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# 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase the binding activity and nuclear level of Oct-1 in mononuclear cells

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**Abstract**

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are drugs very effective to decrease low-density lipoprotein (LDL) cholesterol. In addition, a number of studies suggest that statins have other beneficial clinical effects beyond cholesterol lowering. We recently reported that statins decrease nuclear factor kappa B (NF-κB) binding activity in monocytes and vascular smooth muscle cells. We now explored the effect of two different statins, simvastatin and atorvastatin, in the activation of the octamer transcription factor Oct-1 on the monocytic cell line THP-1. Oct-1 is a nuclear factor that represses the transcription of proinflammatory genes such as interleukin-8, CD11c/CD18, vascular cell adhesion molecule-1 (VCAM-1) and platelet endothelial cell adhesion molecule-1 (PECAM-1). Low concentrations of both statins increased Oct-1 DNA binding activity (electrophoretic mobility shift assay) that was resolved into two specific bands. The upper one was supershifted by preincubation of nuclear extracts with anti-Oct-1 antibody. The lower one was supershifted by preincubation of nuclear extracts with an anti-Oct-2 antibody, also partially competed with 100 mol/l excess of cold activator protein-1 (AP-1) and attenuated by anti-c-Jun antibody. Both statins increased Oct-1 and Oct-2 nuclear protein levels (Western blot). In contrast, neither had any effect on PMA-differentiated cells, suggesting a distinct sensitivity between circulating monocytes and resident tissular macrophages. In addition, statins did not increase Oct-lipoprotein lipase binding activity that contains an Oct-1 binding element. The mRNA expression of interleukin-8, a chemokine containing Oct sites in its promoter, was diminished by statin pretreatment. Our results indicate that simvastatin and atorvastatin increase the activity of the transcriptional repressor Oct-1 in mononuclear cells, and could thus contribute to decrease the activation of these cells. These data suggest a possible novel mechanism supporting a certain anti-inflammatory effect of these two 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors.


*Keywords:* Oct-1; HMG-CoA reductase inhibitor; Nuclear factor; Atherosclerosis

## 1. Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) reduce cardiovascular mortality and the incidence of ischemic events in patients with hypercholesterolemia (Brown and Fuster, 1996). Although it was reasonable to attribute most of these therapeutic benefits to reduction of the serum lipid profile, recent studies suggest that these drugs may have additional effects. These clinical benefits, apparently unrelated to the hepatic effect of statins in reducing LDL cholesterol, have been explained by several mechanisms, the so-called pleiotropic effects. Thus, statins induce certain well-known effects such as inhibition of vascular smooth muscle cell proliferation and migration and cause apoptosis in these cells (Hidaka et al., 1992; Raiteri et al., 1997; Guijarro et al., 1998). Recently, the antioxidant effect of statin therapy for preventing cardiac hypertrophy has also been described (Takemoto et al., 2001). Previously, we found that in cultured vascular smooth muscle cells and macrophages in a rabbit model of atherosclerosis and in in vitro experiments, some statins reduce the activation of NF-κB, a nuclear factor that regulates the transcription of some inflammatory genes (Bustos et al., 1998, Ortego et al., 1999). However, the role of statins in the regulation of other nuclear factors is not well known.

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Oct binding factors are a family of proteins composed of different members that share a common domain called POU domain. The octamer sequence ATTTGCAT was identified as a regulatory element within the immunoglobulin genes and is involved in high-affinity Oct-1 binding. Oct-1 is a ubiquitous transcription factor expressed in all dividing cells and thus essential for proliferation, differentiation and other key cell processes (Verrijzer and Van der Vliet, 1993). Oct-1 is phosphorylated by protein kinase A at $Ser^{385}$, leading to inhibition of mitosis (Segil et al., 1991). Target genes positively controlled by Oct-1 include histone 2B (Sturm et al., 1988), iNOS (Xie, 1997), interleukin-3, interleukin-5, granulocyte–macrophage colony stimulating factor (Kaushansky et al., 1994), lipoprotein lipase (Previato et al., 1991), TIE1 (Boutet et al., 2001) and those negatively controlled such as interleukin-8 (Wu et al., 1997), pituitary transcription factor (PIT1/GHF1) (Delhase et al., 1996), Von Willebrand (Schwachtgen et al., 1998), CD11c/CD18 (López-Rodríguez et al., 1997), platelet endothelial cell adhesion molecule-1 (PECAM-1) (Thum et al., 2000), vascular cell adhesion molecule (VCAM-1) (Iademarco et al., 1992), Osteopontin (Wang et al., 2000) and interleukin-4 (Cron et al., 2001).

Oct-2 is expressed in B-lymphocytes, where it is required for lipopolysaccharide-induced proliferation and maturation. Oct-2 is also expressed in neuronal cells and has been implicated as a regulator of herpes virus latency (Kristie et al., 1989).

Lipoprotein lipase is a hydrolytic enzyme, which is synthesized in different animal tissues, but acts at the luminal surface of the vascular endothelium. Lipoprotein lipase plays an important role in lipoprotein metabolism by hydrolyzing triglycerides contained in chylomicrons and very-low-density lipoprotein (VLDL) particles and by providing fatty acids to tissues for storage or combustion. The proximal lipoprotein lipase promoter region upstream of the transcription start site contains several potential binding sites for known transcription factors, including cAMP-responsive and Oct-1 elements (Yang et al., 1995). In addition, recently, it has been reported that Oct-1 is activated in response to DNA damage (Zhao et al., 2000).

Since Oct-1 is a repressor of molecules implicated in the recruitment of inflammatory cells into the vessel wall, statins could diminish the presence of macrophages in the plaque through the modulation of octamer transcription factors. The purpose of this study was to test if low concentrations of simvastatin and atorvastatin could affect the Oct-1 DNA binding activity in cultured human mononuclear cells and macrophages.

## 2. Materials and methods

Rosweli Park Memorial Institute (RPMI) 1640, Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum were from BioWhittaker (Walkersville, MD). All the chemicals were purchased from Sigma (Spain) unless specified otherwise, and radiochemicals were from Amersham Ibérica. Plastics were from Bibby Esteriling. Atorvastatin (sodium salt) (At) and simvastatin (lactone) (Sv) were from Pfizer (Spain) and Merk Sharp & Dohme (MSD, Spain). Simvastatin was hydrolyzed before use. Anti Oct-1 (sc-232), Oct-2 (sc-233X), c-Jun (sc-44), c-Fos (sc-413X) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Lipoprotein lipase-Oct sequence was obtained from mouse promoter sequence of lipoprotein lipase gene (from 1928 to 1957 bp gene bank reference M63335).

### 2.1. Cell culture

THP-1 cells were purchased from American Type Culture Collection (ATCC) (TIB 202) and were cultured in RPMI 1640 medium (BioWittaker) supplemented with 10% fetal bovine serum. Cells were maintained at a cell density of $2-10 \times 10^5$ cells/ml and were growth-arrested by incubation in medium without serum for 48 h, and then incubated with the corresponding stimuli. THP-1 cells were differentiated by phorbol myristate acetate (PMA) treatment in serum-free RPMI 1640 by incubation with $10^{-6}$ M PMA during 48 h, then washed with fresh serum-free medium and stimulated with the corresponding agents, thus the cells have many of the structural and functional characteristics of monocyte-derived macrophages (Schwende et al., 1996).

### 2.2. Protein extraction

Cells were collected, washed with cold phosphate buffer saline (PBS) and resuspended in five cell-pellet volumes of buffer A [10 mM HEPES (pH 7.8), 15 mM KCl, 2 mM/l $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 1 mM phenylmethanesulfonyl fluoride]. After 10 min on ice, the cells were pelleted, resuspended in two volumes of buffer A and homogenized. The homogenate was centrifuged at $1000 \times g$ for 10 min and the cytosolic fractions were collected and stored at $-80$ °C. Nuclei were resuspended in buffer A and 3 M KCl was added drop by drop to reach 0.39 M KCl. Nuclear proteins were extracted for 45 min on ice and centrifuged at $10{,}000 \times g$ for 30 min. Supernatants were dialyzed in buffer C (50 mM HEPES (pH 7.8), 50 mM KCl, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride, 0.1 mM EDTA and 1 mM DTT) and then cleared by centrifugation and stored at $-80$ °C. Protein concentration was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL).

### 2.3. Electrophoretic mobility shift assays (EMSA)

Oct-1 consensus oligonucleotide (5′-TGTCGAATGCAAATCACTAGAA-3′), NF-κB (5′-AGTTGAGGGGACTTTCCCAGGC-3′) and Oct-lipoprotein lipase (5′-TCGACGATGAGTCTTATTTGCATATTTCCAGTCAG-

3′ ) were [$^{32}$P] end-labeled by incubation for 10 min at 37 °C with 10 U of T4 polynucleotide kinase (Promega, Madison, WI) in a reaction containing 10 μCi of [γ-$^{32}$P]ATP, 70 mM Tris–HCl, 10 mM $MgCl_2$ and 5 mM DTT. The reaction was stopped by the addition of EDTA to a final concentration of 0.05 M. Nuclear proteins (5 μg) were equilibrated for 10 min in a binding buffer containing 4% glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 10 mM Tris–HC (pH 7.5) and 50 μg/ml of poly (dI-dC) (Pharmacia LKB, Uppsala, Sweden). Labeled probe (0.35 pmol) was added to the reaction and incubated for 20 min at room temperature. Competition assays were performed by adding 100 M excess of cold probe 10 min prior to the addition of the labeled probe. For supershift assays, 1 μg of antibodies was added and incubated for 1 h. Negative controls were done setting the reaction without nuclear extract. The reaction was stopped by adding gel loading buffer (250 mM Tris–HCl, 0.2% bromophenol blue, 0.2% xylene cyanol and 40% glycerol) and run on a nondenaturing, 4% acrylamide gel at 100 V at room temperature in Tris–borate–EDTA buffer. The gels were dried and exposed to X-ray film. The retarded bands were densitometered by two independent investigators and the mean value was used for the calculations.

### 2.4. Western blot

The protein levels of Oct-1 and Oct-2 were determined by Western blot analysis with specific polyclonal antibodies (Santa Cruz Biotechnology). Twenty micrograms of nuclear proteins was separated on a 7.5% or 12% SDS-PAGE and transferred to PVDF membrane (Immobilon P, Millipore). The membrane was blocked in washing solution (Tris 0.01 M, NaCl 0.1 M, 0.1% Tween 20; pH 7.5) with 5% nonfat dried milk, for 30 min at 37 °C. It was first incubated overnight with 1 μg/ml of primary antibody at 4 °C and then with a peroxidase-conjugated secondary antibody for 30 min at 37 °C. The bands were detected with a chemiluminiscent system (ECL, Amersham, Arlington Heights, IL) and exposed to X-ray film.

### 2.5. RNA extraction and reverse transcriptase polymerase chain reaction

Total RNA from different experimental conditions was obtained by the Trizol method (Life technologies) and quantified by absorbance at 260 nm in duplicate. For RT-PCR, 100 ng of RNA from different experimental conditions was applied to the access RT-PCR System (Promega). The following primers were used for interleukin-8: antisense: 5′ -

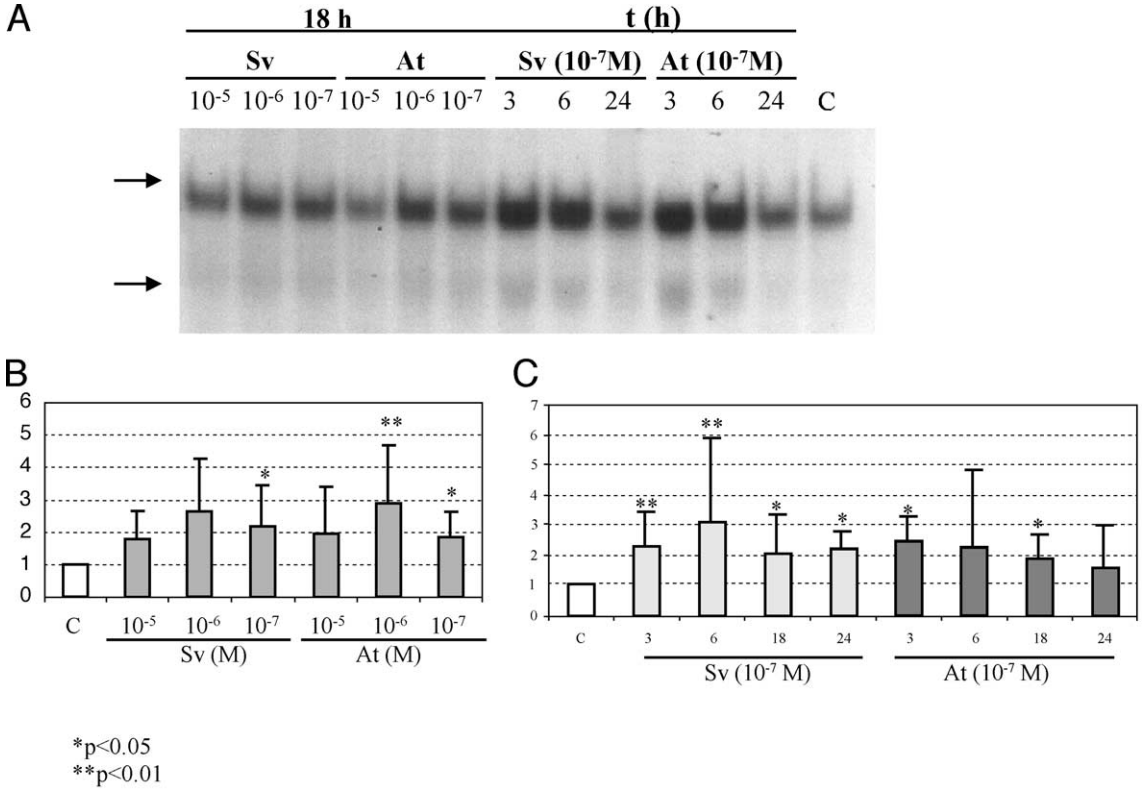


Fig. 1. Time and dose response of Oct-1 activation after incubation with statins. Cells were growth-arrested by serum depletion and incubated with simvastatin (Sv) or atorvastatin (At) ($10^{-5}$–$10^{-7}$ M) for different periods of time (3, 6, 18 and 24 h). Then cells were collected and nuclear proteins were assayed for Oct-1 activity. The figure shows an experiment representative of five.

TGAATTCTCAGCCCTCTTCAAAAACTTCTC-3′ and sense: 5′ -ATGACTTCCAAGCTGGCCGTG-3′ , which yielded products of 297 bp (1 min at 60 °C for annealing of the primers, 21 cycles), and G3PDH: antisense: 5′ -AATGCATCCTGCACCACCAA-3′ and sense: 5′ -ATACTGTTACTTATACCGATG-3′ , which yielded products of 515 bp (1 min at 57 °C for annealing primers, 25 cycles). The DNA products from RT-PCR reactions were analyzed on a 4% polyacrylamide/urea gel in the same buffer. The polyacrylamide gels were dried, exposed to X-ray film, and scanned using the ImageQuant densitometer.

### 2.6. Statistical analysis

Arbitrary units were calculated in relation to the basal value in every experiment. A two-tailed Mann–Whitney test was performed. Results are expressed as the means ± S.D. Significance was established using GraphPAD InStat (GraphPAD Software). Differences were considered significant when $p < 0.05$.

## 3. Results

### 3.1. Oct-1 binding activity is induced in mononuclear cells by simvastatin and atorvastatin

THP-1 mononuclear cells were serum-starved for 48 h to avoid interference with the growth factors contained in serum and then incubated with several concentrations of atorvastatin and simvastatin ($10^{-5}$–$10^{-7}$ M) for 18 h and

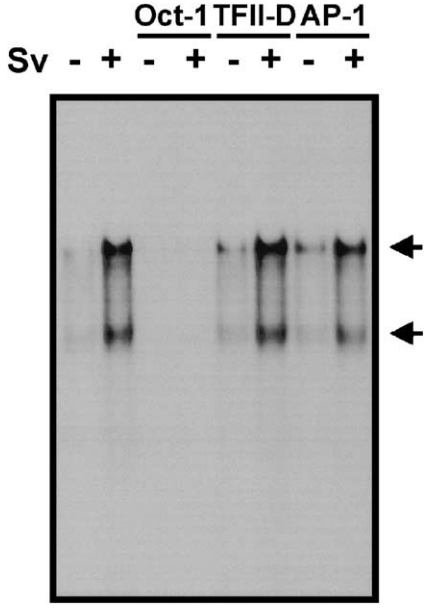


Fig. 2. Competition experiments with related and unrelated oligonucleotides. Cells were incubated for 18 h with medium alone (−) or with simvastatin $10^{-7}$ M (Sv). Competition experiments were done by preincubating for 1 h the nuclear extracts with a 100-fold excess of the corresponding cold oligonucleotide.

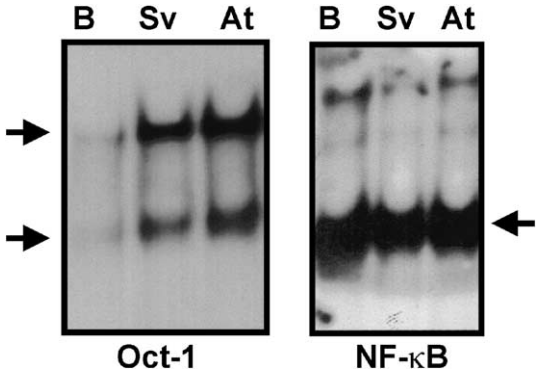


Fig. 3. Atorvastatin and simvastatin induce Oct-1 activation, but neither modulates NF-κB in unstimulated cells. Cells were incubated for 18 h with medium alone (B), $10^{-7}$ M simvastatin (Sv) or $10^{-7}$ M atorvastatin (At). EMSA were performed with an Oct-1 and a NF-κB consensus oligonucleotide. The figure shows an experiment representative of three.

with atorvastatin and simvastatin ($10^{-7}$ M) for different times (3, 6 and 24 h). In control cells, there was basal Oct-1 binding activity. Simvastatin and atorvastatin induced an increase in Oct-1 DNA binding activity from 3 to 24 h with the appearance of two main specific retarded complexes. Simvastatin $10^{-7}$ M induced an increase at 6 h (3 ± 1.7-fold; $p < 0.01$) and at 18 h (2 ± 1.3-fold; $p < 0.05$) in the upper band (Fig. 1A and C). Atorvastatin $10^{-7}$ M induced a similar increase at 6 and 18 h (2.3 ± 1.5-fold, ns; at 18 h, 1.8 ± 0.8-fold; $p < 0.05$). Different concentrations of both statins were tested. Both simvastatin and atorvastatin induced an increase of Oct-1 DNA binding activity at all doses tested at 18 h (Fig. 1A and B).

The complexes proved to be specific since they disappeared when the nuclear extracts were preincubated with a 100 M excess of cold Oct-1 oligonucleotide, but were not affected by preincubation with the same molar excess of cold transcription factor IID (TFIID) oligonucleotide. Some reduction of the intensity in the lower band was achieved when a 100-fold excess of cold activator protein (AP-1) oligonucleotide was included in the reaction (Fig. 2). These results are in accordance with previous data, where Fos and Jun proteins can be associated with Oct proteins (De Grazia et al., 1994). No changes in the activation of other nuclear factors such as NF-κB (Fig. 3) or AP-1 (not shown) were observed.

### 3.2. Oct-1 and Oct-2 are the factors induced in mononuclear cells by statins

We next determined the composition of the retarded bands by preincubation of the nuclear extracts with different antibodies. Cell preincubation with 1 μg anti-Oct-1 antibody elicited the disappearance of the upper band, while the lower band remained unaltered. Anti-Oct-2 antibody caused the disappearance of the lower complex, without affecting the upper one (Fig. 4). These results indicate that the Oct-1 protein is present in the upper complex while Oct-2 is a part

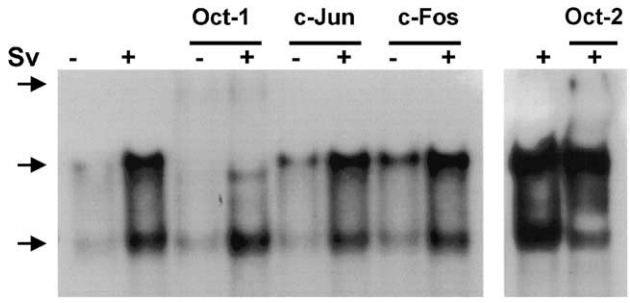


Fig. 4. Characterization of the retarded complexes. EMSA were performed with the nuclear extracts of cells stimulated for 18 h with $10^{-7}$ M simvastatin (Sv) (+) or medium alone ( − ). Antibodies were added to the reaction 1 h prior to addition of the labeled oligonucleotide.

of the lower band. These results are in accordance with similar observations of other authors (Pevzner et al., 2000).

Since we had noticed that an excess of AP-1 oligonucleotide induced the reduction of the intensity of the lower complex, we explored whether Fos and Jun proteins could be associated with Oct-2. Preincubation of the nuclear extracts with the anti-c-Jun antibody, but not with the anti-cFos antibody, caused a reduction of band intensity (Fig. 4). These results suggest that the upper complex is composed mainly of Oct-1 protein while the lower band is formed by both Oct-2 and Jun proteins.

### *3.3. The augmentation of Oct DNA binding is, at least in part, due to the increased presence of nuclear Oct-1 and Oct-2 proteins*

To test if the increased activity of the Oct DNA binding factors could be due to an increase in the presence of Oct proteins, we performed Western blot on nuclear protein extracts to determine the presence of Oct-1 and Oct-2 in the nucleus of the treated cells. In control cells, both Oct-1 and Oct-2 proteins were present in the nucleus. Simvastatin induced a marked increase in the amount of Oct-1 and Oct-2 proteins at 18 h of incubation (Fig. 5), but lower than that seen with atorvastatin. These results indicate that the increase in the DNA binding is due, at least in part, to a higher amount of protein within the nucleus.

### *3.4. Differentiation of the mononuclear cells by incubation with PMA alters the effects of statins on Oct-1 activation*

Since it has been described that the differentiation stage of cells can modify the binding of these factors (Nakshatri et al., 1995), we studied the effect of both statins on Oct-1 activation in THP-1 cells differentiated with PMA. THP-1 cells were incubated for 48 h with $10^{-6}$ M PMA similar to that described by other authors (Schwende et al., 1996). THP-1 cells become adherent and resemble macrophage cells. Then we added the statin for another 18 h. As seen in Fig. 6, in the differentiated cells, the upper band disappeared and the intensity of the lower band was reduced. Moreover, a third

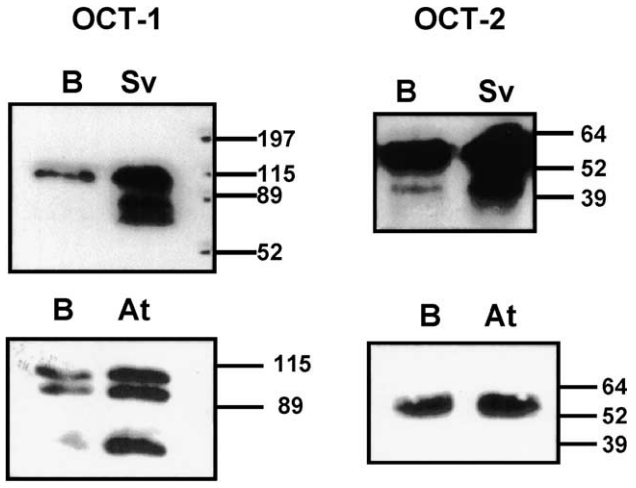


Fig. 5. Western blot analysis of the nuclear proteins. Nuclear proteins from cells incubated in medium alone (B), $10^{-7}$ M simvastatin (Sv) or $10^{-7}$ M atorvastatin (At) for 18 h were separated on a 7.5% (for Oct-1) or 12% (for Oct-2) SDS-PAGE gel, transferred to a PVDF membrane and hybridized with an anti-Oct-1 or anti-Oct-2 antibody. The molecular weights are 97–100 kDa for Oct-1 and 49.5–66 kDa for Oct-2.

lower complex weakly present in the undifferentiated cells appeared more markedly (arrowhead). The treatment with simvastatin and atorvastatin did not modify the intensity of this third complex. These results indicate that the effect of statins on mononuclear cells may differ depending on the differentiation stage, suggesting a potential difference between circulating monocytes and resident tissue macrophages.

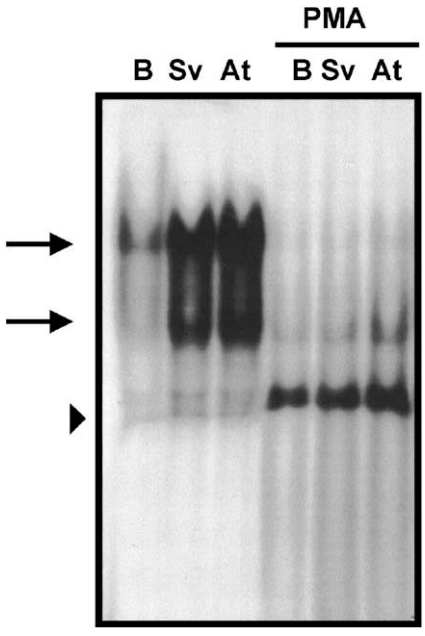


Fig. 6. Effect of PMA differentiation on Oct-1 activity. Nuclear extracts were prepared from PMA-differentiated and undifferentiated cells treated with $10^{-7}$ M simvastatin (Sv), $10^{-7}$ M atorvastatin (At) or medium alone (B) for 18 h, and EMSA was performed with an Oct-1 oligonucleotide consensus.

### 3.5. The pattern of DNA binding is different when a part of the lipoprotein lipase promoter is used as a consensus oligonucleotide

Lipoprotein lipase promoter region contains several potential binding sites for transcription factors, including three octamer sites (Nakshatri et al., 1995). We also studied the effects of statins on the binding of nuclear factors to an oligonucleotide from the promoter of the lipoprotein lipase gene containing the binding sequence of Oct-1. We found three DNA–protein complexes, the lower band was similar but more intense than that found when we used Oct-1 consensus oligonucleotide (Fig. 7). This band competed with neither a cold-specific (Oct-lipoprotein lipase) nor with unspecific oligonucleotide (Oct-1), therefore probably being an unspecific band. In control cells, we observed that Oct-lipoprotein lipase had a binding activity greater than that of the Oct-1 alone. Statins did not increase the basal binding to this oligonucleotide. The band disappeared when the competition assays were performed with both the lipoprotein lipase and the consensus oligonucleotides, but not when incubation was carried out in the presence of an excess of cold AP-1 (not shown). Moreover, preincubation of the nuclear extracts with anti-Oct-1 antibody induced a reduction in the intensity of the band and the appearance of a super-retarded complex, while anti-cFos and anti-c-Jun antibodies did not alter the results (not shown).

### 3.6. Interleukin-8 expression induced by TNF-α is reduced by statins

To test if the statins can reduce the expression of interleukin-8, one of the genes containing OCT-1 sites, we

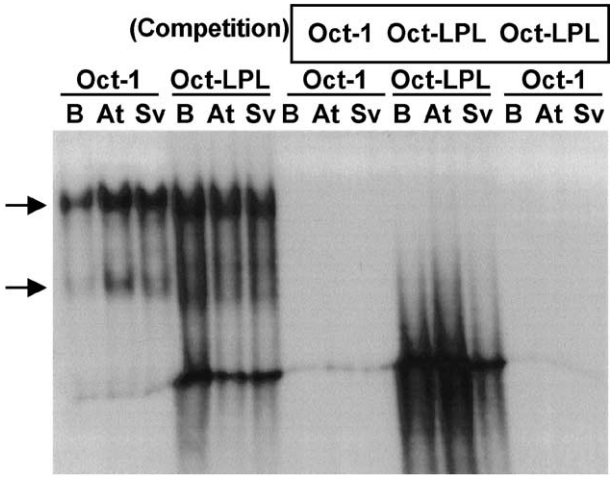


Fig. 7. Effect of statins on the Oct-lipoprotein lipase promoter activity. Nuclear proteins from cells treated with medium alone (B), $10^{-7}$ M simvastatin (Sv) or $10^{-7}$ M atorvastatin (At) were used for EMSA with an Oct-1 oligonucleotide consensus (Oct-1) and an Oct-containing oligonucleotide from the promoter of the lipoprotein lipase (Oct-LPL). Competition assays were performed by preincubation of the extracts with a 100-fold excess of the corresponding cold probe.

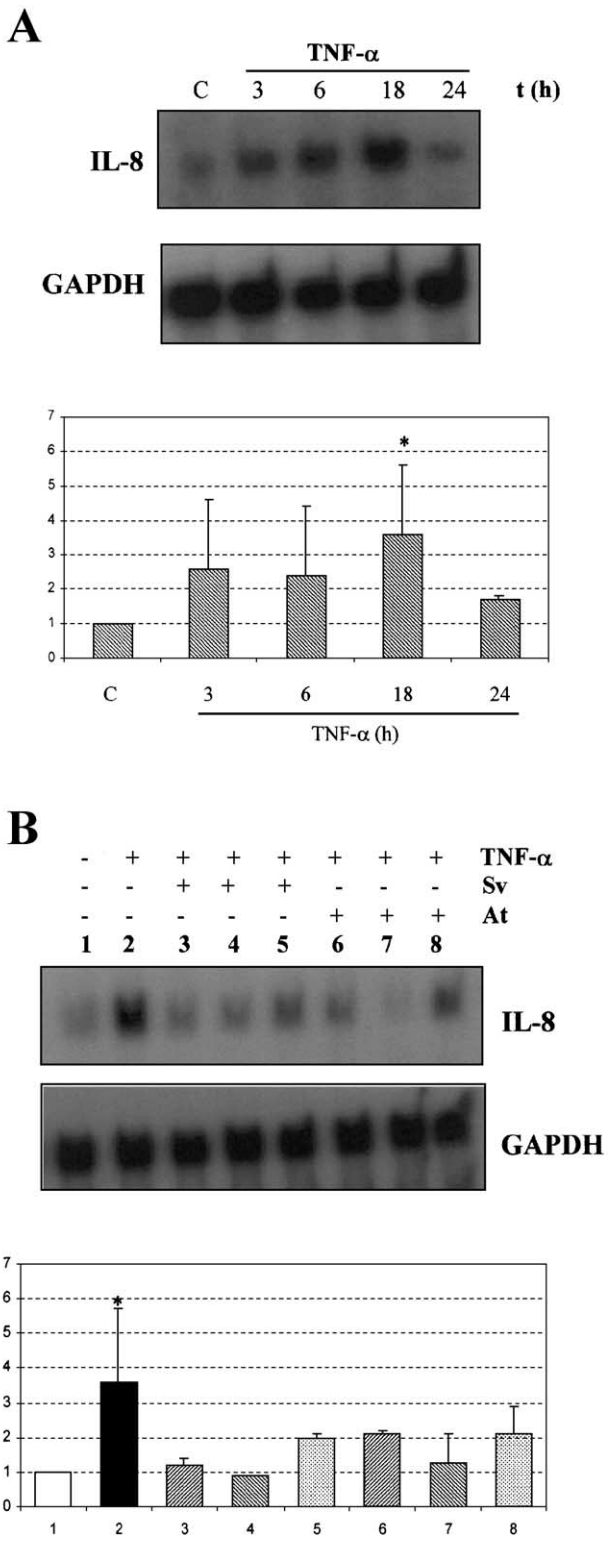


Fig. 8. Effect of statins treatment on interleukin-8 mRNA expression. Cells were incubated 18 or 24 h with TNF-α (100 U/ml) and the analysis of mRNA was performed by RT-PCR. (A) Time course of interleukin-8 induction by TNF-α (3, 6, 18 and 24 h). (B) Effect of statins preincubation. Different doses were tested: (3) simvastatin (Sv) $10^{-5}$ M; (4) simvastatin $10^{-6}$ M; (5) simvastatin $10^{-7}$ M; (6) atorvastatin (At) $10^{-5}$ M; (7) atorvastatin $10^{-6}$ M; (8) atorvastatin $10^{-7}$ M. Densitometric data were corrected by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Figure shows an experiment representative of three.

preincubated THP-1 cells with atorvastatin or simvastatin for 1 h, and afterwards, we added TNF-α (100 U/ml) for an additional 18 or 24 h. As can be seen in Fig. 8A, TNF-α induced an increase of interleukin-8 expression peaking at 18 h ($3.4 \pm 2$; $p < 0.05$) and diminishing at 24 h ($2.6 \pm 1.5$; $p < 0.05$). The incubation with different concentrations of statins induced a dose-dependent interleukin-8 expression inhibition. Thus, the preincubation with simvastatin reduced interleukin-8 expression at 18 h: 68% of inhibition with simvastatin $10^{-5}$ M ($1.1 \pm 0.2$); 53% of inhibition with simvastatin $10^{-7}$ M ($1.6 \pm 1.2$) and 41% with simvastatin $10^{-9}$ M ($2 \pm 0.1$) (Fig. 8B; bands and bars 3, 4 and 5) and with atorvastatin: 41% of inhibition with atorvastatin $10^{-5}$ M ($2 \pm 0.7$); 59% with atorvastatin $10^{-7}$ M ($1.4 \pm 0.7$) and 40% with atorvastatin $10^{-9}$ M ($2.1 \pm 1.1$) (Fig. 8; bands and bars 6, 7 and 8).

## 4. Discussion

In this work, we showed for the first time that the lipophilic statins, simvastatin and atorvastatin, increase Oct-1 DNA binding in mononuclear cells. We also show that the complexes that appear are formed by Oct-1, Oct-2 and Jun proteins, and that the augmented binding activity is due in part to an increase of Oct-1 and Oct-2 proteins in the nucleus. In addition, we have demonstrated that statins reduce the expression of interleukin-8 mRNA, a chemokine whose promoter contains binding sites for octamer elements. Our experiments were performed in serum-starved cells for 48 h. Though this restriction is not physiological, it has been demonstrated that Oct-1 DNA binding was reduced in mitotic cells (Segil et al., 1991). However, other authors have described that 10% serum induced a basal activity of Oct-1 binding in control cells (Wu et al., 1997). Under our conditions Oct-1 in basal cells was hardly expressed.

We have observed that the Oct transcription factor complex activity, which increased in response to statins, seems to be composed of different proteins. It has been described that Oct-2 strongly synergies with several members of the Jun family for transactivating the interleukin-2 octamer motif (De Grazia et al., 1994). Simvastatin and atorvastatin induced the binding of a complex containing Oct-1, Oct-2 and Jun to Oct-1 consensus octamer site, whose functional cooperation is probably required to fully enhance transcription.

However, we have shown that mononuclear cells PMA-differentiated to macrophages did not induce Oct-1 binding activation. A weak increase of Oct-2 complex appears with respect to the basal one, while the nonspecific complex was increased, indicating a different transcription regulation depending on the state of cell differentiation. In this sense, similar results were found earlier. Thus, in murine myeloid M1 cells, differentiated to macrophages by interleukin-6, Oct-1 binding activity was decreased, while Oct-2 binding activity was increased (Sawada et al., 1997). These authors suggest that cell differentiation resulted in differential regulation of several transcriptional activators or proteins that interact with the NF-κB/Rel, NF-interleukin-6 and [Pituitary (Pit), Octamer (Oct), developmental control protein unc86 of *Caernorhabditis elegans* (Unc)] (POU family) consensus sequences. On the other hand, in Jurkat T cells treated with PMA for 8 to 62 h, neither Oct-1 nor Oct-2 mRNA expression was increased (Bhargava et al., 1993).

Oct-1 gene expression has been implicated in cellular replication. In addition, statin treatment is known to stop the cellular cycle in $G_0$ and to diminish cellular proliferation (Guijarro et al., 1998). Oct-1 activation is essential, but not sufficient, for cellular proliferation. It has been reported that Oct-1 binding activity was decreased in mitotic cells, with phosphorylation of Oct-1 by protein kinase A (PKA) (Segil et al., 1991). Phosphorylation of Oct proteins by PKA, or a similar nuclear enzyme, inhibits its DNA-binding activity, while its dephosphorylation by calcineurin promotes its binding to the DNA, thus statin treatment probably was inducing this dephosphorylation by calcineurin (Roberts et al., 1991).

Oct-1 is also involved in the regulation of lipoprotein lipase gene transcription (Previato et al., 1991). It has been described that 1 out of 20 unrelated patients with familial combined hyperlipidemia having diminished levels of plasma lipoprotein lipase showed a regulatory mutation in the lipoprotein lipase gene promoter, coinciding with the Oct-1 binding sites (Nakshatri et al., 1995). Thus, we investigated the effect of statins on Oct-1 binding to lipoprotein lipase promoter. We noted that the two statins did not modify the complex that binds to the Oct site of the lipoprotein lipase promoter. Neither simvastatin nor atorvastatin increased the binding activity of Oct-1 to lipoprotein lipase promoter. It has been reported that recombinant proteins, Oct-1 and Oct-2, bound only weakly to the lipoprotein lipase octamer site. However, the addition of transcription factor IIB (TFIIB) enhanced 12-fold the binding of recombinant Oct-1 protein to the lipoprotein lipase octamer site (Yang et al., 1995), while in whole nuclear extracts, no Oct-1 binding activity was observed, possibly due to the presence of other nuclear factors. Our data show that there is Oct-1 binding to lipoprotein lipase octamer site in whole nuclear extracts from THP-1 cells, which does not increase with the treatment, probably due to the lack of stimulation of TFIIB DNA binding required for the increase of lipoprotein lipase.

On the other hand, the induction of interleukin-8 mRNA expression by TNF-α in THP-1 cells was reduced by statins, an effect in accordance with that previously reported by other authors for THP-1 cells treated with lovastatin or compactin (Terkeltaub et al., 1994).

Wu et al. have described the tight regulation of interleukin-8 gene transcription. Thus, in the absence of stimuli, the transcriptional activity of interleukin-8 is repressed by Oct-1 binding. In the presence of stimuli, NF-κB element, present in interleukin-8 promoter, regulates its inducible activity and AP-1 and CCAAT/enhancer-binding protein

(C/EBP) influence in both basal and inducible transcriptional activity. Our results indicate that interleukin-8 activation could be repressed by statins through Oct-1 binding activity induction.

On the whole, our results indicate that simvastatin and atorvastatin increase the activity of the transcriptional repressor Oct-1 in mononuclear cells, and could thus contribute to a decrease in the activation of these cells. These data suggest a possible novel mechanism supporting the anti-inflammatory effect of these two HMG-CoA reductase inhibitors.

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