

Review

Immunomodulatory Effects of HMG-CoA Reductase Inhibitors

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Abstract. 3-Hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are competitive inhibitors of the rate-limiting enzyme in cholesterol synthesis. Several clinical trials have shown a marked reduction in cholesterol levels associated with decreased cardiovascular mortality in patients treated with statins. However, more recent observations have suggested that the clinical benefits of statins may be, at least in part, independent of the effect of statins on cholesterol synthesis. These so-called pleiotropic or cholesterol-independent effects of statins could be the result of reduction in the formation of intermediaries in the mevalonate pathway as statins, by inhibiting L-mevalonic acid synthesis, also prevent the production of isoprenoids in the cholesterol biosynthetic pathway. Isoprenoids serve as important lipid attachments for the posttranslational modification of a variety of proteins such as small GTP-binding proteins of the Ras superfamily implicated in intracellular signaling. The list of different pleiotropic effects of statins is still growing and includes, among others, direct effects of statins on modulating endothelial function, decreasing oxidative stress and, more recently, anti-inflammatory and immunomodulatory actions of statins. For instance, statins decrease T cell activation, the recruitment of inflammatory cells into atherosclerotic lesions, and inhibit IFN- γ expression of MHC II on antigen-presenting cells. This review article summarizes the anti-inflammatory and immunomodulatory effects of statins and thus provides a new rationale to use statins as a new class of immunosuppressive agents.

Key words: HMG-CoA reductase inhibitors; immunomodulation; transplantation; inflammation.

Introduction

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are potent inhibitors of cholesterol biosynthesis that are extensively used in the treatment of patients with hypercholesterolemia^{5, 15, 21, 38, 78}. Statins impair cholesterol synthesis by inhibiting the activity of the enzyme HMG-CoA reductase, the rate-limiting step in cholesterol synthesis (Fig. 1).

Moreover, inhibition of cholesterol biosynthesis is accompanied by an increase in low-density lipoprotein (LDL) receptors in the liver, leading to increased uptake and clearance of cholesterol from the plasma^{21, 78}.

Since 1976, when ENDO et al.¹⁷ reported the discovery of mevastatin, the first member of the statin family, several statins have been launched by different pharmaceutical companies^{30, 44, 80, 91}. Currently two types of statins are available: the fermentation-derived or natural

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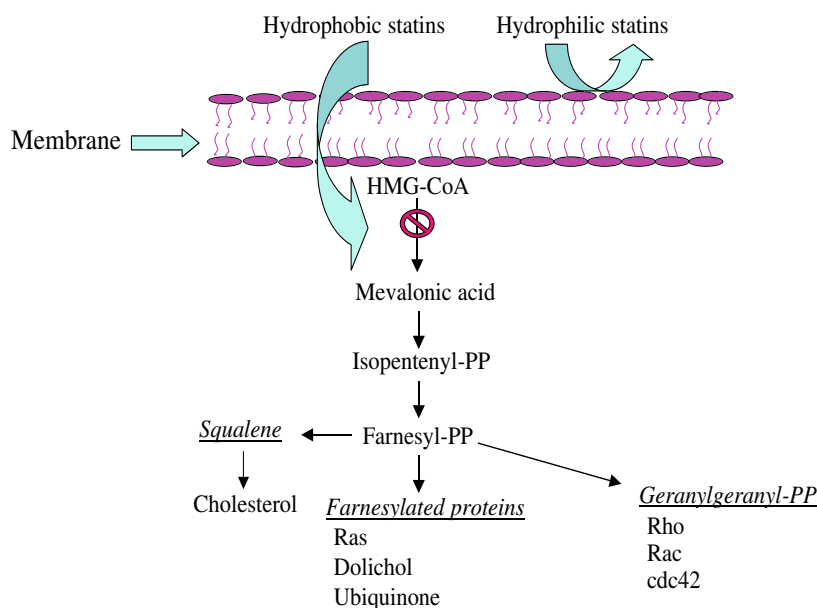


Fig. 1. Diagram of the cholesterol biosynthesis pathway indicates that statins, by decreasing isoprenylation of signaling molecules such as Ras, Rho, and Rac, lead to modulation of various signaling pathways. These effects are more pronounced with hydrophobic statins, as hydrophilic statins have a more limited ability to enter non-hepatic cells

statins (e.g. simvastatin) and the synthetic statins (e.g., atorvastatin). The chemical structures of the natural statins are very similar however, the pharmacokinetics of pravastatin is quite different. For instance, simvastatin and lovastatin are lactone prodrugs that undergo hydrolysis by non-specific carboxyesterases found in the liver, intestinal wall, and plasma³⁰. In contrast, pravastatin is administered as an active drug, and undergoes extensive microsomal metabolism by cytochrome p450. Furthermore, contrary to other statins, pravastatin is enzymatically transformed in the liver cytosol and thereafter undergoes significant renal clearance^{26, 31}. Statins are usually well tolerated, the major adverse effect being myopathy, defined as muscle pain or weakness associated with significant elevation of creatinine kinase levels¹⁸. The lipophilic statins (e.g. simvastatin, atorvastatin) are much more widely taken up by passive diffusion into a broad range of tissues and cells as compared with hydrophilic statins (e.g. pravastatin)³⁴. Thus, an important distinction among statins may arise from the ability of non-hepatic cells to transport the different members of the statin family into the cell based on their hydrophobicity (Fig. 1)^{12, 32, 34, 39}.

In the last decade, several large trials have demonstrated the beneficial effects of statins in lowering cardiovascular-related morbidity and mortality in patients with coronary artery disease^{13, 28, 50, 71, 73, 74}. Studies such as the Scandinavian Survival Study or 4S, the West of Scotland Study or WOSCOPS, and the recently

published Heart Protection Study or HPS, with over 30,000 participants have clearly established the essential role of statins in primary and secondary prevention of cardiovascular diseases^{28, 73, 74}. Because of the strong association between serum cholesterol levels and coronary disease, it was initially assumed that the cardiovascular benefits of statin therapy is solely due to its lipid-lowering effect⁶. However, in these large clinical studies, the observed clinical benefits occurred as early as 6 months after the initiation of statin therapy whereas in other studies using other antilipidemic agents such as niacin, it took almost 16 years before a mortality benefit could be observed^{3, 25}. Furthermore, subgroup analysis of the 4S, WOSCOPS, HPS, and cholesterol and recurrent events (CARE) trials also showed significantly lower cardiovascular events in statin-treated individuals compared with others with comparable cholesterol levels^{28, 66, 71, 73, 74}.

Another recent area of research interest is the impact of statin therapy on the incidence of stroke^{23, 54}. While several large multi-center trials have consistently shown a clear reduction in stroke incidence in patients on statins^{23, 29, 54, 75}, epidemiologic data has failed to make any strong association between hypercholesterolemia and stroke⁸¹. In the recently published Prospective Pravastatin Pooling Project, a systematic review of several large clinical trials, such as Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID), CARE and WOSCOPS, involving more than 100,000

person-years, BYINGTON et al.⁸ showed a significant reduction in total stroke incidence with pravastatin therapy. Almost all of the prevented stroke events were atheroembolic. This was in stark contrast to previous trials using non-statin lipid-lowering therapy, which have never shown statistically significant impact on the incidence of stroke².

Therefore, while the beneficial effects of statins are assumed to result from competitive inhibition of cholesterol synthesis, there is a growing body of literature, including several large clinical studies, that support the pleiotropic effects of statins independent of their ability to lower serum cholesterol levels.

Pleiotropic Effects of Statins

Recent clinical and experimental studies suggest that some of these cholesterol-independent or „pleiotropic” effects of statins might be the result of reduction in the formation of intermediaries in the mevalonate pathway (Fig. 1). By inhibiting L-mevalonic acid synthesis, statins also prevent the production of other isoprenoids in the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP)²². Both FPP and GGPP serve as important lipid attachments for the posttranslational modification of a variety of proteins, such as small GTP-binding proteins of the Ras superfamily⁸³.

By cycling between inactive GDP- and active GTP-bound states, small GTP-binding proteins function as critical relays in the transduction of signals originating from membrane receptors. Despite extensive cross-talk among small GTPase proteins, each one of them has a specific role and mediates specific downstream targets. For example, Ras interacts with protein kinases c-Raf and phosphatidylinositol 3'-kinase, whereas Rac1 activates p21-activated kinases, and Rho regulates Rho-kinase and myosin light chain^{22, 83}. Ras proteins are post-translationally modified by farnesylation, whereas the Rho family is activated by the attachment of geranylgeraniol. These post-translational lipid modifications are necessary for the translocation of inactive GTPase from the cytosol to the membrane. Therefore, as statins block the mevalonate pathway by inhibiting geranylgeranilation and farnesylation of small GTPase proteins, they also prevent membrane translocation and activity of small GTPases and may interfere in a number of cellular processes such as apoptosis^{4, 42}, differentiation^{11, 51, 57} and cellular proliferation^{40, 53, 63, 79, 85, 89, 92}.

Our own work on diabetic nephropathy elucidates one of the pleiotropic effects of statins. We postulated

that statins may ameliorate the detrimental effects of high glucose (HG)-induced proliferation of mesangial cells, a feature of early stages of diabetic nephropathy, by preventing Rho isoprenylation¹¹. Simvastatin inhibited HG-induced mesangial cell proliferation as measured by [³H] thymidine incorporation. This inhibitory effect was reversed with GGPP. Exposure of mesangial cells to HG was associated with an increase in membrane-associated Rho GTPase protein expression. Co-treatment of cells with simvastatin reversed HG-induced Rho membrane translocation and activation. At the cell cycle level, the HG-induced proliferation of mesangial cells was associated with a decrease in cyclin-dependent kinase inhibitor p21 protein expression. Simvastatin reversed the down-regulation of p21 protein expression. Thus, our study unraveled a novel pleiotropic effect of statins on HG-induced Rho GTPase/p21 signaling and provided a molecular basis for the use of statins, independent of their cholesterol-lowering effect, in the early stages of diabetic nephropathy.

Also of interest is the pleiotropic effect of statins on reducing the risk of bone fracture^{16, 73}. MUNDY et al.⁵⁵ has shown that statins enhance new bone formation *in vitro* and in rodents. This effect was associated with increased expression of the bone morphogenetic protein-2 gene in bone cells. Co-treatment of cells with lovastatin and simvastatin increased bone formation when injected subcutaneously over the calvaria of mice and increased cancellous bone volume when orally administered to rats. While the details of the mechanism(s) behind this effect are still not completely understood, IZUMO et al.³⁴ suggested that these effects may be mediated by intermediaries of the mevalonate pathway.

Statins and Transplantation

The ability of statins to modulate immune response has been recently added to the growing list of pleiotropic effects of statins. Since the first clinical observation suggesting a beneficial effect of pravastatin on the incidence of acute rejection in heart transplant patients⁴³, the use of statins in transplantation as adjunctive therapy has gained much attention. More recently, in a prospective randomized study of kidney transplant recipients, a reduction in the incidence of acute rejection episodes with pravastatin therapy has been demonstrated³⁷. The underlying molecular mechanism of the immunomodulatory effects of statins, however, is less well known. A number of investigators have examined the cholesterol biosynthesis pathway as it relates to a possible immune mechanism^{35, 37, 54}.

Hypercholesterolemia is common in transplant patients and a number of studies have associated hyperlipidemia in the pathogenesis of allograft rejection³⁵. For instance, in a study to evaluate the role of pravastatin in reducing cardiac allograft vasculopathy (CAV), 97 heart transplant patients were randomized to pravastatin or placebo within 2 weeks after transplantation⁴³. Twelve months after transplantation, not only the pravastatin group had significantly lower mean cholesterol levels than the control group, but also less frequent cardiac rejection events, better overall survival, and a lower incidence of CAV. Furthermore, in a sub-analysis of study patients, the cytotoxicity of natural killer cells was significantly lower in the pravastatin group (9.8 vs. 22.2% specific lysis). In support of an immunomodulatory effect of statins, DOWNWARD¹⁴ suggested that the immunosuppressive effects of statins might be independent of their cholesterol-lowering effects, as they showed that Ras-related proteins had an important role in T cell activation and function, which were pivotal in the development of allograft rejection during organ transplantation. In addition, a number of more recently published reports indirectly support the notion of an important immunomodulatory effect of statins^{35, 88}. For instance, WEI et al.⁸⁸ showed that perillyl alcohol, a specific inhibitor of protein isoprenylation, can effectively inhibit human T cell proliferation *in vitro* and prevent acute and chronic rejection in a rat model of cardiac transplantation. Perillyl alcohol has also been shown to disrupt the polarized shape and motility of antigen-specific murine 1E5 T cells (murine hen egg lysozyme-restricted CD4⁺ T cells) that are essential in T cell activation and migration. Furthermore, this compound impaired the T cell receptor-mediated calcium response, an important early event in allograft rejection. In addition to the potential effects of statins on the immune response via isoprenylation inhibition, there may be other mechanisms by which statins may modulate immunomodulatory response. MAGGARD et al.⁵² showed that pravastatin not only decreased immunoglobulin G (IgG) alloantibody, but also decreased coronary arterial intimal lesions in a rat model of CAV. Their findings were also consistent with other reports suggesting a role for the humoral immune response in the development of CAV²⁷. In another study, pravastatin-treated rats had significantly fewer graft-infiltrating macrophages, especially within the arterial intima and perivascular areas⁸². This is also consistent with other reports indicating that macrophage infiltration may have a critical role in the pathogenesis of CAV⁴¹.

The recently discovered modulatory effect of statins on the expression of interferon (IFN)- γ -induced class II

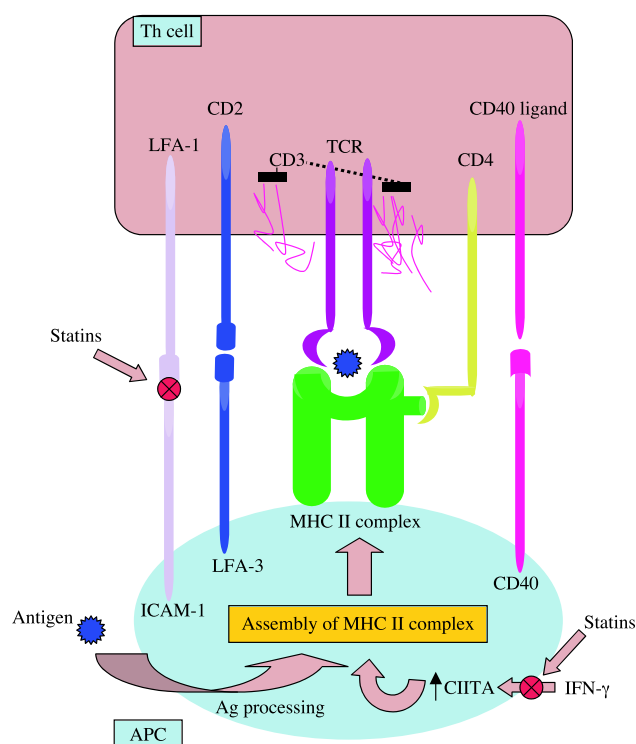


Fig. 2. Modulatory effects of statins on the activation of the CD4 helper (Th) cell. The specific T cell antigen receptor (TCR) binds to the class II MHC peptide of the antigen-presenting cell (APC), leading to a stimulatory signal which is transduced by the TCR-CD4 complex. Accessory T cell adhesion molecules (e.g., LFA-1, LFA-3) facilitate adherence of the Th cell to the APC and influence IL-2 synthesis. Statins modulate this pathway by at least two different means: 1 – by downregulating CIITA, and 2 – by blocking lymphocyte adhesion to ICAM-1 and impaired T cell costimulation via LFA-1 inhibition

MHC expression by KWAK et al.⁴⁹ provides yet another strong scientific rationale for the use of statins as immunomodulatory agents. In this landmark study, the authors showed that a lipophilic statin, atorvastatin, not only inhibited the IFN- γ expression of MHC II on antigen-presenting cells (APCs), but that this effect was a result of the inhibitory effect of statins on the class II transactivator (CIITA). Non-activated endothelial cells (ECs) express minimal or no class II MHC membrane molecules. However, following induction with cytokines such as IFN- γ , these antigens are induced on the endothelium, providing ECs with APC capabilities (Fig. 2). Regulation of endothelial class II MHC molecules occurs primarily at the transcriptional level, and CIITA is a key regulator of MHC class II, as CIITA is the rate-limiting factor for both constitutive and inducible class II MHC expression. The CIITA gene is under the control of four different promoters. Promoter IV is involved in IFN- γ -induced CIITA expression in several

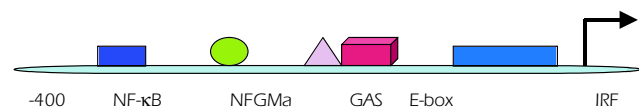


Fig. 3. Promoter IV of the CIITA gene. Several critical regulatory elements, including E-box, to which phospho signal transducer and activator of transduction 1 and upstream stimulatory factor-1 bind, are located 400 base pairs upstream from the transcription start site. IRF – interferon-induced transcription factor site inducible by IRF-1 (from reference by permission)

cell types and includes several functionally important cooperative cis-acting elements (Fig. 3). By monitoring cell-surface expression of MHC II class using fluorescence-activated cell sorting, immunofluorescence and mRNA levels, in this landmark study the authors showed that statins, in a dose-dependent manner, inhibited the activation of IFN- γ -induced MHC II expression. Furthermore, the effect of statins on MHC II complex expression was highly specific for the inducible form of MHC II and did not affect the constitutive expression of MHC II complex. Pretreatment of endothelial cells, macrophages and other APCs, such as dendritic cells and B lymphocytes, with statins ameliorated subsequent T cell proliferation. The authors concluded that the inhibitory mechanism of statins on IFN- γ -induced expression of MHC II complex was based on their down-regulatory effect on the activation of the promoter IV of the CIITA gene. These unexpected immunomodulatory effects of statins were confirmed in other cell types, including fibroblasts and human smooth muscle cells, as well as in cell lines such as ThP1 and HeLa cells⁴¹.

In keeping with these findings, in another recent study not only the inhibitory effect of statins on CIITA was confirmed, but it was also shown that this inhibitory effect was independent of their effect on the signal transducer and activator of transcription 1 phosphorylation⁷². Interestingly, the inhibitory effect of statin was reversed with GGPP and mevalonate but not with squalene, indicating that protein isoprenylation is involved in this phenomenon⁷².

In a more recent study, WEITZ-SCHMIDT et al.⁹⁰ further elucidated the immunomodulatory actions of statins⁹⁰. Their data suggest that statins selectively block lymphocyte function-associated antigen 1 (LFA-1). LFA-1 is involved in adhesion of leukocytes to intracellular adhesion molecule 1 (ICAM-1) and thus effective T cell activation by APCs (Fig. 2). Inhibition of the LFA-1 by statins resulted in lower lymphocyte adhesion to ICAM-1 and impaired T cell costimulation. It has been suggested that this effect occurred via binding of statins to a novel allosteric site within LFA-1. The modulatory effects of several statins on T cells are shown in Table 1.

Statins and Inflammation

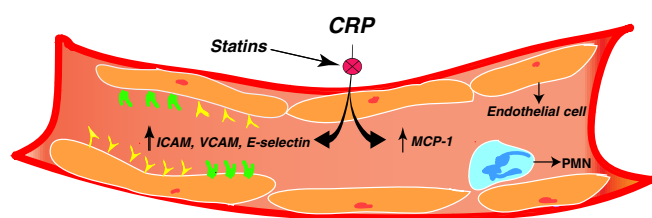
The role of immune response and inflammation in the pathogenesis of atherosclerosis has increasingly been recognized. In fact, histological data has confirmed the presence of classic inflammatory cells in atherosclerotic lesions. Monocytes, other APCs, and T lymphocytes have all been isolated from atheromas⁶⁹. Moreover, elevated levels of interleukin 6 (IL-6), the principal cytokine responsible for the acute phase of the inflammatory response, have also been found in coronary atheromatous lesions in patients with unstable angina⁴⁸. The dynamics of progression of the atherosclerotic lesion may also be significantly influenced by a number of pro-inflammatory cytokines, such as IFN- γ , tumor necrosis factor α (TNF- α), IL-1 β and Fas ligand. Several clinical studies have established inflammatory markers, such as C-reactive protein (CRP), as independent predictors for cardiovascular and cerebrovascular morbidity^{19, 77, 84}. One such study, the CARE study concluded that higher CRP levels after myocardial infarction are associated with an increased risk for recurrent coronary artery events⁷¹. In a similar study, the Air Force/Texas Coronary Atherosclerosis Prevention Study, investigators found that the rate of coronary events increased with higher baseline CRP levels⁶⁴.

Table 1. Pleiotropic effects of statins on T cells

T cell function	Statin
Decrease proliferation	lovastatin ¹⁰ , simvastatin ⁴⁷
Decrease cytokine production: TNF- α IL-8	pravastatin ^{24, 68} , lovastatin ⁵⁹ , simvastatin ⁵⁶ lovastatin ⁵⁹ , simvastatin ³³ , cerivastatin ⁴⁵
Decrease LFA mediated T cell stimulation	pravastatin ⁹⁰ , simvastatin ⁹⁰ , mevastatin ⁹⁰
Decrease expression of MHC II on APCs and on T cell activation	pravastatin ⁴¹ , lovastatin ⁴¹ , atorvastatin ⁴¹

Table 2. Pleiotropic effects of statins on monocytes

Monocyte function	Statin
Decrease expression of surface proteins (ICAM-1, CD11a/CD18, CD11b/CD18)	fluvastatin ⁵⁸ , lovastatin ^{86, 90} , simvastatin ⁹⁰ , mevastatin ⁹⁰ , pravastatin ⁹⁰
Decrease monocyte chemotaxis	pravastatin ⁴⁶
Decrease MCP-1 interference	pravastatin ⁶⁷ , lovastatin ^{61, 67}
Decrease expression of urokinase and urokinase receptor	cerivastatin ²⁰
Decrease expression of matrix metalloproteinases	cerivastatin ²⁰
Decrease proliferation	lovastatin ⁸⁷
Promote differentiation	fluvastatin ⁷⁰

**Fig. 4.** Following release of CRP. The resting state of endothelium is changed by the increased release of monocyte chemotactic protein (MCP) and up-regulation of intracellular adhesion molecules. Statins inhibit up-regulation of ICAM, VCAM, and E-selectin as well as ameliorate the increased release of MCP-1

A number of clinical studies have unraveled a direct correlation between a reduction in inflammation, as measured by a decline in inflammatory markers such as CRP, and a positive clinical outcome^{36, 64, 65}. For instance, in a recent randomized, placebo-controlled trial, pravastatin therapy was shown to lower CRP levels independently of LDL cholesterol and appeared to lower the risk for recurrent coronary events¹. Of note, the benefit of pravastatin therapy was greater among patients with higher levels of CRP, and this effect appeared to be independent of the baseline lipid levels⁶⁵.

Although the underlying mechanisms of how statins exert their anti-inflammatory actions remain to be elucidated, the possible modulatory effects of statins on inflammatory cell signaling pathways seems to be critical in resolution of the atherosclerotic microenvironment. Several recent experimental studies have begun to shed light on this matter. For instance, BUSTOS et al.⁷ induced atherosclerosis in femoral arteries of rabbits via endothelial damage and an atherogenic diet. After 4 weeks of treatment with atorvastatin, rabbits had significant reduction in serum lipids and atherosclerotic lesion size compared with control animals. Macrophage infiltration in arteries was almost abolished in the atorvastatin-treated group, and nuclear factor κ B (NF- κ B) expression was significantly down-regulated in macrophages infiltrated in atherosclerotic lesions in the treated group.

The works of SPARROW et al.⁷⁶ on mice, however, provided even more convincing data that statins have an independent anti-inflammatory property. Using the classic model of acute inflammation, carrageenan-induced foot pad edema, SPARROW et al.⁷⁶ showed that simvastatin reduced acute inflammation and the extent of the edema in a dose-dependent fashion, comparable to the anti-inflammatory effects of indomethacin. Simvastatin therapy did not significantly affect serum cholesterol levels, further supporting statins' new properties as anti-inflammatory agents.

In pursuit of a rationale for the anti-inflammatory effects of statins, a key piece of evidence came from a study by PASCERI et al.⁶², who recently detailed how CRP-induced expression of monocyte chemoattractant protein 1 (MCP-1) in cultured human ECs. This study revealed that CRP is not a mere marker of the underlying inflammatory process, but might be directly involved in the inflammatory pathogenesis of atherosclerosis (Fig. 4). The authors showed that CRP in concentrations ≥ 5 μ g/ml induced the expression of adhesion molecules such as ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), and E-selectin in human ECs. Furthermore, CRP induced expression of MCP-1. MCP-1 is a potent chemokine that facilitates the infiltration of monocytes/macrophages and stimulates monocyte migration into the intima of arterial walls, a necessary step in the development of atherosclerotic plaque. Interestingly, co-treatment of cells with simvastatin partially inhibited the induction of MCP-1 by CRP on ECs. The same group also showed that MCP-1 expression and NF- κ B activity induced by tumor TNF- α were both down-regulated by atorvastatin in VSMC, confirming the modulatory effects of statins in preventing the formation of atherosclerotic plaque. The modulatory effect of several statins on monocytes is shown in Table 2.

Some recent studies have demonstrated that statins inhibit proinflammatory cytokines IL-1 β , IL-6 and cyclooxygenase-2 by up-regulating the peroxisome prolifer-

erator-activated receptor α (PPAR- α) in ECs⁹. While evidence for an anti-atherogenic effect of PPAR- α is still lacking, a significant anti-atherogenic effect associated with the down-regulation of several proinflammatory genes induced by synthetic PPAR- α has been established.

In conclusion, considerable clinical and experimental data suggest that HMG-CoA reductase inhibitors modulate a variety of pathophysiological processes that extend beyond their lipid-lowering properties. The immunomodulatory effects of statins are now widely recognized. Given the importance of HLA molecules in immune response, statins represent a novel class of immunosuppressive agents that may ultimately be used in diseases associated with activation of the immune system. Moreover, statins exert important anti-inflammatory activity by altering leukocyte-EC interactions. The net result of this important function is an endothelial protective action that we have just begun to understand and to explore.

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