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Human mesangial cell production of monocyte chemoattractant protein-1: Modulation by lovastatin

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Human mesangial cell production of monocyte chemoattractant protein-1: Modulation by lovastatin. Macrophages play a critical role in the progression of clinical and experimental glomerular injury. Serum-stimulated human fetal mesangial cells in culture produce a chemotactic factor that is monocyte-selective. This chemotactic factor is most likely monocyte chemoattractant protein-1 (MCP-1) as a monoclonal antibody directed against MCP-1, but not an irrelevant antibody, suppressed the mesangial cell-derived chemotactic activity. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by lovastatin resulted in a reduction of the mesangial cell-derived chemotactic activity as well as MCP-1 mRNA expression. The inhibitory effects of lovastatin in the presence of exogenous cholesterol were reversed by mevalonate, suggesting a role for isoprenoid intermediates of the mevalonate pathway and/or isoprenylated proteins in mesangial cell MCP-1 regulation. These findings suggest an additional mechanism by which HMG-CoA reductase inhibition *in vivo* may reduce glomerular injury.

The development of focal glomerulosclerosis in experimental models of progressive renal disease is frequently characterized by the presence of monocytes and monocyte-derived foam cells [1–8]. Different maneuvers that deplete circulating monocytes have been shown to reduce the degree of glomerular injury in several experimental models [7–10], underscoring the relevance of monocyte-derived macrophage infiltration. The factors that govern migration of monocytes into the glomerular mesangium and their subsequent activation are incompletely understood. Mesangial cells are known to produce a variety of chemotactic substances, including leukotrienes, and cytokines such as monocyte chemoattractant protein-1 (MCP-1) [11–20]. Recent studies have shown that MCP-1 is the major monocyte chemoattractant secreted by stimulated mesangial cells [14, 19].

Inhibitors of HMG-CoA reductase, such as lovastatin, have been shown to reduce glomerular injury in different models of progressive renal disease [21, 22]. The salutary effect of these agents have been generally attributed to their ability to reduce circulating lipids [21]. Preliminary reports suggest, however, that HMG-CoA inhibitors may reduce glomerular as well as vascular injury *in vivo* in the absence of significant effects on blood cholesterol levels [23–25]. It should be emphasized that these agents, which block the synthesis of mevalonate, also inhibit the synthesis of many other compounds derived from mevalonate [26]. Mevalonate metabolism yields a series of isoprenoid compounds which are incorporated into isopentenyl adenine, isoprenylated proteins, dolichol and other end products essential for cell signalling and cell growth [26, 27]. We recently reported that HMG-CoA reductase inhibition results in inhibition of mesangial cell proliferation independently from the availability of cholesterol [28], and others have confirmed this finding [29]. It is conceivable that some mechanisms of cellular activation and cytokine production may also be affected by HMG-CoA reductase inhibition. In the present study, we examined whether human fetal mesangial cell production of chemotactic factors for monocytes might be modulated by lovastatin.

In the present experiments, we demonstrated that serum-stimulated mesangial cells produced a chemotactic factor that is monocyte selective. This factor was identified as MCP-1. Importantly, inhibition of mesangial cell HMG-CoA reductase by lovastatin resulted in reduction of a secreted chemotactic protein as well as a marked reduction in mesangial cell expression of MCP-1 mRNA.

Methods

Human mesangial cell studies

Mesangial cell cultures. Human fetal mesangial cells were obtained from an aborted human fetus. Permission to use this kidney tissue was obtained from the Committee on Use of Human Subjects in Research, University of Minnesota. Glomerular cores were seeded on plastic tissue culture flasks and incubated at 37°C in an atmosphere of 95% air and 5% CO_2. The culture medium was RMPI 1640 supplemented with 20% fetal bovine serum (FBS). Fresh media was added every three to four days. For selective passage of mesangial cells, ridges were removed and replated in the same media. Details of the mesangial cell isolation and culture techniques used in our laboratory as well as their morphological characteristics have been reported [28, 30].

Experimental design. In all experiments 2 to 3 $\times$ 10^4 mesangial cells from the 4th to 6th passages were seeded on tissue culture flasks and allowed to attach and grow to a semiconfluent state over three to four days. Mesangial cells were then synchronized to

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quiescence in serum-free RPMI 1640 for 48 hours. After synchronization, mesangial cells were exposed to 10% FBS as a mitogen in the presence or absence of the HMG-CoA reductase inhibitor lovastatin, a gift from Merck, Inc. (West Point, PA, USA). In some experiments, mesangial cells were pretreated with lovastatin for 16 hours before serum stimulation. Mesangial cell were harvested at different times for RNA extraction and supernates were harvested at 24 hours for evaluation of polymorphonuclear (PMN) cell and monocyte chemotactic activity. In some experiments, mevalonate was added at the time of serum stimulation to assess the reversibility of HMG-CoA reductase inhibition as previously described [28]. Lovastatin and mevalonate salts were prepared as previously described [28].

Evaluation of chemotaxis

Leukocyte preparation. Heparinized blood obtained from healthy donors was mixed with dextran, and red blood cells were allowed to sediment to produce a leukocyte-rich supernatant. Isolation of PMNs was performed as we have previously described [31]. Briefly, the dextran-sedimented, leukocyte-rich supernatant was centrifuged on discontinuous Ficoll-Hypaque (equal volumes of specific gravity 1.080 and 1.120) and the cells were collected at the interface of the bottom layer. Monocytes were obtained by a modification of the Recalde method [32]. After recovering the white cell fraction by centrifugation, they were incubated in a slightly hypertonic solution for approximately 30 minutes at 37°C. The monocytes were then separated using Ficoll-Hypaque and density centrifugation [32].

Chemotaxis assay. Assessments of mesangial cell supernates for chemotactic activity were performed in 48-well Neuroprobe® chemotaxis chambers (Neuro Probe Inc., Cabin John, MD, USA). The lower wells were loaded with 28 μl of the test solution and covered with a 5 μm pore size polycarbonate filter. Upper wells were loaded with 50 μl of the cell suspension containing 5×10^4 monocytes or PMNs. The chambers were incubated at 37°C for 1 or 1½ hours to assess chemotaxis of PMNs and monocytes, respectively. The filters were then removed, fixed in methanol, and stained with Diff-Quick (Baxter Healthcare Inc., Miami, FL, USA). Migrating cells were evaluated by dividing a well into four quadrants and adding the counts from one 200× field in each quadrant. A total of three to five replicate wells were counted and used for determination of the mean value of each data point. Formyl-methionyl-leucyl-phenylalanine (FMLP; Sigma, St. Louis, MO, USA) at a concentration of 1×10^{-8} M was used as the standard chemoattractant for monocytes and PMN.

In certain experiments, the supernate chemotactic activity was assayed after treatment with an affinity-purified IgG_1 mouse monoclonal antibody against human MCP-1, which was kindly provided by T. Yoshimura, M.D., National Cancer Institute (Frederick, MD, USA) [33]. Mesangial cell supernates were incubated with varying dilutions of the MCP-1 antibody for one hour at 37°C. Antibody and complexes were then removed from the sample by ultrafiltration using an Amicon Centricon 100 filter (Amicon Inc., Beverly, MA, USA) with an average molecular cut-off of 100,000 daltons. As a control, similar protein concentrations of an irrelevant mouse IgG_1 monoclonal antibody against von Willebrand factor (Cappel Worthington, Malvern, PA, USA) were added to supernates and processed as described above. Due to the variability of the assay in different days, since the monocytes came from different donors, the results are expressed as the percentage of the appropriate control.

Results from one of two to four different experiments with essentially identical results will be presented.

Molecular techniques

cDNA probes. The cDNA for human MCP-1 was a gift from T. Yoshimura, M.D., and has been previously characterized [34]. The full length cDNA insert contains 739 base pairs and has been shown to recognize a ~0.8 kb mRNA from human mononuclear and smooth muscle cells [34]. The cDNA insert was cloned in an *Eco*RI site in pBluescript SK(−) plasmid. A cDNA for human HMG-CoA reductase was obtained from American Type Culture Collection (Rockville, MD, USA). The cDNA insert contains 4.3 kb, the full sequence for human HMG-CoA reductase that was obtained from a fetal adrenal cDNA library [35]. The full length cDNA was cloned in a pcDV 1 plasmid. After digestion with *Bgl*II, a fragment of about 2.5 kb was isolated and used as a template for radiolabeling. This fragment recognizes a mRNA of ~4.2 kb from human mesangial cells. A cDNA probe for 28s ribosomal RNA was kindly provided by Dr. M.L. Iruela Arispe, University of Washington (Seattle, WA, USA) [36]. The 280 bp fragment between two *Eco*RI sites was used. Between 10 and 50 ng of a cDNA template were labeled with 50 μCi of [^{32}P]-dCTP using Prime It II, a random primer DNA labeling system (Stratagene Cloning System, La Jolla, CA, USA). The cDNAs were purified on a Nuctrap probe purification column (Stratagene Cloning System). The specific activity varied between 1 and 2×10^9 cpm/μg cDNA.

Northern blot analysis. Total RNA was isolated from mesangial cells by a modification of the single step guanidinium-thiocyanate-phenol-chloroform extraction method [37]. The RNA was quantified by absorbance at 260 nm and its purity assessed by the ratio of absorbance at 260:280 being greater than 1.8 to 2.0. Ten to 20 μg samples of denatured mesangial cell RNA together with 10 μg of a RNA ladder (BRL Inc., Gaithersburg, MD, USA) were electrophoresed in a 1.0% agarose gel containing 2.2 M formaldehyde, 20 mM MOPS, 8 mM sodium acetate and 1 mM EDTA, stained with ethidium bromide and photographed to assess degradation, molecular size and equivalent RNA loading. The electrophoresed RNA was transferred to a Zeta Probe membrane (Bio-Rad, Richmond, CA, USA). The membrane was air dried and baked at 80°C for 30 to 60 minutes in a vacuum oven and stored at room temperature until needed. Blotted membranes were incubated with 1.5×10^6 cpm/ml of a labeled cDNA probe in standard hybridization solution for 12 to 24 hours, subjected to a series of stringency washes and exposed on Kodak X-OMAT XAR5 at −70°C. To assess that equivalent amounts of RNA were loaded, the membranes were stripped and reprobed for 28s ribosomal RNA. Autoradiograms of the probed RNA were scanned by densitometry and normalized to the respective 28s values. A representative Northern blot analysis from two to four different experiments is shown.

Statistical analysis

Results were expressed as mean ± SEM. The significance of differences between the means of two groups was tested by the unpaired Student's *t*-test. When more than two groups were compared, significance was tested using analysis of variance. These analyses were performed using the Statistical Package for

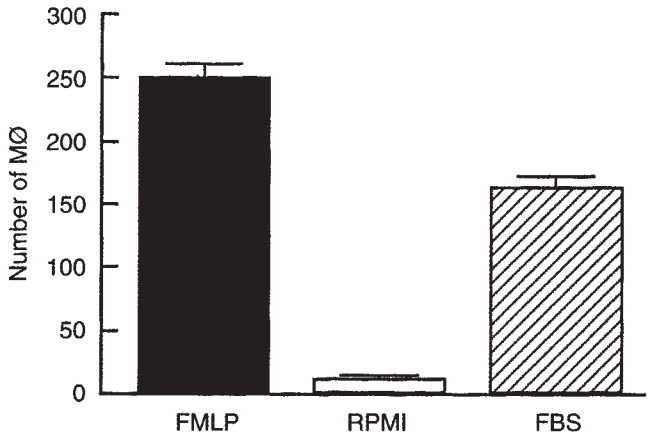

Fig. 1. *Quiescent human fetal mesangial cells when stimulated to proliferate with 10% fetal bovine serum secreted a factor that stimulated chemotaxis of normal peripheral blood monocytes (hatched bar).* Quiescent mesangial cells maintained in serum-free RPMI (open bar) did not secrete a significant amount of this factor. As a positive control the effect of 10^{-8} M FMLP is also displayed (solid bar). Abbreviations are: Mϕ, monocytes; FMLP, formyl-methionyl-leucyl-phenylalanine; FBS, fetal bovine serum.

Social Sciences [38]. Differences were considered significant for $P < 0.05$.

Results

Mesangial cell production of a chemotactic factor

Quiescent human fetal mesangial cells, when exposed to 10% FBS for 24 hours, secreted a factor that stimulated chemotaxis of normal human peripheral blood monocytes. This effect was quantitatively similar to that seen when the chemotactic peptide FMLP was used to stimulate monocyte chemotaxis (Fig. 1). In contrast, no effect on chemotaxis of PMNs was seen with supernates from serum-stimulated mesangial cells (data not shown). Quiescent mesangial cells maintained in serum-free RPMI did not secrete significant amounts of chemotactic factor during the 24-hour incubation (Fig. 1). Similarly, 10% FBS alone in the chemotaxis chamber did not demonstrate any significant chemotactic activity (data not shown). Ultrafiltration of mesangial cell supernates after incubation with a serial dilution of an IgG_1 mouse monoclonal antibody against human monocyte MCP-1 resulted in a dose-response reduction in the supernate chemotactic activity (Fig. 2). Ultrafiltration alone (not shown) or after incubation with a comparable concentration of an irrelevant IgG mouse monoclonal antibody (anti-von Willebrand factor) had no effect on monocyte chemotaxis of the mesangial cell supernates (Fig. 2).

Effect of inhibition of HMG-CoA reductase on production of mesangial cell MCP-1

Lovastatin, in a concentration-dependent manner, inhibited mesangial cell production of a monocyte chemotactic factor (Fig. 3). Between 20 and 40% of chemotactic activity in the supernates was inhibited by 1 to 20 μM lovastatin. The addition of the MCP-1 monoclonal antibody to these supernates followed by ultrafiltration virtually abolished the remaining chemotactic activity (data not shown). The mesangial cell viability, as assessed by trypan blue exclusion, was similar in control cells and in lovastatin treated cells (>85%). In separate experiments, the addition of lovastatin to the chemotaxis chambers did not alter the chemotaxis of monocytes to FMLP nor did it affect the chemotactic response to the mesangial cell-derived chemotactic factor (data not shown). The addition of mevalonate to the mesangial cell incubation medium at the time of serum stimulation completely reversed the inhibitory effect of lovastatin and restored mesangial cell production of the chemotactic factor (Fig. 4). The addition of mevalonate to the medium in the absence of lovastatin did not modify the monocyte chemotactic activity (not shown).

Effect of HMG-CoA reductase inhibition on MCP-1 mRNA expression

To evaluate whether lovastatin influenced the steady state level of mRNA for MCP-1 after 24 hours, total RNA was obtained from serum-stimulated mesangial cells in the presence of lovastatin with or without mevalonate. In these experiments, total RNA was also probed for HMG-CoA reductase mRNA to ascertain the effectiveness of lovastatin inhibition of HMG-CoA reductase. Quiescent mesangial cells had minimal constitutive expression of MCP-1 (Fig. 5). This was consistent with our results demonstrating virtually no detectable chemotactic activity in the supernates from quiescent mesangial cells (Fig. 1). In contrast, serum-stimulation of mesangial cells resulted in a significantly increased expression of MCP-1 mRNA (Fig. 5). This effect was virtually suppressed by lovastatin, while mevalonate completely reversed the effects of HMG-CoA reductase inhibition (Fig. 5). Consistent with the effect of lovastatin to inhibit HMG-CoA reductase, lovastatin markedly up-regulated mRNA expression for this enzyme, while the addition of mevalonate significantly down-regulated the expression of this mRNA (Fig. 6).

Time-dependent effect of HMG-CoA reductase inhibition on MCP-1 mRNA expression

To assess the apparent discrepancy between the total suppression of MCP-1 mRNA at 24 hours and the only partial (20 to 40%) inhibition of chemotactic activity in 24 hour supernates, MCP-1 mRNA expression was evaluated at earlier time points after serum stimulation. Serum-stimulated mesangial cells exhibited a marked up-regulation of MCP-1 mRNA expression that reached a peak between three and six hours and declined thereafter (Fig. 7). Lovastatin inhibited MCP-1 mRNA expression only after six hours of incubation, and had little effects at earlier times. This late inhibition of MCP-1 mRNA expression by lovastatin may have permitted an early MCP-1 production and accumulation in the 24 hour supernates that were used for chemotactic assays. The delayed effects of lovastatin suggest that an incubation period is required for the action of the drug. Alternatively, it may reflect a specific effect on the time dependent events of MCP-1 mRNA up-regulation following serum stimulation, that is, lovastatin might produce a late down-regulation without affecting the initial response. To discern between these two possibilities, mesangial cells were preincubated with lovastatin for 16 hours before serum stimulation. When mesangial cells were preincubated with lovastatin, MCP-1 mRNA up-regulation was prevented from the beginning (Fig. 8). Therefore, these results are consistent with the necessity of an incubation period of several hours for lovastatin effects. In either case, when the cells were exposed to mevalonate for at least three hours, the inhibitory effects of lovastatin were

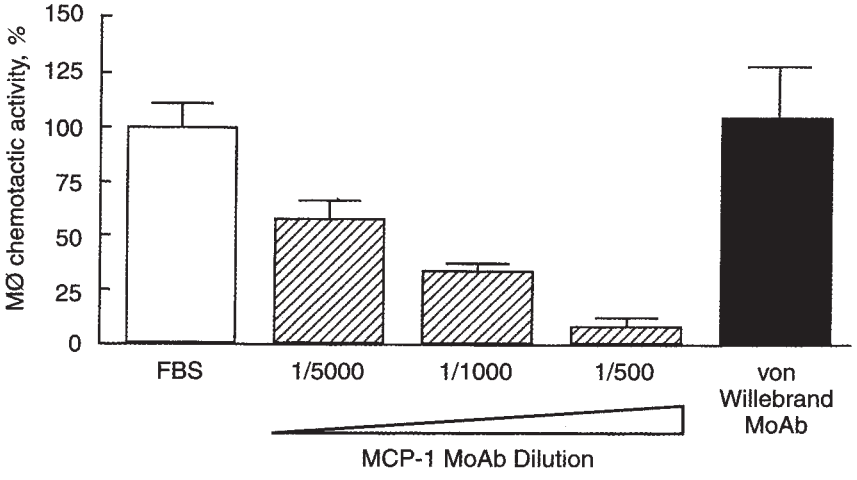

Fig. 2. *Quiescent human fetal mesangial cells were stimulated with 10% fetal bovine serum and the 24 hour supernates were assayed for monocyte chemotactic activity as described in Methods (open bar).* Aliquots of supernates were incubated with different concentrations of a monoclonal IgG antibody against human MCP-1 (hatched bars) or an irrelevant IgG antibody (von Willebrand, solid bar), ultrafiltered and assayed for monocyte chemotactic activity. Ultrafiltration alone had no effect on chemotactic activity (not shown). Abbreviations are: Mϕ, monocytes; FBS, fetal bovine serum; MoAb, monoclonal antibody; MCP-1, monocyte chemoattractant protein-1.

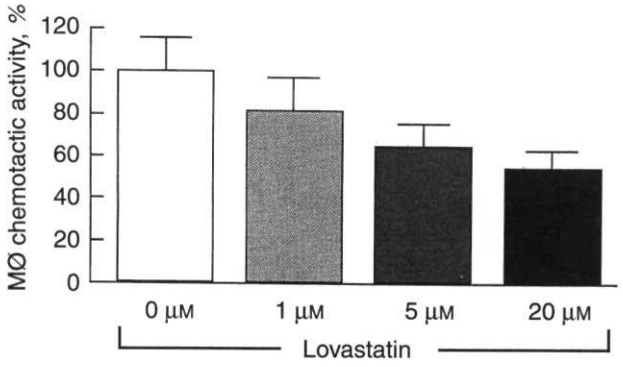

Fig. 3. *Lovastatin (1 to 20 μM) caused a significant ($P < 0.01$, ANOVA) dose-dependent reduction in 24 hour production of a monocyte chemotactic factor by serum-stimulated human fetal mesangial cells.* Mø is monocytes.

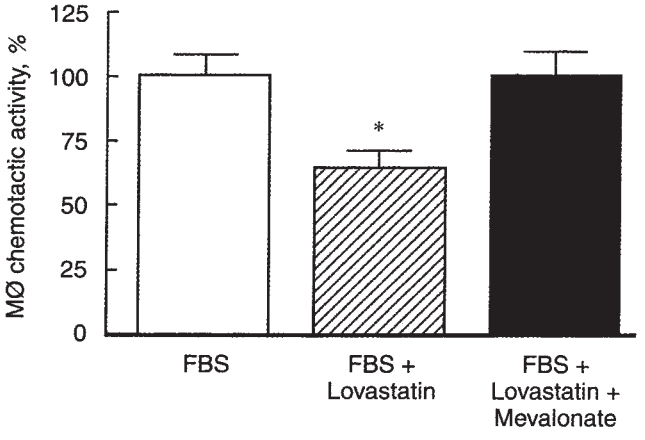

Fig. 4. *In these experiments 5 M lovastatin significantly reduced (hatched bar) serum-stimulated human fetal mesangial cell production of a chemotactic factor for monocytes.* This was completely restored by the addition of 100 μM mevalonate (solid bar). *$P < 0.001$. Abbreviations are: Mϕ, monocytes; FBS, fetal bovine serum.

substantially reversed (Figs. 7 and 8). It is conceivable that a minimum time is required for cell uptake and metabolism of mevalonate to reverse lovastatin effects. In the absence of lovastatin, mevalonate did not affect mesangial cell MCP-1 mRNA expression (data not shown).

Discussion

MCP-1 production has been recently demonstrated in a variety of human and rodent cells [34, 39–44]. Increased glomerular expression of MCP-1 has been shown within minutes of induction of anti-Thy 1.1 glomerulonephritis [45], a model of mesangial cell injury associated with early mesangial monocyte infiltration [45]. Preliminary studies have also found an association between increased renal expression of MCP-1 and monocyte infiltration in a variety of immunologic and non-immunologic models of renal injury [46–51]. A number of growth promoting and inflammatory cytokines have been shown to increase the expression of MCP-1 mRNA as well as the secreted peptide [14, 34, 40, 42–44, 52]. Glomerular mesangial cells have also been shown to produce MCP-1 in response to a variety of stimuli [13–20, 52].

The present results confirm previous reports suggesting that mitogen-stimulated mesangial cells produce monocyte chemoattractants. The chemotactic activity was selective for monocytes, as no chemotactic activity was detected for PMNs. Virtually all the monocyte chemotactic activity was suppressed by an IgG monoclonal antibody against MCP-1, but not by an irrelevant IgG antibody. It is reasonable to conclude that this chemotactic activity was indeed due to mesangial cell secretion of MCP-1. Mesangial cells can produce several products with chemotactic activity such as transforming growth factor (TGF) β1 and platelet-derived growth factor (PDGF). However, their contribution to the monocyte chemotactic activity in our experimental conditions appears to be negligible. Other investigators have also found that most of the monocyte chemotactic activity secreted by cytokine or lipopolysaccharide (LPS) stimulated mesangial cells can be suppressed by antibodies against MCP-1 [14, 19]. Similar results have been obtained in cultures of smooth muscle cells, cells that exhibit many similarities with mesangial cells, after stimulation with oxidatively-modified lipoproteins [53, 54]. It is, therefore, possible that some of the mesangial cell monocyte chemotactic activity previously attributed to different cytokines may be in fact due to MCP-1 production. Indeed, PDGF, TGF-β1, LPS and modified lipoproteins are all potent inducers of MCP-1 expression [19, 43, 44, 53–55].

Recent experimental evidence suggests that some beneficial

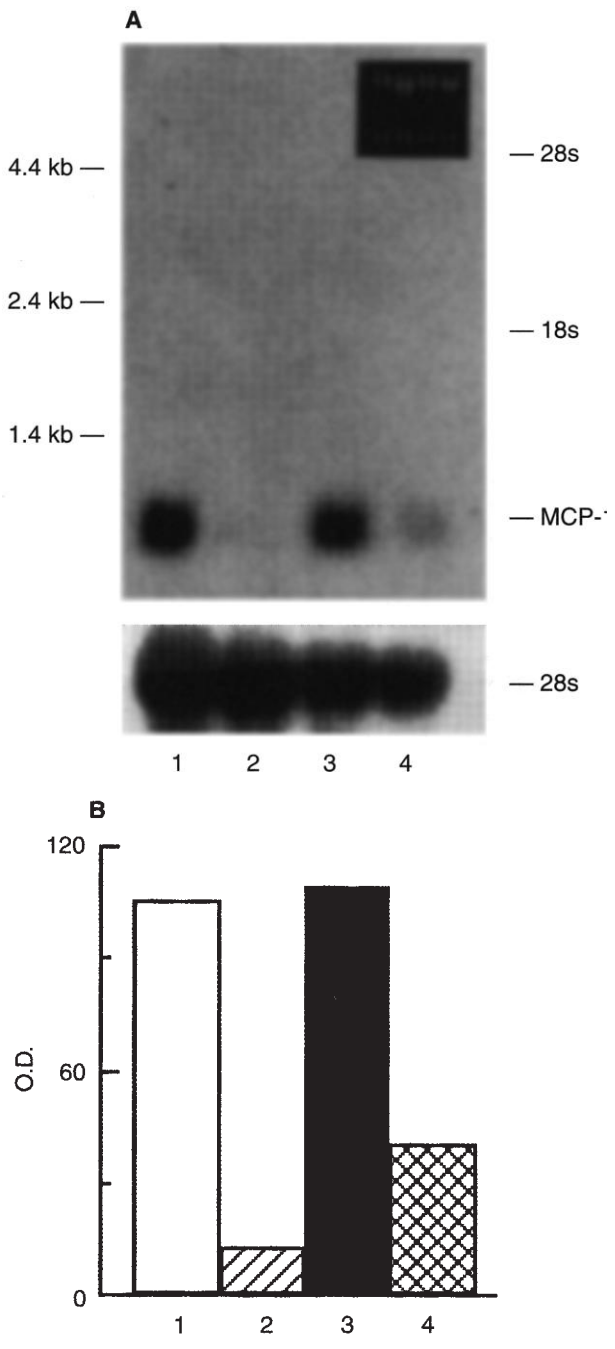

Fig. 5. *Northern analysis (A) and densitometric evaluation (B) of MCP-1 mRNA in serum-stimulated human fetal mesangial cells (lane 1, open bar).* The presence of 5 μM lovastatin dramatically reduced MCP-1 mRNA expression (lane 2, hatched bar). The presence of 100 μM mevalonate completely reversed the inhibitory effect of lovastatin (lane 3, solid bar). Quiescent mesangial cells maintained in serum-free RPMI demonstrated a low constitutive expression of MCP-1 (lane 4, double hatched bar). The ethidium bromide picture of the gel is shown as an insert. The membrane reprobed for 28s ribosomal RNA is also shown. B. Densitometric evaluation of the Northern blot after normalization with 28s ribosomal RNA. Abbreviations are: O.D., optical density, arbitrary units; FBS, fetal bovine serum; MCP-1, monocyte chemoattractant protein-1. Symbols are: (□) FBS; (▨) Lovastatin; (■) Lovastatin + Mevalonate; (▩) RPMI.

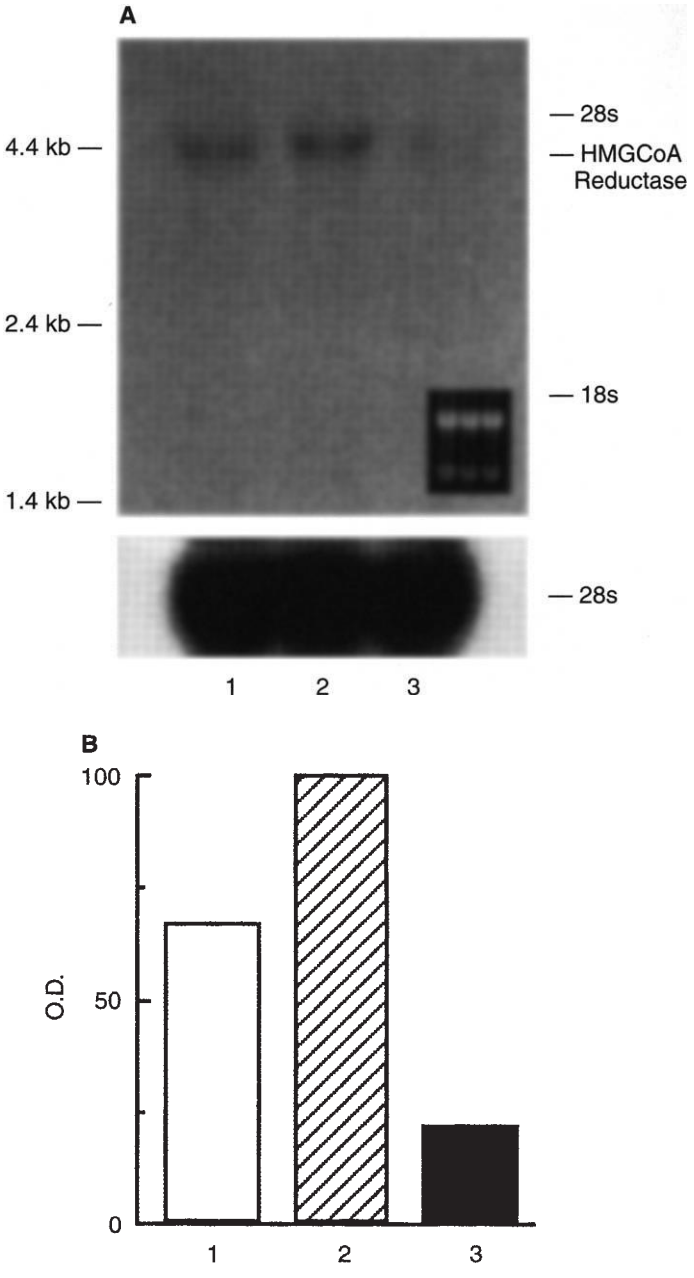

Fig. 6. *Northern analysis of HMG-CoA reductase mRNA in serum-stimulated human fetal mesangial cells (lane 1, open bar).* In accordance with the metabolic effects of lovastatin and mevalonate, HMG-CoA reductase mRNA expression was significantly up-regulated by 5 μM lovastatin (lane 2, hatched bar) and markedly down-regulated after the addition of 100 μM mevalonate (lane 3, solid bar). The ethidium bromide picture of the gel is shown as an insert. The membrane reprobed for 28s ribosomal RNA is also shown. **B.** Densitometric evaluation of the Northern blot after normalization with 28s ribosomal RNA. Abbreviations are: HMG-CoA R, 3-hydroxy-3-methylglutaryl coenzyme A reductase; O.D., optical density, arbitrary units; FBS, fetal bovine serum. Symbols are: (□) FBS; (▨) Lovastatin; (■) Lovastatin + Mevalonate.

effects of HMG-CoA reductase inhibition can be obtained in the absence of significant changes in circulating cholesterol [23–25]. Our data suggest another potential mechanism by which lovastatin affords glomerular protection, namely, inhibition of mesangial cell production of MCP-1 and subsequent monocyte recruitment. The reduction of MCP-1 production by lovastatin involves, at least, the inhibition of MCP-1 mRNA up-regulation. Although the inhibition of MCP-1 mRNA expression may account completely for the lovastatin inhibition of MCP-1 production, other

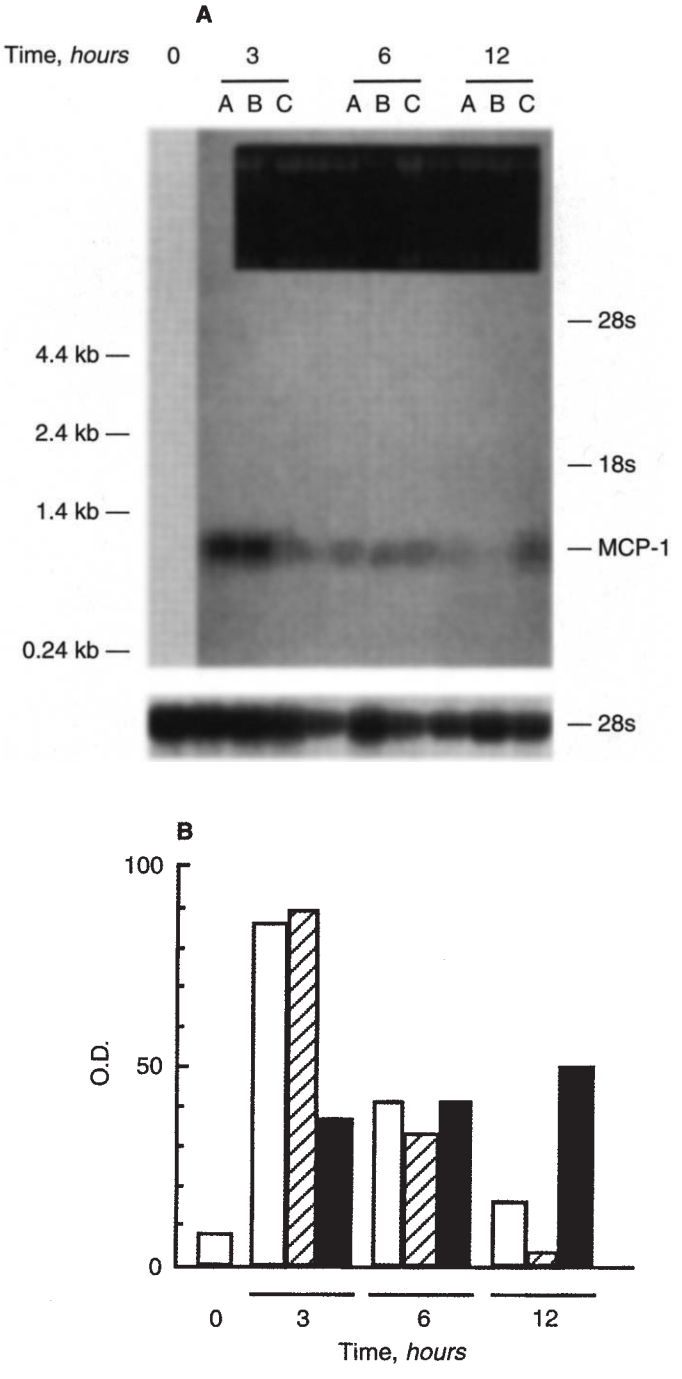

Fig. 7. *Time-dependent effects of lovastatin on MCP-1 mRNA expression.* Quiescent human fetal mesangial cells were stimulated with 10% fetal bovine serum in the absence (lanes A, open bars) or presence (lanes B, hatched bars) of 5 μM lovastatin. Some lovastatin-treated cells were exposed to 100 μM mevalonate (lanes C, solid bars). Total RNA was extracted at different times following serum stimulation, blotted, and probed for MCP-1 as described in **Methods**. Lovastatin inhibited MCP-1 mRNA up-regulation only after six hours of incubation. This late lovastatin inhibition was reversed by mevalonate. The ethidium bromide picture of the gel is shown as an insert. The membrane reprobed for 28s ribosomal RNA is also shown. The lower panel shows the densitometric evaluation of the Northern blot after normalization with 28s ribosomal RNA. Abbreviations are: MCP-1, monocyte chemoattractant protein-1; O.D., optical density, arbitrary units; FBS, fetal bovine serum. Symbols are: (□) FBS; (▨) Lovastatin; (■) Lovastatin + Mevalonate.

potential effects of lovastatin on mesangial cell translation, post-translational regulation and secretion of MCP-1 cannot be ruled out with the present data. The lovastatin inhibition of MCP-1 production and mRNA expression appears to be specific and not a toxic effect for several reasons. First, the number of mesangial cells as well as their viability were not significantly affected by lovastatin treatment. Second, mesangial cells were able to up-regulate HMG-CoA reductase mRNA levels in response to lovastatin inhibition of this enzyme. In case of a non-specific toxic effect this up-regulation would also likely have been inhibited. Third, in similar studies lovastatin did not affect the expression of other unrelated genes such as the α_1 chain of type IV collagen, TGF-β1 and PDGF-B [56–58]. Grandaliano et al have also found that a similar concentration of simvastatin, another HMG-CoA reductase inhibitor, did not affect mesangial cell mRNA expression for PDGF-B [29]. Finally, mevalonate, the first product of the pathway inhibited by lovastatin, reversed lovastatin effects, suggesting that the lovastatin effect was indeed specific to the inhibition of mevalonate production.

Increasing experimental data suggest that, besides cholesterol, different products of the mevalonate pathway play important roles in mesangial cell biology [27, 59]. We and others have reported that mesangial cell proliferation is dependent on the isoprenoid metabolites of this pathway and that HMG-CoA reductase inhibition could therefore result in glomerular protection by the inhibition of mesangial cell proliferation [28, 29]. The present results suggest that mevalonate metabolites are also required for mesangial cell mRNA expression and production of MCP-1. Cholesterol depletion cannot be implicated for the lovastatin inhibition of MCP-1 production, as an exogenous source of cholesterol was provided by the serum-supplemented medium in all of the experiments.

Of particular interest was the observation that an incubation period of several hours was required to detect the effects of lovastatin on MCP-1 mRNA. Few studies have addressed the time-dependent changes after the addition of lovastatin to the cell culture. Repco and Maltese reported that, in the MEL cell line, lovastatin depletes the cellular pool of isoprenoids in less than one hour [60]. However, seven hours of incubation with lovastatin were required to produce a significant (20 to 35%) reduction in the cellular content of isoprenylated proteins [60]. The time course of lovastatin effects on the cellular levels of isoprenylated proteins in MEL cells is remarkably similar to the effects of lovastatin on MCP-1 mRNA expression in our mesangial cell system. If the effects of lovastatin on the mevalonate pathway in mesangial cells follow a similar sequence to that described in MEL cells, a direct role for isoprenoids themselves in mesangial cell regulation of MCP-1 does not seem likely. Rather, an involvement of isoprenylated proteins or other long-lived mevalonate derived compounds is suggested. However, the specific roles of the isoprenoids of the mevalonate pathway and/or isoprenoid-modified proteins in the regulation of mesangial cell MCP-1 production were not assessed in the present study and warrant further investigation.

In summary, our data confirm previous reports suggesting that stimulated mesangial cells secrete chemotactic substances for monocytes and that most of this chemotactic activity can be attributed to the production of MCP-1. The present results also indicate that the mesangial cell production of MCP-1 is dependent on metabolites of the mevalonate pathway. Thus, our

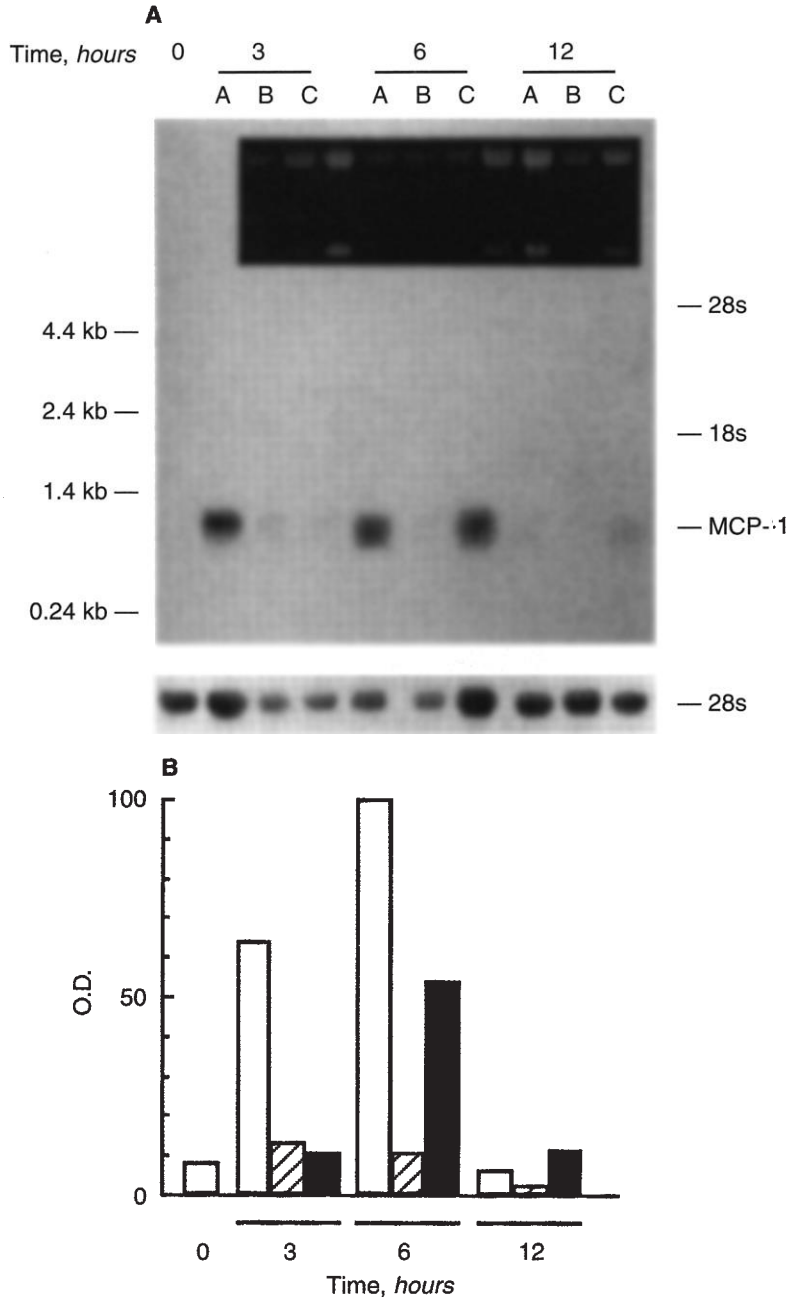

Fig. 8. *Effects of lovastatin preincubation on MCP-1 mRNA expression.* Human fetal mesangial cells were rendered quiescent by incubation in serum-free medium for 48 hours. During the last 16 hours of this period, mesangial cells were exposed to 5 μM lovastatin. At time 0, the cells were extensively washed and stimulated with 10% fetal bovine serum in the absence (lanes A, open bars) or presence (lanes B, hatched bars) of 5 μM lovastatin. Some cells were exposed to 100 μM mevalonate in addition to 5 μM lovastatin (lanes C, solid bars) at the time of serum stimulation. Total RNA was extracted at different times, blotted, and probed for MCP-1 as described in Methods. Lovastatin inhibited MCP-1 mRNA up-regulation from the beginning. When mevalonate was present in the cell culture for more than three hours, it essentially reversed lovastatin inhibition. The ethidium bromide picture of the gel is shown as an insert. The membrane reprobed for 28s ribosomal RNA is also shown. The lower panel shows the densitometric evaluation of the Northern blot after normalization with 28s ribosomal RNA. Abbreviations are: MCP-1, monocyte chemoattractant protein-1; O.D., optical density, arbitrary units; FBS, fetal bovine serum. Symbols are: (□) FBS; (▨) Lovastatin; (■) Lovastatin + Mevalonate.

findings provide another potential mechanism whereby HMG-CoA reductase inhibitors may exert beneficial effects to ameliorate progressive renal injury.

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References

1. Rovin BH, Schreiner GF: Cell-mediated immunity in glomerular disease. *Annu Rev Med* 42:25–33, 1991
2. Wolthuis A, van Goor H, Weening JJ, Grond J: Pathobiology of focal sclerosis. *Curr Opin Nephrol Hypertens* 2:458–464, 1993
3. Schreiner GF: The role of the macrophage in glomerular injury. *Semin Nephrol* 11:268–275, 1991
4. Saito T, Atkins: Contribution of mononuclear leucocytes to the progression of experimental focal glomerular sclerosis. *Kidney Int* 37:1076–1083, 1990

5. VAN GOOR H, FIDLER V, WEENING JJ, GROND J: Determinants of focal and segmental glomerulosclerosis in the rat after renal ablation. *Lab Invest* 64:754–765, 1991
6. KASISKE BL, O'DONNELL MP, SCHMITZ PG, KIM Y, KEANE WF: Renal injury of diet-induced hypercholesterolemia in rats. *Kidney Int* 37:880–891, 1990
7. PESEK-DIAMOND I, DING GH, FRYE J, DIAMOND JR: Macrophages mediate adverse effects of cholesterol feeding in experimental nephrosis. *Am J Physiol* 263:F776–F783, 1992
8. VAN GOOR H, VAN DER HORST MLC, FIDLER V, GROND J: Glomerular macrophage modulation affects mesangial expansion in the rat after renal ablation. *Lab Invest* 66:564–571, 1992
9. DIAMOND JR, PESEK-DIAMOND I: Sublethal X-irradiation during acute puromycin nephrosis prevents late renal injury: Role of macrophages. *Am J Physiol* 260:F779–F786, 1991
10. DIAMOND JR, PESEK I, RUGGIERI S, KARNOVSKY MJ: Essential fatty acid deficiency during acute puromycin nephrosis ameliorates late renal injury. *Am J Physiol* 257:F798–F807, 1989
11. SEDOR JR, KONIECZKOWSKI M, HUANG S, GRONICH JH, NAKAZATO Y, GORDON G, KING CH: Cytokines, mesangial cell activation and glomerular injury. *Kidney Int* 39:S65–S70, 1993
12. FLOEGE J, JOHNSON RJ: Cytokines in renal inflammation. *Curr Opin Nephrol Hypertens* 2:449–457, 1993
13. ZOJA C, WANG JM, BETTONI S, SIRONI M, RENZI D, CHIAFFARINO F, ABBOUD HE, VAN DAMME J, MANTOVANI A, REMUZZI G: Interleukin-1 beta and tumor necrosis factor-alpha induce gene expression and production of leukocyte chemotactic factors, colony-stimulating factors, and interleukin-6 in human mesangial cells. *Am J Pathol* 138:991–1003, 1991
14. ROVIN BH, YOSHIUMURA T, TAN L: Cytokine-induced production of monocyte chemoattractant protein-1 by cultured human mesangial cells. *J Immunol* 148:2148–2153, 1992
15. HORA K, SATRIANO JA, SANTIAGO A, MORI T, STANLEY ER: Receptors for IgG complexes activate synthesis of monocyte chemoattractant peptide 1 and colony-stimulating factor 1. *Proc Natl Acad Sci USA* 89:1745–1749, 1992
16. SATRIANO JA, HORA K, SHAN Z, STANLEY ER, MORI T, SCHLÖNDORFF D: Regulation of monocyte chemoattractant protein-1 and macrophage colony-stimulating factor-1 by IFN-gamma, tumor necrosis factor-alpha, IgG aggregates, and cAMP in mouse mesangial cells. *J Immunol* 150:1971–1978, 1993
17. BROWN Z, STRIETER RM, NEILD GH, THOMPSON RC, KUNKEL SL, WESTWICK J: IL-1 receptor antagonist inhibits monocyte chemotactic peptide 1 generation by human mesangial cells. *Kidney Int* 42:95–101, 1992
18. ROVIN BH, COSIO F, TAN L: Induction of monocyte chemoattractant protein-1 in human mesangial cells by in situ immune complex formation. (abstract) *JASN* 3:612, 1992
19. GRANDE JP, JONES ML, SWENSON CL, KILLEN PD, WARREN JS: Lipopolysaccharide induces monocyte chemoattractant protein production by rat mesangial cells. *J Lab Clin Med* 124:112–117, 1994
20. GRANDALIANO G, VALENTE AJ, ABBOUD HE: A novel biologic activity of thrombin—Stimulation of monocyte chemotactic protein production. *J Exp Med* 179:1737–1741, 1994
21. KEANE WF, KASISKE BL, O'DONNELL MP, SCHMITZ PG: Therapeutic implications of lipid-lowering agents in the progression of renal disease. *Am J Med* 87:21N–24N, 1989
22. GUIJARRO C, KEANE WF: Lipid-induced glomerular injury. *Nephron* 67:1–6, 1994
23. GRONE HJ, MILLER B, WALLI AK, EISENHAUER T, GRONE E, SEIDEL D: Amelioration of lipid glomerulopathy by the HMG-CoA reductase inhibitor lovastatin in guinea pigs. An effect without pronounced reduction in plasma cholesterol. (abstract) *JASN* 3:739, 1992
24. SOMA MR, DONETTI E, PAROLINI C, MAZZINI G, FERRARI C, FUMAGALLI R, PAOLETTI R: HMG-CoA reductase inhibitors—In vivo effects on carotid intimal thickening in normocholesterolemic rabbits. *Arterioscler Thromb* 13:571–578, 1993
25. KAUFFMAN RF, BEAN JS, BENSCH WR: Reduction of intimal thickening by lovastatin in the absence of reductions in serum cholesterol. (abstract) *FASEB J* 8:A319, 1994
26. GOLDSTEIN JL, BROWN MS: Regulation of the mevalonate pathway. *Nature* 343:425–430, 1990
27. MASSY ZA, GUIJARRO C, KASISKE BL: Plasma mevalonate and its importance in nephrology. *Nephol Dial Transplant* 9:1716–1718, 1994
28. O'DONNELL MP, KASISKE BL, KIM Y, ATLURU D, KEANE WF: Lovastatin inhibits proliferation of rat mesangial cells. *J Clin Invest* 91:83–87, 1993
29. GRANDALIANO G, BISWAS P, CHOUDHURY GG, ABBOUD HE: Simvastatin inhibits PDGF-induced DNA synthesis in human glomerular mesangial cells. *Kidney Int* 44:503–508, 1993
30. KEANE WF, O'DONNELL MP, KASISKE BL, KIM YK: Oxidative modification of low-density lipoproteins by mesangial cells. *JASN* 4:187–194, 1993
31. KEANE WF, COMTY CM, VERBRUGH HA, PETERSON PK: Opsonic deficiency of peritoneal dialysis effluent in continuous ambulatory peritoneal dialysis. *Kidney Int* 25:539–543, 1984
32. FOGELMAN AM, ELAHI F, SYKES K, VAN LENTEN BJ, TERRITO MC, BERLINER JA: Modification of the Recalde method for the isolation of human monocytes. *J Lipid Res* 29:1243–1247, 1988
33. YOSHIMURA T, TAKEYA M, TAKAHASHI K, KURATSU J, LEONARD EJ: Production and characterization of mouse monoclonal antibodies against human monocyte chemoattractant protein-1. *J Immunol* 147: 2229–2233, 1991
34. YOSHIMURA T, YUHKI N, MOORE SK, APPELLA E, LERMAN MI, LEONARD EJ: Human monocyte chemoattractant protein-1 (MCP-1). Full length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. *FEBS Lett* 244:487–493, 1989
35. LUSKEY KL, STEVENS B: Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. Conserved domains responsible for catalytic activity and sterol-regulated degradation. *J Biol Chem* 260:10271–10277, 1985
36. IRUELA-ARISPE ML, DIGLIO CA, SAGE EH: Modulation of extracellular matrix proteins by endothelial cells undergoing angiogenesis in vitro. *Arterioscler Thromb* 11:805–815, 1991
37. CHOMCZYNSKI P, SACCHI N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
38. NORUSIS MJ: *Statistical Package for the Social Sciences. (SPSS/PC+™ V2.0): Base Manual.* Chicago, SPSS Inc. 1988
39. YOSHIMURA T, LEONARD EJ: Human monocyte chemoattractant protein-1: Structure and function. *Cytokines* 4:131–152, 1992
40. HANAZAWA S, TAKESHITA A, AMANO S, SEMBA T, NIRAZUKA T, KATOH H, KITANO S: Tumor necrosis factor-alpha induces expression of monocyte chemoattractant JE via *fos* and *jun* genes in clonal osteoblastic MC3T3-E1 cells. *J Biol Chem* 268:9526–9532, 1993
41. LIEBLER JM, KUNKEL SL, BURDICK MD, STANDIFORD TJ, ROLFE MW, STRIETER RM: Production of IL-8 and monocyte chemotactic peptide-1 by peripheral blood monocytes. Disparate responses to phytohemagglutinin and lipopolysaccharide. *J Immunol* 152:241–249, 1994
42. SHYY YJ, WICKHAM LL, HAGAN JP, HSIEH HJ, HU YL, TELIAN SH, VALENTE AJ, SUNG KL, CHIEN S: Human monocyte colony-stimulating factor stimulates the gene expression of monocyte chemotactic protein-1 and increases the adhesion of monocytes to endothelial monolayers. *J Clin Invest* 92:1745–1751, 1993
43. MARMUR JD, POON M, ROSSIKHINA M, TAUBMAN MB: Induction of PDGF-responsive genes in vascular smooth muscle. Implications for the early response to vessel injury. *Circulation* 86:III-53–III-60, 1992
44. ROLLINS BJ, MORRISON ED, STILES CD: Cloning and expression of JE, a gene inducible by platelet-derived growth factor and whose product has cytokine-like properties. *Proc Natl Acad Sci USA* 85: 3738–3742, 1988
45. STAHL RAK, THAISS F, DISSER M, HELMCHEN U, HORA K, SCHLONDORFF D: Increased expression of monocyte chemoattractant protein-1 in anti-thymocyte antibody-induced glomerulonephritis. *Kidney Int* 44:1036–1047, 1993
46. DIAMOND JR, KEESFOLTS D, DING GH, FRYE JE, RESTREPO NC: Macrophages, monocyte chemoattractant peptide-1, and TGF-beta 1 in experimental hydronephrosis. *Am J Physiol* 266:F926–F933, 1994
47. TAM WK, KARKAR AM, SMITH J, WANG Y, YOSHIMURA T, THOMPSON EM, REES AJ: Differential glomerular chemokine expression and leukocyte influx in nephrotoxic nephritis. (abstract) *JASN* 4:637, 1993
48. RUMANCIK M, ROVIN BH: Glomerular expresssion of monocyte cemoattractant protein-1 (MCP-1) in experimental and human glomerulonephritis. (abstract) *JASN* 4:630, 1993

49. SCHILLER-MORAN B, DELEO M, WARREN E, KUMANO K, MORAN J: Modulation of monocyte chemoattractant protein (MCP-1) expression in the remnant kidney model by a low protein diet. (abstract) *JASN* 4:632, 1993
50. ALLISON AE, WARREN JS: Expression and production of monocyte chemoattractant protein-1 (MCP-1) in puromycin aminonucleoside nephrosis. (abstract) *JASN* 4:600, 1993
51. MOXEY-MIMS M, NIELSEN L, LWEBUGA-MUKASA J, NOBLE B: Expression of monocyte chemoattractant protein 1 (MCP-1) parallels disease progression in chronic proliferative immune complex nephritis. (abstract) *FASEB J* 8:A222, 1994
52. SATRIANO JA, SHULDINER M, HORA K, XING Y, SHAN Z, SCHLÖNDORFF D: Oxygen radicals as second messengers for expression of the monocyte chemoattractant protein, JE/MCP-1, and the monocyte colony-stimulating factor, CSF-1, in response to tumor necrosis factor-alpha and immunoglobulin G-Evidence for involvement of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase. *J Clin Invest* 92:1564–1571, 1993
53. CUSHING SD, BERLINER JA, VALENTE AJ, TERRITO MC, NAVAB M, PARHAMI F, GERRITY R, SCHWARTZ CJ, FOGELMAN AM: Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc Natl Acad Sci USA* 87:5134–5138, 1990
54. NAVAB M, IMES SS, HAMA SY, HOUGH GP, ROSS LA, BORK RW, VALENTE AJ, BERLINER JA, DRINKWATER DC, LAKS H: Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J Clin Invest* 88:2039–2046, 1991
55. HANAZAWA S, TAKESHITA A, TSUKAMOTO Y, KAWATA Y, TAKARA KO, KITANO S: Transforming growth factor-beta-induced gene expression of monocyte chemoattractant JE in mouse osteoblastic cells, MC3T3-E1. *Biochem Biophys Res Commun* 180:1130–1136, 1991
56. GUIJARRO C, KEANE WF: Effects of lipids in the pathogenesis of progressive renal failure. Role of HMG-CoA reductase inhibitors in the prevention of glomerulosclerosis. *Miner Electrol Metab* (in press)
57. GUIJARRO C, O'DONNELL MP, KASISKE BL, KIM Y, ATLURU D, KEANE WF: Differential effects of lovastatin on human mesangial cell mRNA for cytokines involved in proliferation and matrix turnover. (abstract) *JASN* 4:770, 1993
58. GUIJARRO C, O'DONNELL MP, KASISKE BL, KIM Y, MASSY ZA, KEANE WF: Opposite effects of lovastatin on human mesangial cell mRNA expression for IL-6 and PDGF-B. (abstract) *JASN* 5:943, 1994
59. O'DONNELL MP, KASISKE BL, KIM Y, ATLURU D, KEANE WF: The mevalonate pathway—Importance in mesangial cell biology and glomerular disease. *Miner Electrol Metab* 19:173–179, 1993
60. REPKO EM, MALTESE WA: Post-translational isoprenylation of cellular proteins is altered in response to mevalonate availability. *J Biol Chem* 264:9945–9952, 1989