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STATE-OF-THE-ART PAPERS

Innate and Adaptive Inflammation as a Therapeutic Target in Vascular Disease

The Emerging Role of Statins

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Atherosclerosis, the main pathophysiological condition leading to cardiovascular disease (CVD), is now considered to be a chronic inflammatory condition. Statins are the most widely used and promising agents in treating CVD and are renowned for their pleiotropic lipid-lowering independent effects. Statins exert their anti-inflammatory effects on the vascular wall through a variety of molecular pathways of the innate and adaptive immune systems, their impact on the circulating levels of pro-inflammatory cytokines, and their effect on adhesion molecules. By inhibiting the mevalonate pathway and isoprenoid formation, statins account for the increase of nitric oxide bioavailability and the improvement of vascular and myocardial redox state by multiple different mechanisms (directly or indirectly through low-density lipoprotein [LDL] lowering). A large number of randomized control trials have shown that statins help in the primary and secondary prevention of cardiovascular events, not only via their lipid-lowering effect, but also due to their anti-inflammatory potential as well. In this paper, we examine the molecular pathways in which statins are implicated and exert their anti-inflammatory effects, and we focus specifically on their impact on innate and adaptive immunity systems. Finally, we review the most important clinical data for the role of statins in primary and secondary prevention of cardiovascular events. (J Am Coll Cardiol 2014;63:2491–502) © 2014 by the American College of Cardiology Foundation

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide, with atherosclerosis being the main pathophysiological condition leading to CVD. Atherosclerosis, once thought to be a lipid storage disease, is now considered a chronic low-grade inflammatory condition that affects the vascular wall. It is characterized by the deposition of cholesterol and lipids followed by infiltration of T cells and macrophages, all as a result of an endothelial injury response (1) (Fig. 1). Oxidative stress is also a key factor in the development of atherosclerosis. Reactive oxygen species (ROS) are capable of not only damaging the cellular components of the vascular wall but also affecting several redox-sensitive transcriptional pathways, shifting the transcriptomic profile to a proatheromatic state. ROS are responsible for the oxidation of the endothelial nitric oxide synthase (eNOS) cofactor tetrahydrobiopterin (BH4) to dihydrobiopterin (BH2). BH2 is an inactive cofactor for eNOS, promoting eNOS uncoupling. This results in the production of superoxide, further promoting endothelial dysfunction (2). Furthermore, in the subendothelial space, ROS oxidize low-density lipoprotein (LDL) to oxidized low-density lipoprotein (ox-LDL). The LDL oxidation hypothesis was first proposed in 1984 (3), and it is presently widely accepted as the hallmark event for the initiation of atherosclerosis. Each LDL particle contains several particles, such as triglycerides, free cholesterol, phospholipids, and cholesteryl esters. LDL oxidation by ROS can occur at several sites of inflammation as well as in the artery wall. Lipids and most importantly polyunsaturated fatty acids can undergo oxidation from ROS and yield several byproducts such as aldehydes, which in turn react with lysine and tyrosine of apolipoprotein B-100, resulting in loss of function (4). This leads to the formation of minimally modified LDL, exerting proatherogenic effects as it is recognized by LDL receptors but not from any ligands of scavenger receptors. The ox-LDL activates endothelial cells to produce pro-inflammatory molecules, promoting formation of foam cells while inhibiting NO-induced vasodilation (5). Meanwhile, oxidation of high-density lipoprotein diminishes its anti-atherogenic properties, contributing to the development of atherosclerosis. As the lesion matures, atherosclerotic plaques gradually lead to narrowing of the lumen and occlusion of the

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Abbreviations and Acronyms

AP = activator protein
HMG-CoA = hydroxymethylglutaryl-coenzyme A
ICAM = intracellular cell adhesion molecule
IL = interleukin
MMP = matrix metalloproteinase
NF-κB = nuclear factor-kappa B
ROS = reactive oxygen species
TNF = tumor necrosis factor
Treg = regulatory T cell

vessel. Plaque ulceration or rupture may also occur, leading to acute thrombosis and occlusion of the vascular lumen. This manifests as acute myocardial infarction (MI), stroke, or acute ischemia of any organ perfused by that arterial branch depending on the site of occlusion (6).The complete mechanism for the formation of atherosclerosis has not been fully elucidated yet, but the theory of atherogenesis continues to evolve as new evidence underlying its pathophysiology is obtained.

Role of Redox-Sensitive Transcriptional Pathways: Nuclear Factor-kappa B and Activator Protein-1

Nuclear factor-kappa B. Nuclear factor-kappa B (NF-κB) consists of a family of 7 transcriptional factors, all sharing a Rel homology domain, and can homo- or heterodimerize. These factors are present in almost all mammalian cell types (7). NF-κB activation can occur as a cellular response to several stimuli, namely, ROS, DNA damage, ultraviolet radiation, ox-LDL, cytokines, and bacterial and viral antigens. As a gene network expression regulator, NF-κB is implicated in several physiological and pathophysiological processes such as response to stress, cardiovascular growth, cancer, innate/adaptive immunity, cell survival, and others. Although all of its subunits are ubiquitously expressed, several distinct responses can be obtained depending on the cell type and induced stimuli with NF-κB activation, leading to a transcription of over 400 genes. Because it is a redox-sensitive transcription factor, the redox balance within the cells is a critical element of NF-κB activation (8). While inactive, it is bound to its inhibitor (I-κBα/β) within the cytoplasm (Fig. 2).

Activation of NF-κB following response to pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1 and IL-18, has been identified as the key component in atherosclerosis development and progression. Such activation results in up-regulation of genes encoding pro-inflammatory cytokines, chemokines, adhesion molecules, inducible nitric oxide synthase (iNOS), growth factors, and enzymes, thus switching to a proatherogenic profile. These act via 2 signaling pathways, resulting in activating an IκB kinase complex containing IKKα and IKKβ kinases and NF-κB essential modifier (NEMO), a scaffold protein that plays a regulating role (9,10). IkBα/β phosphorylation at NH_2-terminal serine residues is then initiated. The phosphorylated product is then ubiquitinated and undergoes degradation by proteasome 26S. This releases the dimers from the cytoplasmic complex and enables their nuclear translocation. Once inside, they bind to specific genes, resulting in the ensuing transcription. Macrophages, endothelial cells (ECs), and smooth muscle cells (SMC) of human atherosclerotic lesions have been reported to exhibit activated NF-κB function (11–13).

Activator protein-1. Activator protein-1 (AP-1) is a transcription factor consisting of the Jun (c-Jun, Jun-B, Jun-D) and Fos (c-Fos, Fos-B, Fra-1, Fra-2) families of transcriptional factors, binding to the 12-*O*-tetradecanoylphorbol-13-acetate or cAMP response elements (14). The gene products of this pathway can mitigate or amplify oxidative stress and inflammatory responses. Phosphorylation of c-Jun by the stress-induced family of c-jun NH_2-terminal kinases (JNK) has been identified as a critical step for the increased transcriptional activity of AP-1 (15). The JNK pathway regulates a variety of pro-inflammatory genes encoding cytokines, adhesion molecules, and metalloproteinases (MMPs) (16).This regulation is achieved via interaction of JNK with AP-1 pathways along with other transcription factors (10).

In atherosclerotic plaques, ROS production by cells can have a critical effect on both of the transcriptional pathways. ROS may oxidize NF-κB subunits, rendering them incapable of binding with DNA and subsequently impairing their transcriptional activities. On the other hand, excess ROS production can lead to increased activation of the JNK/AP-1 pathway, thus creating a new interaction state between these 2 pathways, which has a great impact on pro-inflammatory molecule production (17).

Pro-Inflammatory Cytokines and Adhesion Molecules in Atherosclerosis

Cytokines are small, cell-signaling protein molecules that may have autocrine or paracrine actions that mediate short-range intracellular communication (Table 1). The cytokine family consists of more than 100 factors subcategorized into several smaller clusters, such as ILs, interferons (INFs), colony-stimulating factors, TNFs, and chemokines (18). Cellular sources of cytokines include vascular cells, leukocytes, platelets, and mast cells (10). Pro-inflammatory cytokines owe their proatherogenic potential to several biological effects. At the very first stages of atherosclerosis, endothelial function can be greatly altered by cytokine release. TNF-α causes increased cytosolic Ca^{2+} and activation of myosin light chain kinase, as well as Ras homolog gene family member A (*RhoA*), causing disruption of endothelial cell junctions, which facilitates leukocyte transmigration. TNF-α along with INF-γ inhibit the formation of F-actin stress fibers by altering the cadherin-catenin complex in the vascular endothelium (18).

In later stages of the disease, cytokines such as TNF-α, INF-γ, and IL-1 may induce macrophage and SMC apoptosis, resulting in destabilization of the atheromatic plaque, making it prone to rupturing. Plaque destabilization is further promoted by matrix degradation, which is accelerated by pro-inflammatory cytokine release. The latter

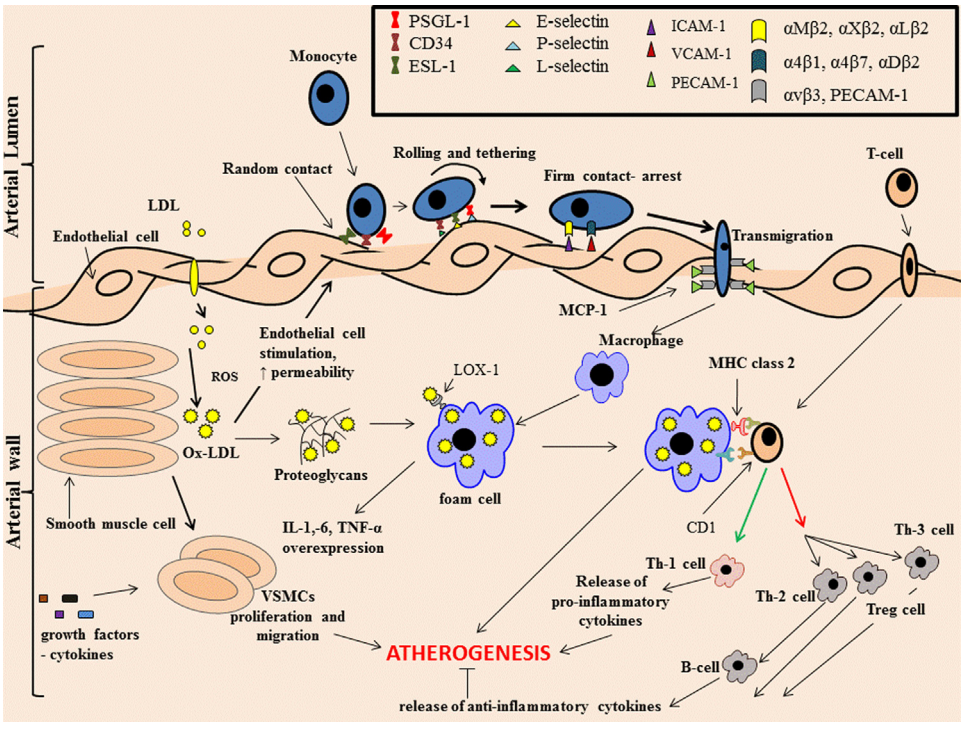

Figure 1 **Overview of Mechanisms Involved in Atherosclerosis**

Low-density lipoprotein (LDL) is oxidized in the presence of reactive oxygen species (ROS) and binds to proteoglycans (heparin sulfate) while simultaneously stimulating the endothelium, leading to adhesion molecule overexpression and increasing its permeability. Apart from this action, oxidized low-density lipoprotein (ox-LDL) inhibits nitric oxide (NO) production, prohibiting vasodilation. Furthermore, cytokines and other chemoattractant molecules, such as MCP-1, are secreted, favoring leukocyte adhesion. Leukocytes come into random contact with the activated endothelium and, due to interactions with adhesion molecules, roll and tether and are subsequently firmly arrested. In addition, leukocytes transmigrate into the subendothelial space, where they differentiate into macrophages, which in turn take up ox-LDL, forming foam cells. Ox-LDL antigens are presented by macrophage major histocompatibility complex class II (MHC-II) proteins and are recognized by $CD4^+$ T cells. These preferentially differentiate into Th-1 cells, pro-inflammatory cytokine production. Finally, smooth muscle cell (SMC) proliferation and migration are induced as a result of cytokine and growth factor secretion.

action has a great impact on the expression of MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), which act synergistically along with other molecules to exert the remodeling of the extracellular matrix (16). Cytokines also contribute to cell apoptosis. Macrophage apoptosis leads to the enlargement of the lipid core, while plaque-present SMC apoptosis contributes to the thinning of the fibrous cap, rendering it rupture prone. In addition, cytokines and, more specifically, TNF-α and IL-1 have a direct prothrombotic potential while inhibiting endogenous fibrinolytic/antithrombotic mechanisms (13). This results in precipitation of thrombus formation, promoting the development of acute coronary syndromes (ACS) (18). Finally cytokines not only attenuate NO synthesis, a physiological regulator of vascular tone, but they also induce synthesis of acute-phase proteins such as C-reactive protein (CRP), serum amyloid A, plasminogen, and fibrinogen, which in turn, amplifies inflammatory responses (13).

Cytokines are also responsible for up-regulation of adhesion molecules on the vascular endothelium, which in turn, mediate leukocyte adhesion, greatly facilitating their rolling and subendothelial migration (13). INF-γ can greatly accelerate transformation of macrophages to foam cells as it up-regulates the scavenger receptor of ox-LDL and the phosphatidyl serine SR-PSOX. In each stage of this process, several distinct adhesion molecules are involved. There are P-, E-, and L-selectins and immunoglobulin-like molecules (intracellular cell adhesion molecule [ICAM]), ICAM-1, ICAM-2, and ICAM-3; vascular cell adhesion molecule (VCAM)-1; and platelet endothelial cell adhesion molecule-1 (PECAM-1). Following endothelial activation from cytokine release, P-selectin glycoprotein ligand-1 (PSGL-1), CD34, and E-selectin ligand-1 (ESL-1) favor the recruitment and binding of leukocytes after random contact with the activated endothelium. Subsequently E-, P-, and L-selectins promote rolling and tethering of leukocytes whereas ICAM-1 and VCAM-1 mediate their firm arrest. Finally, PECAM-1 facilitates leukocyte transmigration into the subendothelial space (13).

Innate and Adaptive Immunity in Atherosclerosis

From the start of the 19th century, researchers proposed that there might be a link between atherogenesis and the immune

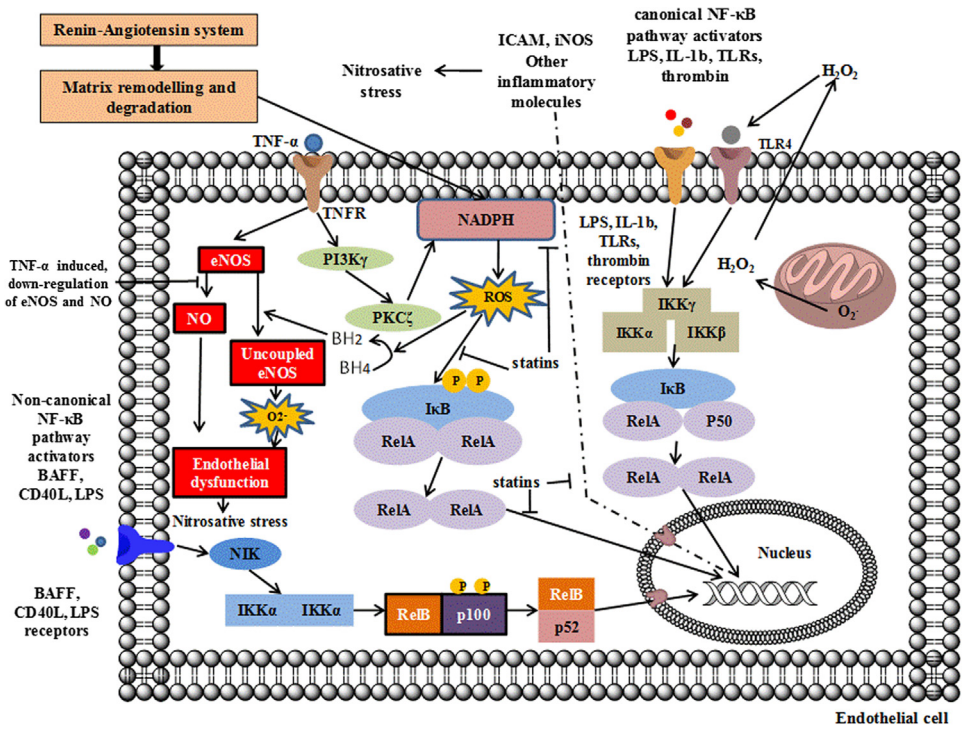

Figure 2 **Overview of the NF-κB Redox-Sensitive Transcriptional Pathway in Endothelial Cells**

Pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1b, and others induce activation of the canonical nuclear factor-kappa B (NF-κB) pathway with the activation of Iκκβ. TNF-α also down-regulates eNOS expression, resulting in reduced NO bioavailability. Tetrahydrobiopterin (BH4) undergoes oxidation to dihydrobiopterin (BH2), resulting in endothelial nitric oxide synthase (eNOS) uncoupling and superoxide production. Other cytokines such as B-cell activating factor (BAFF), lipopolysaccharides (LPS), and CD40 ligand (CD40L) induce activation of the noncanonical NF-κB pathway via activation of NF-κB-inducing kinase (NIK).

system (19). As our knowledge about the mechanisms and the processes involved in atherosclerosis evolves, it is now well documented that the immune system is a key mediator in the initiation and progression of atherosclerosis. First, immune cells are present in atherosclerotic lesions, resulting in specific pro-inflammatory gene expression. In addition, endothelial dysfunction results, not only in loss of antithrombotic ability, but also in an imbalance of the immune system, with the proatheromatic profile prevailing over the antiatheromatic state (20). Furthermore, most of the aforementioned pro-inflammatory cytokines (ILs, TNFs, INFs) along with several anti-inflammatory ones are all secreted from immune cells throughout the atherosclerotic plaques.

Innate immunity. Innate, or nonspecific, immunity is the host's first line of defense against pathogens, in a generic way, lasting briefly compared with the long-lasting action of adaptive immunity. It is composed of endothelial or epithelial barriers and circulating cells. Several receptors responsible for both signaling and pattern recognition participate in the innate immune system. A very interesting category is the Toll-like receptors (TLRs). They are a family of structurally conserved proteins able to recognize pathogen-associated molecular patterns on viral and bacterial components and products (21). Binding of TLRs to their ligands results in the recruitment and activation of adapter proteins to propagate the signal, which in turn activates the NF-κB and interferon pathways (21).

Dendritic cells (DCs) are considered messengers between the innate and adaptive immune systems. They act as antigen-presenting cells, processing antigen material and presenting it on their surface for other cells to recognize it. In early atherosclerosis, disturbance in normal endothelial function enhances DC migration and adhesion, resulting in activated DCs (22). These may create clusters with T cells inside atherosclerotic lesions, or they may migrate to lymphoid organs and in turn induce T-cell activation, triggering cytokine release.

Adaptive immunity. The adaptive or specific immune system is a highly specialized defense mechanism responsible for countering pathogens. Adaptive immunity participates in the development of atherosclerosis in multiple ways: 1) via interactions between antigen-presenting cells (macrophages, DCs, B cells) and naive T cells, which creates a T-cell response; 2) T-cell cytokine release; and 3) antibody secretion.

Most T cells involved in the atherogenic process demonstrate a Th1 profile, followed by production of high amounts of INF-γ, resulting in MMP overexpression,

Table 1 Summary of the Most Important Cytokines, Proteins, and Enzymes Involved in the Atherosclerotic Process

Cytokine	Role
IL-6	Pro-inflammatory cytokine activates several adhesion molecules
IL-1β	Pyrogenic cytokine is produced mostly by monocytes
IL-10	Exhibits significant anti-inflammatory effects and acts via a feedback loop as a response to excessive inflammatory effects; inhibits macrophage activation via NF-κB pathway
IL-18	Significant inflammatory effects; key role in orchestrating the host defense systems against various infections
Proteins and Enzymes	
High-sensitivity CRP	Pivotal role in innate immunity; very reliable biomarker for general inflammation
Serum amyloid A	Synthesized in the liver as a response to TNF, IL-1, and IL-6 cytokine stimulation; elevated levels are associated with low-grade inflammation
Lipoprotein-associated phospholipase A_2	Unique roles in cleaving oxidized phospholipids and creating pro-inflammatory and proapoptotic lipid products
Fibrinogen	Regulator of blood viscosity and flow; ligand of ICAM-1

CRP = C-reactive protein; ICAM = intracellular cell adhesion molecule; IL = interleukin; TNF= tumor necrosis factor.

reduced collagen production, and thinning of the fibrous cap (23). In addition, INF-γ activates antigen-presenting cells, creating an ever-continuing circle of Th1 responses. Also, IL-12 produced by DCs activates STAT4 and T-box transcription factors expressed in T cells, leading to overexpression of INF-γ and attenuation of IL-4 and IL-5. Interactions between CD40, expressed by cells of the immune system and mostly in B cells, with its ligand CD40L, promote Th1 responses, and it has been proposed that inhibition of this pathway reduces atherosclerotic lesion development and causes a shift to a more plaque-stable profile (24,25). Furthermore, ox-LDL, responsible for foam cell formation, is also recognized as an antigen by the immune system. Anti–ox-LDL antibodies have been detected in patients with peripheral artery disease and CAD (26), whereas increased levels of these antibodies may be used to predict the severity of the disease. Human and microbial heat shock protein (HSP) cross-reaction via molecular mimicry may induce TLR-4 production in macrophages, further linking innate and adaptive immunity with the atherosclerotic process (27). Th2 cells are able to secrete several ILs (i.e., IL-4, -5, and -10) and stimulate B cells. Although Th2 cells are scarcely detected in atherosclerotic lesions, their production is stimulated in hyperlipidemic conditions. Up-regulation of IL-4 and IL-5 from differentiated Th2 cells suppresses INF-γ production, and it has been proposed that Th2 responses counter the proatherogenic potential of Th1-mediated responses (23). However there is still a debate about the exact role of Th2 cells in atherogenesis.

T regulatory cells (Tregs) can recognize specific self-antigens, which thus helps to prevent autoimmune responses by destroying pathogenic lymphocytes. Tregs are developed in the thymus and are maintained though simultaneous stimulation of the CD28-CD80/CD86 pathway. Tregs express CD4, CD25, and Foxp3. Furthermore, transforming growth factor (TGF)-β is a critical mediator of immune system homeostasis because it inhibits T-cell differentiation to Th1 and Th2 phenotypes and helps maintain Treg function.

Targeting Inflammation for the Treatment of Atherosclerosis

The most widely used and promising agents in cardiovascular disease, hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, are renowned for their "pleiotropic" lipid-lowering independent effects. Even though this concept has recently been challenged, data from large meta-analyses confirm that the beneficial effects exerted by statins in vascular disease states are independent of their lipid-lowering properties (Table 2). Statin's inhibition of the mevalonate pathway and its isoprenoid formation have been proposed to account for many of these non–lipid-lowering effects, because protein isoprenylation is implicated in intracellular signaling events (28). Asymmetrical dimethyl arginine (ADMA) is believed to be an important mediator of inflammation-induced endothelial dysfunction, leading to atherogenesis. Statins suppress the expression of pro-inflammatory mediators, resulting in reduced circulating ADMA levels (29).

It is well established that agents such as angiotensin-converting enzyme inhibitors (ACEI) and Ang-II receptor blockers are more than simple blood pressure-lowering drugs. Data suggest that these antihypertensive agents are able to modulate vascular redox state because they actively block angiotensin effects on the vascular wall. It is well known that Ang-II exerts pro-oxidant, pro-inflammatory, and proliferative effects on the vasculature mainly via the constitutively expressed AT1R, whereas AT2R is considered to have anti-inflammatory properties. The sulfhydryl ACEI zofenopril seems to have unique properties compared with other ACEIs; zofenopril reduces oxidative stress and enhances NO bioavailability in vitro in cell studies and in vivo in hypertensive patients (30). In addition, losartan inhibits Ang-II–induced expression of vascular remodeling agents (31) and differentiation of adventitial fibroblasts to myofibroblasts (32).

Statins and Their Anti-Inflammatory Effects: General Role of Statins in Inflammation

HMG-CoA is the precursor for cholesterol synthesis. HMG-CoA is formed by condensation of acetyl-CoA and acetoacetyl-CoA, catalyzed by HMG-CoA synthase. HMG-CoA reductase catalyzes the production of mevalonate from HMG-CoA, in which the HMG-CoA reductase reaction is the rate-limiting step for cholesterol

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Table 2 Overview of Diverse Statin Action Through Several Different Targets

Target	Mechanism of Action
Inflammation	• Inhibition of mevalonate synthesis pathway • Lowering of thrombus formation and LDL levels • Reduction of circulating ADMA levels
Redox-sensitive transcriptional pathways	• Inhibition of vascular Rac1-mediated activation of NADPH-oxidase • Improvement of NO bioavailability • Attenuation of vascular superoxide reduction • Endothelin-1 down-regulation • COX-2 and prostacyclin up-regulation • NF-κB, AP-1, and HIF-1 pathway down-regulation
Innate immunity system	• Reduction of MCP-1 • Up-regulation of ERK5
Adaptive immunity	• Differentiation toward Tregs and not Th-17 • Attenuation of Smad6 and Smad7; inhibition of TGF-β signaling • Blocking of CD4 T-cell–mediated endothelial cell apoptosis • Reduced T-cell expression of CD69 and TRAIL
Cytokines	• Attenuation of IL-6, IL-8, and MCP-1 production • Reduction of expression of several MMPs
Adhesion molecules	• Inhibition of Rho post-transcriptional modifications, thus attenuating the expression of ICAM, PECAM, and VCAM

ADMA = asymmetrical dimethyl arginine, AP = activator protein; COX-2 = cyclooxygenase-2; ERK = extracellular signal-regulated kinase; HIF = hypoxia-inducible factor; ICAM = intracellular cell adhesion molecule; IL = interleukin; LDL = low-density lipoprotein; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; NADPH = nicotinamide adenine dinucleotide; NO = nitric oxide; PECAM = platelet endothelial cell adhesion molecule; Tregs = regulatory T cells; VCAM = vascular cell adhesion molecule.

synthesis. Mevalonate is phosphorylated by 2 phosphate transfers from ATP, yielding the pyrophosphate derivative. Pyrophosphomevalonate decarboxylase catalyzes ATP-dependent decarboxylation, with dehydration, to yield isopentenyl pyrophosphate (isoprenylation). Isopentenyl pyrophosphate is the first compound in the mevalonate pathway. The pleiotropic effects of statins are mediated by inhibition of protein isoprenylation, altering several signal transduction molecules in the cardiovascular pathways. By inhibiting mevalonate synthesis, statins also prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (33). These isoprenoid intermediates serve as important lipid attachments for a variety of proteins, including the small guanosine triphosphate (GTP) binding proteins Ras, Rho, and Rac. Rho proteins are involved in the expression of pro-inflammatory cytokines; Rac proteins modulate ROS generation, whereas Ras proteins are involved in cell proliferation, apoptosis, and cell adhesion (28). Inhibition of Ras and Rho isoprenylation by statins renders them inactive. It has been demonstrated that co-incubation of human vessels with statins and mevalonate reverses the beneficial effects of statins on vascular redox state (34,35); such findings highlight the importance of the mevalonate pathway in mediating the pleiotropic effects of statins in humans. Statins lower LDL cholesterol by inhibiting HMG-CoA reductase. The lipid-lowering effect of statins on the reduction of cardiovascular events has been well established over the years through large randomized trials (36,37). Furthermore, statin treatment exhibited a reduction in thrombus formation along with lowering of LDL levels in hyperlipidemic patients (38).

Effect of Statins on Primary and Secondary Prevention of Cardiovascular Events

As discussed previously, statins are widely used for their lipid-lowering effects because their benefits have been well established through large clinical trials. However, the emerging role of statins in primary and secondary prevention of coronary events is the issue for discussion. Numerous large randomized clinical trials have demonstrated a variety of the non–lipid-lowering effects of statins (39–45). A brief summary of the most important randomized clinical trials is given in Table 3. In the MRC/BHF Heart Protection Study of more than 20,000 participants with coronary heart disease (CHD), occlusive arterial disease, or diabetes, simvastatin reduced all-cause and coronary mortality as well as vascular events. Participants were randomly allocated to receive either 40 mg of simvastatin or matching placebo. All-cause mortality was significantly reduced in the simvastatin group due to a highly significant proportional reduction in the coronary death rate. There were highly significant reductions of approximately one-quarter in the first-event rate for nonfatal MI or coronary death. For the first occurrence of any of these major vascular events, there was a definite reduction in the event rate. During the first year, there was no significant reduction in major vascular events, but subsequently, it was highly significant during each separate year. The proportional reduction in the event rate was similar (and significant) even in those who presented with LDL cholesterol concentrations below 3.0 mmol/l (116 mg/dl) or total cholesterol below 5.0 mmol/l (193 mg/dl) (42).

In addition, it was demonstrated in the TNT (Treating to New Targets) trial with a total of 10,001 participants with CHD, high doses of atorvastatin reduced the total risk for major adverse cardiovascular events (MACE) among these patients. The patients were randomly assigned to receive either 10 mg or 80 mg of atorvastatin and were followed for a median of 4.9 years. There was an absolute reduction in MACE for the group of patients who received a higher dose of atorvastatin (43), reflecting, not only the benefits of atorvastatin treatment, but also that of the dosage as well.

It is now well established that statins exert a crucial role in the primary prevention of acute coronary events. Results from the AFCAPS/TexCAPS (Air Force/Texas Coronary

Table 3 Summary of Key Clinical Trials Examining the Role of Statins in Primary and Secondary Prevention of Cardiovascular Events

Study (Ref. #)	Population	Intervention	Outcome
Primary Prevention			
JUPITER (39)	17,802 subjects (LDL <130 mg/dl, CRP >2.0 mg/l	Rosuvastatin vs. placebo	Rosuvastatin reduced risk for MACE (HR: 0.56; 95% CI: 0.46–0.69)
WOSCOPS (45)	6,595 men without CHD	Pravastatin vs. placebo	Pravastatin reduced coronary event(s) by 31% (95% CI: 17%–43%), and coronary mortality by 32% (95% CI: 3%–53%)
SPARCL (85)	4,731 patients with stroke or TIA, without known CHD	Atorvastatin 80 mg/day vs. placebo	Reduced stroke risk (HR: 0.84; 95% CI: 0.71–0.99) Reduced MACE risk (HR: 0.80; 95% CI: 0.69–0.92)
AFCAPS/Tex-CAPS (44)	6,605 subjects without CHD	Lovastatin vs. placebo	Lovastatin reduced incidence of first acute major coronary event(s) (RR: 0.63; 95% CI: 0.50–0.79)
Secondary Prevention			
Stable Coronary Heart Disease			
HPS (42)	20,536 CHD patients with, occlusive arterial disease, or diabetes	Simvastatin vs. placebo	Simvastatin reduced all-cause and coronary mortality, and vascular event(s) by 24% (95% CI: 19%–28%)
TNT (43)	10,001 CHD patients	Atorvastatin (high vs. low dose)	Reduced risk for MACEs with high dose treatment (HR: 0.78; 95% CI: 0.69–0.89)
4S (40)	4,444 CHD patients	Simvastatin vs. placebo	Simvastatin reduced risk of death (RR: 0.70; 95% CI: 0.58–0.85) and coronary death (RR: 0.58; 95% CI: 0.46–0.73)
CARE (86)	4,159 patients with MI	Pravastatin 40 mg/day vs. placebo	Reduced risk for coronary or nonfatal MI by 24% (95% CI: 9%–36%)
LIPS (87)	1,677 patients with stable or unstable angina or silent ischemia after PCI	Fluvastatin 80 mg/day vs. placebo	Reduced risk for MACE (RR: 0.78; 95% CI: 0.64–0.95)
LIPID (41)	9,014 CHD patients	Pravastatin vs. placebo	Pravastatin reduced coronary mortality risk by 24% (95% CI: 12%–35%)
Patients With or at High Risk For Developing Coronary Heart Disease			
ASCOT-LLA (88)	10,305 hypertensive patients, with at least 3 other risk factors for CHD	Atorvastatin 10mg/day vs. placebo	Reduced risk for primary event(s) (HR: 0.64; 95% CI: 0.50–0.83)
ALLHAT-LLT (89)	10,355 hypercholesterolemic, hypertensive patients (>55 yrs) with at least 1 additional CHD risk factor	Pravastatin 40 mg/day vs. usual care	No effect on all-cause mortality nor CHD
Patients With Acute Coronary Syndrome			
A to Z trial (90)	4,497 ACS patients	Simvastatin 40 mg/day for 1 month and then 80 mg/day vs. placebo for 4 month and then simvastatin 20 mg/day	Intensive simvastatin treatment demonstrated a favorable trend toward reduction of MACE
PROVE IT–TIMI 22 (91)	4,162 ACS patients	Atorvastatin 80 mg/day vs. pravastatin 40 mg/day	Atorvastatin significantly reduced primary endpoint by 16% (95% CI: 5%–26%).
Patients With Heart Failure			
CORONA (92)	5,011 ischemic, systolic HF patients (age ≥60 yrs, NYHA classes II–IV)	Rosuvastatin 10 mg/day vs. placebo	No difference in coronary event(s) or death.

ACS = acute coronary syndrome(s); ACOTT-LLA = Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm; AFCAPS/TexCAPS = Air Force/Texas Coronary Atherosclerosis Prevention Study; ALLHAT-LLT = Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial; A-to-Z = Aggrastat to Zocor; CABG = coronary artery bypass graft; CARE = Cholesterol and Recurrent Events; CHD = coronary heart disease; CI = confidence interval; CORONA = Controlled Rosuvastatin Multinational Study in Heart Failure; CRP = C-reactive protein; HF = heart failure; HPS = Heart Protection Study; HR = hazard ratio; JUPITER = Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; LDL = low-density lipoprotein; LIPID = Long-Term Intervention with Pravastatin in Ischaemic Disease; LIPS = Lescol Intervention Prevention Study; MACE = major adverse cardiac event(s); MI = myocardial infarction; NYHA = New York Heart Association; PCI = percutaneous coronary intervention; PROVE IT–TIMI = Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction; RR = relative risk; SPACL = Stroke Prevention by Aggressive Reduction in Cholesterol Levels; TIA = transient ischemic attack; TNT = Treating to New Targets; WOSCOPS = West of Scotland Coronary Prevention Study; 4S = Scandinavian Simvastatin Survival Study.

Atherosclerosis Prevention Study) (44) demonstrated just that with participants who had no clinical evidence of atherosclerotic cardiovascular disease. The subjects were randomized to receive either lovastatin or placebo and were followed for a median of 5.2 years. Treatment with lovastatin reduced the incidence of MI, unstable angina, coronary revascularization procedures, and cardiovascular events. Furthermore, in the WOSCOPS (West of Scotland Coronary Prevention Study), pravastatin reduced the coronary events and coronary mortality among 6,595 men with no history of MI, who received either 40 mg of atorvastatin or placebo (45).

The JUPITER trial (39) was the first trial that tried to address the question of whether statins' anti-inflammatory effects could alter the clinical outcome in apparently healthy men and women with low LDL levels. A total of

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17,802 participants who had no history of CVD and an LDL cholesterol level <130 mg/dl (3.4 mmol/l) and a high-sensitivity CRP level of 2.0 mg/l or more during the first visit were eligible for the trial. The patients were randomized in a 1:1 ratio to receive either 20 mg of rosuvastatin or placebo. Rosuvastatin reduced LDL cholesterol levels by 50% and high-sensitivity CRP levels by 37% (hazard ratio [HR] for rosuvastatin, 0.56; 95% confidence interval [CI]: 0.46 to 0.69; $p < 0.00001$) for the combined primary endpoint. The study demonstrated that rosuvastatin reduced the incidence of major cardiovascular events in apparently healthy, normolipidemic subjects with elevated CRP levels (39). The study highlighted the fact that even in low- or intermediate-risk patients without hypercholesterolemia, as established by their Framingham risk score, statin-induced attenuation of inflammation prevented CVD development and improved clinical outcome (39). Although in clinical trials, it can be only speculated that statin treatment can exert clinical benefits through the suppression of background inflammation, JUPITER's data are in accordance with data from mechanistic studies demonstrating the LDL-independent beneficial effects of statins. Therefore, the results from JUPITER come to facilitate data interpretation from basic science experiments into a clinical setting and cannot directly support the pleiotropic effects of statins as a stand-alone dataset. Other studies such as PRINCE (Pravastatin Inflammation/CRP Evaluation) demonstrated that oral administration of 40 mg/day pravastatin for a period of 24 weeks resulted in a significant reduction of serum CRP levels in subjects with and without CVD. These effects were independent of any changes in LDL levels (46). PRINCE confirmed the anti-inflammatory effects of statins in primary and secondary prevention, although it was not designed to access clinical outcome. In addition, other smaller studies have verified the anti-inflammatory properties of statins through reduction in CRP and pro-inflammatory cytokines levels in patients with metabolic syndrome, diabetes mellitus, and hypercholesterolemia (47–49). However, at a clinical level, it is still hard to dissect the contribution of LDL-independent mechanisms to the statin-induced reduction of cardiovascular risk.

Impact of Statins on Redox-Sensitive Transcriptional Pathways

Inhibition of the mevalonate pathway and isoprenoid formation by statins has been proposed to account for many of these non–lipid-lowering effects because protein isoprenylation is implicated in intracellular signaling events (28). Experimental data have demonstrated that statins have a favorable effect on vascular redox state by both reducing ROS production and enhancing antioxidant defense mechanisms. A major effect on vascular redox state is the blunting of Ang-II effects on the vascular wall by down-regulating AT1R expression and the reduction of NADPH oxidase (NOX)-derived ROS when stimulated by Ang-II. This might involve inhibition of Rac1 isoprenylation and membrane translocation, which is responsible for NOX activation (50). Heme oxygenase-1, an overall vasoprotective molecule, is up-regulated in human umbilical vein and aortic endothelial cells with atorvastatin treatment in vitro. Gene expression and activity of cellular antioxidant enzymes such as catalase, SOD, and Trx-1 have also been shown to be up-regulated by statins (50–52). In addition, statins improve endothelial function in many ways. NO bioavailability is increased, not only by statin-induced reduction in ROS production, but also by direct effects on eNOS enzyme. We have recently shown that short-term treatment with 40 mg of atorvastatin per day before coronary artery bypass graft improves redox state in saphenous vein grafts by inhibiting vascular Rac1-mediated activation of NOX independently of LDL levels (35). These antioxidant effects of atorvastatin were reversed by mevalonate and support the notion that even in patients with relatively low LDL, statins have direct beneficial antioxidant effects on blood vessels. We also demonstrated that statins exert a direct effect on the vascular wall through a BH4-mediated eNOS coupling, by improving NO bioavailability and vascular superoxide reduction (34). Statin-induced activation of PI3 kinase-Akt protein kinase pathway also increases NO production and inhibits endothelial cell apoptosis. In addition, by suppressing the expression of pro-inflammatory mediators, statins reduce circulating ADMA plasma levels and improve NO bioavailability (29). Furthermore, Rho kinase inhibition by statins reduces pre-proendothelin-1 mRNA expression and endothelin (ET)-1 bioavailability (28). On the other hand, cyclooxygenase (COX)-2 and prostacyclin, 2 key mediators of vascular redox, are also up-regulated by statin treatment (53). These combined effects favor endothelium-dependent vasodilation. Additionally, simvastatin-induced vascular endothelial growth factor (VEGF) production helps endothelial healing in injured hamster arteries (54). More importantly, studies have shown that all the well-known redox-sensitive pro-inflammatory transcription pathways, such as NF-κB, AP-1, and HIF-1, are down-regulated by statin treatment in VSMC (55), thus reducing proliferative and migrative responses (56). In summary, experimental data are in agreement with the positive findings of large clinical trials regarding statins in vascular disease states.

Effect of Statins on Innate Immune Responses

Endothelial injury triggers innate immune responses through leukocyte adhesion, transmigration, and movement of proteins out of the capillaries. Several studies have shown that statins confer favorable properties on ECs through the induction of the myeloid transcription factor KLF2 and its target genes. A more recent study implicated KLF4, another member of the Kruppel-like family, in mediating the anti-

inflammatory effects of statins on ECs (57). The authors demonstrated that statins up-regulate extracellular signal-regulated kinase (ERK5) through the MEK5/mitogen-activated protein kinase pathway. Endothelial oxidative stress can induce an inflammatory phenotype and senescence. We recently demonstrated that atorvastatin treatment for 3 days improves vascular redox state in saphenous vein grafts of patients undergoing coronary artery bypass graft surgery by inhibiting Rac-1–mediated activation of NADPH oxidase independently of LDL levels (35). IL-6 is key mediator of innate immunity affecting the JAK/STAT3 signaling pathway. A recent study showed that statins prevented the induction of monocyte chemoattractant protein (MCP)-1 and monocyte chemotaxis by suppressing IL-6 on human aortic endothelial cells (58).

Effects of Statins on Adaptive Immunity System

Tregs are a component of the immune system that suppresses immune responses of other cells. Tregs come in many forms, with the most well-understood being those that express CD4, CD25, and Foxp3. $CD4^+Foxp3^+$ Tregs play an essential role in regulating T-cell responses to both self and foreign antigens. It has been suggested that statins may promote the differentiation of Treg cells in the periphery while blocking the differentiation of pro-inflammatory helper T (Th17) cells (59). Statins divert T-cell differentiation toward Tregs and away from Th17 cells via a mechanism dependent on protein geranylgeranylation. $Foxp3^+$ T cells develop in the thymus but can also be induced in peripheral sites in the presence of TGF-β. Kim et al. (60) demonstrated that simvastatin blockade of the mevalonate pathway can mediate induction of mouse $Foxp3^+$ T cells and that simvastatin can synergize with low levels of TGF-β to induce $Foxp3^+$ T cells. The effects of simvastatin were secondary to a blockade of protein geranylgeranylation, were mediated at late time points after T-cell activation, and were associated with demethylation of the Foxp3 promoter. As demonstrated, 1 major effect of simvastatin was inhibition of the induction of Smad6 and Smad7, inhibitory Smads that inhibit TGF-β signaling. The investigators suggested that 1 mechanism responsible for the immunosuppressive effects of statins is the ability to promote the generation of $Foxp3^+$ T regulatory cells (60). Direct inhibitory effects of statins on T-cell activation and function, independent of antigen-presenting cells or Tregs, have been reported over the past years, although the mechanisms involved are not well characterized. Furthermore, the induction of Kruppel-like factor 2 (KLF2) may be the key to how statins alter T-cell function. KLF2 regulates the expression of molecules essential for naive T-cell recirculation and maintenance of T-cell quiescence. Bu et al. (61) showed that statins blocked activation-induced down-regulation of KLF2 in naive mouse and human T cells, and elevated KLF2 expression in effector T cells in a mevalonate-dependent fashion.

In addition, statins blocked proliferation and T-cell IFN-γ expression in a KLF2-dependent manner. In a mouse model of myocarditis involving adoptive transfer of heart antigen-specific T cells, statin treatment of transferred cells had effects similar to ectopic KLF2 expression in attenuating disease severity. One possible mechanism by which statins might enhance KLF2 expression is by inhibiting the mammalian target of rapamycin complex 1 (MTORC1) activation, a known inhibitor of KLF2 expression in activated T cells (62). In this regard, it is of interest that Ras homolog enriched in brain (Rheb), an isoprenylated Ras family GTPase, is required for MTORC1 activation. A recent study has revealed that statin inhibition of Rheb activation blocks MTORC1 activation in VSMCs, leading to impaired differentiation (63). Statins are known to reduce T-cell cytotoxicity in various in vitro assays. The number of $CD4^+$ T cells with TGF-related apoptosis-inducing ligand (TRAIL)-dependent cytotoxic activity against ECs and SMCs is increased in patients with ACS, and these cells are implicated in plaque destabilization. Recent experimental evidence showed that $CD4^+$ T cells from statin-treated patients with ACS were less effective in TRAIL-dependent killing of ECs than T cells from patients not treated with statins. In in vitro assays, rosuvastatin, fluvastatin, and pitavastatin directly blocked CD4 T-cell-mediated EC apoptosis and reduced T cell-expression of CD69 and TRAIL through TCR-induced ERK activation (64).

Effect of Statins on Circulating Pro-Inflammatory Cytokines

Early-stage atherogenesis entails leukocyte/endothelial interaction and accumulation of inflammatory cells; statins were shown to inhibit adhesion of leukocytes to ECs (65). Furthermore, HMG-CoA reductase inhibitors regulate the expression of chemokines controlling the migration of leukocytes to subendothelial sites of inflammation, such as MCP-1 and IL-8. Simvastatin was shown to reduce the expression of pro-inflammatory cytokines such as IL-6, IL-8, and MCP-1 in peripheral blood mononuclear cells from patients with hypercholesterolemia both in vitro and in vivo (66). MMPs were also described to be implicated in the process of monocyte/macrophage migration, and it has been revealed that statins diminished their expression. Thus, HMG-CoA reductase inhibitors lower the expression and function of a broad range of MMPs, including interstitial collagenases (MMP-1, MMP-13), gelatinases (MMP-2, MMP-9), and stromelysin (MMP-3).

Effect of Statins on Adhesion Molecules

Leukocyte transendothelial migration is regulated by the cooperative actions of ICAM-1 and VCAM-1 on both ECs and the leukocytes. Leukocytes cross ECs either through intercellular junctions (paracellular pathway) or through

the EC body (transcellular pathway). Paracellular transendothelial migration involves PECAM-1 and members of the JAM family (67–69).

The Rho family consists of 22 genes encoding at least 25 proteins in humans, of which the Rho, Rac, and Cdc42 proteins have been studied in the most detail (70,71). Within the Rho family, there are subgroups of closely related isoforms including Rho (A, B, C) and Rac (1, 2, 3). All Rho family members bind GTP, and most exhibit GTPase activity, cycling between an inactive GDP-bound form and an active GTP-bound form. Rho GTPase function is also regulated by their localization. Rho is required for leukocyte adhesion to ECs, for clustering of ICAM-1 and VCAM-1, and for formation of docking structures around adherent leukocytes (72,73). Most Rho family proteins undergo posttranslational modification by prenylation of a cysteine residue located 4 amino acids from the C terminus, followed by methylation of this cysteine and proteolytic removal of the last 3 amino acids. The prenyl group helps anchor the protein to membranes.

Isoprenoids are intermediates in the pathway to cholesterol synthesis, and their production is reduced by statins. As mentioned previously, some of the pleiotropic effects of statins are due to the inhibition of farnesyl-pyrophosphate and geranylgeranyl-pyrophosphate synthesis, the isoprenoids required for prenylation of Rho proteins. Statins thereby modulate Rho family protein subcellular localization, and this affects their stability and activity. Statins also alter Rho expression at the transcriptional level, although the mechanistic basis for this is not clear (74,75). Indirect evidence suggests that Rho GTPases regulate PECAM-1 expression on the EC surface. Statins not only affect the total levels of PECAM-1 in different EC lines (76,77) but also modulate PECAM-1 localization (76). Moreover, C3 transferase treatment mimics the effect of statins on PECAM-1 expression (76).

Statin Effects on Coenzyme Q10

Coenzyme Q10 is a lipid molecule composed of a 1,4-benzoquinone along with 50 carbon atoms and is an essential component of the inner membrane of mitochondria, acting as an electron transporter during ATP synthesis (78). The reduced form of coenzyme Q10, ubiquinol, has been demonstrated to prevent oxidation of key biological molecules such as proteins, lipids, and DNA. In addition, it is almost exclusively bound to the lipoprotein transport of LDL. Many studies have revealed that coenzyme Q10 has anti-inflammatory effects due to its ability to prevent LDL oxidation (79). Because coenzyme Q10 is synthesized from the mevalonate pathway, it is evident that statin administration inhibits its synthesis, often resulting in some adverse effects. Often, depletion of coenzyme Q10 can cause adverse effects, namely, myalgia and fatigue (80). Several studies confirm the fact that statin administration reduces serum levels of coenzyme Q10 (81,82). However, there is still a debate about whether this depletion can actually affect mitochondrial function (83). Other factors affecting circulating levels of coenzyme Q10 are age and exercise.

In a substudy of the CORONA (Controlled Rosuvastatin Multinational Study in Heart Failure) trial, coenzyme Q10 levels were assessed in 1,191 patients and were found lower in older patients with more severe signs of heart failure. Reduction in coenzyme Q10 levels was associated with worse clinical outcome in heart failure, suggesting that coenzyme Q10 can be used as a marker of advanced disease state, but not as an independent prognostic marker. Furthermore, statin treatment resulted in reduced levels of coenzyme Q10, although statin therapy has not been associated with worse clinical outcome, perhaps due to lack of statistical power or simply because its clinical significance is balanced by the beneficial effects of statin treatment on cardiovascular risk in these patients (84).

Conclusions

Given the fact that atherosclerosis is a multivariable disease, with several molecules involved in each stage, it is very difficult to find an effective treatment. However, statins prove to be the most effective treatment so far because they interfere with most of the critical components of the atherosclerotic process and have been proven to have beneficial effects. Further to their well-established impact on nonspecific low-grade inflammation, statins also appear to have significant effects on innate and adaptive immunity that have been underestimated so far. It is certain that their full potential has not yet been revealed and that they will remain the agent of choice for combating atherosclerosis and its related diseases.

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