Human mesangial cells express inducible macrophage scavenger receptor

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**Background.** Type A scavenger receptors (Scr) mediate the uptake of modified low-density lipoproteins by macrophages. The accumulation of lipids via this process is thought to lead to foam cell formation in atherosclerotic plaques. Human mesangial cells (HMCs) have not been previously shown to express Scr in normal culture. We therefore investigated whether there is an inducible form of Scr in a human mesangial cell line (HMCL).

**Methods.** Scr activity was analyzed by cellular uptake of fluorescently labeled acetylated low-density lipoprotein using a flow cytometer. Scr mRNA expression was examined using reverse transcription-polymerase chain reaction, followed by Southern blotting. To investigate the molecular mechanism of Scr expression, several reporter gene constructs were designed. The first contained a full Scr promoter, the second a part of the Scr promoter that has both AP-1 and ets transcription factor binding sites. Other constructs were identical to the second, except that they contained either AP-1 or ets motif mutations.

**Results.** Phorbol 12-Myristate 13-acetate (PMA) and angiotensin II (Ang II) increased both the percentage of Scr-positive cells and the Scr mean fluorescence intensity. PMA and Ang II also increased Scr mRNA and promoter activity in a time- and dose-responsive manner. Protein kinase C and calmodulin transduction pathways were involved in Scr up-regulation induced by PMA and Ang II. Additionally, a serine/threonine kinase was involved in PMA stimulation. Functional analysis showed that both AP-1 and ets motifs were specific response elements to PMA stimulation in HMCLs.

**Conclusions.** This study suggests that HMCs may express an inducible Scr, by which cells can acquire lipids and convert to foam cells in developing glomerulosclerosis.

Scavenger receptors are integral membrane proteins that bind and internalize a broad array of ligands, including acetylated low-density lipoprotein (Ac-LDL) and oxidized low-density lipoprotein (Ox-LDL) [1–3]. Two types of scavenger receptors have been identified. The first of these to be purified and cloned was the type A scavenger receptor (Scr) [4]. Subsequently, the CD36 cell surface protein family was shown to bind Ox-LDL and was defined as type B scavenger receptors [5]. Unlike the LDL receptor, the activity of Scr is not suppressed by rising intracellular cholesterol concentration, thus providing a mechanism for unregulated cholesterol uptake. Two isoforms of the Scr (type A-I and A-II) represent late gene products that are highly restricted to macrophages and that are related cell types in their pattern of expression [6]. A single gene that gives rise to an alternatively spliced primary transcript encodes these two forms of the Scr [7, 8]. Although their normal physiological role remains uncertain, biochemical studies have demonstrated that both isoforms of Scr are capable of binding and internalizing Ac-LDL and Ox-LDL [7, 9]. Nonmacrophage cells transfected with cDNAs encoding Scr accumulate cholesterol and develop foam cell characteristics when incubated with modified LDL, whereas mock-transfected cells do not [9]. These findings suggest that the expression of Scr protein might be both necessary and sufficient to account for foam cell formation in vivo. On the basis of their ability to internalize and degrade Ox-LDL, Scr also appears to provide a mechanism for the development of cholesterol-engorged mesangial cells.

Previous studies characterizing the expression of Scr on human mesangial cells (HMCs) have been conflicting. *In vitro*, Gröne et al reported that Ac-LDL and Ox-LDL were not taken up specifically, suggesting no active Scr in cultures of HMCs [10]. Lee and Koh also reported that there was very low Ox-LDL binding and internalizing activity in normal cultures of HMCs compared with rat mesangial cells [11]. However, Takemura et al demonstrated Scr on the membranes of mesangial cells of renal biopsy tissues from patients with several types of glomerular diseases and that the Scr expression was increased in glomeruli with marked mesangial proliferation [12].

**Key words:** glomerulosclerosis, gene transcription regulation, foam cells, lipids, atherosclerotic plaque.

Received for publication June 23, 1998 and in revised form February 22, 1999
Accepted for publication March 1, 1999
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One explanation for these discrepancies is that growth conditions in culture led to alterations in Scr gene expression that would not occur in vivo. Accordingly, there may be inducible expression of Scr in HMCs.

Phorbol 12-myristate 13-acetate (PMA), which activates the protein kinase C (PKC) pathway, induces differentiation of human THP-1 monocyte cells into macrophages, and both Scr activity and Scr mRNA appear to be up-regulated during this transformation [13]. AP-1 and ets (AP-1/ets) transcription factors are necessary for Scr induction by PMA [14]. Angiotensin II (Ang II) is known to activate PKC in HMCs and has been shown to possess atherogenic properties [15, 16]. It increases the activity of the macrophage Scr, enhances macrophage lipid peroxidation both in vivo and in vitro, and also binds to LDL, forming modified LDL, which is taken up by macrophage Scr at an enhanced rate [17]. These studies suggest that Ang II may have an additional effect in the development of atherosclerosis, which is independent of its hypertensive action. As HMCs exhibit macrophage-like function, this study was undertaken to determine whether PMA and Ang II could induce Scr expression in HMCs, and to investigate the role of AP-1/ets transcription factors in Scr expression.

**METHODS**

**Cell culture**

An established stable HMC line (HMCL) was used in all experiments (kindly donated by Dr. J.D. Sraer, Hôpital Tenon, Paris, France). HMCs were immortalized by transfection with T-SV40 and H-ras oncogene. It retains many morphological and physiological features of normal HMCs [18, 19]. HMCLs were cultured in growth medium containing RPMI, 5% fetal calf serum (FCS), 2 mmol/liter glutamine, 10^5 unit/liter penicillin, 0.1 g/liter streptomycin, 2.5 × 10^5 g/liter amphotericin, 5 × 10^-3 g/liter insulin, 5 × 10^-3 g/liter human transferrin, and 5 × 10^-6 g/liter sodium selenite. Experiments were carried out in serum-free RPMI medium containing 0.2% bovine serum albumin (BSA; Sigma, Poole, Dorset, UK). All reagents for cell culture were obtained from GIBCO BRL (Paisley, UK).

**Preparation of acetylated lipoprotein**

Plasma was collected from healthy human volunteers. LDL was isolated by sequential ultracentrifugation [20] and was acetylated (Ac-LDL) by the method as described by Innerarity, Pitas and Mahley [21]. Ac-LDL was passed through a 0.2 μm filter before use and was not used beyond 10 days. Ac-LDL labeled with 1,1'-dioctadecyl-3,3',3',3'-tetramethylindocarbocyanine perchlorate (DiI-Ac-LDL) was obtained from Biogenesis (Poole, UK).

**Cell labeling for flow cytometry**

The use of DiI-Ac-LDL as a fluorescent probe to detect Scr protein expression is an established method in studies of lipoprotein–receptor interactions and atherosclerosis [22, 23]. HMCLs were cultured in serum-free medium containing 16 nmol/liter PMA or 1 μmol/liter Ang II for various times before the addition of 10 μg/ml of DiI-Ac-LDL in the presence or absence of a 100-fold excess of unlabeled Ac-LDL at 37°C. After three hours, the cells were detached from the plates by incubation with 0.5% trypsin-ethylenediaminetetraacetic acid (EDTA) and were fixed in 5% formalin solution in phosphate-buffered saline (PBS). Each experiment was carried out in duplicate.

**Flow cytometry analysis**

The fixed cells were washed three times in PBS and were analyzed by fluorescence-activated cell sorter analysis (FACS) using a flow cytometer (EPICS XL-MCL; Coulter, Hialeah, FL, USA). Forward angle and 90° light-scatter gates were established to exclude dead cells and cell debris from analysis. Fluorescence signals from the accumulated DiI in the cells were collected at 555 to 600 nm by a photomultiplier, converted to digital format, and processed for storage and display in one-parameter, log scale frequency histograms. Five thousand cells were analyzed in each sample. The data were evaluated by two parameters of the histograms: the percentages of DiI-labeled positive cells and the mean fluorescence intensity (MFI). Autofluorescence signals from unlabeled cells were used as negative controls in each experiment. The percentage of DiI-labeled positive cells was calculated by counting labeled cells that exceeded the upper limit of the autofluorescence of unlabeled cells. The MFI of DiI-labeled cells was calculated by subtracting the autofluorescence intensity from the observed MFI of labeled cells. The average of the duplicate determination was used for statistical analyses.

**Establishment of a stable cell line expressing a high level of Scr**

Human mesangial cell lines were transfected with expression vector pXhSR1, which contains cDNA encoding Scr (kindly donated by Dr. Kodama, University of Tokyo, Tokyo, Japan) using SuperFect Reagent (Qiagen, Crawley, West Sussex, UK). Two days after transfection, G418 was added to a final concentration (400 μg/ml), and resistant cells were selected over two weeks. Transfected cells (HMCL-Scr) expressing high levels of Scr activity were detected using reverse transcription-polymerase chain reaction (RT-PCR) and FACS. HMCL-Scr were subjected to morphological studies.

**Morphological examination**

Human mesangial cell lines or HMCL-Scr were plated in chamber slides for tissue culture (Nunc Inc., Naper-
ville, IL, USA) and incubated in serum-free RPMI medium or serum-free medium in the presence of 16 mmol/liter PMA or 1 μmol/liter Ang II. After 72 hours of incubation, the cells were further incubated with 50 μg/ml Ac-LDL for 48 hours at 37°C. The cells were washed three times with PBS, fixed for 30 minutes with 5% formalin solution in PBS, stained with Oil Red O for 30 minutes, and counterstained with hematoxylin for another 5 minutes. Finally, the cells were examined by light microscopy. For fluorescence microscopy, both HMCLs and primary cultures of HMCs treated with 16 mmol/liter PMA for 72 hours were incubated for 14 hours with 1 μmol/liter of colchicine to synchronize populations and then cultured for 5 hours with 10 μg/ml of Dil-Ac-LDL at 37°C. The cells were then washed in PBS and fixed in 5% formalin solution in PBS. Slides were examined on a Leitz fluorescence microscope.

**Reverse transcription-polymerase chain reaction**

Total RNA (500 ng) was used as a template for RT-PCR. All reagents were obtained from Perkin-Elmer (PE Applied Biosystems Ltd., Warrington, Cheshire, UK). The RT reaction was set up in a 20 μl mixture containing 50 mmol/liter KCl, 10 mmol/liter Tris/HCl, 5 mmol/liter MgCl₂, 1 mmol/liter of each dNTPs, 2.5 μmol/liter random hexamers, 20 U RNAsin, and 50 U of M-MLV reverse transcriptase. Incubations were performed in a DNA Thermal Cycler (Perkin-Elmer 9600) for 10 minutes at room temperature, followed by 30 minutes at 42°C and 5 minutes at 99°C. After cDNA synthesis by RT, the incubation mixture was split into two 10 μl aliquots for separate amplification of the Scr cDNA and the GAPDH cDNA using specific primers: Ser 5’ primer (nucleotide position 75 to 94), TCGCTCAATGACAGCTTTGC; 3’ primer (nucleotide position 345 to 364), CCATGTTGCACTCATGTTGCC; GAPDH, 5’ primer (nucleotide position 73-92) TCATAGACAGATGGTGGAAG; 3’ primer (nucleotide position 303-327), TGACCGGAGTC TCGCTCCTGGGAAGAT [24]. For PCR, the final concentrations of the 50 μl PCR reaction mixture were 50 mmol/liter KCl, 10 mmol/liter Tris/HCl, 2 mmol/liter

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**Fig. 1. Scavenger receptor (Scr) promoter and reporter gene constructs used in this study.**

(A) The Scr 5’ upstream region. (B) The nucleotide sequences of the Scr promoter AP-1 and ets motifs. (C and D) Corresponding sequences containing mutations in the AP-1 or ets. (E) Reporter gene construct (ESL) containing the full Scr promoter (−696 to +46) and luciferase gene. (F) A minimal reporter gene construct of the minimal prolactin gene promoter and luciferase gene. (G) Reporter gene constructs that contain minimal reporter gene plus one or three copies of AP-1 or ets motifs with or without mutations.
probed with $[\gamma^{32}\text{P}]$ATP (3000 Ci/mmol; Amersham, Little Chalfont, Buckinghamshire, UK)-labeled oligonucleotides: Scr probe (nucleotide position 128-157), GTGCA GCTTGAAGGACTTCTCTCTCT; GAPDH probe (nucleotide position 170-191), AATGAAGGGG TCGTTGATGGCA [24]. The DNA probes were labeled using the 5'-end labeling system (Promega, Southampton, Hampshire, UK). Membranes were irradiated by ultraviolet stratalinker for three minutes, prehybridized in 50% formamide, 5X Denhardt's solution, 5X SSPE, 100 mg/ml herring sperm (hs) DNA, 50 mmol/liter sodium phosphate (pH 6.8) at 42°C for four hours, and hybridized overnight at 42°C with at least $1 \times 10^6$ cpm/ml of the labeled probe in the same solution as for prehybridization but without Denhardt's reagents. The membranes were washed twice for 15 minutes at room temperature with 5X SSC and then washed for 10 minutes at 42°C with 5X SSC. The blots were exposed to x-ray films (Kodak, Rochester, NY, USA) for four hours. The relative radiation density (the ratio of Scr to GAPDH) was calculated for each sample to adjust for the differences in RNA mass between templates and was used for the quantitative comparisons.

**Plasmid constructs**

To determine the molecular mechanisms responsible for PMA and Ang II-dependent Scr transcriptional activation in HMC, the Scr promoter-luciferase fusion gene ESL was constructed. This contains Scr promoter 5' upstream (2696 to 146) from the Scr gene (Fig. 1) [14]. The 5' upstream of Scr gene (−4.5kb to +46) was found to lack a conventional TATA box and initiated transcription from a cluster of closely spaced start sites. These sequence motifs include three near consensus transcription factor AP-1 binding sites (AP-1 motifs), two of which are juxtaposed with binding sites for members of the transcription factor ets family (ets motifs) [13, 14]. To further examine the relative roles of the AP-1 and ets (AP-1/ets) motifs in mediating PMA and Ang II-dependent transcriptional activation, luciferase reporter genes were made in which either one or three copies of the AP-1/ets motifs or the corresponding sequences were introduced upstream of the TATA box of the rat prolactin gene promoter (Fig. 1) [14].

**Transient expression assay**

Human mesangial cell lines at a density of $6 \times 10^7$ cells/ml were transfected with 100 µg of supercoiled reporter genes and 100 µg control plasmid of psv-β-galactosidase (Internal standard) by electroporation at 340 V and 125 µF with a Gene Pulser (Bio-Rad, Hemel Hempstead, Herefordshire, UK). After electroporation, the cells were placed in six-well plates with growth medium. Approximately 24 hours after replating, cells were washed
with PBS, and the medium was replaced by fresh serum-free RPMI medium in the presence or absence of different concentrations of PMA or Ang II for various times. The cells were then washed twice with PBS and lysed using a lysis buffer (Promega). The luciferase and β-galactosidase enzyme activity in the supernatants were assayed using Promega luciferase and β-galactosidase assay systems.

Effect of various signal transduction inhibitors on PMA and Ang II-mediated activity of Scr promoter

Using the transient expression assay system described earlier in this article, the reporter gene ESL was transfected into HMCLs. Transfected cells were incubated for 24 hours in serum-free RPMI medium or serum-free medium with 16 nmol/liter of PMA or 1 μmol/liter of Ang II in the presence or absence of different signal transduction pathway inhibitors: 25 μmol/liter W-7 (calmodulin antagonist), 25 μmol/liter genistein (receptor tyrosine kinase inhibitor), 100 nmol/liter calphostin C (PKC inhibitor), 1 nmol/liter staurosporine (serine/threonine kinase inhibitor), and 10 ng/ml pertussis toxin (G-protein inhibitor). Scr promoter activity was estimated by measuring luciferase luminescence.

Data analysis

In all experiments, groups of data were evaluated for significance by one-way analysis of variance using Mini-tab software. Data were considered significant if the P value ≤ 0.05.

RESULTS

We assessed the binding and internalization of DiI-labeled Ac-LDL in HMCLs treated with PMA and Ang II by FACS analysis. The parameters used to evaluate data were the percentage of fluorescent-positive cells and the MFI of DiI-labeled cells. The results showed that PMA and Ang II increased the percentage of DiI-labeled positive cells in a time-dependent manner (Fig. 2A). We also investigated whether PMA and Ang II affected the density of Scr on HMCLs. The data showed that PMA and Ang II increased Scr density on HMCLs. The specificity of the analysis was confirmed by showing that excess amounts of unlabeled Ac-LDL inhibited the uptake of DiI-Ac-LDL (Fig. 3). These results suggest that the enhanced metabolism of Ac-LDL results from an increase in both the number of Scr-positive cells and Scr density in HMCLs.

Staining of HMCLs with Oil Red O before (Fig. 4A) and after PMA (Fig. 4B) and Ang II (Fig. 4C) stimulation showed that both PMA and Ang II increased the number of intracellular Oil Red O-stained lipid droplets. The stable cell line HMCL-Scr showed a stronger pattern of
Fig. 4. Visualization of acetylated low density lipoprotein (Ac-LDL) uptake and lipid droplets in human mesangial cell lines (HMCLs) after phorbol 12-myristate 13-acetate (PMA) or angiotensin II (Ang II) treatment. HMCLs were incubated for 72 hours in serum-free medium in the absence (A) or presence (B) of 16 nmol/liter PMA or 1 µmol/liter of Ang II (C). The cells (A–C) and cultured HMCL-Scr (D) were further incubated with 50 µg/ml Ac-LDL for 48 hours at 37°C and then were examined for lipid inclusions by Oil Red O staining. For fluorescence microscope examination, primary cultures of HMCs (E) and HMCLs (F) were incubated with 16 nmol/liter PMA for three days and were then treated with 1 µmol/liter of colchicine for 14 hours and were finally labeled with 10 µg/ml DiI-Ac-LDL for five hours at 37°C. The cells were fixed and examined for fluorescence. The results are typical of those observed in four experiments.

Oil Red O staining (Fig. 4D), which confirmed that Scr had a functional relevance in mesangial cells cultured in the appropriate environment. The uptake of DiI-Ac-LDL by HMCLs and primary cultures of HMCs following PMA stimulation was also visualized by fluorescence microscopy. HMCLs and primary cultures of HMCs were treated by 16 nmol/liter of PMA for 72 hours and were then arrested by incubation with colchicine, which maintains mitotic cells in metaphase by blocking the spindle apparatus production needed for the chromo-

some separation. The synchronized HMCLs and primary cultures of HMCs were used to evaluate DiL-Ac-LDL uptake. Result showed that the majority of cells displayed high fluorescence after stimulation by PMA. However, some PMA-treated cells were low fluorescent or nonfluorescent, even after synchronization with colchicine (Fig. 4 E, F). These data suggest that not all cells in this population express Scr to the same degree when stimulated by PMA and that the nonhomogeneous behavior of HMCLs with respect to DiI-Ac-LDL uptake
is not a function of the cell cycle, with temporary suspension of the cells during certain phases of the cycle. It is also excluded that heterogeneity results from different functional specificity within the cell line.

Reverse transcription-PCR, followed by Southern blotting, showed that HMCLs had a low Scr mRNA level under normal tissue culture conditions. However, HMCLs expressed an inducible form of Scr when stimulated by PMA and Ang II. PMA (16 nmol/liter) induced Scr expression in a time-dependent manner. Ang II (1 μmol/liter) also induced Scr expression (Fig. 5 A, B). Both PMA at 1.6 to 160 nmol/liter and Ang II at 1 to 1000 nmol/liter induced Scr mRNA expression in HMCLs in a dose-responsive manner (Fig. 6 A, B).

To investigate whether enhanced Scr mRNA resulted from increased gene transcription in HMCLs, we analyzed the Scr promoter activity. The reporter gene (ESL) containing the full Scr promoter was transfected into HMCLs. The transfected HMCLs were stimulated by different concentrations of PMA and Ang II at various times, and the promoter activity was measured. The results showed that PMA and Ang II induced Scr promoter activity in a time- and dose-dependent manner, which was consistent with the induction of Scr mRNA (Fig. 7 A, B).

To determine the role of signal transduction pathways in the regulation of Scr gene expression in PMA or Ang II-stimulated HMCLs, we evaluated the effects of various signal transduction pathway inhibitors on Scr transcription using the luciferase system. At nontoxic concentrations of inhibitors, we observed that the promoter activity of Scr in PMA or Ang II-treated HMCLs in the presence of PKC inhibitor (calphostin C) or calmodulin inhibitor (W-7) was significantly lower than in the absence of calphostin C or W-7, suggesting that both PKC and calmodulin pathways were involved in Scr upregulation. Additionally, a serine/threonine kinase inhib-
Fig. 6. Effect of different concentrations of phorbol 12-myristate 13-acetate (PMA) and angiotensin II (Ang II) on scavenger receptor (Scr) mRNA expression. Human mesangial cell lines (HMCLs) were incubated in serum-free medium (control) or serum-free medium containing various concentrations of PMA (1.6, 16, and 160 nmol/liter) for 72 hours or Ang II (10, 100, and 1000 nmol/liter) for 48 hours. (A) Scr mRNA expression was examined using reverse transcription-polymerase chain reaction (RT-PCR) followed by Southern blotting as described in the Methods section. (B) The histogram represent means ± sd of the densitometric scans of the Scr mRNA band from three experiments, normalized by comparison with GAPDH mRNA, and expressed as a percentage of control.

itor (staurosporine) significantly inhibited Scr promoter activity induced by PMA, suggesting that the serine/threonine kinase pathway was also involved in up-regulation of Scr induced by PMA (Table 1).

The molecular mechanism of gene transcription was further investigated using several reporter gene constructs described in Figure 1. Functional analysis showed that the minimal prolactin promoter exhibited very little activity either before or after PMA and Ang II treatment (Fig. 8). However, when either one or three copies of the AP-1/ets motifs were introduced upstream of the minimal prolactin promoter, this promoter could respond to PMA stimulation in HMCLs. Three copies of the AP-1/ets motifs increased basal activity by twofold to threefold in response to PMA stimulation. This suggested that AP-1/ets motifs are specific response elements for PMA stimulation (Fig. 8). Mutation of either the AP-1 or the ets motifs decreased its ability to respond to PMA, suggesting that both AP-1 and ets motifs are necessary response elements for gene transcription induced by PMA (Fig. 8). However, the reporter gene containing three copies of AP-1/ets responsive elements responded slightly to Ang II stimulation, suggesting that AP-1/ets motifs are not specific response elements for Ang II (Fig. 8).

DISCUSSION

Scavenger receptors mediate the uptake of modified LDLs by macrophages. The accumulation of lipids via this process is thought to lead to foam cell formation in developing atherosclerotic plaques and in glomerulosclerosis [25–27]. These experiments showed that PMA and Ang II increased Scr protein activity by increasing both the number of Scr-positive cells and the density of Scr per cell in HMCLs. We also analyzed the mRNA expression of Scr and showed that there was an inducible ex-
expression of Scr mRNA in cultures of HMCLs. These results imply that there is limited expression of Scr under physiological conditions, but when HMCLs were stimulated by PMA and Ang II, they expressed an inducible Scr. Ang II has been shown to have an important role in progressive glomerulosclerosis in various animal models and clinical studies [28]. We therefore suggest that Ang II contributes to atherogenesis \textit{in vivo} at least in part through the induction of Scr. This may explain why HMCs that have not previously been shown to express Scr in normal culture can express Scr \textit{in vivo} [10, 12].

There is some degree of heterogeneity of Scr expression within the HMCL population. A proportion of the cell population was negative for receptor, as judged by cell-sorting analysis and by fluorescence microscopy, regardless of the length of exposure to PMA. This may explain several aspects of the phenotypical conversion of HMCs, including cell proliferation, transformation from smooth muscle cells to phagocytes, and foam cell formation. Synchronized HMCLs and primary cultures of HMCs after colchicine arrest also showed a nonhomogeneous staining pattern with DiI-Ac-LDL, suggesting that the nonhomogeneous behavior of HMCLs with respect to DiI-Ac-LDL uptake did not result from different functional specificities within the cell line used nor was it a function of the cell cycle, with temporary suspension of the cells during certain phases of the cycle.

The mechanism by which PMA induces the expression of Scr remains unclear. Both PMA and Ang II are activators of the PKC pathway. The experiments using signal transduction pathway inhibitors showed that the PKC and calmodulin pathways were involved in the induction of Scr by PMA and Ang II during the first 24 hours of culture. PMA-induced down-regulation of PKC has been examined in various cell types in long-term incubation. Different cell lines displayed a range of sensitivities to tetradecanoyl phorbol acetate (TPA)-induced down-regulation of PKC, suggesting that there could be cell-type-specific differences in the pattern of down-regulation [29]. In these HMCL experiments, we could not exclude PMA-induced down-regulation of PKC. However, the activation of the PKC pathway may be an early event, followed by a more sustained phosphorylation of signaling proteins.

Additionally, we focused on the molecular mechanism of Scr up-regulation by PMA and Ang II. A reporter gene containing the Scr promoter (−696 to +46) was transfected into HMCLs. The results showed that PMA and Ang II could increase Scr promoter activity in a dose-responsive manner. This result suggests that PMA and Ang II increase Scr expression by enhancing Scr gene transcription. Scr has been shown to have an important role in progressive glomerulosclerosis in various animal models and clinical studies [28]. We therefore suggest that Ang II contributes to atherogenesis \textit{in vivo} at least in part through the induction of Scr. This may explain why HMCs that have not previously been shown to express Scr in normal culture can express Scr \textit{in vivo} [10, 12].

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Table 1. Effects of phorbol 12-myristate 13-acetate (PMA) and angiotensin II (Ang II) on scavenger receptor (Scr) promoter activity in the presence of various signal transduction pathway inhibitors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>PMA</th>
<th>Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>100 ± 6.32</td>
<td>195.92 ± 20.7</td>
<td>172.20 ± 13</td>
</tr>
<tr>
<td>Calmodulin inhibitor (w-7)</td>
<td>100 ± 15.71</td>
<td>134.66 ± 18.92^a</td>
<td>119.68 ± 16.81^a</td>
</tr>
<tr>
<td>Tyrosine kinase inhibitor (genistein)</td>
<td>100 ± 10.86</td>
<td>253.04 ± 44.29</td>
<td>226.44 ± 61.57</td>
</tr>
<tr>
<td>PKC inhibitor (calphostin C)</td>
<td>100 ± 18.39</td>
<td>148.53 ± 30.50^b</td>
<td>124.65 ± 21.52^b</td>
</tr>
<tr>
<td>Serine/threonine kinase inhibitor (staurosporine)</td>
<td>100 ± 6.91</td>
<td>164.76 ± 16.69^b</td>
<td>158.86 ± 18.71</td>
</tr>
<tr>
<td>G-protein inhibitor (pertussis toxin)</td>
<td>100 ± 15.13</td>
<td>190.88 ± 41.36</td>
<td>176.86 ± 49.61</td>
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^a P < 0.05, ^b P < 0.001

Fig. 8. Functional analysis of the Scr promoter and AP-1/ets motifs in the context of the minimal prolactin gene promoter. The reporter gene constructs shown in Figure 1 were transfected into human mesangial cell lines (HMCLs). Transfected cell were incubated in serum-free RPMI medium in the absence (control) or presence of 16 nmol/liter phorbol 12-myristate 13-acetate (PMA) or 1 μmol/liter angiotensin II (Ang II) for 24 hours, and the promoter activity was determined. Results represent the mean of duplicate determinations from six experiments.

3 copies of AP-1/ets mutations + MRG
3 copies of AP-1 mutations/ets + MRG
3 copies of AP-1/ets + MRG
1 copy AP-1/ets + MRG
Minimal reporter gene (MRG)
ESL

Relative luciferase activity

minimal promoter derived from the rat prolactin gene. Using this established method, Wu et al reported that combinatorial interactions between AP-1 and ets domain proteins contribute to the developmental regulation of the macrophage Scr gene in the THP-1 cell line [14]. AP-1/ets activity could be induced by and were necessary for PMA-induced monocyte differentiation [14]. Our results showed that there was a similar regulatory effect of AP-1/ets in HMCLs. AP-1/ets motifs were specific response elements to PMA stimulation in HMCLs. A mutation of either the AP-1 or the ets motifs decreased its ability to respond to PMA, suggesting that AP-1/ets motifs are necessary response elements for gene expression induced by PMA. These results imply that PMA induces Scr promoter activity by increasing the binding of AP-1/ets transcription factors to the Scr promoter region and that AP-1/ets transcription factors are necessary for Scr expression induced by PMA in HMCLs. The reporter gene containing three copies of AP-1/ets responsive elements responded slightly to Ang II stimulation, suggesting that AP-1/ets motifs are not specific response elements for Ang II. This probably implies that different transcription factors are involved in Scr induction induced by PMA and Ang II.

AP-1 proteins regulate the expression of a diverse set of immediate/early genes that exhibit very rapid and transient transcriptional responses to signals transmitted from cell surface receptors [30]. These immediate re-
sponses reflect post-translational modifications of AP-1 proteins, which have been particularly well studied in the case of c-jun [31, 32]. They may increase transcriptional activity independent of new protein synthesis [31]. In contrast to immediate/early genes, Moulton et al reported that TPA maximally stimulated macrophage Scr gene transcription between 8 and 16 hours after TPA treatment. This induction was dependent on new protein synthesis and could be blocked by staurosporine [13]. Consistent with these observations, we found that transcription activation of the Scr gene in response to PMA did not become maximal until 24 hours following PMA treatment in HMCLs. This suggests that PMA induces Scr expression in HMCLs by increasing transcription and translation of AP-1/ets protein rather than by post-translational modification of AP-1/ets protein.

Therefore, during inflammation, HMCs may express an inducible Scr, through which cells can acquire lipids and convert to foam cells observed in glomerulosclerosis. The induction of Scr expression in HMCs could be a useful marker of HMCL activation during the development of glomerulosclerosis. The importance of AP-1/ets in inducing Scr suggests that the AP-1/ets binding sites in the Scr promoter region may become useful targets for therapeutic manipulation.

ACKNOWLEDGMENTS

We thank Dr. Christopher K. Glass (University of California, San Diego, CA, USA) for his kind gift of the human scavenger receptor promoter, Professor J.D. Sraer (Hôpital Tenon, Paris, France) for providing the immortalized HMC line, and Dr. R. Fernando (Royal Free Hospital, London, UK) for helpful discussions.

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APPENDIX

Abbreviations used in this article are: Ac-LDL, acetylated low density lipoprotein; Ang II, angiotensin II; Dil-Ac-LDL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled acetylated low density lipoprotein; FACS, fluorescence-activated cell sorter; HMCL, human mesangial cell line; HMCs, human mesangial cells; LDL, low density lipoprotein; MFI, mean fluorescence intensity; Ox-LDL, oxidized low density lipoprotein; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; Scr, scavenger receptors; TPA, tetradecanoyl phorbol acetate.

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