Review

Inflammation and the Development of Atherosclerosis

— Effects of Lipid-Lowering Therapy

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Atherosclerosis is a progressive disease causally associated with multiple cardiovascular risk factors, including dyslipidemia. Without effective intervention, atherosclerosis becomes evidenced clinically as coronary artery and cerebrovascular disease, both of which remain the leading causes of death worldwide. Multiple lines of investigation indicate a central role for inflammation in atherosclerotic plaque progression, vulnerability and thrombogenicity. Randomized clinical trials have documented the benefit of lipid-lowering therapy for both primary and secondary prevention of cardiovascular events. Statins, a class of drugs that lower cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, have been shown to slow the progression of the atheroma and the frequency of associated clinical events to an extent that cannot be attributed solely to LDL reduction. The non-LDL or pleiotropic effects of statins are attributed to anti-inflammatory activity, enhanced endothelial function, and inhibition of oxidative stress. In this review, we discuss the role of inflammation in atherogenesis along with the effects of statins in slowing this process through LDL-dependent and -independent mechanisms.

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Introduction

Despite marked advances in medical research over the past several decades, cardiovascular disease continues to be the primary cause of morbidity and mortality worldwide. According to the World Health Organization, approximately one-third of all deaths globally are attributed to coronary heart disease. Atherosclerosis is the underlying cause of cardiovascular disease and can be characterized as an inflammatory disease linked to certain risk factors, such as dyslipidemia, hypertension, and cigarette smoking. Atherosclerotic plaque progression and vulnerability are influenced by plaque cell and lipid composition rather

than by the extent of arterial stenosis¹⁻⁴. Vulnerable plaques in the coronary arteries tend to have a thin fibrous cap, a large lipid core, and a high macrophage content⁵. These plaques reside almost entirely in the intravascular space and, as a result of compensatory expansion of the diseased artery, are typically not detected by conventional angiographic approaches⁴. Indeed, the majority of coronary occlusions and myocardial infarctions evolve from areas characterized by previous angiography as being mildly to moderately stenotic⁶.

The evolution of the atherosclerotic plaque has been well characterized with respect to molecular and inflammatory processes⁷. In the initiation phase of plaque development, endothelial cells are stimulated by various risk factors to express adhesion and chemoattractant molecules that recruit inflammatory monocytes into the vascular wall. Endothelial dysfunction at this stage of atherogenesis is also associated with the introduction of extracellular lipid into the in-

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timal layer of the arterial wall. In the lipid accumulation phase, monocytes that have migrated into the arterial wall differentiate into macrophages and begin to express scavenger receptors that bind and facilitate the uptake of modified lipoproteins. Over time, these macrophages become lipid-laden and are transformed into large, phenotypically distinct foam cells. These and other cells in the vascular wall release multiple cytokines and growth factors that stimulate smooth muscle cell (SMC) migration and proliferation. As the lesion progresses, inflammatory mediators evoke the expression of platelet tissue factor (TF), a potent procoagulant and matrix-degrading proteinase that weakens the fibrous cap. Eventually, the fibrous cap may rupture, exposing tissue factors in the lipid core to circulating coagulation factors and promoting thrombosis, which, in some cases, may be occlusive, resulting in acute coronary syndromes (ACS).

Initiation and Progression of Atherosclerotic Plaque

Monocyte recruitment into the vascular wall is an early and important event in atheroma development. Local inflammation is attributed to various factors produced by leukocytes, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), monocyte chemoattractant protein 1 (MCP-1), monocyte colony stimulating factor (M-CSF), and interleukin 8 (IL-8), which stimulate the transendothelial migration of monocytes into the arterial wall⁸. In the subendothelial space, macrophages up-regulate various receptors, including CD36 and the scavenger receptor A, which internalize modified LDL^{9, 10}. Enhanced levels of intracellular adhesion molecules and L-selectin are associated with early stages of ACS even in the absence of myocardial necrosis.

Macrophage foam cells contribute to the formation of early and mature fatty streaks. These activated foam cells release mitogens and chemoattractants that accelerate plaque development by recruiting additional macrophages and vascular SMCs into the injured vessel—an essential early event in plaque formation. The ratio of the number of macrophages to SMCs has been shown to be an important indicator of plaque vulnerability¹¹. Macrophages are implicated in the eventual destabilization of atherosclerotic lesions by their production of matrix metalloproteinases (MMPs), which cause plaque disruption and thrombus formation by digesting various structural components of the extracellular matrix^{12, 13}.

Monocytes vary with respect to their inflamma-

tory properties. Those with low inflammatory activity contribute to metabolic tissue repair by activating angiogenic mediators. Highly inflammatory monocytes express increased levels of immunogenic factors such as Toll-like receptors (TLR) and cytokines, including tumor necrosis factor (TNF) and interleukin 1 (IL-1). *In vitro* studies have shown that monocytes are transformed by the binding of CD14 and TLR 4 to lipopolysaccharides—a process that is accelerated in the presence of C-reactive protein (CRP) and heat shock protein¹⁴. Although mechanisms of plaque destabilization are attributed primarily to intravascular events, circulating monocytes are also able to produce the major factors involved in plaque destabilization and thrombogenesis. Circulating monocytes positive for TLR 4, as well as CD14, have been shown to increase in patients with ACS¹⁵. Monocytes have also been observed to overexpress TLR 4 following plaque rupture during an atherothrombotic event¹⁵. In the recent PRavastatin Or atorVastatin Evaluation Infection Therapy-Thrombolysis In Myocardial Infarction (PROVE IT-TIMI 22) trial, neopterin, a soluble marker of monocyte activation, was shown to increase in patients at risk for death or recurrent acute coronary events after ACS¹⁶.

Plaque rupture is caused by a structural defect in the fibrous cap that results in exposure of the highly thrombogenic necrotic core to the bloodstream. Rupture of an atherosclerotic plaque occurs most frequently where the fibrous cap has thinned due to a loss of collagen. The most vulnerable region of an unstable plaque is often the proximal shoulder region between the plaque and the adjacent vessel wall^{17, 18}. Atherectomy specimens from patients with ACS show that macrophages are disproportionately concentrated in these areas⁵. Macrophages are known to release proteolytic enzymes, such as interstitial collagenase and gelatinase, which degrade collagen, elastin, and other extracellular matrix proteins, ultimately inducing plaque destabilization¹⁹⁻²³. Neutrophil infiltration at the inflammatory site also contributes to plaque instability and rupture as these cells release reactive oxygen species (ROS), including superoxide anion and hypochlorous acid derived from myeloperoxidase, resulting in further lipid modification and an increase of proteolytic enzymes²⁴. Finally, a reduction in SMC levels, with subsequent deficiency in collagen production, has been shown to compromise the integrity of the fibrous cap and is associated with an increased risk for plaque rupture and atherothrombotic events^{11, 25, 26}. A ruptured plaque typically contains connective tissue (collagen, proteoglycans, fibronectin elastic fibers), lipids (crystalline cholesterol, cholesteryl esters, phos-

pholipids), inflammatory cells (monocyte-derived macrophages, T-lymphocytes, neutrophils), SMCs, calcium, and thrombotic deposits^{18, 27}.

Atherothrombosis

Thrombosis is associated with acute complications of atherosclerosis, most notably unstable angina and acute myocardial infarction. Angiographically small coronary lesions account for the majority of clinical events in patients who develop these complications^{4, 6}. Cardiovascular risk factors, including elevated LDL cholesterol, cigarette smoking, and hyperglycemia, are associated with increased blood thrombogenicity²⁸. Inflammation also contributes to plaque thrombogenicity in patients with ACS.

Thrombus formation on disrupted atherosclerotic lesions plays a fundamental role in the onset of ACS. TF, an integral membrane glycoprotein, is the main initiator of the coagulation cascade and a major regulator of hemostasis and thrombosis²⁹. Increased expression of TF is observed in plaque material as well as plasma collected from patients with ACS³⁰. Coronary atherectomy specimens from patients with unstable angina showed a strong relationship between TF and macrophage levels, suggesting a role for TF in cell-mediated thrombogenicity^{31, 32}. TF has also been observed to co-localize with macrophages that have undergone apoptotic death rather than with macrophages that are still biologically active³⁰. This association is most apparent in the lipid core, which also represents the most thrombogenic component of unstable plaque^{31, 32}. The thrombogenicity of disrupted human atherosclerotic plaque can be predicted by its TF content and activity. Circulating levels of TF are also associated with increased blood thrombogenicity in patients with unstable angina and chronic coronary artery disease³³ and are predictive of outcomes in patients with unstable angina³⁴. Specific inhibition of TF by tissue factor pathway inhibitor (TFPI), a circulating, lipoprotein-associated, Kunitz-type protease inhibitor, has been shown to significantly reduce plaque thrombogenicity³⁵.

The Endothelium in Atherosclerosis

The endothelium possesses dynamic autocrine and paracrine properties and plays an essential role in controlling vasomotion and homeostasis through the release of various anti-thrombotic factors as well as nitric oxide (NO) and other vasodilating agents³⁶. Many pro-inflammatory and atherogenic factors, such as oxidative stress, low shear stress, advanced glycation

end products, and smoking, contribute to endothelial dysfunction^{37, 38}. In addition, chronic injury to the arterial endothelium disturbs blood flow, particularly near arterial tree bifurcations. The endothelium responds to these mechanical and chemical signals by releasing mediators that modulate vascular tone, platelet function, coagulation, and monocyte adhesion. Endothelial NO biosynthesis is also reduced in atherosclerosis, which leads to impaired vasodilation and increased expression of adhesion molecules that facilitate monocyte and platelet migration into the vessel wall².

Endothelial injury promotes platelet aggregation and the release of platelet-derived growth factor (PDGF), which stimulates the proliferation of SMCs in the arterial wall. Endothelial dysfunction is also associated with the increased production of endothelin-1 and the increased activation of pro-inflammatory signaling pathways such as nuclear factor kappa B (NF κ B)^{39, 40}. As endothelial cells have a central role in controlling vascular homeostasis, a dysfunctional endothelium will produce a pro-thrombotic environment. Endothelial cells also express metalloproteinases that are regulated by inflammatory cytokines and tissue factor pro-coagulants that are released in response to inflammatory mediators and bacterial products such as endotoxins^{41, 42}.

Ultimately, endothelial dysfunction facilitates the internalization of circulating lipids, the adhesion and differentiation of monocytes, and the increased release of cytokines³⁶. Some cases of occlusive thrombi arise not from fracture of the fibrous cap but from superficial erosion of the endothelial layer, which results in mural thrombus, leading to acute myocardial infarction⁷. In addition to reducing platelet aggregation, NO reduces endothelial expression of adhesion molecules and proinflammatory cytokines. NO release augments production of NF κ B, a transcription factor involved in the expression of genes encoding proteins involved in numerous pro-inflammatory functions in the vascular wall⁴³⁻⁴⁵. Collectively, these findings confirm the central role that endothelial dysfunction and inflammation have in the initiation and progression of atherosclerotic disease.

Oxidized Lipid and Inflammation

Oxidized phospholipids derived from modified lipoproteins are a major contributor to vascular inflammation in ACS. Levels of oxidized lipid, detected as lipid hydroperoxides, thiobarbituric acid reactive substances (TBARS), or monoclonal antibodies against oxidized LDL (oxLDL), have been shown to

correlate with the severity of ACS and to predict cardiovascular events⁴⁶⁻⁴⁸). Oxidized LDL and other lipid particles stimulate increased monocyte expression of urokinase and the urokinase receptor, which promote the secretion of metalloproteinase MMP-9^{49, 50}). Cellular exposure to oxLDL also contributes to increased mononuclear cell NF κ B activity while expression of lectin-like oxLDL receptor-1 in liver slows atherosclerotic progress in apoE-deficient mice by reducing oxLDL levels, as recently reviewed by Ishigaki *et al.*⁵¹). In the absence of effective intervention, these events lead to plaque destabilization and intracoronary thrombus formation through degradation of the extracellular matrix and eventual rupture of the fibrous cap. Reduction of circulating LDL levels has therefore become an important therapeutic goal in the management of cardiovascular disease.

Lipid Lowering and Reduced Inflammation with Statins

The use of statins to treat hyperlipidemia has been shown, in large randomized trials, to reduce cardiovascular events in patients at risk^{52, 53}). At high doses, statins slow or even reverse plaque progression in coronary and carotid arteries as demonstrated with intravascular ultrasound⁵⁴⁻⁵⁷). The effects of statins on carotid atherosclerosis could not be reproduced with other lipid-lowering therapies despite similar methods and patient profiles⁵⁸). These broader vascular benefits are attributed to the anti-inflammatory actions of statins, which are differentially influenced by their distinct physico-chemical and metabolic properties⁵⁹). In a number of clinical trials, statins have been shown to reproducibly lower circulating levels of CRP, an inflammatory biomarker associated with ACS^{55, 57, 60-63}). Reducing inflammation may therefore be a key mechanism by which statins alter the biology of the plaque and slow disease progression. Statins also reduce reactive oxygen species while increasing interstitial collagen content and promoting SMC maturation^{7, 64-69}). Statins have been further shown to decrease thrombus formation, in parallel with treatment-induced reduction in LDL cholesterol levels, in hypercholesterolemic patients⁷⁰).

Clinical studies have demonstrated that statins, as a class, promote reproducible reductions in CRP levels related to their potency. In the Cholesterol and Recurrent Events (CARE) study, pravastatin was shown to significantly reduce CRP levels, independent of its effects on LDL lowering⁷¹). In the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), lovastatin reduced the occur-

rence of cardiovascular events in patients not eligible for treatment on the basis of conventional LDL lowering-guidelines⁷²). In a separate study, rosuvastatin was shown to reduce the risk of clinical events in patients with elevated CRP but normal LDL levels^{57, 73}).

Statins may stabilize plaque through various mechanisms unrelated to LDL reduction. In particular, statins have been shown to exert anti-proliferative actions through interference with G protein-mediated cell cycle regulation, as observed in cultured SMCs⁷⁴). While inhibition of SMC proliferation might slow plaque development, a reduction in SMC levels may also lead to plaque disruption due to reduced collagen synthesis^{11, 25}). Statins also increase the endothelial expression of the fibrinolytic enzyme, tissue plasminogen activator (tPA), and decrease the expression of its inhibitor, plasminogen activator inhibitor 1 (PAI-1)⁷⁵). Additionally, statins alter macrophage metabolism and inhibit TF expression⁷⁶). Some statins, such as atorvastatin, have been shown to possess potent antioxidant benefits and to improve endothelial-dependent NO release through enhanced eNOS expression and activity under disease conditions^{69, 77-79}).

There is continuing debate as to whether the pleiotropic effects of statins are LDL-dependent or LDL-independent. In a recent meta-analysis of twenty-three randomized, placebo-controlled trials reporting correlative changes in LDL and CRP with LDL-lowering interventions, investigators concluded that most of the anti-inflammatory effects of LDL-lowering therapies were related to the magnitude of change in LDL⁸⁰); however, other reports have provided evidence in support of LDL-independent effects of these agents in reducing vascular disease. In one study, for example, cerivastatin was shown to improve endothelial function in elderly diabetic patients before the detection of any appreciable reduction in serum cholesterol⁸¹). In another study, atorvastatin was shown to reverse endothelial dysfunction in young, normocholesterolemic smokers independent of changes in lipid levels⁸²). These studies emphasize the need for additional research to elucidate the relative contribution of these pleiotropic effects to clinical event reduction. A better understanding of these benefits will also enable clinicians to determine optimal treatment guidelines and to improve selection of patients for treatment using this class of agents. These studies will also contribute to the discovery of more targeted pharmacotherapy that focuses on key aspects of atherogenesis, while improving efficacy and reducing side effects.

Conclusion

Atherosclerosis is a chronic inflammatory disease characterized by the abnormal accumulation of lipids in the arterial wall, leading to myocardial infarction, stroke, and sudden death. The "response to injury" hypothesis proposes that atherosclerosis begins with endothelial damage that produces adaptive and innate immune responses that propagate an arterial lesion, eventually progressing to a vulnerable plaque. Immune cells, such as monocytes, are recruited to the injured vessel wall by adhesion molecules associated with the immune response. A number of circulating inflammatory biomarkers have been identified, including C-reactive protein (CRP), fibrinogen, cytokines and other proteins associated with the immune system. Lipid-lowering therapy with statins reduces the risk for cardiovascular events through LDL-dependent and LDL-independent mechanisms, including various anti-inflammatory actions. Elucidating the basis of the clinical benefit of lipid-lowering therapy is an area of active research that may lead to the development of more effective therapies for the prevention and treatment of cardiovascular disease.

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