Rapid regression of atherosclerosis: Insights from the clinical and experimental literature

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Rapid regression of atherosclerosis: insights from the clinical and experimental literature

Kevin Jon Williams*, Jonathan E Feig and Edward A Fisher*

SUMMARY

Looking back at animal and clinical studies published since the 1920s, the notion of rapid regression and stabilization of atherosclerosis in humans has evolved from a fanciful goal to one that might be achievable pharmacologically, even for advanced plaques. Our review of this literature indicates that successful regression of atherosclerosis generally requires robust measures to improve plasma lipoprotein profiles. Examples of such measures include extensive lowering of plasma concentrations of atherogenic apolipoprotein B (apoB)-lipoproteins and enhancement of 'reverse' lipid transport from atheromata into the liver, either alone or in combination. Possible mechanisms responsible for lesion shrinkage include decreased retention of apoB-lipoproteins within the arterial wall, efflux of cholesterol and other toxic lipids from plaques, emigration of foam cells out of the arterial wall, and influx of healthy phagocytes that remove necrotic debris and other components of the plaque. Unfortunately, the clinical agents currently available cause less dramatic changes in plasma lipoprotein levels, and, thereby, fail to stop most cardiovascular events. Hence, there is a clear need for testing of new agents expected to facilitate atherosclerosis regression. Additional mechanistic insights will allow further progress.

KEYWORDS atherogenesis, atheromata, atherosclerosis, regression

REVIEW CRITERIA

A search for original articles published between 1920 and 2007 and focusing on atherosclerosis regression was performed on PubMed and by hand. The search terms used were "atherosclerosis" and "regression". All papers identified were English-language, full-text papers. We also searched the reference lists of identified articles for further papers.

CME

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Learning objectives

Upon completion of this activity, participants should be able to:

1 Describe animal models that have demonstrated atheroma plaque shrinkage or reversal.
2 Identify the first human interventional model of plaque regression.
3 List the most useful tests of plaque reversal for human clinical use and studies.
4 Describe high-density lipoprotein cholesterol as a therapeutic target for human atheroma regression.
5 Describe the most effective measures to reduce cardiovascular risk by achieving atheroma plaque regression.

Competing interests

KJ Williams is the inventor of a number of US patents on the use of phospholipid liposomes to promote reverse lipid transport *in vivo*. See the article online for full details. JE Feig and EA Fisher declared they have no competing interests. Désirée Lie, the CME questions author, declared no relevant financial relationships.

INTRODUCTION

The idea that human atheromata can regress at all has met considerable resistance over the decades.[1,2] Resistance to the idea of lesion regression is strengthened by 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. In addition, numerous theories have been proposed to explain atherogenesis that include processes thought to be difficult, if not impossible, to reverse including oxidation,[3] injury,[4] and cellular transformations resembling carcinogenesis.[5]

In this Review we summarize the failure of many established and experimental interventions to induce plaque regression, and examine other data indicating that sufficiently drastic changes in the plaque environment can stabilize and cause regression of even advanced lesions.

EVIDENCE FROM ANIMAL STUDIES: RABBITS, NONHUMAN PRIMATES, AND PIGS

In the 1920s, Anichkov and colleagues reported that switching cholesterol-fed rabbits to low-fat chow over 2–3 years resulted in arterial lesions becoming more fibrous with a reduced lipid content.[6] From a modern perspective, these results suggest plaque stabilization.[7] To our knowledge, however, the first prospective, interventional study demonstrating substantial shrinkage of atherosclerotic lesions was performed in cholesterol-fed rabbits and reported in 1957.[8] The dietary regimen raised total plasma cholesterol to around 26 mmol/l (~1,000 mg/dl) and induced widespread lesions involving around 90% of the aorta. To mobilize tissue stores of cholesterol, animals received intravenous bolus injections of phosphatidylcholine (PC). After less than a week and a half of treatment, the remaining plaques were scattered and far smaller than initially, and three-quarters of arterial cholesterol stores had been removed.

Over the next 20 years, similar arterial benefits from the injection of dispersed phospholipids were reported by a number of groups using a variety of atherosclerotic animal models, including primates.[9] 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.[1,2,6,10,11] Perhaps a factor contributing to the lack of recognition of atheromata regression was the publication of a study that showed worsening of arterial lesions in cholesterol-fed rabbits after return of the animals to regular chow.[6] Since then, it has been established that rabbits maintain substantial diet-induced cholesterol reservoirs in the liver that allow hypercholesterolemia to persist long after the atherogenic diet is stopped.[2] Nevertheless, studies that cast doubt on the possibility of regression, particularly of advanced lesions, continued to emerge in other models.[2]

The concept of regression gained support with a short-term study in squirrel monkeys by Maruffo and Portman,[12] and more-extensive work by Armstrong and colleagues. The latter reported that advanced arterial lesions in cholesterol-fed rhesus monkeys underwent shrinkage and remodeling during long-term follow-up after a switch to low-fat or linoleate-rich diets.[10,13] 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 approximately 3.6 mmol/l (~140 mg/dl) and resulting in the loss of approximately two-thirds 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.[10,13] Further work by Wissler and Vesselinovich, and others confirmed and extended these findings.[6,11] Three decades ago, in an overview of this work, Armstrong 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".[10]

Regression of advanced lesions in cholesterol-fed swine after reversion to a chow diet demonstrated an important sequence of events.[14] Histologic 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 approximately 1.8 mmol/l (~70 mg/dl), implying an even lower LDL-cholesterol level. 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 an influx of functioning, healthy phagocytes.[14]

To revive the long-neglected finding of rapid atherosclerosis regression after injections of dispersed phospholipids, we first sought to determine the underlying mechanism of action.[9,15] Aqueous dispersions of PC spontaneously form vesicular structures called liposomes. Initially, cholesterol-free PC liposomes remain intact in the circulation[16] and can mobilize cholesterol from tissues *in vivo*[16–18] by acting as high-capacity sinks into which endogenous HDL cholesterol shuttles lipid.[9,19,20] Bolus injections of PC liposomes

rapidly restore normal macrovascular and microvascular endothelial function in hyperlipidemic animals,[18] remove lipid from advanced plaques in rabbits *in vivo*,[21] and rapidly mobilize tissue cholesterol *in vivo* in humans.[22] Importantly, optimization of liposomal size (~120 nm) allows these particles to gradually deliver their cholesterol to the liver without suppressing hepatic LDL-receptor expression or raising plasma concentrations of LDL cholesterol.[17,23]

Eventually, in 1976, success in atherosclerosis regression was again achieved in rabbits, following reversion to a normal-chow diet in combination with the administration of hypolipidemic and other agents.[6] Decades later, a series of studies achieved shrinkage of atheromata in rabbits via injections of HDL or HDL-like apolipoprotein A-I (apoA-I) and PC disks.[24] At first, regression seemed limited to fatty streaks;[24] advanced lesions remained unaffected, reinforcing the now-obsolete view that regression was possible only in early lesions.[2,24] A lipid-lowering regimen in rabbits was, however, found to diminish local proteolytic and prothrombotic factors in the artery wall—a finding consistent with the remodeling of atheromata into a more stable phenotype.[25]

EVIDENCE FROM ANIMAL STUDIES: MURINE MODELS OF ATHEROSCLEROSIS

Unlike humans, mice have a naturally high plasma HDL:LDL ratio, providing a strong intrinsic resistance to atherosclerosis. Drastic manipulations of plasma lipoproteins are, therefore, required to induce arterial lipoprotein accumulation and sequelae. A revolution in murine atherosclerosis research began in the 1980s when Breslow's laboratory began applying transgenic techniques to create mouse models of human lipoprotein metabolism.[26] 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 apolipoprotein E (apoE)-null (*Apoe–/–*) mouse,[27] and the LDL-receptor-null (*Ldlr–/–*) mouse.[28] In these models, the normally low plasma apoB-lipoprotein levels are increased to atherogenic levels through the elimination of either a ligand (*Apoe–/–*) or a receptor (*Ldlr–/–*) for lipoprotein clearance. Feeding these modified mice on a cholesterol-enriched and fat-enriched diet (dubbed the 'Western' diet; WD) increased plasma apoB-lipoprotein levels to an even greater degree, resulting in accelerated plaque formation in the major arteries. A large number of atherosclerosis studies followed; here we review the comparatively small number of these studies that focused on regression.

Gene transfer and recombinant protein complexes

Gene transfer was the first strategy used to achieve plaque regression in mice. For example, injection of *Ldlr–/–* mice that had developed fatty streak lesions after a 5-week WD, with an adenoviral vector containing a human apoA-I cDNA, caused a significant increase in HDL-cholesterol levels and, importantly, regression of fatty streak lesions at a sampling point 4 weeks later.[29] Efficacy of this treatment for more advanced lesions was not evaluated. The ability of HDL-like particles to rapidly remodel plaques in mice was also shown by infusion of recombinant apoA-I_{Milano}/PC complexes that contained a variant of apolipoprotein A-I identified in individuals who exhibit very low HDL cholesterol levels. Infusion of this complex reduced foam-cell content in arterial lesions in *Apoe–/–* mice within 48 h.[30] This finding was corroborated by a specific transplantation model that we reported in 2001,[31] described later. Although another HDL protein, apolipoprotein M, has been overexpressed in mice with the intention of retarding plaque progression,[32] evaluation of its role in regression has not yet been reported.

Another major gene transfer strategy aimed at achieving regression in mice is hepatic overexpression of apoE, which increases the clearance of plasma atherogenic lipoproteins through receptors in the liver for LDL[28] and postprandial lipoprotein remnants.[33–35] Following successful slowing of atherosclerosis progression in *Apoe–/–* mice with short-term adenoviral-mediated expression of apoE,[36] a number of laboratories capitalized on the greater duration of apoE expression afforded by 'second-generation' viral vectors.[37] For example, in *Ldlr–/–* mice fed on a WD for 14 weeks to develop plaques rich in foam cells (~50% macrophage content), increased expression of apoE resulted in considerable plaque regression, despite having no discernable effect on fasting plasma lipoprotein levels.[38] These findings were attributed in part to the entry of expressed apoE into the vessel wall, consistent with other studies;[39,40] however, we believe that expressed apoE might have also improved clearance of atherogenic lipoproteins in the postprandial state.

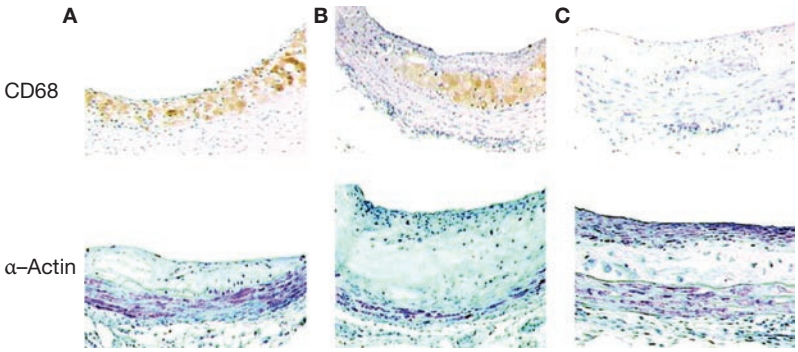

Figure 1 Regression of advanced atherosclerotic plaques in the mouse transplantation model. Apolipoprotein E (apoE)-null mice (*Apoe*–/–) were fed on a Western diet for 40 weeks to develop advanced atherosclerosis. Aortic arches from these mice were either (**A**) harvested and analyzed by histochemical methods, or they were transplanted into (**B**) *Apoe*–/– ('progression') or (**C**) wild-type ('regression') recipient mice. Nine weeks later, the same analyses were performed. Shown are the histochemical results for the foam-cell marker CD68 (brown), and the vascular smooth-muscle-cell marker, α-actin (purple). The pictures show the immunostaining of representative aortic lesions in cross section. The virtual absence of foam cells and the presence of a fibrous cap can be seen in the 'regression' group. In contrast with the 'regression' results, the 'progression' group showed persistence of foam cells and no development of a fibrous cap. Permission obtained from Lippincott Williams & Wilkins © Trogan E *et al.* (2004) Serial studies of mouse atherosclerosis by *in vivo* magnetic resonance imaging to detect lesion regression after correction of dyslipidemia. *Arterioscler Thromb Vasc Biol* **24:** 1714–1719.

In 2002, apoE was overexpressed long-term in *Apoe*–/– mice.[41] Adenoviral therapy improved plasma HDL and apoB-lipoprotein levels significantly in 10-month-old mice with advanced plaques. Aortic en face lipid staining 1 month after *ApoE* transfer showed loss of lipid mainly in the thoracic and abdominal aortic segments. Lesion development in these areas is slower than in the aortic root and arch.[42] The lack of success in reducing lipid staining in the arch compared with thoracic and abdominal aortic segments could, therefore, reflect increased plaque complexity in the arch area. Despite these promising results, plaque regression after gene transfer occurs only under sustained, high-level apolipoprotein expression, which is yet to be achieved in any clinical setting.

Transplantation model

To further explore cellular and molecular mechanisms of atherosclerosis regression in murine models, we and others have developed new approaches to rapidly induce robust improvements in the plaque environment and trigger lesion remodeling. Our study group developed the technique of transplanting a segment of plaque-containing aorta from a (WD-fed) hyperlipidemic *Apoe*–/– mouse (an extremely pro-atherogenic milieu consisting of high plasma apoB-lipoprotein levels and low HDL-cholesterol levels), into a wild-type recipient (i.e. rapidly improving the lipoprotein environment, which is sustainable indefinitely).[31,43] This approach allows analysis of plaques of any degree of complexity.

We found that transplanting early[44,45] or advanced, complicated[43,46] plaques into wild-type recipients substantially reduced lesion foam-cell content, while also increasing the number of smooth muscle cells present, particularly in the cap. These findings are consistent with plaque stabilization and regression (Figure 1).[43,46] The loss of foam cells from early lesions was surprisingly rapid, with large decreases evident as early as 3 days after transplantation.[44,45] With advanced lesions, all features regressed after 9 weeks, including necrosis, cholesterol clefts and fibrosis.[43,46] This evidence of regression, in even advanced lesions, indicates that the negative results of numerous previous regression attempts could reflect a failure to sufficiently improve the plaque environment.

Cellular and molecular biology of regression

By using our transplantation model, we characterized cellular and molecular features of the regressing plaque. 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 by emigration? In collaboration with Gwendalyn Randolph, we used fluorescence-activated cell-sorting based on a nonimmunogenic polymorphism of leukocyte common antigen (CD45) to distinguish between macrophages from the donor and those from the recipient. We showed that the rapid loss of foam cells was largely accounted for by their emigration into regional and systemic lymph nodes,[44,45] and interestingly, that many retained their foamy appearance (EA Fisher, unpublished data).

With impaired emigration identified as a key, but reversible, derangement of macrophage function within atheromata, the direction of our research changed. Again in collaboration with Randolph, we implicated candidate lipoprotein-derived lipids that impaired macrophage emigration *in vitro*.[44,47] We found that the wild-type milieu provoked foam cells to display markers characteristic of both macrophages and, surprisingly, dendritic cells, which enabled emigration.[44,45] The dendritic cell phenotype is

associated with a severe inflammatory response,[48] which initially seems inconsistent with a favorable outcome;[49] however, consistent with our findings, dendritic cells are known to sample native antigens in tissues and emigrate to lymph nodes as part of their role in the innate immune system.[50]

Using laser microdissection to remove foam cells from regressing and nonregressing plaques,[51] analyses revealed the presence of mRNA for CCR7[44] (chemokine [C-C motif] receptor 7), which is required for dendritic cell emigration.[52] Interestingly, injection of wild-type recipient animals with antibodies against the two 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.[45] We are currently focusing on mechanisms of *Ccr7* induction in foam cells from regressing plaques, as well as identifying other factors that block or facilitate foam-cell emigration.

As expected, mRNA concentrations of several well-known proteins implicated in atherothrombosis, such as vascular cell adhesion protein 1, monocyte chemotactic protein 1 and tissue factor, are decreased in foam cells during regression.[45] Contrary to expectation, however, the level of mRNA for the nuclear oxysterol liver X receptor α (LXRα)—known to be induced *in vitro* by oxidized sterols[53]—significantly increased *in vivo*, as did its antiatherogenic target, the ATP-binding cassette transporter A1 (Abca1).[45] Systemic administration of an LXR agonist caused lesion regression in *Ldlr–/–* mice,[54] although the concomitant development of fatty liver has dampened enthusiasm for this approach in humans.[55] Of note, activation of LXR stimulates *Ccr7* expression *in vitro*[56] and we hypothesize that increased LXR activity promotes regression partly by stimulating CCR7-dependent foam-cell emigration. Overall, these findings indicate that regression does not simply comprise 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.

Apolipoprotein B and functional HDL in atheromata regression

In transplantation models, wild-type recipients have low levels of apoB-lipoproteins and high HDL levels relative to the *Apoe–/–* donors. Both lipoprotein factors could facilitate lesion regression, and we have used the transplant model to distinguish their contributions. Diseased aortic segments from *Apoe–/–* donors were transplanted into human apoA-I transgene expressing *Apoe–/–* mice[31] that have wild-type levels of HDL cholesterol (~1.7 mmol/l [65 mg/dl]) but high apoB-lipoprotein levels. Over 5 months, these transplanted plaques showed considerable foam-cell depletion,[31] but the level of regression seen in this model during follow-up was lower than that observed in previous studies using wild-type recipients.[45] Together these studies suggest that raising plasma functional HDL levels through enhanced apoA-I production is sufficient to favorably remodel atheromata, but that a stronger effect is achieved when high levels of functional HDL are combined with large reductions in the concentration of circulating apoB-lipoproteins.

Nonsurgical models of plaque regression

Other new approaches for rapidly inducing robust improvements in the plaque environment in mice are primarily genetic, thereby enabling results to be tested in nonsurgical models of plaque regression. In collaboration with Stephen Young, we characterized the 'Reversa' mouse, in which inactivation of the gene for microsomal triglyceride transfer protein, which is required for hepatic secretion of apoB, can be induced.[57] Reversal of hyperlipidemia delays progression[57] and induces plaque regression,[58] which in our preliminary studies, was also associated with induction of *Ccr7* expression in foam cells (J Feig *et al.*, unpublished data).

Another nonsurgical model is the hypomorphic *ApoE/Mx1–Cre* mouse,[59] in which apoE levels are only 2–5% of normal because of a floxed neomycin resistance cassette in intron 3 that impairs transcription. These mice have normal lipid levels while maintained on a chow diet, but when placed on a high-fat or high-cholesterol diet, their levels of plasma cholesterol drastically increase (>25.9 mmol/l [1,000 mg/dl]). Either excision of the cassette with Cre recombinase to restore normal *ApoE* transcription, or simply switching the animals back to standard chow quickly normalizes the dyslipidemia. To study regression, *ApoE/Mx1–Cre* mice were fed on an atherogenic diet for 18 weeks, followed by a chow diet for 16 weeks, either with or without reinduction of *ApoE* transcription.[60] En face aortic analyses showed a 40% reduction in the lipid-containing surface area in the uninduced animals, and a further 30% reduction (70% total) in the induced mice compared with controls at baseline. Analysis of plaque cross-sections showed that

the concentration of subendothelial foam cells was reduced in the lesions in both sets of treated mice, with a substantial reduction of neutral lipid from the necrotic cores of the lesions in the induced mice. Thus, the majority of plaque improvement arose from changes in the plasma lipoprotein profiles, with additional, unexplained effects of *ApoE* in the induced mice, consistent with prior work.[39,40]

EVIDENCE FROM CLINICAL STUDIES

Insights into the so-called angiographic paradox

To our knowledge, the first prospective, interventional study to demonstrate plaque regression in humans was carried out in the mid-1960s, in which approximately 10% of patients ($n = 31$) treated with niacin showed improved femoral angiograms.[61] Larger trials of lipid lowering have since shown angiographic evidence of regression; however, though statistically significant, the effects were surprisingly small, particularly in light of large reductions in clinical events.[1,2,62] This 'angiographic paradox' was resolved with the realization that lipid-rich, vulnerable plaques have a central role in acute coronary syndromes. Vulnerable plaques are usually small in size and cause less than 50% occlusion. They are generally full of intracellular and extracellular lipid, rich in macrophages and tissue factor, have low concentrations of smooth muscle cells, and usually have only a thin fibrous cap under an intact endothelial layer.[7,62,63] Rupture of a vulnerable plaque provokes the formation of a robust local clot, and hence vessel occlusion and acute infarction.[64] Lipid lowering, which promoted measurable shrinkage of angiographically prominent but presumably stable lesions, probably had most impact on risk reduction by the remodeling and stabilization of small, rupture-prone lesions.[62,63] 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 the HDL-Atherosclerosis Treatment Study (HATS),[65] administration of simvastatin plus niacin to subjects with low levels of HDL cholesterol, elevated levels of LDL cholesterol and coronary disease, lowered LDL by 42% and raised HDL by 26%, in comparison with treatment with antioxidant vitamins alone or placebo. A 0.4% decrease in angiographically detected stenosis and an 87% reduction in cardiovascular end points were associated with this beneficial effect. Interestingly, adding antioxidant vitamins to simvastatin plus niacin attenuated the beneficial effects on HDL levels, angiographic regression and event reduction. As discussed above, the impressive event reduction from simvastatin plus niacin, despite small angiographic changes, most likely reflects major changes in plaque composition.

Regression documented by direct vessel-wall imaging

Statins

In order to track potentially more important changes in plaque composition, and 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 (e.g. electron-beam CT) and plaque volume (e.g. intravascular ultrasonography; IVUS). A retrospective analysis found that aggressive LDL-cholesterol lowering with statins correlated significantly with reduction in coronary calcium-volume score by electron-beam CT, indicating that coronary artery calcifications can shrink.[66] In the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) study[67] and A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden (ASTEROID),[68] patients with acute coronary syndromes were treated for over a year with high-dose statins and evaluated by IVUS.

The REVERSAL trial compared the 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-cholesterol levels of 2.8 mmol/l (110 mg/dl) which was close to the then-current Adult Treatment Panel III goal.[69] By contrast, the high-dose statin group experienced no significant progression of atheroma volume (average LDL-cholesterol level, 2 mmol/l [79 mg/dl]). Importantly, analysis across the treatment groups found that LDL reduction exceeding approximately 50% was associated with a decrease in atheroma volume. In ASTEROID all patients received the same high-dose therapy for 24 months and pretreatment and post-treatment IVUS findings were

compared. During treatment, LDL cholesterol dropped to an average of 1.6 mmol/l (60.8 mg/dl), and atheroma volume shrank by a median of 6.8%. Hence, in both of these studies, extensive LDL-cholesterol lowering over an extended period caused established plaques to shrink. The greater efficacy seen in ASTEROID could be explained by the lower median LDL-cholesterol level, but also by the longer treatment period and higher HDL cholesterol levels achieved in this study than 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 which is further supported by evidence that robust plasma LDL lowering to 1.0–1.6 mmol/l or below (≤40–60 mg/dl) is associated with further reductions in cardiovascular events.[70]

Statin and niacin

Ultrasonography can be used to measure non-invasively carotid intimal medial thickness (CIMT), validated as a surrogate of coronary artery disease risk.[71] In the Arterial Biology for the Investigation for the Treatment Effects of Reducing Cholesterol (ARBITER) series of studies,[72,73] CIMT was tracked in patients with coronary artery disease and low HDL-cholesterol levels treated either with statin and placebo or statin and extended-release niacin. Treatment with niacin raised HDL-cholesterol levels by 23% compared with placebo. In the statin and placebo group, CIMT increased; in the statin and niacin group, CIMT had decreased by 0.027 mm at 12 months' follow-up and decreased by an additional 0.041 mm at 24 months. The combination of LDL-cholesterol lowering and HDL-cholesterol raising could, therefore, be an effective clinical strategy to regress atherosclerosis. The extended-release niacin formulations with reduced flushing effects available from Abbott Laboratories, and under development at Merck, could lead to the wider use of niacin.

Artificial HDL-like apoA-I/PC complexes

HDL cholesterol as a therapeutic target for achieving atheroma regression was the focus of two recent studies in which patients with acute coronary syndromes completed short courses of four or five weekly infusions of either saline (control) or artificial HDL-like apoA-I/PC complexes. In the first trial the artificial HDL-like apoA-I/PC complex was made from the apoA-I_{Milano} mutant,[74] while the second trial used wild-type apoA-I.[75] In the first study, atheroma volume in the patients receiving apoA-I/PC (either 15 mg/kg or 45 mg/kg weekly) decreased by 4.2% overall compared with baseline volume ($P = 0.02$) despite the short treatment period. Although not a statistically significant result, atheroma volume increased by 0.14% in the placebo group.[74] The second regression study produced similar results: atheroma volume in the apoA-I/PC groups decreased by 3.4% compared with volume at baseline ($P < 0.001$), and by 1.6% in the control group treated with saline injections and standard care. Nevertheless, the primary end point, percentage change in atheroma volume, did not differ statistically between the treated and placebo groups.[75] Given the limitations of these studies, the results are promising but not definitive.

Cholesteryl-ester-transfer-protein inhibition: a bad way to raise 'good' cholesterol?

Not all strategies for raising plasma HDL-cholesterol concentrations have proven successful. The Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) study,[76] the Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) study,[77] and the Rating Atherosclerotic Disease Change by Imaging with a New CETP Inhibitor (Radiance 1) trial[78] compared statin therapy with and without torcetrapib—an orally administered inhibitor of cholesteryl ester transfer protein (CETP)—in high-risk patients. Despite significant increases in HDL-cholesterol levels in the statin–torcetrapib groups, torcetrapib use raised cardiovascular-related events and mortality, as well as noncardiovascular deaths,[76] and failed to improve coronary[77] or carotid[78] anatomy.

These results bring earlier reports to mind, however, such as those studies that found that a genetic CETP deficiency did not provide cardiovascular protection or might even increase risk,[79,80] and that HDL elevation by CETP inhibition fails to increase overall reverse cholesterol transport.[81] Hence, particles that accumulate in the HDL-density range after CETP inhibition might not function as normal HDL cholesterol. Whether the failure of torcetrapib reflects a general failure of CETP inhibition as a therapeutic strategy should be clarified, as early-phase clinical trials of CETP inhibitors without blood pressure effects continue.

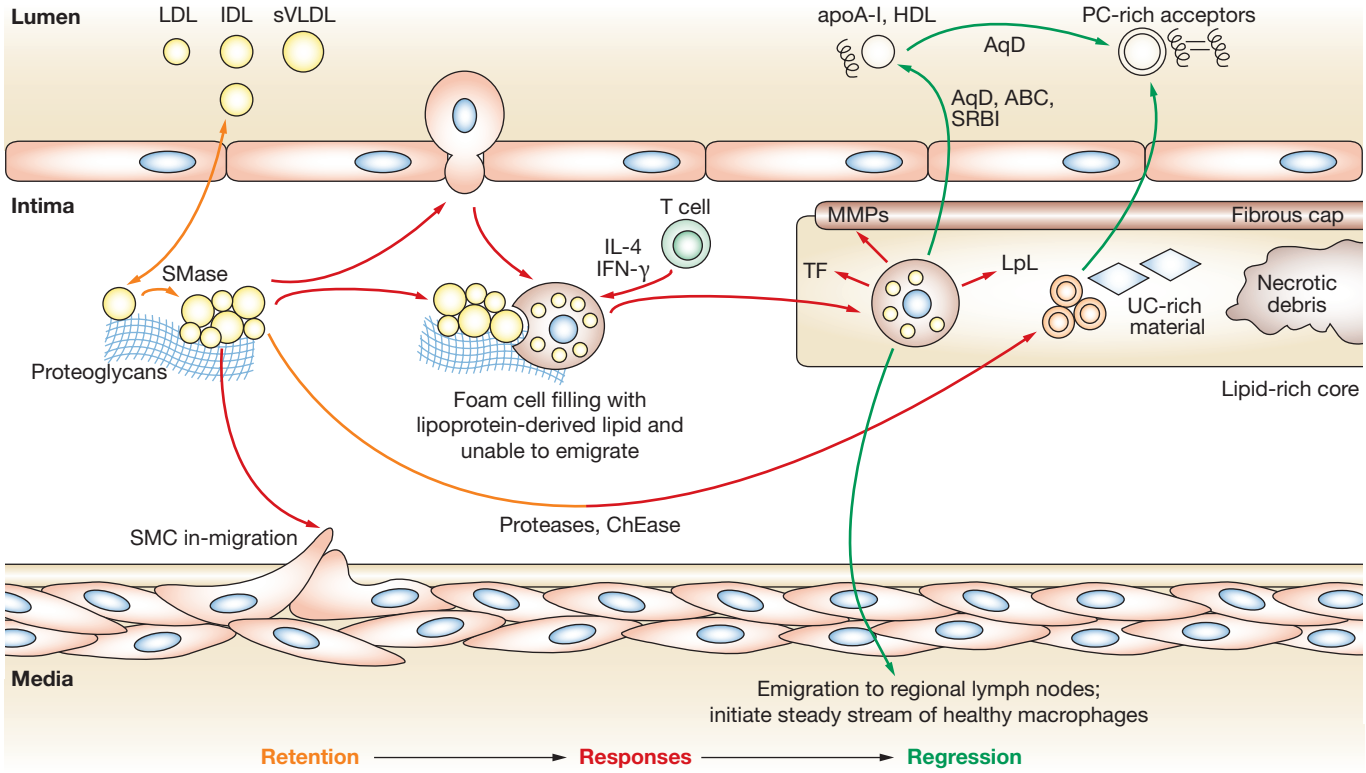

Figure 2 Retention, responses and regression. Arrows are color-coded to indicate crucial mechanisms in the retention of atherogenic apolipoprotein B lipoproteins within the arterial wall: the key initiating step in atherogenesis (orange); local responses to the retained and modified lipoproteins that lead to plaque growth and evolution (red); and 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). Abbreviations: ABC, ATP binding cassette transporters; apoA-I, apolipoprotein A-I; AqD, aqueous diffusion; ChEase, cholesteryl esterase; IDL, intermediate-density lipoprotein; IFN-γ, interferon γ; IL-4, interleukin 4; LpL, lipoprotein lipase; MMPs, matrix metalloproteinases; PC, phosphatidylcholine; SMase, sphingomyelinase; SMC, smooth muscle cell; SRBI, scavenger receptor B-I; sVLDL, small very-low-density lipoprotein; TF, tissue factor; UC, unesterified cholesterol. Permission obtained from Lippincott Williams & Wilkins © Williams KJ and Tabas I (2005) Lipoprotein retention and clues for atheroma regression. *Arterioscler Thromb Vasc Biol* **25:** 1536–1540.

ApoB-lipoproteins and functional HDL in atheromata regression in humans

To date, human vessel-wall imaging studies suggest that intensive lowering of plasma LDL-cholesterol concentrations concomitant with elevation of functional HDL-cholesterol levels could achieve rapid plaque regression. This hypothesis is consistent with well-established epidemiology, the earlier intervention trials that show a positive correlation between clinical end points and low LDL and high HDL levels, a recent meta-analysis of IVUS trial data[82] and our recent, aforementioned, experimental studies in mice. Furthermore, these strategies reduce cardiovascular events in at-risk subjects.

THE RESOLVING PLAQUE: RAPID STABILIZATION AND REGRESSION

As summarized in this Review, for the better part of a century, data from both animal and human studies have indicated that atherosclerotic lesions at all stages of development can regress. Under some circumstances, regression and beneficial remodeling might even occur rapidly. Although cautious about drawing definitive conclusions from these data, we believe that through the use of agents that target specific biological processes, stabilization and regression of established lesions in humans are achievable clinical goals.

The key initiating event in atherosclerosis is the retention, or trapping, of cholesterol-rich lipoproteins within the arterial wall.[15,83,84] The retained lipoproteins become modified, particularly by local enzymes, and they provoke a series of responses that can account for all known features of this disease, including endothelial dysfunction and the development of the lipid-rich, vulnerable plaque. Recruitment of macrophages into early-stage lesions could enable

phagocytosis and disposal of small quantities of retained lipoproteins. Our recent work indicates that downstream products of retained and modified lipoproteins are particularly dangerous because they eventually block the normal emigration of monocyte-derived cells (i.e. macrophages, dendritic cells) from the plaque, thereby accelerating disease progression.[44,47] These cells then become abnormally persistent within the lesion and exhibit a series of strikingly maladaptive responses that include the secretion of lipases, which greatly accelerate further lipoprotein retention and modification,[85,86] proteases, which weaken the overlying fibrous cap,[87] and tissue factor, which ensures vigorous clot formation upon plaque rupture (Figure 2).[88]

Given that retained and modified lipoproteins are atherogenic,[83,84] it follows that removal of the offending material should be beneficial.[9,15] Cholesterol of even advanced human atheromata dynamically exchanges with that in the plasma.[89,90] We presume that other biologically active lipids in plaques, either transported there or locally generated, can also be removed.[15] Even extracellular lipid, a major component of type III and IV lesions[64] and a key feature of lipid-rich vulnerable plaques,[91] might be amenable to mobilization.[6,10,15] HDL cholesterol, the major endogenous mediator of reverse lipid transport, can readily enter the interstitial space,[92] and we recently demonstrated the penetrance of HDL-like particles specifically into atherosclerotic lesions.[93]

These data imply a simple model for plaque regression (Figure 2). Major reduction in plasma apoB-lipoprotein concentrations slows further entry and retention within the arterial wall. Major enhancement of reverse lipid transport removes cellular but also extracellular lipid deposits. An early change seen following these environmental changes is restoration of normal endothelial function.[18,94,95] We hypothesize that at some point, lipoprotein-derived lipids that had been blocking monocyte-derived cell emigration become scarce enough to enable monocytes to leave the plaque, via a process resembling dendritic cell emigration. The emigrating cells take with them their intracellular lipid, and their potential for secretion of unhelpful lipases, proteases and tissue factor.[44,45] Removal of necrotic debris, calcifications and fibrosis also occurs,[6,10,43,66] facilitated, in theory, by new, normally functioning macrophages (i.e. they enter the regressing plaque, phagocytose and digest what they can, and donate exchangeable material to acceptor lipoproteins, but then leave).[14,96] Our study group[18,44,45] and others[30] have shown that these processes can occur surprisingly rapidly.

CONCLUSIONS AND FUTURE DIRECTIONS

For regression of atheromata to become a realistic therapeutic goal, clinical practitioners must be provided with tools that extensively change plasma lipoprotein concentrations and plaque biology while avoiding adverse effects. To date, the animal and human studies that achieved clear-cut plaque regression required large reductions in plasma levels of apoB-lipoproteins, sometimes combined with brisk enhancements in reverse lipid transport. Agents to lower plasma apoB-lipoprotein concentrations include statins and cholesterol-absorption inhibitors. Although these two classes of agents have proven safe in widespread clinical use, most patients will not achieve and sustain the dramatically low LDL-cholesterol levels seen in chow-fed nonhuman primates.[97] Efforts to explore other strategies that lower apoB-lipoprotein levels are underway,[98,99] but progress may be difficult because of potential adverse effects or inconvenient modes of administration. Experimental agents designed to accelerate reverse lipid transport from plaques into the liver include PC liposomes, apoA-I/PC complexes, apoA-I mimetic peptides, and two remaining CETP inhibitors.[15,100,101] These experimental agents are either in clinical trials or preclinical testing.[15,81,101] An extracorporeal device that delipidates HDL and returns the unloaded particles to the circulation is also undergoing testing.[81] Besides the CETP inhibitors, other orally administered small molecules have been investigated preclinically for their potential to enhance HDL-cholesterol levels and reverse lipid transport, such as agonists for LXR and peroxisome proliferator-activated receptors.[81]

On the basis of experimental data summarized above, we expect that the best regression results will be observed when plasma LDL-cholesterol concentrations are reduced and HDL function in reverse lipid transport is enhanced. Additional strategies, such as specific induction of pro-emigrant molecules to provoke the emigration of foam cells from the arterial wall, should also attract pharmaceutical interest. Rapid stabilization and regression of established plaques occurs in experimental settings in animals and humans, and we should know shortly whether plaque regression is within our grasp in the broader clinical setting as well.

KEY POINTS

- Regression (i.e. shrinkage and healing) of advanced, complex atherosclerotic plaques has been clearly documented in animals, and plausible evidence supports its occurrence in humans as well
- The crucial event in atherosclerosis initiation is the retention, or trapping, of apolipoprotein-B (apoB)-containing lipoproteins within the arterial wall; this process leads to local responses to this retained material, including a maladaptive infiltrate of macrophages that consume the retained lipoproteins but then fail to emigrate
- Plaque regression requires robust improvements in the plaque environment, specifically large reductions in plasma concentrations of apoB-lipoproteins and large increases in the 'reverse' transport of lipids out of the plaque for disposal
- Regression is not merely a rewinding of progression, but instead involves emigration of the maladaptive 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
- The challenge we face is making robust improvements in the plaque environment a widely achievable clinical goal. Additional strategies to provoke stabilization and regression of human atheromata, such as direct induction of CCR7 in plaque macrophages, might eventually become clinically feasible as well

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Competing interests

KJ Williams is the inventor of a number of US patents on the use of phospholipid liposomes to promote reverse lipid transport *in vivo*. See the article online for full details. JE Feig and EA Fisher declared they have no competing interests.

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