REVIEW

Pleiotropic vasoprotective effects of statins: The chicken or the egg?

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Abstract: Statins (3-hydroxy-3-methyl glutaryl coenzyme A [HMG-CoA] reductase inhibitors) are the most commonly used lipid-lowering drugs. Their main lipid-lowering effect is achieved by an increase in the expression of low-density lipoprotein cholesterol receptors associated with inhibition of cholesterol synthesis through inhibition of HMG-CoA reductase – the first and rate-limiting step in cholesterol synthesis. However, beyond cholesterol synthesis inhibition, inhibition of the HMG-CoA reductase affects as well the synthesis of other molecules with significant roles in different, yet often intercalating, metabolic pathways. On this basis, and supported by an increasing series of advocating epidemiological and experimental data, an extended dialogue has been established over the last few years regarding the nonlipid or "pleiotropic" actions of statins.

Keywords: statins, immunomodulatory, pleiotropic effects

Introduction

Statins are the most widely prescribed lipid-lowering drugs worldwide. They have been persistently shown to decrease serum low-density lipoprotein (LDL) cholesterol (by as much as 70%), total cholesterol and serum triglycerides and increase serum high-density lipoprotein (HDL) cholesterol. Statins target mainly hepatocytes and inhibit 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor. The mevalonate pathway then branches out before the synthesis of squalene and cholesterol (Figure 1). Other biologically important products are dolichols, which are involved in lipoprotein synthesis, ubiquinone, and the isoprenoids, farnesyl-pyrophosphate (PP) and geranylgeranyl-PP.[1] Isoprenoids can be covalently bound to proteins, a process termed prenylation, and play an important role in the post-translational modification of regulatory proteins, such as G proteins, Ras, Rho, and Rab, that influence the polymerization, the membrane anchoring and intracellular trafficking and thus the biologic activity of these proteins.[2]

Large-scale epidemiologic studies, such as 4S,[3] WOSCOPS,[4] CARE,[5] LIPID,[6] AFCAPS/TexCAPS,[7] REVERSAL,[8] AVERT,[9] PROVE IT-TIMI 22,[10] HPS,[11] and ASCOT-LLA[12] have provided solid data showing statins' preventive effects on the progression of atherosclerosis and its clinical sequelae. The beneficial effects of statins on vascular clinical events had been attributed solely to their lipid-lowering action until recently. However, the results from these and several other recent clinical studies

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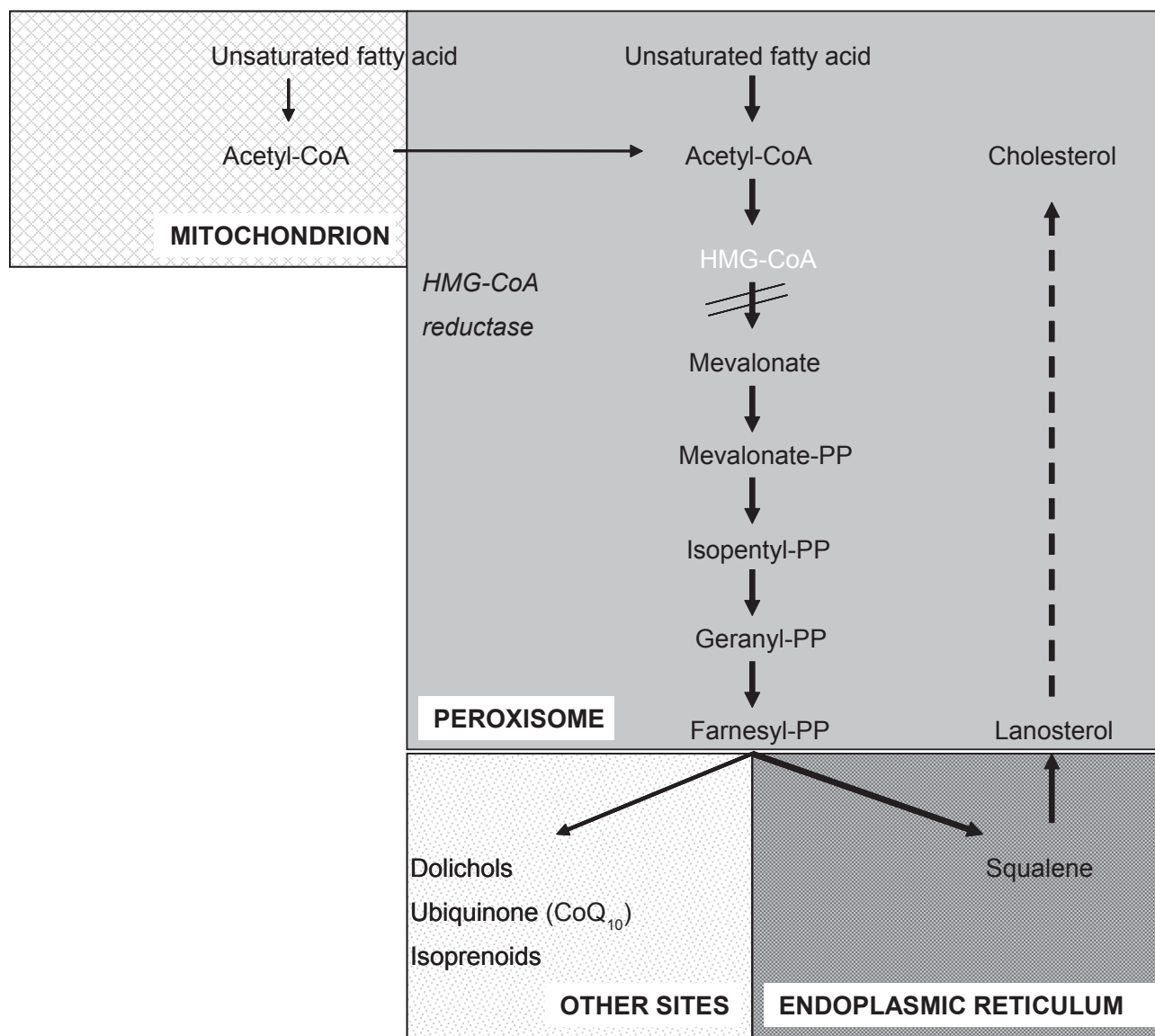Fax +30 23 5103 5555
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191

Figure showing the mevalonate pathway with boxes for MITOCHONDRION, PEROXISOME, OTHER SITES, and ENDOPLASMIC RETICULUM.

Within MITOCHONDRION: Unsaturated fatty acid → Acetyl-CoA (arrow pointing right to Acetyl-CoA in peroxisome region).

Central pathway: Unsaturated fatty acid → Acetyl-CoA → HMG-CoA → (HMG-CoA reductase, blocked) → Mevalonate → Mevalonate-PP → Isopentyl-PP → Geranyl-PP → Farnesyl-PP. Farnesyl-PP branches to Dolichols, Ubiquinone (CoQ₁₀), Isoprenoids (OTHER SITES) and to Squalene (ENDOPLASMIC RETICULUM). Squalene → Lanosterol → Cholesterol.

Figure 1 The mevalonate pathway.
Abbreviations: Acetyl-CoA, acetyl-coenzyme A; HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A; PP, pyrophosphate.

have been surprising and intriguing. In particular, statins' efficacy even in patients with normal cholesterol levels,[11,13] as well as the rapidity and magnitude of their action have suggested that other factors are at play. Careful comparison of the cardiovascular event rates in patients on statins with the rates expected for those with similar lipid levels from epidemiological data has shown that statin treatment decreases the rate below that expected.[4,14] Furthermore, although some regression in lesion size has been achieved with statins,[15] it is not of such a magnitude that could explain their clinical efficacy. The increasing perception of the inflammatory pathology of atherosclerosis has led to further research for the multipotentiality of these drugs. The term "pleiotropic effects" signifies effects of statins other than

cholesterol-lowering, though for many of these effects it has not been proved that they are really independent of their hypocholesterolemic action.

Mechanisms of action
Direct lipid actions
Inhibition of HMG-CoA reductase

Statins target hepatocytes and inhibit HMG-CoA reductase, the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor (Figure 1). Through the inhibition of this rate-limiting step in cholesterol synthesis statins achieve an increase in the expression of LDL cholesterol receptors, which results in increased take up by hepatocytes of LDL cholesterol from the circulation and a decrease in

plasma LDL cholesterol level. Statins do not simply exert a competitive action against the normal substrate in the enzyme active site; instead, they bind reversibly HMG-CoA reductase and result in a conformational change in the enzyme active site, preventing HMG-CoA reductase from attaining a functional structure. The resultant increase in hepatic cell LDL receptors determines the reduction of circulating LDL and of its precursors (intermediate-density [IDL] and very low-density [VLDL] lipoproteins).[16] Statins' efficacy in the reduction of triglyceride concentration parallels LDL cholesterol reduction.[17] HMG-CoA reductase inhibition by statins results also in the inhibition of hepatic synthesis of apolipoprotein B-100 and, thus, in the reduction of the synthesis and secretion of triglyceride rich lipoproteins, as well as in an increase in the production of receptors for apolipoprotein B. Statins have only modest effect on HDL increase and no influence on the concentration of lipoproteins.

Reduction of LDL susceptibility towards oxidation

At least five mechanisms have been proposed to explain statins' antioxidant properties:[18] (a) the reduced lipoprotein cholesterol and reduced level of oxidation substrate, as a result of their hypocholesterolemic effect; (b) the inhibition of the generation of superoxide by macrophages, which results in the decrease of cell oxygen production. The attenuation of the formation of superoxide anion in endothelial cells is also achieved by statins by preventing the prenylation of p21 Rac protein; (c) the preservation of the activity of the endogenous antioxidant system, like superoxide dismutase; (d) the binding of statins to phospholipids on the surface of lipoproteins (fluvastatin and lovastatin bind to LDL phospholipids) preventing the diffusion towards the lipoprotein core of free radicals generated during oxidative stress; and (e) the potent antioxidative potential of the metabolites (ie, atorvastatin and fluvastatin metabolites).

Inhibition of the expression of type A scavenger receptor

The inhibition of the expression of type A scavenger receptor in THP-1 cells and in human monocytes[19] decrease the receptor-mediated degradation of oxidized LDL (oxLDL). Statins also reduce mRNA level and CD36 expression on the cell surface, as well as LDL binding to human U937 monocytes.[20]

Intracellular signaling pathways

Several of the pleiotropic effects of statins, which affect endothelial function and redox equilibrium as well, have been attributed to the prenylation of regulatory proteins, such as the G proteins, and the enzymes involved in this prenylation have been proposed as potential targets for therapeutic intervention.[21] Prenylated proteins may interact with specific membrane-bound receptor proteins and hence prenylation mediates protein–protein interactions. The complex process of cell signaling is very important for intercellular communication. Extracellular signaling molecules, which are water-soluble and have high molecular weight, need to bind to specific receptors on the cell surface, which transduce the extracellular signals into the cell by intracellular signaling pathways. Many intracellular signaling molecules are prenylated proteins. The specific receptors on the cell surface are associated with trimeric G protein, or have Ser/Thr/Tyr kinases activities. The trimeric G protein has a geranylgeranylated subunit (gamma), allowing this signaling protein to be inserted in the cell membrane near specific membrane receptors and to receive extracellular signals, which are then transferred to the secondary signaling molecules in the cell. Another important class of prenylated signaling molecules are the components of Ras family, which are farnesylated and intermediate the Ser/Thr/Tyr kinases activities of membrane receptors from the cell surface. The small monomeric G proteins, such as Rho, are also regulated by prenylation. It has been known for some time that Rho is a regulator of actin-containing stress fibers of the cytoskeleton[22] and more recently of focal adhesion sites, a cell membrane component connected to stress fibers. These sites are foci where integrins congregate and through which a cell makes adhesive contacts with extracellular components, either other cells or extracellular matrix proteins. They also contain the proteins focal adhesion kinase (pp125 FAK), p130, and paxillin.[23] Recent evidence confirmed the essential role of Rho in the assembly of the focal adhesion sites.[24] Other G protein pathways may also be involved in focal adhesion formation, as the lipoxygenase-derived arachidonic acid metabolite 5-hydroxyeicosatetraenoic acid can activate neutrophil self-adhesion via the G protein pathway Raf-1/Mek/ Erk.[25] In the case of the $\beta 1$ integrins, which are involved in leukocyte adhesion to extracellular matrix, there is direct evidence that adhesion of U937 cells involves geranylgeranylated signaling proteins.[26]

Despite the fact that several effects of statins were shown to be independent of cholesterol lowering *in vitro*, extensive evidence links hypercholesterolemia *in vivo* with increased lipid peroxidation and increased oxidative stress,[27] and increased oxygen radical formation accompanying hypercholesterolemia influences many of the same factors that are modulated by statins via inhibition of prenylation.

It is well established that multiple signaling pathways regulating the expression of atherogenic genes are oxidation-sensitive, either because oxLDL activates them by binding to cell surface receptors or because increased extracellular lipid oxidation causes a shift in the intracellular redox balance.[28] Among the many oxidation-sensitive pathways that affect cell growth, secretory activity, and death, three are particularly relevant in inflammation and atherogenesis. The first of these is nuclear factor-κB (NFκB), which regulates adhesion molecules and growth factors, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1), important contributors to monocyte and T cell recruitment into the arterial intima.[29] The second is the apoptotic signaling pathway that is activated through Fas/FasL and tumor necrosis factor (TNF) receptors and regulates the expression of caspases and other effectors of apoptosis.[30] The third oxidation-sensitive pathway is the peroxisome proliferators-activated receptor γ (PPARγ) pathway. PPARγ is a nuclear receptor that regulates fat cell development and glucose. It is also highly expressed in macrophage/foam cells of atherosclerotic lesions. Activation of PPARγ by oxLDL or synthetic ligands upregulates the expression of the ABC-A1 transporter involved in reverse cholesterol transport from peripheral cells, but it also downregulates a number of pro-inflammatory factors, including TNFα, interleukin-1α (IL-1α), IL-6, the inducible nitric oxide synthase (iNOS), and gelatinase B, one of the metalloproteinases thought to promote plaque rupture.[31] Therefore, many of the factors which hypercholesterolemia and its associated oxygen radical formation affect are modulated by statins via inhibition of prenylation. For example, the conversion of NO into the less active peroxynitrate is the mechanism through which hypercholesterolemia induced LDL oxidation interferes with NO-mediated vasodilatation. Statins act on this site by decreasing the activity of NAD(P)H oxidase and therefore by increasing endothelial NO production and decreasing the production of reactive oxygen species (ROS), reducing thus both LDL oxidation and intracellular oxidative stress.[32]

Pleiotropic effects

Recent evidence revealed a multitude of actions of statins, other than lipid-lowering, on different types of cells, which have been addressed with the term "pleiotropic" and are predominantly vasoprotective. These include inhibition of smooth muscle cell growth, inhibition of neointima formation, induction of apoptosis in smooth muscle cells, reduction of leukocyte adhesion to and transmigration

through endothelial cells, induction of endothelial nitric oxide synthase, inhibition of endothelin and MCP-1 expression in endothelial cells, inhibition of MCP-1, tissue factor and matrix metalloprotease-9 expression in macrophages. Figure 2 summarizes the mechanisms through which statins exert their vasoprotective effects.

Effects on endothelial dysfunction and inflammation

Atherosclerosis is a complex inflammatory process characterized by the presence of monocytes, macrophages, and T lymphocytes in the atheroma.[33] Endothelial dysfunction is one of the earliest manifestations of atherosclerosis, occurring well before the presence of any angiographic evidence of disease.[34] Studies in animals and humans have shown that the combination of hemodynamic strain and the accumulation of lipids may initiate an inflammatory process in the artery. Activated endothelial cells express several types of leukocyte adhesion molecules, which cause blood cells rolling along the vascular surface to adhere at the site of activation.[35] An important characteristic of endothelial dysfunction is the paradoxical vasoconstriction caused by acetylcholine because of the impaired synthesis, release, and activity of endothelium-derived nitric oxide (NO). Statins reverse endothelial dysfunction through the reduction of both extracellular LDL oxidation (by reducing substrate availability) and intracellular oxidative stress (by cholesterol-independent effects on NO and, indirectly, by reducing oxLDL). Geranyl-geranylation of the GTP-inding protein Rho decreases endothelial cell nitric oxide synthase (eNOS) expression and inhibits nitric oxide-induced vascular relaxation. By blocking synthesis of geranyl-geranyl-PP, statins decrease geranylation of Rho and upregulate eNOS.[37,38] Nitric oxide generation in endothelial cells is also promoted by another mechanism. Statins activate the protein kinase Akt/PKB, which results in enhanced phosphorylation (activation) of its natural substrate, eNOS.[38] Regarding the effects of statins on Akt activation and nitric oxide, Laufs and colleagues[39] showed that statins prevent the hypoxia-induced downregulation of NOS 3 in human endothelial cells via inhibition of mevalonate synthesis. Furthermore, in human, increased circulating NO was found in response to fluvastatin treatment, which correlated with decreased circulating soluble P-selectin and ICAM-1 levels.[40] Kureishi and colleagues[38] found that simvastatin activates the signaling molecule Akt in human umbilical vein endothelial cells (HUVEC), an effect requiring phosphatidylinositol 3-kinase (PI3-kinase), which indeed is normally the upstream activator of Akt, and being inhibited by the specific PI3-kinase inhibitor wortmannin.
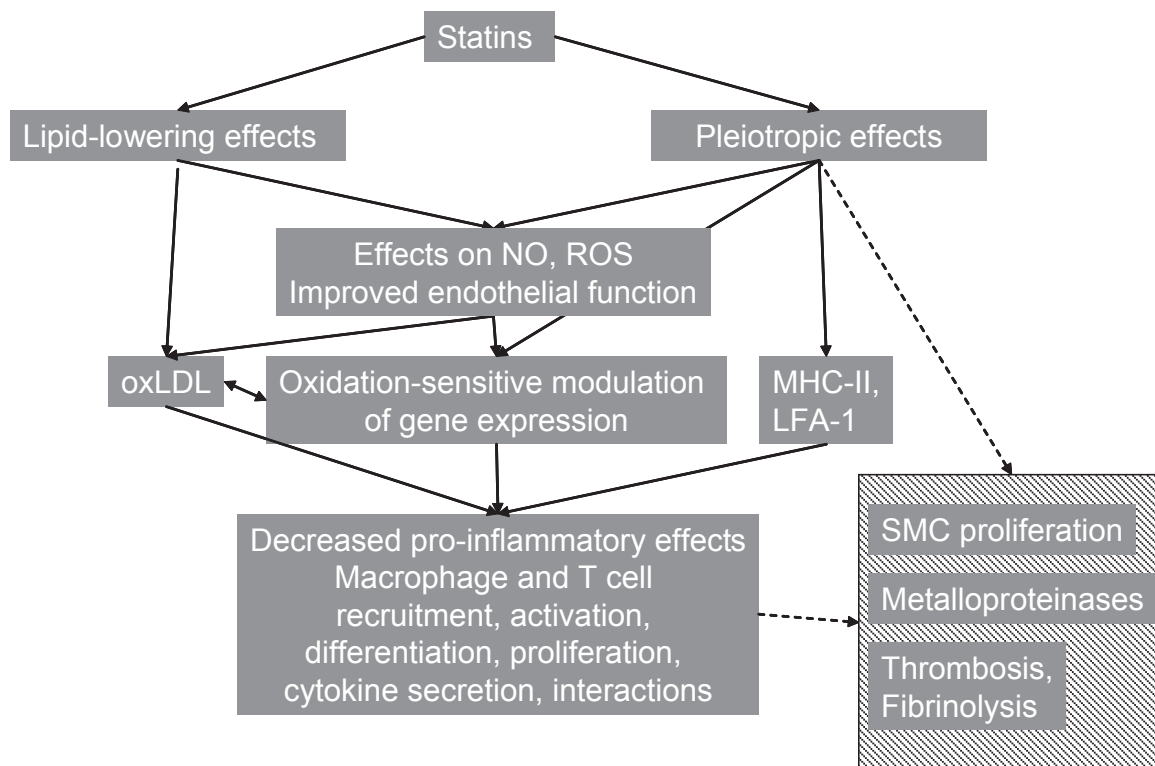
Figure 2 Pathways of the effects exerted by statins.
Abbreviations: LFA-1, leukocyte funtion antigen-1; MHL-II, major histocompatibility antigen-II; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; SMC, smooth muscle cells.

Normally, PI3-kinase activity is suppressed by mevalonate, so that the decrease in mevalonate concentration caused by statin action would be expected to increase PI3-kinase activity. Akt itself undergoes phosphorylation by PI3-kinase, thereby phosphorylating NOS 3 (one of several substrates for Akt) and thus increasing NO production. Enhanced NOS 3 activity, and hence NO production, will give rise not only to vasodilatation but also to other potentially beneficial and vasoprotective effects: inhibition of atherogenesis, inhibition of platelet activation and aggregation, attenuation of endothelial cell apoptosis, and promotion of angiogenesis. Increased formation of NO promotes arterial vasodilatation and inhibits atherogenesis.[41] NO is therefore emerging as a prime target for pharmacologic intervention.[42] Resistance to the inhibitory effect of oxLDL on NOS 3 activity has also been elicited by statin treatment *in vitro*. By contrast with the induction of NOS 3 by statins, which has a generally beneficial action, lovastatin inhibited NOS 2 (the inducible isoform of NOS) expression in rat astrocytes, microglia, and macrophages.[43] This may be advantageous, as the large quantities of NO generated by NOS 2 may not be as benign as the smaller amounts generated through NOS 3. In addition to preventing the Rho-mediated downregulation of eNOS, other

effects of statins have been linked to geranyl-geranylation, such as inhibition of proliferation and induction of apoptosis in SMCs,[44] inhibition of integrin-dependent leukocyte adhesion and increased fibrinolytic activity.[45] The observation of some of these effects, eg, improved vascular function,[46] in the absence of hypercholesterolemia supports the notion that they are cholesterol-independent. Unequivocal evidence that many of the above effects are due to protein prenylation has been provided by the fact that they are reversible by addition of geranylgeranyl-PP (or farnesyl-PP), which does not restore cholesterol synthesis, but not by addition of squalene or cholesterol.

Recent evidence suggests that statins possess anti-inflammatory properties because of their ability to reduce the number of inflammatory cells in atherosclerotic plaques.[47] The mechanisms have yet to be fully elucidated but seem to involve inhibition of adhesion molecules expression, reduction of leukocyte adhesion,[48] as well as inhibition of inflammatory and chemotactic cytokine production. Lovastatin and simvastatin have been found to reduce the production of MCP-1 in human peripheral blood mononuclear cells or endothelial cells following exposure to lipopolysaccharide (endotoxin), other bacterial products, or the inflammatory cytokine IL-1.

Likewise, they reduced the exudates content of MCP-1 and the degree of leukocyte accumulation in a mouse air-pouch inflammation model.[49] The expression of MCP-1 in both endothelial cells and monocyte-derived macrophages of atherosclerotic plaques is believed to be important in mediating monocyte chemotaxis and hence in stimulating atherogenesis. Indeed, it has been found that statins reduce the expression of adhesion molecules on leukocytes,[50] inhibit leukocyte-endothelium interactions,[51] and reduce inflammatory cell number within atherosclerotic plaques.[52] Xenos and colleagues[53] have shown in human iliac artery endothelial cell cultures that fluvastatin caused a substantial reduction in ICAM-1 expression, through the upregulation of eNOS and AMP kinase. Cicha and colleagues[54] in their study on HUVEC cultures found that statins reversed the shear stress-induced expression of adhesion molecules on endothelial cells. Moreover, Lin and colleagues[55] found in human aortic endothelial cell cultures that statins caused a similar reversion of homocystein-induced VCAM-1 expression upregulation. Interestingly, Liang and colleagues[56] found in HUVEC cultures that simvastatin reduced VCAM-1 expression on endothelial cells as well as the adhesiveness of monocytes, through inhibition of NF-κB activation by C-reactive protein (CRP).

Integrins are activated through a conformational change of their molecule and it has been suggested recently that the principal regulation of integrins *in vivo* is by receptor clustering into adhesion complexes.[57] Rho geranylgeranylation by HMG-CoA reductase products is likely to play a crucial role in this procedure.[58] Cerivastatin was shown by Yoshida and colleagues[59] to reduce human monocyte cell line adhesion to endothelial cells under physiological flow conditions via RhoA-dependent mechanisms. Similarly, atorvastatin was found to reduce the adhesion of U937 cells to HUVEC and to decrease RhoA and FAK activation also in those cells.[60] It is likely that HMG-CoA reductase inhibitors can inhibit focal adhesion complex formation and thereby inhibit leukocyte adhesion. In the absence of an activating signal, the β2 integrin leukocyte function antigen-1 (LFA-1) does not associate with lipid rafts. After its activation in T-lymphocytes, LFA-1 is mobilized to the lipid raft domains. The association between LFA-1 and lipid rafts is required for LFA-1-dependent adhesion to occur, and similar results were obtained with α4β1 integrin.[61] The recent work of Lum and colleagues[62] provided direct evidence of the involvement of these events in leukocyte-endothelial adhesion. RhoA molecule, beyond its involvement in these events of integrin activation that take place in leukocytes, with particular relevance to atherosclerosis, it is also involved in endothelial cells in creating receptor clusters, which allow adhesion to monocytes. Association with the actin cytoskeleton is required, but the formation of stress fibers is not.[63] Monocyte adhesion and spreading on human endothelial cells is dependent on Rho regulated receptor coupling in the latter. Therefore, both types of cell involved in the monocyte-endothelial interaction could possibly be affected by statin-mediated modification of Rho signaling, though evidence from cellular adhesion studies suggests that it is mainly the monocyte that is affected *in vivo*. Surprisingly, lovastatin, simvastatin, and other statins were found capable of binding to a novel site on the I-domain of LFA-1.[64] This domain is probably involved in activation changes that allow binding to ICAM-1. Interestingly, even the lactone forms of the statins were capable of binding despite having no activity against HMG-CoA reductase. This binding inhibits the adhesive activity of LFA-1 while, a novel high affinity statin- related compound, LFA703, was found to have powerful anti-inflammatory activity. However, this field remains controversial, as long as statin-LFA-1 interaction was not confirmed by others.

Further parallel evidence was obtained from the suppression of IL-8 and MCP-1 production by cerivastatin in macrophages.[65,66] Similarly, in endothelial cells, the more lipophilic statins upregulate PPARγ, resulting in decreased expression of IL-1, IL-6, and cyclooxygenase (COX)-2.[67] The decrease in IL-6 production could provide an explanation for the decreased production of CRP observed *in vivo* in patients on statin therapy, as IL-6 is the principal inducer of its synthesis in the liver.[68] More recently, it was shown in an *in vitro* system that human adipocytes can release CRP under inflammatory conditions and that this phenomenon may be modulated by statin treatment,[69] a finding which might explain in part the beneficial cardiovascular effects of these drugs.[70] Furthermore, recently it was found that statins reduce IL-6 induced CRP directly in hepatocytes via inhibition of protein geranylgeranylation and of the phsophorylation of the transcription factor STAT3.[71,72] Other probable beneficial effects of statins include an increase in the number and mobilization of circulating endothelial progenitor cells (EPCs), which are cell with reparative action on sites of ischemic injury, the inhibition of the migration and proliferation and the induction of apoptosis of vascular smooth muscle cells (SMCs)[73] and the inhibition of leukocyte–endothelial cell interactions.[74] Statin treatment has also been found to reduce effectively experimental atherosclerosis as well.[75–77]

Effects on immune responses

Although strictly speaking not yet proven, there is substantial evidence that statins may modulate immune responses. These include effects on the intimal recruitment, differentiation, proliferation, and secretory activity of a number of immune cells, mainly monocyte/macrophages and T cells.[78–82] Recently, statins were found to inhibit the expression of class II major histocompatibility antigens (MHC-II) on human macrophages, endothelial cells and SMCs stimulated by interferon γ (IFN-γ).[83,84] Precise regulation of MHC class II gene expression plays a pivotal role in the control of the immune response especially after transplantation. The expression of MHC-II on the surface of antigen-presenting cells together with processed antigens and cellular cofactors results in activation of the T cell receptor. Statins' effect on the expression of MHC-II is exerted by both lipophilic and hydrophilic statins at nanomolar to micromolar concentrations, but it is limited to antigen-presenting cells requiring co-stimulation by IFN-γ, an effect which is dose-dependent for both MHC class II protein as well as mRNA. Whereas a limited number of specialized antigen-presenting cells express MHC class II constitutively, numerous other cells become MHC class II-positive upon induction with IFN-γ.[85] This means that professional antigen-presenting cells constitutively expressing MHC-II, eg, B cells and dendritic cells are not affected. This complex regulation is under the control of the class II transactivator CIITA. Statins were found to inhibit promoter IV of the MHC-II transactivator CIITA, which regulates transcription of MHC-II and thus synthesis of the MHC-II protein.[86,87] In contrast, statins did not affect the expression of MHC class I, pointing to specific actions in the MHC class II signaling cascade. This discovery has been proposed[88] to provide a firm scientific rationale to recommend the use of this drug as an immunosuppressor. The reduction of MHC-II molecules on the vast majority of arterial cells leads to a reduction of T cell proliferation and differentiation. Mixed lymphocyte reactions showed that statin treatment of endothelial cells and SMCs indeed reduced T cell proliferation and IL-2 release. These *in vitro* data provide evidence that the overall effect of reduced activation and proliferation of T cells, mostly the Th1 cell subpopulation which is the one that secretes cytokines such as IFN-γ that promote inflammation, in the arterial wall, would likely be beneficial.

In another study, statins were found to exert a selective blocking of the β2 integrin LFA-1.[89] LFA-1, also known as CD11a/CD18, is expressed on the surface of leukocytes and, when activated, binds to ICAM-1. In addition to its role in leukocyte adhesion and extravasation, LFA-1 is a co-stimulator of T cells. At least one clinically used statin, lovastatin, as well as several modified statin compounds subsequently developed, bound selectively to a novel site of LFA-1 and prevented LFA-1–mediated adhesion and lymphocyte co-stimulation. This effect was unrelated to the statins' inhibition of HMG-CoA.

Antioxidant effects

Another class of actions for statins and a potential mechanism by which statins may improve endothelial function is through their antioxidant effects. In this regard, a recent study showed that pravastatin therapy caused an early significant decrease in serum malondialdehyde concentration, an index of lipid peroxidation and plaque instability, and increase in flow-mediated dilation of the brachial artery, before any substantial reduction in blood lipid levels, in 37 patients with unstable angina.[90] These findings suggest that pravastatin exerts pleiotropic effects on endothelial dysfunction during the early phase of the acute coronary syndrome, that are independent of the degree of plasma cholesterol lowering;[91] however, it has never been unequivocally demonstrated in humans that prolonged statin treatment exerts effects independent of LDL cholesterol lowering because the use of statins always resulted in reduced LDL cholesterol levels. In a recent study, Pretnar-Oblak and colleagues[92] found a significant improvement of the cerebrovascular reactivity to intravenous application of L-arginine, a perceived index of cerebral endothelial function, as well as flow-mediated dilatation of the brachial artery, after a three-month treatment with atorvastatin in patients with lacunar cerebral infarctions, a state of endothelial impairment. In their elegant study, Landmesser and colleagues[93] showed that despite the similar reduction in LDL cholesterol levels caused by simvastatin and ezetimibe, a novel cholesterol absorption inhibitor, only simvastatin resulted in beneficial effects on endothelial function.

Effects on plaque stability

It is now established that atherosclerotic plaques are heterogeneous and vary in their tendency to undergo thrombosis and the consequent acute clinical events. Plaques with a high degree of smooth muscle cell proliferation, giving rise to well-developed tough fibrous caps on the luminal side of the lesions, rarely are complicated by thrombosis and are termed stable plaques. On the other hand, lesions rich in inflammatory macrophages and lipid deposits are mechanically weaker, as metalloproteinase enzymes from macrophages digest the extracellular matrix and weaken the wall.

They frequently crack, so exposing circulating platelets to an abnormal vascular wall that rapidly induces activation and a thrombotic mass.[94] These are unstable plaques, the inflammatory nature of which is central to their clinically hazardous behavior. Coronary events are the result of unstable atherosclerotic lesion rupture and thrombus formation.[95] High circulating cholesterol, apart from promoting atherogenesis, may also give rise to an increase in thrombogenesis through an increase in tissue factor expression.[96] Fluvastatin has been shown to decrease this prothrombotic tendency with an associated reduction in tissue factor, and this may be partly through cholesterol lowering but also through non-cholesterol-mediated actions, such as reduced prenylation of the Rho protein Cdc42. Statins influence plaque stability by preventing macrophage activation, reducing the uptake and endogenous synthesis of cholesterol and the production of metalloproteinases by macrophages.[97] Metalloproteinases are the enzymes responsible for weakening the plaque's fibrous cap, thereby increasing the risk of rupture. In addition, lipid lowering by statins contributes to stability by reducing plaque size, by modifying the physicochemical properties of the lipid core,[98] by combined reduction in lipids, lipid oxidation, matrix-metalloproteinase-2, inflammation, and cell death, or by increases in tissue inhibitor of metalloproteinase-1 and collagen content.[99]

Effects on thrombogenesis and thrombolysis

It is now established that atherosclerotic plaques are heterogeneous and vary in their tendency to undergo thrombosis and the consequent acute clinical events. Plaques rich in inflammatory macrophages and lipid deposits are mechanically weaker ("unstable plaques"), as metalloproteinase enzymes from macrophages digest the extracellular matrix and weaken the wall, they frequently crack, so exposing circulating platelets to an abnormal vascular wall that rapidly induces activation and a thrombotic mass. High circulating cholesterol, beyond promoting atherogenesis, may also give rise to an increase in thrombogenesis through an increase in tissue factor expression, in platelet reactivity, in thromboxane A_2 (TXA_2) synthesis, in platelet α_2 adrenergic receptor density, in platelet cytosolic calcium, and changes in platelet membrane phospholipids and cholesterol. Platelets play a critical role in the development of acute coronary syndromes.[102] Although the precise mechanisms involved are not fully understood, statins have been shown to influence platelet function,[101] probably through reduction in the production of TXA_2, increased synthesis of prostacyclin,[102] and modifications in the cholesterol content of platelet membranes. Notably,

one of the well-characterized effects of endothelial NO is its inhibition of platelet aggregation. Thrombosis on mildly damaged swine arteries at high shear rate was reduced by half with atorvastatin.[103] Likewise, blood from patients on statins showed significant reduction in platelet thrombus formation,[104] and platelet-derived thrombin generation was decreased.[105] Both lipid-lowering and nonlipid-related effects are likely to contribute, as patients with hypercholesterolemia have hyperreactive platelets, which would be normalized with lipid lowering.[106] Furthermore, multiple other routes of action have been detailed.[107] Hypercholesterolaemic patients also have increased circulating coagulation factors and increased soluble CD41 ligand, a cell-activating factor derived from activated platelets; these components are reduced by pravastatin or cerivastatin treatment.[108] Mechanisms involved in the antithrombotic action of statins may include the augmented production of NO from endothelial cells, as described above. In addition, atorvastatin administered to mice was found to enhance platelet production of NO, and this was accompanied by a decrease in circulating markers of platelet activation,[109] which is probably related to the reduction of geranylation of Rap1b, a protein involved in platelet aggregation.[110,111] The effect of statins on other cells involved in thrombosis also appears to play a part. Human aortic smooth muscle cells *in vitro* were found to increase their expression of COX-2 and production of prostacyclin, a platelet inhibitory agent, under the influence of mevastatin or lovastatin.[102] In addition, statins have been shown to mitigate platelet stimulation in a time- and dose-dependent manner, to decrease the prothrombin fragments F1+2 in plasma from patients with type 2 diabetes, independent of cholesterol levels, and to act as inhibitors of tissue factor-dependent thrombin generation.[112] The authors of a recent study hypothesized that statins decrease the exocytosis of Weibel–Palade bodies, which are endothelial cell granules whose contents promote thrombosis and vascular inflammation.[113] Simvastatin decreased thrombin-stimulated Weibel–Palade body exocytosis by 89%, in part by increasing the synthesis of NO, which induced s-nitrosylation of the n-ethylmaleimide-sensitive factor, a critical regulator of exocytosis. Simvastatin treatment also decreased the myocardial infarct size by 58% in wild-type but not eNOS knockout mice. Furthermore, simvastatin decreased endothelial exocytosis and neutrophil infiltration into the ischemic reperfused myocardium, which was mediated in part by the P-selectin contained in the Weibel–Palade bodies. However, simvastatin did not affect the exocytosis and inflammation in the myocardial infarcts of eNOS knockout mice. Inhibition of endothelial exocytosis is

a novel mechanism by which statin inhibitors reduce vascular inflammation, inhibit thrombosis, and protect the ischemic myocardium. Recently, an *in vivo* study investigated the effects of rosuvastatin on vascular remodeling and thrombosis after arterial injury in apoE–/– mice. In the rosuvastatin treated mice, the size of the neointimal area and the severity of the luminal stenosis were significantly reduced, and these effects were independent of systemic lipid lowering.[114]

Furthermore, statins exert antithrombotic effects on monocytes through reduction of the synthesis of plasminogen activator inhibitor (PAI)-1, a change likely to result in enhanced fibrinolysis and thrombus dissolution.[115,116] The same result may be achieved in another route in endothelial cells, as statins cause an increase in expression of tissue plasminogen activator (t-PA). Activation of the coagulation pathway may also be impeded, as tissue factor expression has been found to be prevented by statins in human endothelial cells.[117] There is increasing evidence from *in vitro* studies that statins positively affect the fibrinolytic system of cultured smooth muscle cells as well as endothelial cells. In these studies a decrease in PAI-1 and an increase in t-PA were observed after co-treatment with statins in endothelial cells.[118] It seems likely that further pathways remain to be discovered. Remarkably, statins may have a direct influence on the coagulation pathway itself, as patients treated with simvastatin were seen to have decreased rates of stimulated activation of fibrinogen, prothrombin, factor V, and factor XIII.[119] These changes did not relate to cholesterol lowering. Finally, initiation of thrombosis may be substantially inhibited by the stabilization of plaques by statin therapy. Furthermore, fluvastatin has been shown to decrease prothrombotic tendency with an associated reduction in tissue factor,[121] and this may be partly through cholesterol lowering but also through noncholesterol-mediated actions, such as reduced prenylation of the Rho protein Cdc42.

Clinical studies

Statin therapy has been shown to reduce cardiovascular risk even in patients without vascular disease.[121] Large epidemiologic studies have shown that CRP is a predictor of cardiovascular disease in the general population[122–125] as well as special patient populations, such as patients with diabetes mellitus and patients with end-stage renal disease.[126–129] Statin therapy lowers high sensitivity-CRP (hs-CRP) levels in patients with hypercholesterolemia.[130] Long-term therapy with pravastatin in the Cholesterol and Recurrent Events (CARE) trial also reduced the levels of CRP,[131] a change which was not found to correlate with the reduction in LDL cholesterol levels.

The latter finding seems to be confirmed by recent trials, such as the PRINCE study.[132] In the more recent JUPITER study,[133] a four-year treatment with rosuvastatin resulted in considerable decrease in hs-CRP levels and cardiovascular morbidity in healthy adults without hyperlipidemia. However, any potential clinical benefits conferred by the lowering of hs-CRP levels are difficult to separate from those of the lipid-lowering effects of statins without further clinical studies and, even in the JUPITER study in patients with no hyperlipidemia, hs-CRP lowering was escorted by a significant reduction in LDL cholesterol levels below normal levels during the first year of the study. Lately, the independent effects of statins on CRP are debated.[134] In their extensive meta-analysis, Genser and colleagues[135] found a close correlation between statin-induced reductions in LDL cholesterol and CRP which has not been evident from individual studies. This discrepancy could be attributed to the great variability and the non-normal distribution of the values of CRP. As long as serum CRP originates predominately from the liver and secondly form the sites of inflammation, different degrees of inflammation could probably stimulate different sites of CRP production. If this hypothesis is true, CRP measured in low degrees of inflammation, such as in the general population, could originate from local production in atherosclerotic plaques, while CRP measured in higher degrees of inflammation, such as in end-stage renal disease patients, could originate predominantly from liver production. Statins effects might differ in each situation, and their effects on the CRP produced by the liver might be linked somehow with the inhibition of HMG-CoA reductase, while peripherally produced CRP might be genuinely independent. Unfortunately, in CARE study there has not been a separate analysis of the correlation between CRP and lipid decrease in those patients with the highest levels of CRP, it is however characteristic that these patients enjoyed the maximal cardiovascular benefits. However, in PRINCE study such a correlation was indeed found in the patients with hsCRP values within the highest quartile. Other studies revealed several other beneficial effects of statins, most notably their immunosuppressant effects in heart transplant recipients. These studies have suggested a better transplant outcome in patients taking statin therapy[136,137] as well as improved endothelial function and reduced inflammatory cytokine release.[138] In renal transplant recipients, some evidence for similar beneficial effects of statins on endothelial functions has been noted[139,140] and, probably, on the rate of acute rejection reactions,[141] although the later is still debatable.[142–144]

The effect of serum lipids on monocyte adhesion molecules has not been investigated until recently.

The monocyte adhesion molecules CD11b[145] and CD14 have been found to be elevated and L-selectin to be decreased (usually, this occurs as a consequence of cell activation) in patients with hypercholesterolemia compared with control subjects; furthermore, the levels of each of these adhesion molecules were found to correlate with those of LDL.[146] Simvastatin was found to correct the levels of each of these towards normal. The exposure of normal leukocytes to LDL induced changes similar to those in the hypocholesterolemic patients. It is important to note that changes in monocyte adhesion molecule expression in the patients can be a direct consequence of their exposure to increased LDL levels. Reversal with statins may therefore be attributable both to their hypocholesterolemic effect and to another direct effect on the cells unrelated to lipid lowering. Finally, several *in vivo* studies have also investigated the effect of statin therapy on the regulation of the fibrinolytic system. Although the results are inconsistent, in some studies statins decrease PAI-1 plasma levels.[147]

Conclusions

In recent years several *in vitro* and *in vivo* studies have provided solid evidence suggesting that statins exert multiple vasoprotective effects. Some of them are likely to be independent of the lipid-lowering effects of these drugs. However, caution is needed when an effect is to be disconnected from others, as long as it is now clear that there are multiple interconnections between lipid- and nonlipid-lowering pathways and the endothelium is the center where many different physiologic intercalating pathways converge. Furthermore, hypercholesterolemia itself has an inflammatory effect on the vascular endothelium and liver.[148] To this extent, the correlation between different effects of statins appears to be like the causality dilemma, "which came first, the chicken or the egg?", and it wouldn't be surprising if future research revealed a correlation of effects which nowadays seem independent. Given all the evidence that research has come up to, it is now tempting to question the multiple-dispersed "pleiotropic" effects of statins in favor of a unifying theory that would bind together the pieces of the puzzle.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature*. 1990;343:425–430.
2. Casey PJ. Protein lipidation in cell signaling. *Science*. 1995;268:221–225.
3. The Scandinavian Simvastatin Survival Study (4S). Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease. *Lancet*. 1994;344:1383–1389.
4. West of Scotland Coronary Prevention Study Group. Influence of pravastatin and plasma lipids on clinical events in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation*. 1998;97:1440–1445.
5. Ridker PM, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation*. 1999;100:230–235.
6. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. *N Engl J Med*. 1998;339:1349–1357.
7. Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA*. 1998;279:1615–1622.
8. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA*. 2004;291:1071–1080.
9. Pitt B, Waters D, Brown WV, et al. Aggressive lipid-lowering therapy compared with angioplasty in stable coronary artery disease. Atorvastatin versus Revascularization Treatment Investigators. *N Engl J Med*. 1999;341:70–76.
10. Ray KK, Cannon CP, McCabe CH, et al; PROVE IT-TIMI 22 Investigators. Early and late benefits of high-dose atorvastatin in patients with acute coronary syndromes: results from the PROVE IT-TIMI 22 trial. *J Am Coll Cardiol*. 2005;46:1405–1410.
11. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360:7–22.
12. Sever PS, Poulter NR, Dahlöf B, et al. Reduction in cardiovascular events with atorvastatin in 2,532 patients with type 2 diabetes: Anglo-Scandinavian Cardiac Outcomes Trial – lipid-lowering arm (ASCOT-LLA). *Diabetes Care*. 2005;28:1151–1157.
13. Sacks FM, Pfeffer MA, Moye LA, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med*. 1996;335:1001–1009.
14. Schwartz GG, Olsson AG, Ezekowitz MD, et al. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. *JAMA*. 2001;285:1711–1718.
15. Taylor AJ, Kent SM, Flaherty PJ, et al. ARBITER: Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol: a randomized trial comparing the effects of atorvastatin and pravastatin on carotid intima medial thickness. *Circulation*. 2002;106:2055–2060.
16. Sehayek E, Butbul E, Avner R. Enhanced cellular metabolism of very low density lipoprotein by simvastatin: a novel mechanism of action of HMG-CoA reductase inhibitors, *Eur J Clin Invest*. 1994;24:173–178.
17. Stein EA, Lane M, Laskarzewski P. Comparison of statins in hypertriglyceridemia. *Am J Cardiol*. 1998;81:66B–69B.
18. Stancu C, Sima A. Statins: mechanism of action and effects. *J Cell Mol Med*. 2001;5:378–387.
19. Hrboticky N, Draude G, Hapfelmeier G, et al. Lovastatin decreases the receptor mediated degradation of acetylated and oxidized LDLs in human blood monocytes during early stage of differentiation into macrophages. *Arterioscler Throm Vasc Biol*. 1999;19:1267–1275.
20. Pietsch A, Erl W, Lorenz RL. Lovastatin reduces the expression of the combined adhesion and scavenger receptor CD36 in human monocytic cells. *Biochem Pharmacol*. 1996;52:433–439.

21. Cohen LH, Pieterman E, van Leeuwen RE, et al. Inhibitors of prenylation of Ras and other G-proteins and their application as therapeutics. *Biochem Pharmacol*. 2000;60:1061–1068.

22. Hall A. Rho GTPases and the actin cytoskeleton. *Science*. 1998;279:509–514.

23. Flinn HM, Ridley AJ. Rho stimulates tyrosine phosphorylation of focal adhesion kinase, p130 and paxillin. *J Cell Sci*. 1996;109:1133–1141.

24. Ridley AJ, Hall A. Signal transduction pathways regulating Rho-mediated stress fibre formation: requirement for a tyrosine kinase. *EMBO J*. 1994;13:2600–2610.

25. Capodici C, Pillinger MH, Han G, et al. Integrin-dependent homotypic adhesion of neutrophils. Arachidonic acid activates Raf-1/MEK/Erk via a 5-lipoxygenase-dependent pathway. *J Clin Invest*. 1998;102:165–175.

26. Liu L, Moesner P, Kovach NL, et al. Integrin-dependent leukocyte adhesion involves geranylgeranylated protein(s). *J Biol Chem*. 1999;274:33334–33340.

27. Reilly MP, Praticò D, Delanty N, et al. Increased formation of distinct F2 isoprostanes in hypercholesterolemia. *Circulation*. 1998;98:2822–2828.

28. Napoli C, de Nigris F, Palinski W. Multiple role of reactive oxygen species in the arterial wall. *J Cell Biochem*. 2001;82:674–682.

29. Collins T, Cybulsky M. NF-kB: pivotal mediator or innocent bystander in atherogenesis? *J Clin Invest*. 2001;107:255–264.

30. Napoli C, Quehenberger O, de Nigris F, et al. Apoptosis induced by mildly oxidized LDL involves multiple apoptotic signaling pathways in human coronary cells. *FASEB J*. 2000;14:1996–2007.

31. Li AC, Brown KK, Silvestre MJ, et al. Peroxysome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J Clin Invest*. 2000;106:523–531.

32. Münzel T, Keaney JF Jr. Are ACE inhibitors a "magic bullet" against oxidative stress? *Circulation*. 2001;104:1571–1574.

33. Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J*. 1999;138:S419–S420.

34. Libby P. Molecular bases of the acute coronary syndromes. *Circulation*. 1995;91:2844–2850.

35. Eriksson EE, Xie X, Werr J, et al. Importance of primary capture and L-selectin-dependent secondary capture in leukocyte accumulation in inflammation and atherosclerosis *in vivo*. *J Exp Med*. 2001;194:205–218.

36. Laufs U, La Fata V, Plutzky J, et al. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation*. 1998;97:1129–1135.

37. Hernandez-Perrera O, Perez-Sala D, Soria E, et al. Involvement of Rho GTPases in the transcriptional inhibition of preproendothelin-1 gene expression by simvastatin in vascular endothelial cells. *Circ Res*. 2000;87:616–622.

38. Kureishi Y, Luo Z, Shiojima I, et al. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat Med*. 2000;6:1004–1010.

39. Laufs U, Fata VL, Liao JK. Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase blocks hypoxia-mediated downregulation of endothelial nitric oxide synthase. *J Biol Chem*. 1997;272:31725–31729.

40. Romano M, Mezzetti A, Marulli C, Fluvastatin reduces soluble P-selectin and ICAM-1 levels in hypercholesterolemic patients: role of nitric oxide. *J Investig Med*. 2002;48:183–189.

41. Davis KL, Martin E, Turko IV, et al. Novel effects of nitric oxide. *Annu Rev Pharmacol Toxicol*. 2001;41:203–236.

42. Ignarro LJ, Napoli C, Loscalzo J. Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide: An Overview. *Circ Res*. 2002;90:21–28.

43. Pahan K, Sheikh FG, Namboodiri AM, Singh I. Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. *J Clin Invest*. 1997;100:2671–2679.

44. Guijarro C, Blanco-Colio LM, Ortego M, 3-hydroxy-3-methylglutaryl coenzyme A reductase and isoprenylation inhibitors induce apoptosis of vascular smooth muscle cells in culture. *Circ Res*. 1998;83:490–500.

45. Essig M, Nguyen G, Prié D, Escoubet B, Sraer JD, Friedlander G. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells: role of geranylgeranylation and Rho proteins. *Circ Res*. 1998;83:683–690.

46. Laufs U, Gertz K, Huang P, et al. Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from cerebral ischemia in normocholesterolemic mice. *Stroke*. 2000;31:2442–2449.

47. Vaughan CJ, Gotto AM Jr, Basson CT. The evolving role of statins in the management of atherosclerosis. *J Am Coll Cardiol*. 2000;35:1–10.

48. Scalia R. Statins and the response to myocardial injury. *Am J Cardiovasc Drugs*. 2005;5:163–170.

49. Romano M, Diomede L, Sironi M, et al. Inhibition of monocyte chemotactic protein-1 synthesis by statins. *Lab Invest*. 2000;80:1095–1100.

50. Niwa S, Totsuka T, Hayashi S. Inhibitory effect of fluvastatin, an HMG-CoA reductase inhibitor, on the expression of adhesion molecules on human monocyte cell line. *Int J Immunopharmacol*. 1996;18:669–675.

51. Vaughan CJ, Murphy MB, Buckely BM. Statins do more than just lower cholesterol. *Lancet*. 1996;348:1079–1082.

52. Rossen RD. HMG-CoA reductase inhibitors: a new class of anti-inflammatory drugs? *J Am Coll Cardiol*. 1997;30:1218–1219.

53. Xenos ES, Stevens SL, Freeman MB, Cassada D, Goldman MH. Nitric oxide mediates the effect of fluvastatin on intercellular adhesion molecule-1 and platelet endothelial cell adhesion molecule-1 expression on human endothelial cells. *Ann Vasc Surg*. 2005;19:386–392.

54. Cicha I, Goppelt-Struebe M, Yilmaz A, Daniel WG, Garlichs CD. Endothelial dysfuntion and monocyte recruitment in cells exposed to non-uniform shear stress. *Clin Hemorheol Microcirc*. 2008;39:113–119.

55. Lin CP, Chen YH, Lin WT, et al. Direct effect of statins on homocystein-induced endothelial adhesiveness: potential impact to human atherosclerosis. *Eur J Clin Invest*. 2008;38:106–116.

56. Liang YJ, Shyu KG, Wang BW, Lai LP. Simvastatin inhibits C-reactive protein-induced proinflammatory changes in endothelial cells by decreasing mevolanate pathway products. *Cardiology*. 2008;110:182–190.

57. van Kooyk Y, Figdor CG. Avidity regulation of integrins: the driving force in leukocyte adhesion. *Curr Opin Cell Biol*. 2000;12:542–547.

58. Zhou Q, Liao JK. Statins and cardiovascular diseases : from cholesterol lowering to pleiotropy. *Curr Pharm Des*. 2009;15(5):467–478.

59. Yoshida M, Sawada T, Ishii H, et al. HMG-CoA reductase inhibitor modulates monocyte-endothelial cell interaction under physiological flow conditions in vitro: involvement of Rho GTPase-dependent mechanism. *Arterioscler Thromb Vasc Biol*. 2001;21:1165–1171.

60. Kawakami A, Tanaka A, Nakajima K, et al. Atorvastatin attenuates remnant lipoprotein-induced monocyteadhesion to vascular endothelium under flow conditions. *Circ Res*. 2002;91:263–271.

61. Leitinger B, Hogg N. The involvement of lipid rafts in the regulation of integrin function. *J Cell Sci*. 2002;115:963–972.

62. Lum AF, Green CE, Lee GR, et al. Dynamic regulation of LFA-1 activation and neutrophil arrest on intercellular adhesion molecule 1 (ICAM-1) in shear flow. *J Biol Chem*. 2002;277:20660–20670.

63. Wojciak-Stothard B, Williams L, Ridley AJ. Monocyte adhesion and spreading on human endothelial cells is dependent on Rho-regulated receptor clustering. *J Cell Biol*. 1999;145:1293–1307.

64. Weitz-Schmidt G, Welzenbach K, Brinkmann V, et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med*. 2001;7:687–692.

65. Kothe H, Dalhoff K, Rupp J, et al. Hydroxymethylglutaryl coenzyme A reductase inhibitors modify the inflammatory response of human macrophages and endothelial cells infected with Chlamydia pneumoniae. *Circulation*. 2000;101:1760–1763.

66. Ikeda U, Shimada K. Statins and monocytes. *Lancet*. 1999;353:2070.

67. Inoue I, Goto S, Mizotani K, et al. Lipophilic HMG-CoA reductase inhibitor has an anti-inflammatory effect: reduction of mRNA levels for interleukin-1b, interleukin-6, cyclooxygenase-2, and p22phox by regulation of peroxisome proliferator-activated receptor a (PPARa) in primary endothelial cells. *Life Sci*. 2000;67:863–876.

68. Kluft C, de Maat MP, Gevers Leuven JA, Potter van Loon BJ, Mohrschladt MF. Statins and C-reactive protein. *Lancet*. 1999;353:1274.

69. Calabro P, Chang DW, Willerson JT, et al. Release of C-reactive protein in response to inflammatory cytokines by human adipocytes linking obesity to vascular inflammation. *J Am Coll Cardiol*. 2005;46(6):1112–1113.

70. Calabro P, Yeh ET. Multitasking of the 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor: beyond cardiovascular diseases. *Curr Atheroscler Rep*. 2004;6:36–41.

71. Arnaud C, Burger F, Steffens S, et al Statins reduce interleukin-6-induced C-reactive protein in human hepatocytes. New evidence for direct anti-inflammatory effects of statins. *Arterioscler Thromb Vasc Biol*. 2005;25:1231–1236.

72. Mayer C, Gruber HJ, Landl EM, et al. Rosuvastatin reduces interleukin-6-induced expression of C-reactive protein in human hepatocytes in a STAT3- and C/EBP-dependent fashion. *Int J Clin Pharmacol Ther*. 2007;45:319–327.

73. Erl W. Statin-induced vascular smooth muscle cell apoptosis: a possible role in the prevention of restenosis? *Curr Drug Targets Cardiovasc Haematol Disord*. 2005;5:135–144.

74. Pruefer D, Scalia R, Lefer AM. Simvastatin inhibits leukocyte-endothelial cell interactions and protects against inflammatory processes in normocholesterolemic rats. *Arterioscler Thromb Vasc Biol*. 1999;19:2894–2900.

75. Sukhova GK, Williams JK, Libby P. Statins reduce inflammation in atheroma of nonhuman primates independent of effects on serum cholesterol. *Arterioscler Thromb Vasc Biol*. 2002;22:1452–1458.

76. Niwa S, Totsuka T, Hayashi S. Inhibitory effect of fluvastatin, an HMG-CoA reductase inhibitor, on the expression of adhesion molecules on human monocyte cell line. *Int J Immunopharmacol*. 1996;18:669–675.

77. Pruefer D, Makowski J, Schnell M, et al. Simvastatin inhibits inflammatory properties of Staphylococcus aureus alpha-toxin. *Circulation*. 2002;106:2104–2110.

78. Laufs U, Liao JK. Direct vascular effects of HMG-CoA reductase inhibitors. *Trends Cardiovasc Med*. 2000;10:143–148.

79. Weber C, Erl W, Weber KS, et al. HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. *J Am Coll Cardiol*. 1997;30:1212–1217.

80. Pahan K, Sheikh FG, Namboodiri AM, et al. Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages. *J Clin Invest*. 1997;100:2671–2679.

81. Rudich SM, Mongini PK, Perez RV, et al. HMG-CoA reductase inhibitors pravastatin and simvastatin inhibit human B-lymphocyte activation. *Transplant Proc*. 1998;30:992–995.

82. Katznelson S, Wang XM, Chia D, et al. The inhibitory effects of pravastatin on natural killer cell activity *in vivo* and on cytotoxic T lymphocyte activity *in vitro*. *J Heart Lung Transplant*. 1998;17:335–340.

83. Kwak B, Mulhaupt F, Myit S, et al. Statins as a newly recognized type of immunomodulator. *Nat Med*. 2000;6:1399–1402.

84. Palinski W. Immunomodulation: A new role for statins? *Nat Med*. 2000;6:1311–1312.

85. Reith W, Mach B. The bare lymphocyte syndrome and the regulation of MHC expression. *Annu Rev Immunol*. 2001;19:331–373.

86. Kwak B, Mulhaupt F, Veillard N, et al. The HMG-CoA reductase inhibitor simvastatin inhibits IFNgamma induced MHC class II expression in human vascular endothelial cells. *Swiss Med Wkly*. 2001;131:41–46.

87. Sadeghi MM, Tiglio A, Sadigh K, et al. Inhibition of interferon-gamma mediated microvascular endothelial cell major histocompatibility complex class II gene activation by HMG-CoA reductase inhibitors. *Transplantation*. 2001;71:1262–1268.

88. Palinski W, Tsimikas S. Immunomodulatory effects of statins: Mechanisms and potential impact on arteriosclerosis. *J Am Soc Nephrol*. 2002;13:1673–1681.

89. Weitz-Schmidt G, Welzenbach K, Brinkmann V, et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med*. 2001;7:687–692.

90. Karatzis E, Lekakis J, Papamichael C, et al. Rapid effect of pravastatin on endothelial function and lipid peroxidation in unstable angina. *Int J Cardiol*. 2005;101:65–70.

91. Laufs UWS, Wassmann S, Hilgers S, et al. Rapid effects on vascular function after initiation and withdrawal of atorvastatin in healthy, normocholesterolemic men. *Am J Cardiol*. 2001;88:1306–1307.

92. Pretnar-Oblak J, Sabovic M, Sebestjen M, et al. Influence of atorvastatin treatment on L-arginine cerebrovascular reactivity and flow-mediated dilatation in patients with lacunar infarctions. *Stroke*. 2006;37:2540–2545.

93. Landmesser U, Bahlmann F, Mueller M, et al. Simvastatin versus ezetimibe: pleiotropic and lipid-lowering effects on endothelial function in humans. *Circulation*. 2005;111:2356–2363.

94. Davies MJ, Richardson PD, Woolf N, et al. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J*. 1993;69:377–381.

95. Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation*. 1995;92:657–671.

96. Camera M, Toschi V, Comparato C, et al. Cholesterol-induced thrombogenicity of the vessel wall: inhibitory effect of fluvastatin. *Thromb Haemost*. 2002;87:748–755.

97. Bellosta S, Canavesi VM, Pfister P, et al. HMG-CoA reductase inhibitors reduce MMP-9 secretion by macrophages. *Arterioscler Thromb Vasc Biol*. 1998;18:1671–1678.

98. Koh KK. Effects of statin on vascular wall: vasomotor function, inflammation, and plaque stability. *Cardiovasc Res*. 2000;47:648–657.

99. Crisby M, Nordin-Fredriksson G, Shah PK, et al. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. *Circulation*. 2001;103:926–933.

100. Fitzgerald DJ, Roy L, Catella F, et al. Platelet activation in unstable coronary disease. *N Engl J Med*. 1986;315:983–989.

101. Huhle G, Abletshauser C, Mayer N, et al. Reduction of platelet activity markers in type II hypercholesterolemic patients by a HMG-CoA-reductase inhibitor. *Thromb Res*. 1999;95:229–234.

102. Degraeve F, Bolla M, Blaie S, et al. Modulation of COX-2 expression by statins in human aortic smooth muscle cells. Involvement of geranylgeranylated proteins. *J Biol Chem*. 2001;276:46849–46855.

103. Alfon J, Royo T, Garcia-Moll X, et al. Platelet deposition on eroded vessel walls at a stenotic shear rate is inhibited by lipid-lowering treatment with atorvastatin. *Arterioscler Thromb Vasc Biol*. 1999;19:1812–1817.

104. Thompson PD, Moyna NM, White CM, et al. The effects of hydroxyl-methyl-glutaryl co-enzyme A reductase inhibitors on platelet thrombus formation. *Atherosclerosis*. 2002;161:301–306.

105. Puccetti L, Bruni F, Bova G, et al. Effect of diet and treatment with statins on platelet-dependent thrombin generation in hypercholesterolemic subjects. *Nutr Metab Cardiovasc Dis*. 2001;11:378–387.

106. Athyros VG, Kakafika AI, Tziomalos K, Karagiannis A, Mikhailidis DP. Pleiotropic effects of statins – clinical evidence. *Curr Pharm Des*. 2009;15(5):479–489.

107. Hussein O, Rosenblat M, Schlezinger S, et al. Reduced platelet aggregation after fluvastatin therapy is associated with altered platelet lipid composition and drug binding to platelets. *Br J Clin Pharmacol*. 1997;44:77–84.

108. Cipollone F, Mezzetti A, Porreca E, et al. Association between enhanced soluble CD40L and prothrombotic state in hypercholesterolemia: effects of statin therapy. *Circulation*. 2002;106: 399–402.

109. Laufs U, Gertz K, Huang P, et al. Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from cerebral ischemia in normocholesterolemic mice. *Stroke*. 2000;31:2442–2449.

110. Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins. *JAMA*. 1998;279:1643–1650.

111. Comparato C, Altana C, Bellosta S, et al. Clinically relevant pleiotropic effects of statins: drug properties or effects of profound cholesterol reduction? *Nutr Metab Cardiovasc Dis*. 2001;11:328–343.

112. Fenton JW 2nd, Brezniak DV, Ofosu FA, et al. Statins and thrombin. *Curr Drug Targets Cardiovasc Haematol Disord*. 2005;5: 115–120.

113. Yamakuchi M, Greer JJ, Cameron SJ, et al. HMG-CoA reductase inhibitors inhibit endothelial exocytosis and decrease myocardial infarct size. *Circ Res*. 2005;96:1185–1192.

114. Schafer K, Kaiser K, Konstantinides S. Rosuvastatin exerts favourable effects on thrombosis and neointimal growth in a mouse model of endothelial injury. *Thromb Haemost*. 2005;93:145–152.

115. Ishibashi T, Nagata K, Ohkawara H, et al. Inhibition of Rho/Rho-kinase signaling downregulates plasminogen activator inhibitor-1 synthesis in cultured human monocytes. *Biochim Biophys Acta*. 2002;1590:123–130.

116. Bourcier T, Libby P. HMG-CoA reductase inhibitors reduce plasminogen activator inhibitor-1 expression by human vascular smooth muscle and endothelial cells. *Arterioscler Thromb Vasc Biol*. 2000;20:556–562.

117. Eto M, Kozai T, Cosentino F, et al. Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. *Circulation*. 2002;105:1756–1759.

118. Essig M, Nguyen G, Prie D, Escoubet B, Sraer JD, Friedlander G. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells: role of geranylgeranylation and Rho proteins. *Circ Res*. 1998;83:683–690.

119. Undas A, Brummel KE, Musial J, et al. Simvastatin depresses blood clotting by inhibiting activation of prothrombin, factor V, and factor XIII and by enhancing Va production. *Circulation*. 2001;103: 2248–2253.

120. Colli S, Eligini S, Lalli M, Camera M, Paoletti R, Tremoli E. Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. *Arterioscler Thromb Vasc Biol*. 1997;17:265–272.

121. Ky B, Rader DJ. The effects of statin therapy on plasma markers of inflammation in patients without vascular disease. *Clin Cardiol*. 2005;28:67–70.

122. Sesso HD, Buring JE, Rifai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. *JAMA*. 2003;290:2945–2951.

123. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med*. 2002;347:1557–1565.

124. Di Napoli M, Schwaninger M, Cappelli R, et al. Evaluation of C-reactive protein measurement for assessing the risk and prognosis in ischemic stroke: a statement for health care professionals from the CRP Pooling Project members. *Stroke*. 2005;36:1316–1329.

125. Shankar A, Li J, Nieto FJ, Klein BE, Klein R. Association between C-reactive protein level and peripheral arterial disease among US adults without cardiovascular disease, diabetes, or hypertension. *Am Heart J*. 2007;154:495–501.

126. Parfrey PS, Foley RN, Harnett JD, Kent GM, Murray DC, Barre PE. Outcome and risk factors for left ventricular disorders in chronic uraemia. *Nephrol Dial Transplant*. 1996;11:1277–1285.

127. Yeun JY, Levine RA, Mantadilok V, Kaysen GA. C-reactive protein predicts all-cause and cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis*. 2000;35:469–476.

128. Qureshi AR, Alvestrand A, Divino-Filho JC, et al. Inflammation, malnutrition, and cardiac disease as predictors of mortality in hemodialysis patients. *J Am Soc Nephrol*. 2002;13(Suppl 1): S28–S36.

129. Papagianni A, Kalovoulos M, Kirmizis D, et al. Carotid atherosclerosis is associated with inflammation and endothelial cell adhesion molecules in chronic haemodialysis patients. *Nephrol Dial Transplant*. 2003;18:113–119.

130. Ridker PM, Rifai N, Lowenthal SP. Rapid reduction in C-reactive protein with cerivastatin among 785 patients with primary hypercholesterolemia. *Circulation*. 2001;103:1191–1193.

131. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E, for the Cholesterol and Recurrent Events (CARE) Investigators. Longterm effects of pravastatin on plasma concentration of C-reactive protein. *Circulation*. 1999;100:230–235.

132. Albert MA, Danielson E, Rifai N, et al. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA*. 2001;286:64–70.

133. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195–2207.

134. Shaw SM, Fildes JE, Yonan N, Williams SG. Pleiotropic effects and cholesterol-lowering therapy. *Cardiology*. 2009; 112(1):4–12.

135. Genser B, Grammer TB, Stojakovic T, Siekmeier R, Maerz W. Effect of HMG-CoA reductase inhibitors on low-density lipoprotein cholesterol and C-reactive protein: systematic review and metaanalysis. *Int J Clin Pharmacol Ther*. 2008;46:497–510.

136. Kobashigawa JA, Katznelson S, Laks H, et al. Effect of pravastatin on outcomes after cardiac transplantation. *N Engl J Med*. 1995;333: 621–627.

137. Wenke K, Meiser B, Thiery J, et al. Simvastatin reduces graft vessel disease and mortality after heart transplantation: a four-year randomized trial. *Circulation*. 1997;96:1398–1402.

138. Weis M, Pehlivanli S, Meiser BM, et al. Simvastatin treatment is associated with improvement in coronary endothelial function and decreased cytokine activation in patients after heart transplantation. *J Am Coll Cardiol*. 2001;38:814–818.

139. Asberg A, Hartmann A, Fjeldsa E, et al. Atorvastatin improves endothelial function in renal-transplant recipients. *Nephrol Dial Transplant*. 2001;16:1920–1924.

140. Hausberg M, Kosch M, Stam F, et al. Effect of fluvastatin on endothelium-dependent brachial artery vasodilation in patients after renal transplantation. *Kidney Int*. 2001;59:1473–1479.

141. Katznelson S, Wilkinson AH, Kobashigawa JA, et al. The effect of pravastatin on acute rejection after kidney transplantation – a pilot study. *Transplantation*. 1996;61:1469–1474.

142. Holdaas H, Jardine AG, Wheeler DC, et al. Effect of fluvastatin on acute renal allograft rejection: a randomized multicenter trial. *Kidney Int*. 2001;60:1990–1997.

143. Kasiske BL, Heim-Duthoy KL, Singer GG, et al. The effects of lipid-lowering agents on acute renal allograft rejection. *Transplantation*. 2001;72:223–227.

144. Fellström B, Holdaas H, Jardine AG, et al. Assessment of Lescol in Renal Transplantation Study Investigators. Effect of fluvastatin on renal end points in the Assessment of Lescol in Renal Transplant (ALERT) trial. *Kidney Int*. 2004;66:1549–1555.

145. Weber C, Erl W, Weber KS, Weber PC. HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. *J Am Coll Cardiol*. 1997;30:1212–1217.

146. Serrano Jr CV, Yoshida VM, Venturinelli ML, et al. Effect of simvastatin on monocyte adhesion molecule expression in patients with hypercholesterolemia. *Atherosclerosis*. 2001;157:505–512.

147. Dangas G, Smith DA, Unger AH, et al. Pravastatin: an antithrombotic effect independent of the cholesterol-lowering effect. *Thromb Haemost*. 2000;83:688–692.

148. Liao F, Andalibi A, Qiao JH, Allayee H, Fogelman AM, Lusis AJ. Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice. *J Clin Invest*. 1994;94:877–884.