Inflamm. res. 51 (2002) 058–062
1041-3830/02/020058-05 $ 1.50+0.20/0

Inflammation Research

Atorvastatin activates PPAR-γ and attenuates the inflammatory response in human monocytes

O. Grip, S. Janciauskiene and S. Lindgren

Department of Medicine, Gastroenterology and Hepatology Division, Lund University, University Hospital MAS, SE-20502 Malmö, Sweden, Fax: ++ 4640923272, e-mail: olof.grip@medforsk.mas.lu.se

Received 17 April 2001; returned for revision 28 August 2001; accepted by M.J. Parnham 22 October 2001

Abstract. *Objective:* To investigate the ability of statins to activate the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) in primary human monocytes in culture.
Materials and methods: Human peripheral monocytes were incubated with atorvastatin (0.1–10 μmol/l) for up to 24 hours. PPAR-γ expression was analysed by electrophoretic mobility shift assay. Pro-inflammatory cytokines were measured by enzyme-linked immunosorbent assays, and oxygen consumption was determined polarographically with a Clark-type oxygen electrode.
Results: We found that atorvastatin activates PPAR-γ and inhibits the production of tumour necrosis factor-alpha up to 38% ($p < 0.05$), monocyte chemoattractant protein-1 up to 85% ($p < 0.05$), and gelatinase B up to 73% ($p < 0.05$), in a concentration-dependent manner. Moreover, atorvastatin shows concentration-dependent inhibition of cellular oxygen consumption up to 41%.
Conclusions: These findings contribute to the growing knowledge of the anti-inflammatory effects of statins, and have led us to the suggestion that statins may control inflammatory responses by the regulation of intracellular lipid homeostasis.

Key words: Atorvastatin – Monocytes – Inflammation – PPAR-γ

Introduction

Evidence is accumulating from clinical and experimental studies that the effects of statins extend beyond the reduction of plasma cholesterol levels [1–3]. Statins are competitive hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors of cholesterol synthesis from mevalonate [4]. They also enhance the expression of low-density lipoprotein (LDL) receptors, increase the incorporation of LDL and reduce serum levels of cholesterol [5]. Various studies have shown that statins can inhibit cholesterol accumulation in macrophages, thereby reducing their activity [6, 7].

During monocyte differentiation into macrophages, the highest gene expression observed is that of genes encoding proteins in lipid metabolism, such as apolipoprotein E, osteopontin, CD9, sterol 27-hydroxylase, and liposomal acid lipase, suggesting that alteration of lipid metabolism in mononuclear phagocytes is associated with their differentiation [8]. Nuclear receptors are ligand-dependent transcription factors that regulate gene networks involved in controlling cellular differentiation, growth, morphogenesis, and homeostasis [9]. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily which heterodimerize with the retinoid X receptor (RXR) and regulate transcription of target genes through binding to specific response elements or PPREs, which consist of a direct repeat of the nuclear receptor hexameric DNA core recognition motif spaced by one nucleotide. The target genes of PPARs/RXR are involved in many critical physiological and pathological functions, such as cellular differentiation, lipid metabolism and glucose homeostasis [10]. Three subtypes, α, β/δ, and γ are known, and special interest has been focused on PPAR-γ in macrophage development and function [11–13]. Recently it has also been proposed that PPAR-γ is a key regulator of lipoprotein metabolism, functioning as a co-ordinator between oxidised LDL uptake and the processing and removal of cholesterol in macrophages [14, 15].

Macrophages are important in the host's immunological and inflammatory responses. Macrophages in tissue are derived from circulating, mature monocytes that are believed to be a replacement pool. During inflammation, chemotatic cytokines (chemokines), and various peptide and non-peptide mediators of inflammation are generated locally and stimulate monocytes to migrate into the site of inflammation where they differentiate into macrophages. A mechanism underlying monocyte infiltration during acute inflammatory processes is the increased expression of monocyte chemoattractant protein (MCP)-1 [16]. These newly recruited macrophages secrete cytokines, such as interleukin (IL)-1,

Correspondence to: O. Grip

IL-6, IL-8, IL-12, IL-18 and tumor necrosis factor (TNF)-α, that are important in the pro-inflammatory responses. They also release reactive metabolites of oxygen and nitrogen, and proteases that degrade the extracellular matrix [17, 18]. A suggested signal pathway of statins in regulating pro-inflammatory cytokine and chemokine expression in mononuclear cells is via the inhibition of transcription factor NF-κB activity [19]. We have previously demonstrated in human monocyte cultures that pravastatin not only lowers cholesterol synthesis and lipid accumulation, but also potently inhibits the generation of pro-inflammatory modulators and oxygen consumption in activated monocytes [20].

However, the anti-inflammatory effects of statins still remain largely unknown. We conducted the present study to investigate the ability of atorvastatin to activate PPAR-γ expression with the aim to establish a link between its lipid-lowering and its anti-inflammatory properties.

Materials and methods

Atorvastatin calcium salt was provided by Park-Davis, Sweden. TNF-α was procured from Sigma, Sweden. [γ^{-32}P]ATP was manufactured by Amersham Pharmacia Biotech. PPAR-γ consensus and mutant olig onucleotides were made at the unit of Biomedicine, Lund University, Sweden. The supershift antibody against PPAR-γ was supplied by Calbiochem.

Isolation and culture of monocytes

Human monocytes were isolated by the Ficoll-Hypaque procedure from buffy coats obtained from healthy blood-donors. Cell quality was analysed by autocounter (AC 900^{EO}, Swelab, Sweden). Monocytes were plated at a density of 4×10^6 cells/ml into plastic plates or dishes. After removal of non-adhering cells, monocytes were cultured in RPMI 1640 (Gibco™, Life Technologies, Paisley, Scotland) supplemented with 2 mmol/l N-acetyl-L-alanyl-L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 1% non-essential amino acid, 2% sodium pyruvate and 20 mmol/l HEPES without serum (Fluka™, Chemie AG), at 37 °C in 5% CO_2. In parallel experiments, monocytes were activated with TNF-α (1 ng/ml) for 45 min. All experiments were performed within 24 h, using non-activated and activated monocytes alone or exposed to various concentrations of atorvastatin dissolved in methanol. Control cells were exposed to methanol without atorvastatin.

Preparation of nuclear extracts

Nuclear extracts were prepared essentially as described previously [21]. All buffers were kept on ice unless stated otherwise. Protease inhibitors leupeptin (15 µg/ml), aprotinin (15 µg/ml), and PMSF (1 mmol/l) were added to all buffers just before use. Cells cultured in 90 mm dishes were rinsed with ice-cold phosphate-buffered saline. The cells were resuspended in 60 µl hypotonic buffer (10 mmol/l HEPES (pH 7.3), 10 mmol/l KCl, 1.5 mmol/l $MgCl_2$). After centrifugation (9000 g, 2 min), the cells were lysed by resuspention in 80 µl lysis buffer (10 mmpl/l HEPES (pH 7.3), 10 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.4% Nonidet P-40). After incubation at 4 °C for 10 min, nuclei were collected by centrifugation for 1 min at 9000 g, and the pellets were washed once in 1 ml of 20 mmol/l KCl buffer (20 mmol/l HEPES (pH 7.3), 20 mmol/l KCl, 22% glycerol, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l EDTA). The isolated nuclei were resuspended in 15 µl of 20 mmol/l KCl buffer, and 60 µl of 600 mmol/l KCl buffer (20 mmol/l HEPES (pH 7.3), 600 mmol/l KCl, 22% glycerol, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l EDTA) was added. Nuclear proteins were extracted by incubation on ice for 30 min. After centrifugation at 9000 g (4 °C) for 15 min, the supernatant containing the nuclear proteins was transferred to a pre-cooled microcentrifuge tube. Protein concentration was determined spectrophotometrically according to the following equation: Concentration (µg/ml) = 183 × A (230 nm) – 75.8 × A (260nm), [22] where A denotes absorbance.

Electrophoretic mobility shift assay (EMSA)

Equal amounts of protein from nuclear extracts (1.8 µg) were incubated on ice with 130 µg/ml polydI-dC, and 65 µg/ml acetylated bovine serum albumin in binding buffer (5% glycerol, 20 mmol/l HEPES (pH 7.9), 60 mmol/l KCl, 20 mmol/l $MgCl_2$, 0.5 mmol/l EDTA, 1 mmol/l DTT, 1.5 mmol/l PMSF) for 10 min. The oligonucleotide probe (50.000 cpm in 5 µl) was added, and the reaction mixture was incubated for 30 min at room temperature. DNA-protein complexes were separated from unbound DNA probe on native 7% polyacrylamide gels (acrylamide: bisacrylamide (w/v) 29:1 in low ionic strength buffer (22.5 mmol/l Tris, 22.5 mmol/l borate, 0.5 mmol/l EDTA, pH 8) by electrophoresis at 250 V for 2 h. The sequences of the double stranded oligonucleotide probes ([γ-^{32}P]ATP labelled with T4 kinase and purified using Pharmacia NICK columns) for the detection of PPAR-γ binding was designed according to Tontonoz [11], containing a DR-1 motif in the CD-36 promoter region. Unlabeled competitor oligonucleotides were added in 50 X excess to confirm the specificity of the binding reactions.

TNF-α, monocyte chemoattractant protein-1 and gelatinase B enzyme-linked immunosorbent assays

Triplicates of cell culture supernatants from monocytes treated with atorvastatin (up to 10 µmol/l) for 24 h were analysed to determine TNF-α, monocyte chemoattractant protein-1 (MCP-1), and gelatinase B (MMP-9) release. A quantitative sandwich enzyme immunoassay technique (Quantikine™, R & D Systems, Minneapolis, USA), sensitive to pg/ml levels was used according to the manufacturer's instructions. Each sample was assayed in duplicate.

Measurement of oxygen consumption

Freshly isolated monocytes, activated with TNF-α, were simultaneously treated with atorvastatin (0.1, 1 and 10 µmol/l). Oxygen consumption was measured polarographically with a Clark-type oxygen electrode (CB1-D3, Techtum Lab., AB) in a water-jacketed chamber connected to a circulating bath, at 37 °C. The instrument was calibrated prior to each assay according to the instructions in the manual. Air saturated reaction mixtures containing 0.5 ml, 2×10^5 cells/ml, in monocyte culture medium were used.

[^{3}H] Thymidine incorporation assay

Cells were incubated with 10 µmol/l atorvastatin for 20 h. [^{3}H] Thymidine (Amersham Life Sience) was then added to the cells (0.2 µCi/ml) for a further 4 h of incubation at 37 °C. After the medium had been aspirated, the cells were washed twice with 0.5 mol/l NaCl and incubated for 5 min with 5% trichloroacetic acid. The cells were then washed with water, dissolved in 1 ml 0.5 mol/l NaOH, neutralised with 200 µl HCl and the radioactivity was determined in a β-detector (Winspectral™, 1414 liquid scintillation spectrometer; Wallac)

Statistical analysis

The differences in the means of the experimental results were analysed for their statistical significance with the independent-samples, two-sided t test and/or one-way analysis of variance (ANOVA) combined with a multiple comparisons procedure (Scheffé multiple range test) with the overall significance level of $\alpha = 0.05$.

Results

Activation of monocyte PPAR-γ

Freshly isolated human monocytes were incubated alone or with atorvastatin at concentrations of 1 and 10 μmol/l for 1 h and analysed for PPAR-γ activity. By using EMSA we found that atorvastatin activates PPAR-γ in a dose-dependent manner (Fig. 1). In parallel experiments, we activated monocytes with TNF-α (1 ng/ml) for 45 min prior to the addition of atorvastatin. Under these experimental conditions PPAR-γ levels were lower, and further, the cell response to atorvastatin treatment was also less pronounced (Fig. 1).

Effects of atorvastatin on pro-inflammatory molecules

We examined the levels of pro-inflammatory cytokine, TNF-α and chemokine, MCP-1, in monocyte cultures which were either untreated or treated with atorvastatin for 24 h. Atorvastatin lowered MCP-1 levels by up to 81% ($p < 0.05$), in a dose-dependent manner, but showed no influence on TNF-α. Under these same experimental conditions we also analysed protein expression of gelatinase B (MMP-9), a protease that degrades extracellular matrix, evoking tissue damage in the inflammatory process. We found that atorvastatin at a concentration of 10 μmol/l, significantly suppress MMP-9 levels by 72% ($p < 0.05$), (Fig. 2).

Parallel experiments were conducted with activated monocytes (TNF-α, 1 ng/ml, for 45 min). Basal production of pro-inflammatory molecules in monocytes stimulated with TNF-α was markedly up regulated compared to non-stimulated monocytes (Fig. 2). Addition of up to 10 μmol/l atorvastatin to TNF-α activated cells resulted in a dose-dependent decrease in TNF-α levels, up to 38% ($p < 0.05$), MCP-1 up to 85% ($p < 0.05$) and a reduction in MMP-9 protein levels up to 73% ($p < 0.05$). It should be pointed out that atorvastatin added to both non-activated and activated monocytes showed an inhibitory effect on MCP-1 levels already at a concentration of 0.1 μmol/l, while more pronounced effects on other pro-inflammatory molecules were observed at higher concentrations.

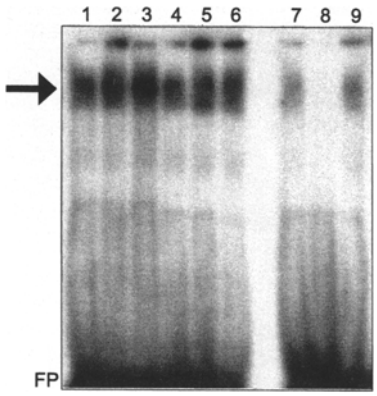

Fig. 1. Atorvastatin dose-dependently activates PPAR-γ in primary human monocytes, as assessed by EMSA. Cells were incubated with atorvastatin for 1 h. Control cells (lane 1), atorvastatin 1 μmol/l (lane 2), atorvastatin 10 μmol/l (lane 3). Parallel experiments with TNF-α-activated (1 ng/ml) cells, control cells (lane 4), atorvastatin 1 μmol/l (lane 5), atorvastatin 10 μmol/l (lane 6). Specificity tests included supershift analysis with an anti PPAR-γ antibody which led to a decrease of the specific band (lane 7), as well as competition assays with a 50 times excess of unlabelled specific or mutant competitor DNA (lanes 8 and 9, respectively), The arrow indicates the specific band. FP denotes free probe. Representative of three independent experiments.

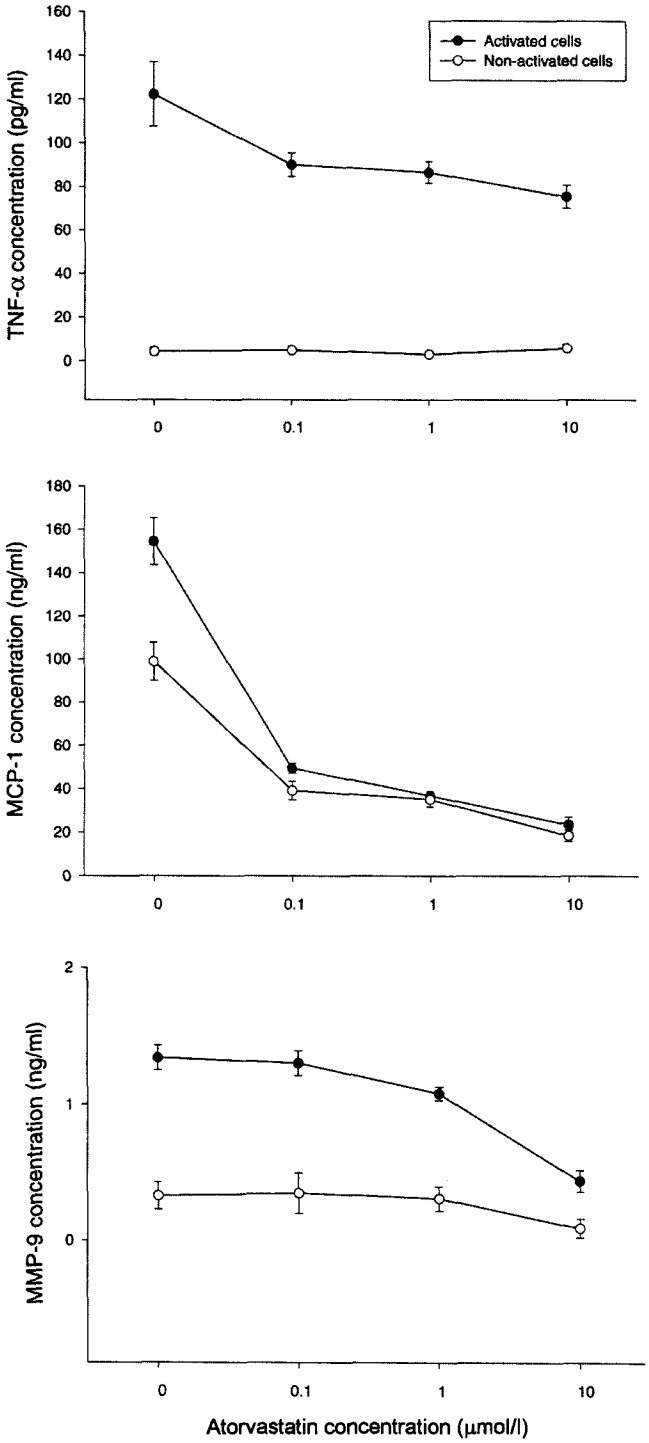

Fig. 2. TNF-α, MCP-1 and MMP-9 produced by non-activated or TNF-α-activated human monocytes (1 ng/ml), alone and with the addition of various concentrations of atorvastatin for 24 h. Results are the mean and SD of triplicate determinations, representative of two independent experiments.

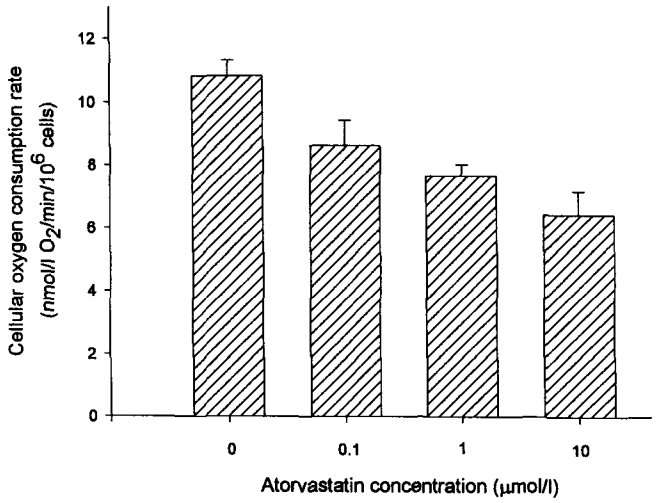

Fig. 3. Inhibition by atorvastatin of TNF-α-induced mitochondrial oxygen consumption in human monocytes. Cellular oxygen consumption was measured polarographically following the activation of the cells with TNF-α (1 ng/ml) in the presence of the concentrations of atorvastatin indicated. Data are the mean and SD from three independent experiments.

Cellular oxygen consumption

Cellular oxygen consumption was used to assess the effect of atorvastatin on mitochondrial respiration. Since cytokines, especially TNF-α, are known to induce a respiratory burst, we stimulated monocytes with TNF-α (1 ng/ml). The oxygen consumption levels measured were dose-dependently decreased by atorvastatin in experiments with simultaneous TNF-α addition, by up to 41% by 10 μmol/l atorvastatin, (Fig. 3).

Cytotoxicity assay

Since it has been shown that PPAR-γ activation induces apoptosis in human monocytes [23], we investigated whether atorvastatin (10 μmol/l) has a cytotoxic effect on monocytes by measuring [^{3}H] thymidine incorporation into monocytes. The radioactivity, determined as counts per minute, in cultured monocytes exposed to atorvastatin (109,5 ± 36.2 SD, n = 4) did not differ from that of controls (110 ± 25.7 SD, n = 4).

Discussion

Studies have indicated that statins, such as lovastatin and atorvastatin, exert anti-inflammatory effects via the inhibition of transcription factor NF-κB [19]. To further assess the anti-inflammatory properties of atorvastatin, and thus link the activation of transcription factor to lipid-lowering properties, we examined pro-inflammatory molecule expression and PPAR-γ activation in non-activated and TNF-α-activated human monocytes. We found atorvastatin to be a highly potent suppresser of the production of the chemokine MCP-1 in monocyte cultures. Since expression of chemokines is largely dependent on the oxidative stress, the observed inhibition on MCP-1 levels suggests that atorvastatin may also inhibit other oxidative, stress-related molecular species. Our finding that activated monocytes treated with atorvastatin significantly reduce TNF-α and MMP-9 levels further confirms that atorvastatin possesses properties which can reduce inflammatory reactions and possibly prevent the degradation of the extracellular matrix by MMP's.

In our experimental model we also show that the addition of increasing amounts of atorvastatin to freshly isolated monocytes rapidly reduces oxygen consumption. Since mitochondria produce reactive oxygen species as the by-products of molecular oxygen consumption in the electron transport chain [24–26] we propose that atorvastatin inhibits the oxidative burst in activated monocytes and therefore prevents tissue damage due to the action of reactive oxygen species. Similar findings have been observed in cultured human umbilical vein endothelial cells, where statins have been shown to decrease the enzyme expression involving superoxide production [27].

To correlate its inhibitory effects on pro-inflammatory mediators, we investigated the capacity of atorvastatin to induce PPAR-γ expression and demonstrated a marked activation of PPAR-γ protein expression by atorvastatin in primary human monocytes. Similar observations have been made in endothelial cells and hepatocytes by using other statins, such as pravastatin, simvastatin, fluvastatin, and cerivastatin, which induced PPAR-α and PPAR-γ mRNA expression, and increased corresponding protein levels [27]. The intracellular effects of HMG-CoA reductase inhibitors can be mediated by the regulation of the content of cellular cholesterol or products of the mevalonate pathway, although not necessarily cholesterol, since mevalonate is an important precursor of many isoprenoids, which play important roles in signal transduction [28].

The cellular expression of PPAR-γ depends on whether cells are subjected to pro- or anti-inflammatory stimuli. Pro-inflammatory cytokines such as TNF-α, IL-1α, IL-1β, IL-6, and leukaemia inhibitory factor, decrease PPAR-γ mRNA expression in adipose tissue [29]. By contrast, IL-4, an interleukin that exerts an anti-inflammatory effect, induces PPAR-γ1 expression in macrophages [30]. We found in TNF-α-stimulated monocytes that PPAR-γ protein expression was suppressed and PPAR-γ activation in response to atorvastatin treatment was less pronounced. This further supports the idea that statin effects of PPAR-γ activation might be largely dependent on the status of the functional activity of the cell. Moreover, recent studies have indicated that the inhibitory effects of PPAR-γ agonists on cytokine production and inflammation may be independent of the receptor [31, 32].

Certain eicosanoides derived from the cyclooxygenase and lipoxygenase pathways, such as 13-HODE, 15-HETE and 15-deoxy PG-J2, have been suggested to act as ligands increasing PPAR-γ expression [33–35]. HMG-CoA reductase inhibitors are known to inhibit cyclooxygenase-2 activity [27], which indicates that they can also inhibit stimulatory effects of eicosanoid on PPAR-γ expression. However, atorvastatin in concentration dependent manner induces PPAR-γ expression that suggests that this effect of statin is most likely related to inhibition of HMG-CoA reductase, but not cyclooxygenase-2 activity. Moreover, our findings that atorvastatin inhibits the generation of pro-inflammatory molecules and oxygen consumption potently in pre-activated cells, and in contrast, shows less pronounced activation of PPAR-γ in these cells, than in non-activated monocytes, has led us to propose that anti-inflammatory properties of atorvastatin are probably independent of PPAR-γ.

Acknowledgements. The authors are grateful to Camilla Orbjörn for expert technical assistance with the experiments. This work has been supported by grants from The Swedish Medical Research Council (13008, 1614), The Swedish Society of Medicine, The Lundström Foundation and The Tore Nilsson Foundation.

References

[1] Kendall MJ, Toescu V. Non-lipid properties of statins. J Clin Pharm Ther 1999; 24: 3–5.
[2] Kwak B, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. Nat Med 2000; 6: 1399–402.
[3] Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S et al. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. Arterioscler Thromb Vasc Biol 2001; 21: 115–21.
[4] Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F. New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther 1999; 84: 413–28.
[5] Ma PT, Gil G, Sudhof TC, Bilheimer DW, Goldstein JL, Brown MS. Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low-density lipoprotein receptor in livers of hamsters and rabbits. Proc Natl Acad Sci USA 1986; 83: 8370–4.
[6] 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: 1212–7.
[7] Bocan TM, Mazur MJ, Mueller SB, Brown EQ, Sliskovic DR, O'Brien PM et al. Antiatherosclerotic activity of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase in cholesterol-fed rabbits: a biochemical and morphological evaluation. Atherosclerosis 1994; 111: 127–42.
[8] Hashimoto S, Suzuki T, Dong HY, Yamazaki N, Matsushima K. Serial analysis of gene expression in human monocytes and macrophages. Blood 1999; 94: 837–44.
[9] Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K et al. The nuclear receptor superfamily: the second decade. Cell 1995; 83: 835–9.
[10] Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature 1992; 358: 771–4.
[11] Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPAR-gamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998; 93: 241–52.
[12] Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998; 391: 79–82.
[13] Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391: 82–6.
[14] Chawla A, Boisvert WA, Lee C, Laffitte BA, Barak Y, Joseph SB et al. A PPAR-gamma-LXR-ABCA1 Pathway in Macrophages Is Involved in Cholesterol Efflux and Atherogenesis. Mol Cell 2001; 7: 161–71.
[15] Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP et al. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med 2001; 7: 53–8.
[16] Rollins BJ. Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease. Mol Med Today 1996; 2: 198–204.
[17] Diaz MN, Frei B, Vita JA, Keaney JF Jr. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997; 337: 408–16.
[18] Nathan CF. Secretory products of macrophages. J Clin Invest 1987; 79: 319–26.
[19] Ortego M, Bustos C, Hernandez-Presa MA, Tunon J, Diaz C, Hernandez G et al. Atorvastatin reduces NF-κB activation and chemokine expression in vascular smooth muscle cells and mononuclear cells. Atherosclerosis 1999; 147: 253–61.
[20] Grip O, Janciauskiene S, Lindgren S. Pravastatin down-regulates inflammatory mediators in human monocytes in vitro. Eur J Pharmacol 2000; 410: 83–92.
[21] Dichtl W, Moraga F, Ares MP, Crisby M, Nilsson J, Lindgren S et al. The carboxyl-terminal fragment of alpha1-antitrypsin is present in atherosclerotic plaques and regulates inflammatory transcription factors in primary human monocytes. Mol Cell Biol Res Commun 2000; 4: 50–61.
[22] Welgus HG, Campbell EJ, Cury JD, Eisen AZ, Senior RM, Wilhelm SM et al. Neutral metalloproteinases produced by human mononuclear phagocytes. Enzyme profile, regulation, and expression during cellular development. J Clin Invest 1990; 86: 1496–502.
[23] Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z et al. Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. J Biol Chem 1998; 273: 25573–80.
[24] Wei YH, Lu CY, Lee HC, Pang CY, Ma YS. Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. Ann NY Acad Sci 1998; 854: 155–70.
[25] Morel F, Doussiere J, Vignais PV. The superoxide-generating oxidase of phagocytic cells. Physiological, molecular and pathological aspects. Eur J Biochem 1991; 201: 523–46.
[26] Fridovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? Ann NY Acad Sci 1999; 893: 13–8.
[27] Inoue I, Goto S, Mizotani K, Awata T, Mastunaga T, Kawai S et al. Lipophilic HMG-CoA reductase inhibitor has an anti-inflammatory effect: reduction of mRNA levels for interleukin-1beta, interleukin-6, cyclooxygenase-2, and p22phox by regulation of peroxisome proliferator-activated receptor alpha (PPAR-alpha) in primary endothelial cells. Life Sci 2000; 67: 863–76.
[28] Casey PJ. Protein lipidation in cell signaling. Science 1995; 268: 221–5.
[29] Tanaka T, Itoh H, Doi K, Fukunaga Y, Hosoda K, Shintani M et al. Down regulation of peroxisome proliferator-activated receptorgamma expression by inflammatory cytokines and its reversal by thiazolidinediones. Diabetologia 1999; 42: 702–10.
[30] Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C et al. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. Nature 1999; 400: 378–82.
[31] Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med 2001; 7: 48–52.
[32] Thieringer R, Fenyk-Melody JE, Le Grand CB, Shelton BA, Detmers PA, Somers EP et al. Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-alpha responses of macrophages to lipopolysaccharide in vitro or in vivo. J Immunol 2000; 164: 1046–54.
[33] Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR-gamma. Cell 1995; 83: 803–12.
[34] Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell 1995; 83: 813–9.
[35] Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR-gamma. Cell 1998; 93: 229–40.