

Statin Inhibition of Fc Receptor–Mediated Phagocytosis by Macrophages Is Modulated by Cell Activation and Cholesterol

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Objectives—An inflammatory response to altered lipoproteins that accumulate in the arterial wall is a major component of the pathogenesis of atherosclerosis. Statins reduce plasma levels of low-density lipoprotein (LDL) and are effective treatments for atherosclerosis. It is hypothesized that they also modulate inflammation. The aim of this study was to examine whether lovastatin inhibits macrophage inflammatory processes and clarify its mechanism of action.

Methods and Results—We examined the effects of statins on phagocytosis of antibody-coated red blood cells by cultured human monocytes and mouse peritoneal macrophages. Lovastatin, simvastatin, and zaragozic acid, a squalene synthase inhibitor, blocked Fc receptor–mediated phagocytosis by cultured human monocytes and mouse peritoneal macrophages. The inhibitory effect of lovastatin on Fc receptor–mediated phagocytosis was prevented completely by addition of mevalonate, farnesyl pyrophosphate, LDL, or cholesterol to the culture medium. The inhibitory effect of zaragozic acid was reversed by addition of LDL, but not by the addition of geranylgeranyl pyrophosphate, to the medium. In addition, the effect of lovastatin on phagocytosis is a function of cell activation because treatment of cells with tumor necrosis factor- α or lipopolysaccharide prevented inhibition of phagocytosis by lovastatin.

Conclusions—The inhibition of Fc receptor–mediated phagocytosis of lovastatin is related to its effect on cholesterol biosynthesis rather than its effect on the formation of isoprenoids. (*Arterioscler Thromb Vasc Biol.* 2004; 24:2051-2056.)

Key Words: lovastatin ■ phagocytosis ■ cell activation ■ monocytes and macrophages

Atherosclerosis is thought to be mediated by an inflammatory response to the deposition of lipoproteins and altered lipids in the arterial wall.¹ Atherogenesis involves the migration of monocytes into the subendothelial space,² where they adhere to matrices containing oxidized low-density lipoprotein (LDL), mature into macrophages, and secrete growth factors, proteases, and reactive oxygen species.³ Since their introduction into clinical practice, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, have had a profound effect on preventing and treating atherosclerosis.⁴ By competitively inhibiting HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, statins block mevalonate generation and subsequent cholesterol synthesis. By lowering cellular cholesterol concentration, statins upregulate hepatic LDL receptors, thereby lowering plasma LDL levels $\leq 50\%$.

However, some of the biochemical effects of statins have been postulated to be independent of their lipid-lowering effects.⁵ Multiple reports document the anti-inflammatory effects of statins under in vivo and in vitro conditions. Statins suppress expression of proinflammatory chemokines, chemokine receptors, cytokines,⁶ reduce plasma levels of C-reactive

protein,⁷ and P-selectin,⁸ inhibit leukocyte adhesion,^{9,10} downregulate scavenger receptor A,¹¹ and blunt the respiratory burst of monocytes.¹² Statins also prolong the survival of lung, renal, and cardiac allografts.^{13,14}

Anti-inflammatory properties of statins may be attributable to their effects on protein isoprenylation. At very high concentrations, statins block formation of the isoprenyl lipids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP),¹⁵ thereby inhibiting the prenylation of proteins such as GTPases.^{16–18} GTPases regulate many macrophage actions such as phagocytosis, and prenylation is required for their activity.^{19,20}

We report here that lovastatin inhibits one inflammatory process: receptor-mediated phagocytosis. Contrary to our expectations, reduction of cellular cholesterol by zaragozic acid A (ZA), a squalene synthase inhibitor,²¹ as well as lovastatin, reduced Fc receptor–mediated phagocytosis of IgG antibody-coated sheep red blood cells [E(IgG)] by human monocytes and mouse peritoneal macrophages. However, the inhibitory effect of lovastatin on Fc receptor–mediated phagocytosis was not a result of its capacity to block prenylation; rather, it was attributable to its capacity to

Original received June 29, 2004; final version accepted August 23, 2004.

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Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

DOI: 10.1161/01.ATV.0000143858.15909.29

inhibit cholesterol biosynthesis. Furthermore, tumor necrosis factor- α (TNF- α) or lipopolysaccharide (LPS) treatment prevented and reversed lovastatin-mediated inhibition of Fc receptor-mediated phagocytosis in the absence of added cholesterol. These findings indicate that cellular cholesterol regulates Fc receptor-mediated phagocytosis, and TNF- α and LPS stimulation overcome inhibition of lovastatin.

Materials and Methods

Human Monocytes

Monocyte-enriched cell preparations were isolated from the blood of healthy volunteers (none were taking statins) after informed consent as described.^{22,23} Briefly, human mononuclear cells were isolated from freshly drawn blood by centrifugation over Histopaque-1077 (Sigma-Aldrich) as described previously.^{22,23} Cells were washed 3 \times in RPMI medium 1640 (Life Technologies BRL) containing penicillin (140 U/mL) and streptomycin (140 μ g/mL; Gibco BRL) by centrifugation at 1500 rpm for 10 minutes. After washing, they were resuspended in RPMI complete medium (RPMI medium 1640, penicillin [140 U/mL], streptomycin [140 μ g/mL], and 10% heat-inactivated FBS [Gibco]). Monocyte-enriched cell preparations were suspended at 2 to 3 million cells per milliliter in RPMI medium 1640 and incubated on glass coverslips (12-mm diameter) at 37°C for 1 hour in 24-well culture dishes. Coverslips then were washed with PBS containing 1 mmol/L CaCl₂ and 0.5 mmol/L MgCl₂ to remove nonadherent cells, incubated with 0.5 mL of RPMI medium 1640, and incubated overnight at 37°C in the same medium. The adherent cells (200 000 to 300 000 per coverslip) were >95% monocytes as measured by their phagocytic capacity to ingest E(IgG).

Mouse Peritoneal Macrophages

Resident peritoneal cells were isolated from C57BL/126 mice 8 to 12 weeks of age as described.²⁴ Twenty-nine percent (\pm 3%) of these cells were macrophages, as indicated by their expression of nonspecific esterase and endocytosis of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-labeled acetylated LDL.²² Cells were allowed to adhere to 12-mm glass coverslips for 2 hours, and all nonadherent cells were removed by washing with RPMI medium 1640 maintained as described for human monocytes. Adherent cells were >99% macrophages as described.²²

IgG-Opsonized Sheep Red Blood Cells

Sheep red blood cells (Hardy Diagnostics) were opsonized with rabbit anti-sheep red blood cell IgG antibody (Cappel) as described.²⁴

Phagocytosis Assay

Freshly isolated human monocytes or mouse peritoneal macrophages maintained on glass coverslips were incubated overnight at 37°C. Coverslips were then washed once in PBS containing 0.1% (wt/vol) human serum albumin and 5.5 mmol/L glucose, overlaid with 15 μ L of E(IgG) in PBS, and incubated at 37°C for 1 to 2 hours, dipped 5 \times in PBS to remove excess E(IgG), 6 \times in distilled water to lyse the extracellular E(IgG), and an additional 5 \times in PBS, all as described,²⁴ mounted on glass slides, and viewed by light microscopy using a \times 40 lens. Intracellular E(IgG) appeared as phase dense spheres as described.²⁴ Phagocytic Index (PI) is the average number of E(IgG) ingested per 100 macrophages. To access the total number of attached and ingested E(IgG) per 100 macrophages, cells were washed only in PBS without lysing the extracellular E(IgG) in water and viewed under the microscope.

Fluid Phase Pinocytosis

Fluid phase pinocytosis was measured using Lucifer yellow as described.²⁵ Briefly, monocytes cultured for 24 hours were further incubated in 300 μ L PBS containing 0.5 mg/mL Lucifer yellow, 0.1% human serum albumin, and 5.5 mmol/L glucose for the indicated time periods. The medium was removed and adherent cells were washed 6 \times in PBS at 4°C. To lyse the cells, 500 μ L of water

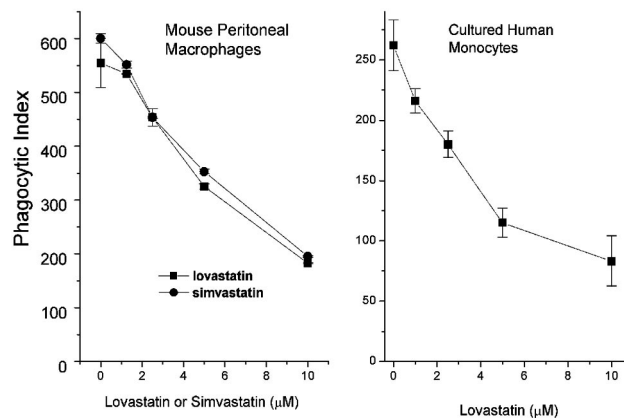


Figure 1. Lovastatin and simvastatin inhibit Fc receptor-mediated phagocytosis. Phagocytic indices of mouse peritoneal macrophages (a) and freshly isolated human monocytes (b) ingesting E(IgG) in response to increasing concentrations of lovastatin and simvastatin. Values represent mean \pm SEM; n=3.

containing Triton X-100 (0.05%) was added to each well, and fluorescence was measured on a cell plate reader using excitation of 485 nm, emission at 530 nm. Standard curves for solutions containing known concentrations of Lucifer yellow were prepared and found to be linear from 0.05 to 100 ng/mL.

Reagents

Lovastatin in its active form was from A.G. Scientific, and simvastatin and ZA were obtained from Merck Sharp & Dohme. Statins were all dissolved and stored in dimethyl sulfoxide (DMSO) and diluted 1000-fold in RPMI medium 1640 immediately before they were added to the cells. TNF- α was obtained from Upstate Biotechnology Co. All other reagents were from Sigma Chemical Co.

Results

Lovastatin and Simvastatin Inhibit Fc Receptor-Mediated Phagocytosis by Mouse Peritoneal Macrophages and Cultured Human Monocytes

Mouse peritoneal macrophages and cultured human monocytes adherent to glass coverslips were incubated at 37°C for 24 hours in medium containing 10% FBS without or with lovastatin or simvastatin at the concentrations indicated (Figures 1 and 2), washed, and then incubated at 37°C in PBS containing 10% FBS, 5.5 mmol/L glucose, and statin as indicated E(IgG), for an additional 2 hours. In medium without statins, mouse macrophages ingested an

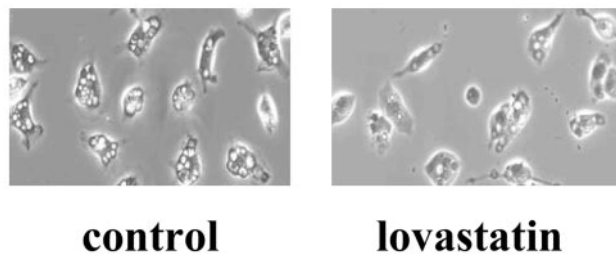


Figure 2. Phagocytosis by mouse peritoneal macrophages. Phase micrographs comparing phagocytosis of E(IgG) by mouse peritoneal macrophages maintained for 24 hours in medium containing no additions (left) and 10 μ mol/L lovastatin (right). Before E(IgG) were added, macrophages were equally spread in both conditions.

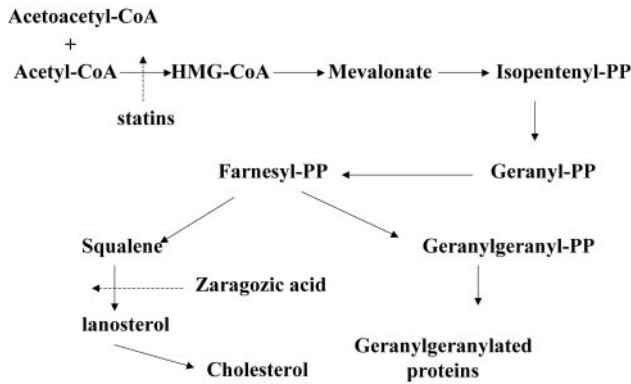


Figure 3. Biosynthesis of cholesterol.

average of ≈ 6 E(IgG) each (ie, PI of ≈ 600) with some macrophages ingesting as many as 10 (Figures 1 and 2). Lovastatin and simvastatin were equally effective in inhibiting E(IgG) ingestion by mouse macrophages. At 5 and 10 $\mu\text{mol/L}$, both drugs inhibited E(IgG) uptake by mouse macrophages $\approx 45\%$ and $\approx 70\%$, respectively (Figure 1). Although most lovastatin-treated murine macrophages ingested ≈ 2 to 3 E(IgG), occasionally, we observed lovastatin-treated macrophages that had ingested 4 to 6 E(IgG) (Figure 2). Untreated cultured human monocytes ingested an average of 2 to 3 E(IgG) each (ie, PI of ≈ 260) and 5 and 10 $\mu\text{mol/L}$ of lovastatin and simvastatin inhibited E(IgG) uptake by cultured human monocytes $\approx 57\%$ and 70%, respectively (Figure 1). Control experiments confirmed that mouse macrophages and human monocytes ingested few, if any, unopsonized erythrocytes (data not shown;²⁴) and that lovastatin (10 $\mu\text{mol/L}$) had no adverse effects on attachment of E(IgG) to these cells, on the number of cells adherent to glass coverslips, and on cell viability, as measured by trypan blue dye exclusion (data not shown).

Lovastatin Has No Effect on Fluid Phase Endocytosis

Because membrane fluidity and movement are required for all endocytic events, we examined whether lovastatin affects fluid phase endocytosis, a process that occurs in macrophages via clathrin-dependent and clathrin-independent mechanisms.²⁶ Human monocytes (10^6 cells per well) were incubated overnight in 24-well plates in RPMI medium 1640 in the absence or presence of lovastatin (1 to 10 $\mu\text{mol/L}$). Lucifer yellow was added to the medium, the cells were incubated for an additional 120 minutes, and the amount of Lucifer yellow taken up by the cells was assayed as described in Materials and Methods. The amount of Lucifer yellow taken up under all conditions was the same (1.7 ± 0.2 for untreated cells and 1.8 ± 0.4 pmol per 2 hours for cells treated with 10 $\mu\text{mol/L}$ lovastatin). Thus, maintenance of cultured human monocytes in medium containing 1 to 10 $\mu\text{mol/L}$ lovastatin for 24 hours had no effect on fluid phase endocytosis (data not shown).

Lovastatin Inhibits Fc Receptor–Mediated Phagocytosis by Blocking HMG-CoA Reductase

Inhibition of HMG-CoA reductase by statins alters a number of downstream pathways, including biosynthesis of isoprenyl

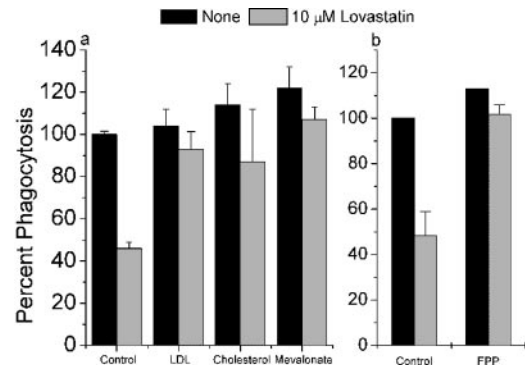


Figure 4. Inhibition of Fc receptor–mediated phagocytosis by lovastatin is reversed completely by mevalonate, LDL, cholesterol, or FPP. a, E(IgG) phagocytosis by freshly isolated human monocytes treated overnight with medium containing DMSO (control), 100 $\mu\text{mol/L}$ mevalonate, 100 $\mu\text{g/mL}$ LDL, or 100 $\mu\text{mol/L}$ cholesterol with or without 10 $\mu\text{mol/L}$ lovastatin. Values represent mean \pm SEM; $n=5$. b, E(IgG) phagocytosis by freshly isolated human monocytes incubated overnight in medium containing DMSO or 10 $\mu\text{mol/L}$ FPP with or without 10 $\mu\text{mol/L}$ lovastatin. Values represent mean \pm SEM; $n=5$. Student t test analysis ($P<0.05$) determined that lovastatin inhibited phagocytosis and that LDL, cholesterol, mevalonate, and FPP significantly blocked the inhibitory effect of lovastatin.

lipids and cholesterol (Figure 3). Therefore, we used various agents to differentiate between these pathways.

To confirm that the effect of lovastatin on phagocytosis was attributable to its inhibitory effect on HMG-CoA reductase and not a result of pleiotropic effects, we incubated human cultured monocytes in medium containing mevalonate, the immediate product of HMG-CoA reductase, or in medium containing FPP, a precursor of cholesterol and isoprenyl lipids (Figure 3). Mevalonate and FPP blocked the inhibitory effect of lovastatin on phagocytosis of E(IgG) (Figure 4a and 4b).

Cholesterol-Containing Lipoproteins, Cholesterol, and Biosynthetic Precursors of Cholesterol Prevent Inhibitory Effects of Lovastatin on Phagocytosis of E(IgG)

Mevalonate and FPP are precursors of cholesterol and of isoprenyl lipids. To determine whether lovastatin inhibited phagocytosis by blocking the synthesis of cholesterol, isoprenyl lipids, or both, we compared E(IgG) phagocytosis by monocytes incubated for 24 hours in medium containing 10 $\mu\text{mol/L}$ lovastatin alone versus medium containing 10 $\mu\text{mol/L}$ lovastatin and 100 $\mu\text{mol/L}$ cholesterol,²⁷ or 10 $\mu\text{mol/L}$ lovastatin and 100 $\mu\text{g/mL}$ LDL. Addition of cholesterol or LDL completely blocked the inhibitory effect of lovastatin on E(IgG) uptake (Figure 4a). These results suggested that lovastatin inhibits Fc receptor–mediated phagocytosis by depriving monocytes of cholesterol.

ZA Inhibits Phagocytosis of E(IgG) by Cultured Monocytes

Squalene synthase catalyzes the formation of squalene, a cholesterol precursor, from FPP (Figure 3). Moreover, the squalene synthase inhibitor ZA stimulates FPP synthesis, thereby increasing its availability, and the availability of other

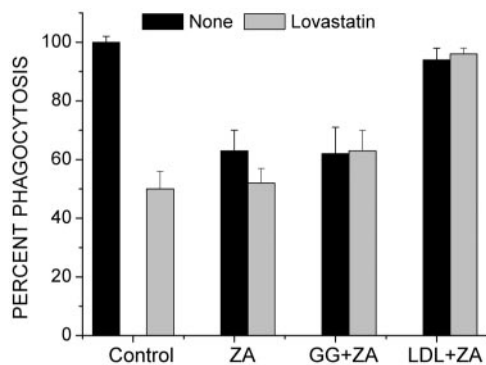


Figure 5. Effects of zaragozic acid on Fc receptor–mediated phagocytosis. Freshly isolated human monocytes were incubated for 24 hours with the indicated inhibitors in the presence or absence of GGPP or LDL as indicated. Cells were washed and further incubated with E(IgG) under the conditions indicated. Phagocytic indices were assessed as described in Materials and Methods. Values shown are the percentage of control \pm SEM; n=3. Student *t* test analysis ($P<0.05$) determined that ZA and lovastatin inhibited phagocytosis and that LDL significantly blocked the effect of ZA on phagocytosis.

isoprenyl lipids derived from it (eg, GGPP), to isoprenylate small GTPases.^{16,19,20} Therefore, we incubated cultured human monocytes in medium containing 5 μ mol/L ZA, a concentration vastly in excess of that required to inhibit rat liver squalene synthase,²¹ to further test the hypothesis that cholesterol is required for E(IgG) phagocytosis. ZA inhibited phagocytosis of E(IgG) by 40% (Figure 5), a value similar to that obtained with lovastatin alone. The finding that 5 μ mol/L ZA in combination with 10 μ mol/L lovastatin was not a better inhibitor of E(IgG) phagocytosis than ZA alone (Figure 5) suggests that increased availability of isoprenyl lipids in ZA-treated cells played no role in the extent to which ZA inhibited E(IgG) uptake (Figure 5). Rather, cholesterol is required for Fc receptor–mediated phagocytosis of E(IgG). The conclusion that ZA inhibits Fc receptor–mediated phagocytosis of E(IgG) by blocking cholesterol synthesis was further buttressed by the finding that the inhibitory effect of ZA on E(IgG) phagocytosis was unaffected by the addition of 100 μ mol/L GGPP to the medium but was completely prevented by addition of 100 μ g LDL to the medium (Figure 5). GGPP does not contribute to cholesterol biosynthesis but is a precursor for the production of isoprenyl products (Figure 3).

TNF- α and LPS Prevent and Reverse the Inhibitory Effect of Lovastatin on Fc Receptor–Mediated Phagocytosis

The experiments described up to this point used monocytes cultured in medium lacking added growth factors and cytokines. However, monocytes and macrophages are exposed to bacterial products (eg, LPS) and cytokines (eg, TNF- α , interleukin-1, interferon- γ [INF- γ]) when they enter sites of infection or inflammation in vivo. Therefore, we examined the effects of LPS and TNF- α on lovastatin-mediated inhibition of E(IgG) phagocytosis. We observed that monocytes incubated in medium containing 10 μ mol/L lovastatin and either 10 ng/mL LPS or 100 ng/mL TNF- α for 24 hours phagocytosed E(IgG) as efficiently as cells treated with LPS

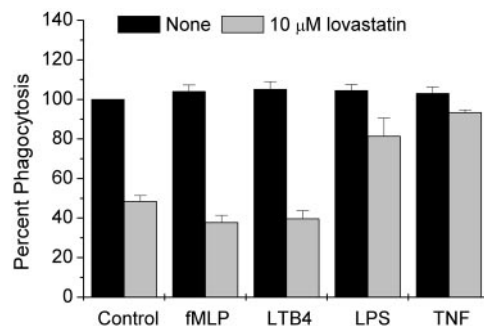


Figure 6. TNF- α and LPS prevent the inhibitory effect of lovastatin on Fc receptor–mediated phagocytosis of E(IgG) by human monocytes. Freshly isolated human monocytes were incubated for 24 hours in medium containing either 10⁻⁷ mol/L fMLP, 10⁻⁷ mol/L LTB4, 100 ng/mL of TNF- α , or 10 ng/mL of LPS in the presence or absence of 10 μ mol/L lovastatin, as indicated. Cells then were washed and reincubated under the indicated conditions with the addition of E(IgG), and their phagocytic indices were assessed. Values shown are the percentage of control \pm SEM; n=3. Student *t* test analysis ($P<0.05$) determined that lovastatin inhibited phagocytosis and that LPS and TNF significantly blocked the inhibitory effect of lovastatin on phagocytosis.

or TNF- α alone (Figure 6). In contrast, other proinflammatory substances such as formyl-methionyl-leucyl-phenylalanine (fMLP) and leukotriene B4 (LTB4), which stimulate monocytes by activating their heptahelical G α -coupled receptors, had no effect on the ability of lovastatin to inhibit phagocytosis (Figure 6). In addition, when cells were treated with lovastatin (5 μ mol/L) for 24 hours and then further incubated in medium containing lovastatin and either TNF- α or LPS for 4 hours, we observed complete reversal of the inhibitory effect of lovastatin on phagocytosis (data not shown).

Discussion

Statins have a number of important pharmacological effects in addition to reducing plasma LDL levels. Pursuing the widespread belief that statins exert anti-inflammatory effects, we found that lovastatin and simvastatin inhibit macrophage phagocytosis of E(IgG) but do not affect endocytosis of soluble substances. Three lines of evidence indicate that lovastatin inhibits Fc receptor–mediated phagocytosis by blocking cellular cholesterol synthesis and not by blocking synthesis of isoprenyl lipids. First, the inhibitory effect of lovastatin is prevented by addition of LDL or cholesterol (Figure 4) but not of GGPP (Figure 5) to the medium. Second, ZA, which inhibits synthesis of cholesterol but not of isoprenyl lipids, blocked Fc receptor–mediated phagocytosis to about the same extent as lovastatin (Figure 5). The inhibitory effect of ZA also was prevented and reversed by addition of LDL but not of GGPP to the medium (Figure 5). Third, ZA and lovastatin in combination were no more potent in inhibiting phagocytosis than either drug alone (Figure 5), suggesting they act by a common mechanism (ie, by inhibiting cholesterol synthesis).

Macrophages often function in regions that are rich in proinflammatory cytokines (eg, atherosclerotic plaques). For this reason, we tested the effects of TNF- α and LPS,

proinflammatory substances found in blood.¹⁹ Bacterial infection is associated with increased risk of coronary heart disease,^{28,29} and TNF- α is found within atherosclerotic lesions.³⁰ TNF- α and LPS prevented and reversed the inhibitory effect of lovastatin on E(IgG) phagocytosis (Figure 6; data not shown), suggesting that these substances activate pathways that override the effects of cholesterol depletion.

Endogenous Synthesis Is the Primary Source of Cholesterol in Monocytes/Macrophages Maintained in Medium Containing FBS

Mammalian cells, including monocytes and macrophages, obtain cholesterol from 2 sources: circulating lipoproteins and de novo synthesis. In our experiments, monocytes and macrophages were maintained in medium containing 10% FBS. Because FBS contains very little cholesterol, monocytes and macrophages maintained in FBS rely primarily on synthesis to obtain the cholesterol they require. Indeed, it seems likely that even in the absence of inhibitors, endogenous cholesterol synthesis was less than optimal. This is probably the reason that addition of mevalonate to the culture medium enhanced E(IgG) phagocytosis by 20% to 30% (Figure 4a).

Fc receptor-mediated phagocytosis of E(IgG) requires the coordinated movement of membranes and cytoplasm.³¹ Cholesterol is required for plasma membrane formation and for the activity of many plasma membrane signaling pathways, including Fc receptor signaling.³² There are 2 principle mechanisms by which reductions in cellular cholesterol might affect Fc receptor-mediated phagocytosis. First, by depleting mononuclear phagocytes of cholesterol, lovastatin and ZA may impede Fc receptor redistribution into cholesterol-rich lipid rafts. By this means, these drugs may reduce Fc receptor cross-linking, tyrosine kinase activation, and actin filament assembly,³² and thereby inhibit phagocytosis. In fact, recent studies by Hillyard et al show that fluvastatin at micromolar concentrations inhibited Fc receptor signal transduction in human monocytes at the level of tyrosine kinase activation in a time- and dose-dependent manner.³³

A second mechanism by which lovastatin and ZA may inhibit Fc receptor-mediated phagocytosis is by interfering with plasma membrane biosynthesis, thereby reducing the availability of macrophage plasma membrane stores. Greenberg et al³¹ have reported that insertion of plasma membrane from internal stores is required for efficient phagocytosis of E(IgG) by macrophages. Our finding that TNF- α and LPS reverse the inhibitory effect of lovastatin on phagocytosis within a few hours of their addition to the medium (Figure 6; data not shown) suggests that lack of availability of internal plasma membrane stores is not the principal mechanism by which lovastatin blocks phagocytosis of E(IgG).

Our studies provide several insights that are relevant to the reported inhibitory effects of statins on mononuclear phagocyte functions in vitro and in vivo. First, they show that statins exert a powerful inhibitory effect on one of the cardinal functions of mononuclear phagocytes (Fc receptor-mediated phagocytosis) when these cells are maintained in medium lacking cholesterol or LDL. Second, they demonstrate that in medium containing cholesterol or a subphysiological concentration of LDL (ie, 100 μ g/mL), lovastatin

does not inhibit phagocytosis, even when used at 10 μ mol/L (Figure 4). The peak plasma lovastatin concentration in patients taking this drug orally ranges between 0.2 and 1 μ mol/L. At these concentrations, lovastatin had an insignificant effect on E(IgG) phagocytosis (Figure 1). This suggests that under physiological conditions in which LDL concentrations generally exceed 1 mg/mL, statin-mediated inhibition of endogenous cholesterol synthesis by mononuclear phagocytes is unlikely to affect the functions of these cells. Third, they show that even in LDL-free medium, proinflammatory cytokines (eg, TNF- α) and bacterial products (eg, LPS) can overcome the inhibitory effect of lovastatin on phagocytosis. TNF- α and LPS are likely to exert similar effects in vivo in tissues containing very low concentrations of LDL (eg, cerebrospinal fluid, airways, and joint spaces). This is consistent with clinical observations that patients taking statins show no defects in wound healing or in combating bacterial infections.

Do Statins Affect Inflammation?

It has been postulated that statins provide beneficial anti-inflammatory effects exclusive of cholesterol lowering;³⁴ however, this conclusion is controversial.³⁵ There are limited data that other more clearly defined anti-inflammatory drugs affect the progression of atherosclerosis. Comparisons of multiple intervention trials for coronary artery disease show declines in disease that correlate with the degree of cholesterol reduction, whether this is achieved by statins or other methods.³⁶ The reduction in plasma C-reactive protein, produced primarily in the liver, that occurs in patients treated with statins has been interpreted as evidence of an anti-inflammatory effect of these drugs.¹ It is uncertain whether this reduction is due specifically to statins or the concomitant cholesterol reduction. Reduction of plasma cholesterol or LDL itself may reduce some inflammatory processes.³⁴

Acknowledgments

This work was supported by National Institutes of Health grants HL-62301 and HL-56984 (I.J.G.), National Heart, Lung, and Blood Institute grants AI20651 and NIA-A619772 (S.C.S.), Alzheimer's Association grant IIRG-02-3510 (J.H.), and by Merck and Co. (I.J.G., J.D.L.).

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Arteriosclerosis, Thrombosis, and Vascular Biology



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Arterioscler Thromb Vasc Biol. 2004;24:2051-2056; originally published online September 2, 2004;

doi: 10.1161/01.ATV.0000143858.15909.29

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:

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