothelial layer.[10,11,83,84] Rupture of a vulnerable plaque provokes the formation of a robust local clot, and hence vessel occlusion and acute infarction.[85] Lipid lowering, which promoted measurable shrinkage of angiographically prominent but presumably stable lesions, probably had a greater effect on risk reduction by the remodeling and stabilization of small, rupture-prone lesions.[83,84] Regression studies in animal models strongly support this interpretation, given that macrophage content, a key hallmark of instability, can be rapidly corrected with robust improvements in the plaque lipoprotein environment.

In order to track potentially more important changes in plaque composition, to avoid the confounding effects of lesion remodeling on lumen size, arterial wall imaging is required. Recent human trials have switched from quantitative angiography, which images only the vascular lumen, to techniques that image plaque calcium (eg, electron-beam computed tomography [CT]) and plaque volume (eg, IVUS). A retrospective analysis found that aggressive LDL-C lowering with statins correlated significantly with reduction in coronary calcium-volume score by electron-beam CT, indicating that coronary artery calcifications can shrink.[86] In the REVERSAL (Reversal of Atherosclerosis with Aggressive

Lipid Lowering) study[87] and in ASTEROID (A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden),[88] patients with acute coronary syndromes were treated for more than 1 year with high-dose statins and evaluated by IVUS. The REVERSAL trial compared high-dose statin therapy with a conventional, less-potent statin regimen. During 18 months of treatment, patients treated with the conventional regimen exhibited statistically significant progression of atheroma volume (+2.7%), despite achieving average LDL-C levels of 2.8 mmol/L (110 mg/dL) and, therefore, meeting the then-current Adult Treatment Panel III goal.[89] By contrast, the high-dose statin group experienced no significant progression of atheroma volume (average LDL-C level, 2 mmol/L [79 mg/dL]). Importantly, analysis across the treatment groups found that LDL reduction exceeding ~50% was associated with a decrease in atheroma volume. In ASTEROID, all patients received the same high-dose therapy for 24 months, and IVUS findings pre- and post-treatment were compared. During treatment, LDL-C dropped to 1.6 mmol/L (60.8 mg/dL), and atheroma volume decreased by a median of 6.8%. Thus, in both of these studies, extensive LDL-C lowering for extended periods caused established plaques to decrease. The greater efficacy seen in ASTEROID could be explained by the lower median LDL-C level, but also by the longer treatment period and higher HDL-C levels achieved than those in REVERSAL. As in earlier angiographic studies, we believe that these reductions in plaque volume are accompanied by favorable alterations in plaque biology, a theory that is further supported by evidence that robust plasma LDL lowering to 1.0 to 1.6 mmol/L or below (≤40−60 mg/dL) is associated with further reductions in cardiovascular events.[90]

In addition to the preclinical studies reviewed here, there are a limited number of human studies in which HDL levels have been manipulated by infusion, and the effects on plaques assessed. In the first,[91] patients at high risk for cardiovascular disease (CVD) were infused with either an artificial form of HDL (apoA-I$_{Milano}$/PC) or saline (placebo) once a week for 5 weeks. By IVUS, there was a significant reduction in atheroma volume (−4.2%) in the combined (high- and low-dose) treatment group, although no dose response was observed with a higher versus lower dose of the artificial HDL. There was no significant difference in atheroma volume compared with the placebo group, but the study was not powered for a direct comparison. In the second infusion study, high-risk patients received 4 weekly infusions with reconstituted HDL (rHDL; containing wild-type apoAI) or saline (placebo).[92] As in the previous study, there was a significant decrease in atheroma volume (−3.4%) (as assessed by IVUS) after treatment with rHDL compared with baseline, but not compared with placebo (which the study was not powered for). However, the rHDL group had statistically significant improvements in plaque

characterization index and in a coronary stenosis score on quantitative coronary angiography compared with the placebo group. In the third infusion trial,[93] a single dose of reconstituted human HDL was infused into patients undergoing femoral atherectomies, with the procedure performed 5 to 7 days later. Compared with the control group (receiving saline solution), in the excised plaque samples in the HDL infusion group, macrophage activation state (ie, diminished VCAM-1 expression) as well as cell size (owing to diminished lipid content) were reduced.

In addition to the aforementioned meta-analysis of statin trials in which the relationships among LDL, HDL, and plaque regression were analyzed, there are also a number of other drug studies in which effects on plaques were ascribed to the raising of HDL levels. This includes the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) study, in which coronary events were reduced by 11% with gemfibrozil for every 5-mg/dL increase in HDL-C.[94] In another series of studies (Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol [ARBITER][95-98]), high-risk patients were placed on statins or statins plus niacin. Over 18- to 24-month observation periods, carotid intimal-medial thickness measurements were obtained as a surrogate for coronary artery plaque burden. As expected, when niacin was part of the treatment, HDL-C levels were increased (by 18.4%), and the authors attributed the improvement in carotid intimal-medial thickness particularly to this change. It is important to note that niacin does more than just raise HDL-C levels; it also decreases plasma triglyceride levels, makes LDL size increase, and possesses anti-inflammatory properties, all of which have the potential to limit plaque progression.[99-101] These pleiotropic effects obviously confound the interpretation of both the ARBITER and another statin-niacin clinical trial: the HDL-Atherosclerosis Treatment Study (HATS).[102] In the latter study, the addition of niacin to statin treatment resulted not only in a reduction in coronary artery stenosis, but also in events. The encouraging results with niacin, however, were recently called into question by the early termination of the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL Cholesterol/High Triglyceride and Impact on Global Health Outcomes (AIM-HIGH) study, which failed to show a benefit in the treatment group.[103] AIM-HIGH has been criticized, however, as being underpowered and for the fact that both the treatment group and the control group in the study received statin therapy, making additional benefits harder to detect, as well as for the placebo that the control patients received was actually a low dose of niacin.[104]

Recently, cholesteryl ester transfer protein (CETP) inhibitors have been investigated as pharmacological agents to raise HDL levels. Surprisingly, torcetrapib, the first CETP inhibitor tested in a clinical trial, increased the

all-cause mortality and cardiovascular events, which led to the premature ending of the Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial.[105] Subsequent studies indicated that the observed off-target effects of torcetrapib (increased blood pressure and low serum potassium by stimulation of aldosterone production) were rather molecule-specific, unrelated to CETP inhibition, and thereby might have overshadowed the beneficial effects of the raised HDL-C levels. Importantly, post hoc analysis of ILLUMINATE showed that patients with greater increases of HDL-C or apoAI levels had lower rates of major cardiovascular events within the torcetrapib group.[106] Despite the general failure of torcetrapib, in the post hoc analysis of the Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) study, regression of coronary atherosclerosis (as assessed by IVUS) was observed in patients who achieved the highest HDL-C levels with torcetrapib treatment.[107] In vitro studies showed an improved functionality of HDL-C particles under CETP inhibition, as HDL-C isolated from patients treated with torcetrapib and anacetrapib exhibited an increased ability to promote cholesterol efflux from macrophages.[108,109] Indeed, the CETP inhibitors anacetrapib, dalcetrapib, and evacetrapib increase HDL-C levels between 30% and 138%, and have not shown the off-target effects of torcetrapib in recent clinical Phase II trials, confirming the premise of a non—class-related toxicity of torcetrapib.[110-113] Thus, raising HDL-C by CETP inhibition or modulation remains a potential therapeutic approach for atherosclerotic CVD. Large clinical outcome trials were initiated for dalcetrapib (dal-OUTCOMES) and anacetrapib (Randomized EValuation of the Effects of Anacetrapib Through Lipid- modification [REVEAL]) including ~45,000 patients. Surprisingly, in May 2012, Roche stopped the complete dal-HEART program for dalcetrapib after an interim analysis of dal-OUTCOMES due to a lack of clinically meaningful efficacy. The failure of dal-OUTCOMES might have been a result of the rather moderate increases in HDL-C levels (30%) and minor effect on LDL-C levels induced by dalcetrapib, a fate that does not necessarily apply for anacetrapib, which has been shown to increase HDL-C levels by 138% accompanied by more robust reductions in LDL-C levels.[114] Whether the failure of dal-OUTCOMES challenges the benefits of raising HDL-C, in general, or rather the underlying mechanisms of how HDL-C is to be raised will be answered by the Phase III study with anacetrapib, which is expected over the next few years.

Novel Imaging Modalities

Although IVUS has provided important coronary anatomic information, there is still a need for imaging modalities that provide more details. Optical coherence tomography (OCT) has revolutionized intracoronary imaging. The unprecedented spatial resolution of this technique (15 μm) provides unique insights on the microstructure of the coronary wall. OCT is increasingly used in clinical practice and also constitutes an emerging, highly robust, research tool. OCT allows detailed visualization of atherosclerotic plaques and provides reliable information on plaque composition (lipid, fibrous, calcified). Importantly, OCT is the only technique allowing accurate measurements of the thickness of the fibrous cap, a classical marker of plaque vulnerability, and readily detects thin-cap fibroatheromas. In patients with acute coronary syndromes, plaque ruptures, with associated red or white thrombus, are nicely identified.[115]

The lipid core is an important plaque component and its relationship with macrophages and the vulnerable plaque has been established in animal models. Near-infrared spectroscopy is a technique that can identify the lipid core burden in the coronary arteries. It works by light of discrete wavelengths from a laser being directed onto the tissue sample via glass fibers. Light scattered from the samples is then collected in fibers and launched into a spectrometer. The plot of signal intensity as a function of wavelength is subsequently used to develop chemometric models to discriminate lipid cores from non-atherosclerotic tissue.[116]

Ideally, it is the early detection and characterization of atherosclerotic lesions susceptible to sudden rupture and thrombosis that need to be identified. Plaque development has been extensively studied using magnetic resonance imaging (MRI) in animal models of rapidly progressing atherosclerosis. MRI permits the accurate assessment of atherosclerotic plaque burden and the differentiation between the lipid and fibrous content of individual plaques, thus providing a noninvasive approach to serially monitor the evolution of individual plaques. In addition, 18F-fluorodeoxyglucose positron emission tomography is a relatively new noninvasive tool for inflammation functional imaging. Low spatial resolution is now compensated by coregistration with CT or MRI. One can envision having novel contrast agents that target specific plaque components or diverse set of molecules within the plaque, which would elucidate the changes at the cellular and molecular levels during plaque progression and regression. The feasibility of this concept was demonstrated in a study in which the detection of macrophages using a nanoparticulate contrast agent was achieved.[118] The aforementioned has important implications because pharmaceutical companies are looking for early surrogate markers that could be evaluated in a small number of patients to predict the beneficial effects of new drugs on atherosclerotic plaques before moving to costly clinical trials with a large number of patients.[117-119]

CONCLUSION

The crucial event in atherosclerosis initiation is the retention, or trapping, of apoB-containing lipoproteins within the arterial wall; this process leads to local responses to this retained material, including a maladaptive
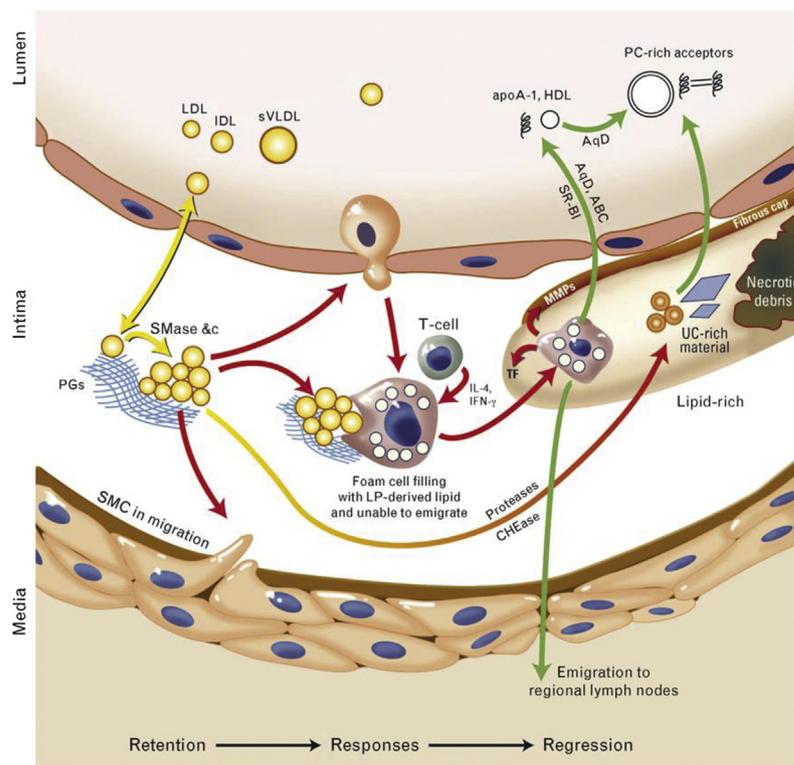
Figure 2. Retention, responses, and regression. Arrows are color-coded to indicate crucial mechanisms in the retention of atherogenic apolipoprotein B within the arterial wall, the key initiating step in atherogenesis (yellow); local responses to the retained and modified lipoproteins that lead to plaque growth and evolution (red); and then regression of all plaque components after robust improvements in the plasma lipoprotein profile, such as increased concentrations of natural and artificial mediators of reverse lipid transport (green). Reproduced from Williams KJ, Feig JE, Fisher EA. Cellular and molecular mechanisms for rapid regression of atherosclerosis: from bench top to potentially achievable clinical goal. *Curr Opin Lipidol*. 2007;18:443-450. **Abbreviations**: ABC, ATP binding cassette transporters; AqD, aqueous diffusion; HDL, high-density lipoprotein; IDL, intermediate density lipoprotein; LDL, low-density lipoprotein; LpL, lipoprotein lipase; MMPs, matrix metalloproteinases; PC, phosphatidylcholine; PGs, proteoglycans; SMase, sphingomyelinase; SMC, smooth muscle cell; SR-BI, scavenger receptor B-I; sVLDL, small very low-density lipoprotein; TF, tissue factor; UC, unesterified cholesterol.

infiltrate of macrophages that consume the retained lipoproteins but then fail to emigrate. Regression (ie, shrinkage and healing) of advanced, complex atherosclerotic plaques has been clearly documented in animals, and plausible evidence supports its occurrence in humans as well. Data have shown that plaque regression requires robust improvements in the plaque environment, specifically large reductions in plasma concentrations of apoB-containing lipoproteins and large increases in the reverse transport of lipids out of the plaque for disposal. Furthermore, it is important to note that regression is not merely a rewinding of progression, but instead involves a coordinated series of events such as emigration of the macrophage infiltrate, followed by the initiation of a stream of healthy, normally functioning phagocytes that mobilize necrotic debris and all other components of advanced plaques (Fig. 2).

For regression of atheromata to become a realistic therapeutic goal, clinicians must be provided with tools that extensively change plasma lipoprotein concentrations and plaque biology while avoiding adverse effects. To date, animal and human studies that achieved plaque regression required large reductions in plasma levels of

apoB, sometimes combined with brisk enhancements in reverse cholesterol transport. Unfortunately, most patients who take statins, for example, will not achieve and sustain the dramatically low LDL-C levels seen in chow-fed nonhuman primates. Efforts to explore other strategies that lower apoB levels are currently underway (ie, PCSK9 inhibitors). Experimental agents designed to accelerate reverse cholesterol transport from plaques, into the liver include PC liposomes, apoA-I/PC complexes, and apoA-I mimetic peptides. Other small molecules have been investigated preclinically for their potential to enhance HDL-C levels and reverse lipid transport, such as agonists for LXR and peroxisome proliferator-activated receptors. On the basis of experimental data summarized here, we expect that the best regression results will be observed when plasma LDL-C concentrations are reduced and HDL-C function in reverse lipid transport is enhanced. Indeed, years of work have demonstrated that the plaque and its components are dynamic. Most recently, by performing microarrays, we have discovered that regression of atherosclerosis is characterized by broad changes in the plaque macrophage transcriptome with preferential expression of

genes that reduce cellular adhesion, enhance cellular motility, and overall act to suppress inflammation.[120] Additional strategies, such as specific induction of pro-emigrant molecules to provoke foam cells to leave the arterial wall (eg, via CCR7), should attract pharmaceutical interest. Furthermore, there is a need for clinical trials that use the imaging modalities described previously to identify the specific effects of novel agents on plaque components rather than just atheroma size. This review provided evidence that plaque is dynamic and depending on the conditions macrophages, which play a crucial role in atherogenesis, can exit the lesions, proving that indeed regression is possible. However, there is still much work to be done and ultimately, the insights gained will lead to new therapeutic targets against CVD.

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