Transcriptional Inhibition by Interleukin-6 of the Class A Macrophage Scavenger Receptor in Macrophages Derived From Human Peripheral Monocytes and the THP-1 Monocytic Cell Line

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Abstract—Expression of the class A macrophage scavenger receptor (MSR) contributes to the uptake of modified low density lipoproteins (LDL) by macrophages and transformation of these cells into lipid-laden foam cells, which characterize atherosclerosis. Many environmental factors, in particular, proinflammatory cytokines and growth factors, can exert regulatory effects on MSR expression, whereas intracellular accumulation of cholesterol itself does not influence MSR levels to any considerable extent. In the present study, by using an in vitro model, we examined whether stimulation with interleukin-6 (IL-6), an immunoregulatory, multipotential cytokine, modulates the expression and activities of the MSR in macrophages. When treated with IL-6, macrophages derived from peripheral monocytes and phorbol 12-myristate 13-acetate (PMA)–differentiated THP-1 monocytic cells showed significantly reduced uptake and/or binding of the MSR ligand, acetylated LDL. This effect was paralleled by a reduction in the expression of MSR protein and mRNA. Analysis of MSR promoter activity in THP-1 cells transfected with an MSR promoter–reporter gene construct demonstrated decreased activity of the MSR promoter in IL-6–treated THP-1 macrophages. Electrophoretic mobility gel shift assay also showed a reduction in the binding of a transcription factor to the MSR promoter AP-1/ets elements in IL-6–treated cells. Thus, exposure to IL-6 may inhibit expression of the class A MSR in differentiated macrophages at transcriptional levels. This result suggests that this cytokine may modulate foam cell formation during atherogenesis. (Arterioscler Thromb Vasc Biol. 1999;19:1872-1880.)

Key Words: scavenger receptors n atherosclerosis n cytokines n foam cells n lipoproteins

Atherosclerosis is characterized by the intimal accumulation of lipids, mainly cholesterol and cholesterol esters, and the infiltration of inflammatory cells, particularly macrophages and T cells, in addition to migration and proliferation of medial smooth muscle cells. The macrophage scavenger receptor (MSR) pathway plays a major role in the internalization of chemically modified lipoproteins, such as oxidized and acetylated (AcLDL) LDL by macrophages, leading to the transformation of macrophages into lipid-laden foam cells.

Expression of the class A MSR is limited to tissue macrophages and related cell types.1–3 Peripheral monocytes express the class A MSR at low levels.4,5 However, on penetrating the arterial intima and differentiating into macrophages, MSR expression dramatically increases.4,6,7 Many environmental factors that influence the development and differentiation of monocytes/macrophages can regulate expression of the class A MSR,8,9 even though, in contrast to native-LDL receptors, class A MSR expression is not affected by intracellular cholesterol accumulation. It has been reported that proinflammatory cytokines produced by activated immune cells (eg, T cells and macrophages) can modulate MSR expression. For example, macrophage colony-stimulating factor enhances,9 but transforming growth factor-β1 (TGF-β1), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) inhibit, MSR expression.10–13 The molecular mechanisms underlying cytokine regulation of MSR expression remain largely unclear. Several lines of evidence indicate that those cytokines may exert their regulatory effects at transcriptional levels of the MSR.13,14 Recent studies of the MSR gene promoter have documented that at least 2 transcription factor families, AP-1/ets and Pu1, may be critical for MSR transcription.15

It is known that interleukin-6 (IL-6), a multifunctional cytokine produced by immune cells as well as smooth muscle cells,16,17 can regulate the development and differentiation of...
monocytes/macrophages in vivo\textsuperscript{18-20} as well as in vitro.\textsuperscript{21} IL-6 has been found to serve as a survival factor for certain lymphoma and hybridoma cells, and therefore it contributes to the synthesis of immunoglobulins.\textsuperscript{19,22,23} Challenged by antigens and proinflammatory factors, immune cells can elaborate substantial amounts of IL-6, which in turn promotes production of several important acute-phase proteins. Although IL-6 exerts a regulatory effect on monomyeloid cell differentiation and acute-phase protein production,\textsuperscript{19,21} little is known about whether IL-6 can modulate expression of the MSR.

Atherosclerotic lesions with features of chronic inflammation are comprised of cells that are able to produce IL-6 and the MSR, respectively.\textsuperscript{24-27} It is important to examine whether IL-6 influences the macrophage-scavenging function, as do other cytokines such as IFN-γ,\textsuperscript{12} TGF-β,\textsuperscript{10} and TNF-α.\textsuperscript{13} In this study, we employed an in vitro model to address (1) whether treatment with IL-6 affects MSR activity; (2) whether IL-6 regulates MSR gene expression; and (3) which signaling transduction pathway or transcription factor is involved in IL-6-mediated regulation of MSR expression.

**Methods**

**Reagents**

Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma Chemical Co and dissolved in dimethyl sulfoxide in a stock concentration of 200 μmol/L. 1,1\textsuperscript{-}Dioctadecyl-3,3,3,3\textsuperscript{-}tetrachloroacarbocyanine perchlorate (DiI) AcLDL, Dil-LDL, native LDL, and AcLDL were purchased from PerkinElmer, Inc. Recombinant IL-6 was obtained from R&D Systems. Actinomycin D was purchased from Gibco-BRL. Human monoclonal antibody against MSR I (MH1) was prepared in Dr Kodama’s laboratory, University of Tokyo, Tokyo, Japan.\textsuperscript{28} Antibodies against AP-I/Jun B and AP-1/c-Jun were obtained from Santa Cruz Laboratories.

**Cell Culture and Cytokine Treatment**

The human monocytic leukemia cell line THP-1 was obtained from the American Type Culture Collection (Manassas, Va) and maintained in RPMI-1640 (GIBCO-BRL) medium supplemented with heat-inactivated 10% FBS (MultiSer), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine at 37°C under 5% CO\textsubscript{2}. THP-1 cells were treated with or without PMA (200 nmol/L) or a combination of PMA and IL-6 by the acid guanidinium thiocyanate–phenol-chloroform extraction method.\textsuperscript{29} The MSR I–specific DNA fragment (278 bp, nucleotides 1106 to 1384 in the human MSR cDNA sequence) and the MSR II–specific DNA fragment (229 bp, nucleotides 1433 to 1662 of the human MSR cDNA sequence) were amplified by the polymerase chain reaction (PCR, Perkin Elmer) from the human MSR cDNA clone.\textsuperscript{30} These fragments were subcloned into a pCR II vector (Invitrogen) and confirmed by sequencing. These plasmids containing MSR I– and MSR II–specific DNA fragments were linearized, treated with proteinase K, and extracted with phenol-chloroform–isoamyl alcohol (25:24:1, vol/vol/vol) to remove RNase activity. The riboprobes were prepared by in vitro transcription with the linearized plasmid constructs [α\textsuperscript{32}P]UTP (NEN Research Products) and T7 or SP6 RNA polymerase with a Riboprobe Gemini kit (Promega). The labeled transcripts were treated with DNase I and purified by extraction with phenol-chloroform–isoamyl alcohol. The RNase protection assay was performed with an RPA II kit (Ambion). Levels of mRNA were quantified by estimation of the corresponding band with a BAS-2000 densitometer (Fuji Film). The intensity of the bands was normalized with respect to the radioactivities of \textsuperscript{32}P-labeled uridines in the protected fragments. All experiments were carried out in triplicate, and the GAPDH mRNA level was used as an internal standard. To evaluate the half-life (t\textsubscript{1/2}) of MSR mRNA, THP-1 cells pretreated with 200 nmol/L PMA for 48 hours were washed twice with PBS, and actinomycin D was added to the cultures at 5 μg/mL in the presence or absence of IL-6 (20 ng/mL). Total RNA was isolated at multiple time points (0, 1, 2, 4, 8, and 16 hours) and analyzed for MSR mRNA. For analysis of MSR mRNA in cultured human peripheral monocytes treated with or without IL-6 (20 ng/mL), a reverse transcription (RT)–PCR assay was performed with a set of primers for MSR types I and II as reported previously.\textsuperscript{4,5} As the internal control, β-actin mRNA was assessed with a set of specific primers for the actin cDNA. The resulting PCR products were analyzed by electrophoresis on 2% agarose gels.

**Isolation and Treatment of Monocytes From Peripheral Blood**

Mononuclear cells were isolated from peripheral blood by Ficoll-Paque centrifugation (Sigma) and suspended in RPMI-1640 medium supplemented with 10% FBS and 10% pooled human serum (Sigma). Monocytes were enriched by adherence during a 1-hour incubation at 37°C in 90-mm polystyrene culture dishes. Nonadherent lymphocytes were removed by washing the dishes 3 times with PBS. Adherent monocytes were gently collected by scraping the dishes with a rubber policeman and cultured in 6-well culture plates with a set of primers for MSR types I and II as reported previously.4,5 As the internal control, β-actin mRNA was assessed with a set of specific primers for the actin cDNA. The resulting PCR products were analyzed by electrophoresis on 2% agarose gels.
fragments from our previous preparation were generated as previously described. THP-1 cells were cotransfected with luciferase constructs and the chloramphenicol acetyltransferase (CAT)–control plasmid (as an internal control) by electroporation. In brief, THP-1 cells at 5 × 10⁶/mL were plated 16 to 24 hours before transfection. Electroporation was then carried out at room temperature under 960 μF and 350 V in 700 μL of the reaction mixture containing 2 × 10⁶ cells, 30 μg of the indicated luciferase construct, and 10 μg of CAT–control plasmid DNA in a 0.4-cm electroporation cuvette (Bio-Rad). After electroporation, the cuvette was placed on ice for 10 minutes. Transfected cells were divided into 3 dishes containing 5 mL of RPMI-1640 medium and incubated at 37°C under 5% CO₂. The cells were treated with or without PMA in the presence or absence of IL-6 and then harvested 48 hours after treatment for the luciferase and CAT assays. Relative luciferase activity was determined in 20 μg of total protein of cell extracts. All experiments were carried out in triplicate.

**Electrophoretic Mobility Gel Shift Assay**

Cell extracts were prepared from THP-1 cells treated with or without PMA in the presence or absence of IL-6. THP-1 cells were collected by centrifugation, washed twice with cold PBS, and then suspended in 400 μL of cold lysis buffer A (10 mmol/L HEPES, pH 7.9; 10 mmol/L KCl; 0.1 mmol/L EDTA; 0.1 mmol/L EGTA; 1 mmol/L DTT; and 1 mmol/L PMSF). After incubation for 15 minutes on ice, 25 μL of 10% NP-40 was added and the mixture was vortexed for 15 seconds. These cell lysates were collected by centrifugation at 10 000 g for 10 minutes at 4°C. The cell nuclei were resuspended in 50 μL of cold buffer C (20 mmol/L HEPES, pH 7.9; 0.42 mmol/L NaCl; 1 mmol/L EDTA; 1 mmol/L EGTA; 1 mmol/L DTT; and 1 mmol/L PMSF) and rotated for 30 minutes at 4°C. After the cell debris was removed by centrifugation, the supernatants were collected. The concentrations of nuclear proteins were determined by a debris was removed by centrifugation, the supernatants were collected. The concentrations of nuclear proteins were determined by a protein assay (Bio-Rad). After electroporation, the cuvette was placed on ice for 10 minutes. Transfected cells were divided into 3 dishes containing 5 mL of RPMI-1640 medium and incubated at 37°C under 5% CO₂.

**Statistical Analysis**

Data were analyzed by 1-way ANOVA, followed by the assessment of differences by Duncan’s multiple range tests. Significant differences were established when probability value was less than 0.05.

**Results**

**IL-6 Inhibits Uptake and Binding of DiI-AcLDL**

The MSR ligand uptake and binding activities were examined by flow cytometry of human THP-1 monocytic cells exposed to DiI-AcLDL. After incubation with DiI-AcLDL under baseline conditions, we observed no significant fluorescent signals for DiI-AcLDL in the cells (Figure 1). However, treatment with PMA induced differentiation of THP-1 cells into macrophages, which was accompanied by a marked increase in the cellular fluorescent intensity of DiI-AcLDL, indicating enhanced uptake of the MSR ligand by the cells. The DiI-AcLDL uptake was receptor mediated because it could be blocked by incubation with excess amounts of unlabeled AcLDL but not native LDL in the PMA-differentiated THP-1 macrophages (Figure 1), indicating the specificity of DiI-AcLDL uptake by these cells.

To determine whether IL-6 can affect MSR activity in PMA-differentiated and undifferentiated THP-1 cells, we treated THP-1 cells with PMA in the presence of IL-6 and then evaluated DiI-AcLDL uptake by the cotreated cells with the use of a flow cytometer. We observed no DiI-AcLDL uptake by undifferentiated THP-1 cells treated with IL-6 alone. However, in PMA-treated, differentiated THP-1 macrophages, exposure to the same concentrations of IL-6 reduced the uptake of DiI-AcLDL by nearly 40% in a concentration-dependent manner (Figure 2). In contrast, no significant changes in the uptake of DiI-LDL were found in the PMA-treated THP-1 cells, and addition of IL-6 did not alter the uptake of DiI-LDL by these cells either, indicating that IL-6 had little effect on expression of the native LDL receptor (Figure 3).

To confirm the inhibitory effect of IL-6, monocytes were isolated from peripheral blood and induced to mature into macrophages in vitro. As reported previously, freshly isolated monocytes expressed lower levels of MSR activity. However, when they differentiate into macrophages, the cells exhibit a marked enhancement of their MSR activities. To examine the effects of IL-6 on MSR expression, we first examined the uptake of DiI-AcLDL by human monocyte–derived macrophages. We observed that treatment with IL-6 significantly reduced the uptake of DiI-AcLDL by the cells (Figure 4). Macrophages derived from both the THP-1 monocytic cell line and normal monocytes responded to IL-6 by internalizing less of the MSR ligand, AcLDL.

To determine whether the inhibitory effect of IL-6 on the uptake of DiI-AcLDL was the consequence of reduced AcLDL binding, an assay for evaluating DiI-AcLDL binding...
was conducted in the PMA-differentiated THP-1 cells at 4°C, because lowering the temperature can inhibit endocytosis of MSR ligands. We observed by flow cytometry that exposure to IL-6 significantly reduced the binding of DiI-AcLDL to PMA-differentiated THP-1 cells (Figure 5). IL-6 treatment appeared to inhibit both uptake and binding of DiI-AcLDL in the PMA-differentiated THP-1 macrophages.

**IL-6 Inhibits Expression of MSR Protein and mRNA**

We further examined whether IL-6 treatment inhibits the expression of MSR proteins and mRNA. Immunoblotting...
assay showed little or no MSR protein in PMA-untreated THP-1 monocytes (Figure 6). However, in PMA-differentiated THP-1 macrophages, there was a marked increase in expression of the MSR proteins (Figure 6). Treatment with IL-6 clearly reduced the density of the MSR protein bands (Figure 6), indicating the suppression of MSR protein by IL-6 treatment in the cells.

Examination of MSR mRNA levels by an RNase protection assay demonstrated that stimulation with IL-6 also reduced the levels of both MSR I and II mRNAs in the PMA-differentiated THP-1 macrophages (Figure 7). The levels of MSR I and II mRNAs were simultaneously reduced by 50% to 60% by addition of IL-6 (Figure 7), indicating that IL-6 might decrease the expression of the MSR at the transcriptional level. The inhibitory effect of IL-6 was independent of PMA, because 14 hours after PMA withdrawal, the cells could respond to IL-6 stimulation by a reduction in MSR transcripts (data not shown). In contrast to undifferentiated THP-1 cells, freshly isolated peripheral monocytes expressed lower but significant levels of MSR type II mRNA as determined by RT-PCR (Figure 8). However, similar to differentiated THP-1 cells, monocyte-derived macrophages produced MSR type I as well as type II mRNA, and IL-6 treatment also inhibited expression of both MSR mRNA isoforms in the normal monocyte-derived macrophages (Figure 8). We evaluated the $t_{1/2}$ of MSR mRNA in the cells treated with the transcription inhibitor actinomycin D. The MSR I mRNA $t_{1/2}$ was estimated to be 16.4 ± 3.8 hours in the cells treated with actinomycin D and 15.8 ± 4.5 hours with actinomycin D plus IL-6. Similarly, the MSR II mRNA $t_{1/2}$ was 14.5 ± 2.2 hours with actinomycin D and 14.6 ± 4.6 hours with actinomycin D plus IL-6. No difference in the $t_{1/2}$ of both MSR I and II transcripts was observed between IL-6–treated and untreated THP-1 cells in the presence of actinomycin D, indicating that IL-6 has little effect on posttranscriptional modulation of MSR mRNA stability.

**Figure 6.** Inhibitory effect of IL-6 on MSR protein expression in PMA-differentiated THP-1 macrophages. Total proteins isolated from THP-1 cells treated with or without PMA (200 nmol/L) in the presence or absence of IL-6 (20 ng/mL) for 48 hours were separated by electrophoresis on 7.5% SDS-PAGE gels. After electrophoresis, the proteins were transferred onto membranes, blocked, and incubated with a human monoclonal antibody to the MSR. Peroxidase-conjugated anti-mouse IgG was used as a second antibody. The blots were developed with an enhanced chemiluminescence kit from Amersham. Lane 1, untreated THP-1 cells; 2, THP-1 cells treated with PMA; and 3, THP-1 cells treated with PMA and IL-6.

**Figure 7.** Inhibition by IL-6 of both MSR I and MSR II mRNAs in PMA-differentiated THP-1 macrophages. Total RNA isolated from THP-1 cells treated with or without PMA (200 nmol/L) in the presence or absence of IL-6 (20 ng/mL) for 48 hours was hybridized with MSR I and MSR II cRNA riboprobe synthesized from linearized plasmids containing the type I and II cDNA, respectively. The MSR was detected by electrophoresis on denatured gels after autoradiography. a, RNase protection assays for MSR mRNA isoforms and the GAPDH “housekeeping” control. b, Densitometry of the bands of MSR mRNA isoforms and GAPDH mRNA. The data represent mean ± SD of 3 independent experiments. Lane 1, untreated THP-1 cells; 2, THP-1 cells treated with IL-6 alone; 3, THP-1 cells treated with PMA alone; and 4, THP-1 cells treated with IL-6 and PMA. *Significantly different from IL-6–untreated THP-1 cells (P < 0.05, ANOVA).

**IL-6 Inhibits MSR Promoter Activity**

To determine whether the IL-6–induced reduction in expression of MSR mRNA in PMA-differentiated THP-1 macrophages resulted from an attenuation of MSR gene promoter activity, we analyzed the luciferase activity driven by a promoter of the MSR gene in THP-1 cells transiently transfected with MSR promoter–luciferase reporter constructs by electroporation. Two forms of the MSR-luciferase constructs were generated, which contained the sequence from −630 to −150 bp and from −10 to −150 bp, with most of the cis-elements responsible for transcription of the MSR gene (Figure 9). After transfection, luciferase activity was measured in THP-1 cells treated with or without PMA in the presence or absence of IL-6. In untreated THP-1 cells, only low luciferase activity was observed, but treatment with PMA markedly increased luciferase activity in the cells transfected with both longer and shorter forms of the luciferase reporter constructs, indicating that both sequences in the MSR promoter contain the responsive elements to PMA stimulation.
This finding is consistent with that by Moulton et al., who showed that the short promoter in the human MSR gene promoter has an AP-1/ets-like binding site. Treatment with IL-6 reduced luciferase activities in PMA-differentiated THP-1 cells transfected with either the long or short form of the MSR promoter–luciferase construct (Figure 9). Deletion of the sequence from −630 to −10 nucleotides of the MSR (long construct) reduced the luciferase activity but did not appear to abolish the inhibitory effect of IL-6 (Figure 9), suggesting that the AP-1/ets element in the short-form construct might be responsible for IL-6 inhibition of MSR transcription.

To characterize the transcription factors responsible for IL-6–induced suppression of MSR promoter activity in PMA-differentiated THP-1 macrophages, we performed the electrophoretic mobility gel shift assay with double-stranded oligonucleotides for various nuclear transcription factors. We determined by using antibodies against AP-1/c-Jun and AP-1/Jun-B that the addition of anti-c-Jun or anti-Jun-B in the reaction mixtures of nuclear proteins and the radioactive probe partially blocked the binding of PMA-induced complex to its cis-formation of the complexes between the transcription factor and the probe for the AP-1/ets domain (Figure 11). There was no supershifted band appearing in the gel shift assay, suggesting that the binding sites for the AP-1/ets probe were blocked by the antibodies, in agreement with a finding by Moulton et al. These results indicated that IL-6–mediated reduction in transcription of the MSR gene might be at least partially due to a reduction in activity of a transcription factor that interacts with the AP-1/ets cis-elements.

**Discussion**

The MSR is a membrane glycoprotein responsible for macrophage uptake of an unusually diverse array of ligands, including modified lipoproteins and bacterial lipopolysaccharides. This receptor has been shown to represent a marker for monocyte-to-macrophage differentiation. Progress in understanding the structure and expression of the MSR has provided important information on how it is involved in the pathogenesis of atherogenesis. Recent work by Suzuki et al. has shown that targeted disruption of the MSR-A gene can reduce the size of atherosclerotic lesions in mice that are deficient in both apolipoprotein E and the MSR, indicating the significance of this gene for atherogenesis.

In this study, we utilized an in vitro model to address whether IL-6, a well-defined cytokine in immune and inflammatory responses, exerts a regulatory effect on expression of the class A MSR in macrophages derived from human peripheral monocytes and the THP-1 monocytic cell line. The observations that IL-6 inhibited MSR ligand binding and uptake activities, protein expression, and mRNA transcription (Figure 10b), AP-1/ets and Pu1 were reported to be required for transcription of the MSR gene. We therefore determined the effect of IL-6 on the binding activity of AP-1/ets–like transcription factors by using a corresponding cis-element of AP-1/ets in the human MSR promoter. We observed little AP-1/ets–like activity in untreated, MSR-negative, THP-1 cells. During PMA-induced differentiation of THP-1 cells into macrophages, the activity of AP-1/ets–like transcription factors dramatically increased (Figure 11). However, addition of IL-6 to the cultures significantly inhibited PMA-induced AP-1/ets–like transcription factor activity (Figure 11). The specificity of the AP-1/ets–like transcription factor was determined by using antibodies against AP-1/c-Jun and AP-1/Jun-B. We observed that the addition of anti-c-Jun or anti-Jun-B in the reaction mixtures of nuclear proteins and the radioactive probe partially blocked the binding of PMA-induced complex to its cis-formation of the complexes between the transcription factor and the probe for the AP-1/ets domain (Figure 11). There was no supershifted band appearing in the gel shift assay, suggesting that the binding sites for the AP-1/ets probe were blocked by the antibodies, in agreement with a finding by Moulton et al. These results indicated that IL-6–mediated reduction in transcription of the MSR gene might be at least partially due to a reduction in activity of a transcription factor that interacts with the AP-1/ets cis-elements.
strongly suggest that IL-6 might serve as a negative regulator of MSR gene expression. Apparently, IL-6 shares several features with other cytokines in terms of downregulation of class A MSR transcription. Previous reports indicate that treatment with IFN-γ and TGF-β reduces MSR mRNA expression as well as MSR-mediated uptake and binding of AcLDL. Hsu et al reported that TNF-α decreased transcription of both the MSR and MSR mRNA steady-state levels in PMA-differentiated THP-1 macrophages. The inhibitory effects of IL-6 are similar to those of IFN-γ, TNF-α, and TGF-β on class A MSR gene expression. Such similarity indicates that activation of macrophages by these cytokines may actually lead to attenuation of MSR activities and the reduction of lipid accumulation in the cells.

This in vitro model has several limitations. For instance, data from the in vitro studies may not precisely reflect the situation observed in vivo, particularly in atherosclerotic lesions where macrophages express high levels of the MSR, despite the presence of proinflammatory cytokines. This controversial phenomenon may be explained by an imbalance between MSR-promoting and -inhibiting factors in the microenvironment. This concept of how IL-6 affects in vivo expression of the MSR deserves further study. Additionally, IL-6 treatment does not appear to completely block uptake and binding of DiI-AcLDL by PMA-differentiated THP-1 cells, even though reductions in MSR mRNA and protein are substantial. One explanation for this is that in addition to the class A MSR, other membrane proteins expressed in differentiated monocytic cells may mediate endocytosis of DiI-AcLDL as well. Recently, new type of MSR, structurally different from the class A MSR, has been cloned. Macrophages from mice lacking the class A MSR also maintain to a certain degree the capacity to take up and bind AcLDL. Future studies are needed to clarify whether IL-6 stimulation affects expression of other forms of the MSR. Nonetheless, the reduced AcLDL uptake and binding in IL-6-treated cells with decreased expression of the class A MSR mRNA and protein point to a role for IL-6 in the regulation of MSR-
mediated degradation of chemically modified lipoproteins in macrophages.

Our current observation indicates that IL-6 inhibited MSR promoter activity by reducing the binding of the AP-1/ets-like transcription factor to the MSR promoter. Comparison of sequences upstream from the 5' ends of bovine, human, and murine MSR genes shows a high similarity in the 300 bp proximal to the start site of transcription, but it does not appear to have a popular TATA box in the region ∼30 bp upstream from the transcription start site.30 Moulton et al.32 have shown that AP-1/ets and Pu1 elements located in the MSR promoter regions are critical for expression of the class A MSR gene in PMA-differentiated THP-1 cells. Our data indicate that new elements located from −504 to −399 bp in the human MSR gene promoter are also required for the greatest expression of the MSR gene in murine macrophages.31 These results indicate that the proximal promoter regions at <600 bp from the initial site play a critical role in the regulated expression of the MSR gene. When THP-1 cells differentiate into macrophages on exposure to PMA, the luciferase activity of both −630 to +50 bp and −10 to +50 bp constructs was markedly enhanced. The results indicate that the responsive element to PMA is located at −10 to +50 bp in the promoter region, in agreement with the findings by Wu et al.13 and Moulton et al.32 Our current data indicate that IL-6 treatment can inhibit by 30% and 40%, respectively, the promoter activities of the 2 constructs. These findings suggest that IL-6 inhibits transcription of MSR mRNA by decreasing the promoter activity of the MSR gene.

It is unclear which signal transduction pathway is involved in IL-6–mediated downregulation of MSR expression. Binding of transcription factors to cis-elements triggers transcription of many different genes, including the MSR gene. So far, no IL-6–responsible cis-elements have been identified in the human MSR gene promoter regions. Therefore, we examined whether IL-6 affects other transcription factors that may bind to the cis-elements. Examination of the binding activities of well-characterized transcription factors such as NF-κB, AP-1, AP-3, and CREB by electrophoretic mobility gel shift assays demonstrated that IL-6 increased the binding activity of NF-κB and AP-3 but decreased that of CREB, Oct-1, NF-1, and AP-1. There are several AP-1/ets cis-elements similar to the popular AP-1 cis-element in the human MSR promoter.13,32 The anti–c-Jun and Jun B antibodies have been shown to inhibit the formation of a complex between transcription factors and AP-1/ets cis-elements.32 This result is consistent with our current observation (Figure 11) that anti–c-Jun and Jun-B antibodies blocked the binding of a nuclear transcription factor to the AP-1/ets probe. Our observation that IL-6 inhibited the activity of an AP-1/ets–like transcription factor(s) suggests that a transcription factor recognizing AP-1/ets elements may be at least partially responsible for the inhibitory effect of IL-6 on MSR gene transcription. This notion is also in agreement with the recent finding by Horvai et al.,14 who showed that IFN-γ inhibits transcription of the MSR gene by antagonizing the Ras-dependent activities of AP-1 and cooperating ets domain transcription factors, apparently as a result of competition between AP-1/ets factors and activated STAT (signal transducers and activators of transcription)-1, leading to a limitation in the amounts of CREB binding protein and p300.

MSR-mediated lipid loading into macrophages and transformation of these cells into foam cells represent a critical event during the pathogenesis of atherosclerosis. The results of our current study shed new insight on the biological function of IL-6, pointing to complex interactions between lipid-carrying proteins, inflammatory cells, and vascular cells. The MSR is expressed predominantly by macrophages, and IL-6 is produced by smooth muscle cells and macrophages in atherosclerotic lesions. Locally produced IL-6, together with other cytokines such as IFN-γ, TNF-α, and TGF-β1, may exert regulatory effects on the formation of lipid-laden foam cells during the development of atherogenesis.

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**References**


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