Journal of the American College of Cardiology
© 2014 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 63, No. 23, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.01.054

STATE-OF-THE-ART PAPERS

# Innate and Adaptive Inflammation as a Therapeutic Target in Vascular Disease

## The Emerging Role of Statins

Dimitris Tousoulis, MD, PhD,* Costas Psarros, MSc,* Michael Demosthenous, MD,* Rikhil Patel, MD,† Charalambos Antoniades, MD, PhD,*† Christodoulos Stefanadis, MD*

*Athens, Greece; and Oxford, United Kingdom*

**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

From the *First Cardiology Department, Athens University Medical School, Athens, Greece; and the †Radcliffe Department of Medicine, Cardiovascular Medicine Division, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom. This study was supported by the Hellenic Cardiological Society. All authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Tousoulis and Psarros contributed equally to this work and should be considered joint first authors.

Manuscript received November 4, 2013; revised manuscript received January 10, 2014, accepted January 29, 2014.

JACC Vol. 63, No. 23, 2014
June 17, 2014:2491–502

**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