Atherogenic Lipoproteins Stimulate Mesangial Cell p42 Mitogen-Activated Protein Kinase

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Abstract. Previously, it has been shown that atherogenic lipoproteins, through the activation of glomerular cells, stimulate pathobiological processes involved in monocyte infiltration into the mesangium. This study examined the role of LDL and its oxidatively modified variants (mildly oxidatively modified LDL [mm-LDL] and oxidatively modified LDL [ox-LDL]) on the activation of mesangial cell p42 mitogen-activated protein kinase (MAP kinase), a key intracellular signaling mechanism associated with cell proliferation. The incubation of mesangial cells with either LDL, mm-LDL, or ox-LDL induced the activation of MAP kinase dose dependently. The activation of MAP kinase by these lipoproteins in mesangial cells occurred biphasically: initially at 15 min of incubation period and at later time points of 8 to 24 h. No activation of MAP kinase was noted between 30 min (except in LDL) and 6 h. The induction of MAP kinase by both mm-LDL and ox-LDL was greater by 1.5- to 2-fold when compared with LDL. Similarly, these atherogenic lipoproteins stimulated mesangial cell proliferation. Lysophosphatidylcholine, a component of both oxidatively modified variants of LDL, markedly stimulated mesangial cell MAP kinase activity at early incubation times (5 to 30 min) but not at later time points (3 to 24 h), suggesting that lysophosphatidylcholine may, at least in part but not solely, act as an active component of ox-LDL-mediated effects. These data define putative key signal transduction events associated with lipoprotein-mediated induction of mesangial cell proliferation. (J Am Soc Nephrol 9: 488–496, 1998)

Disturbances in lipid and lipoprotein metabolism have been recognized to play a central pathobiological role in the development of glomerular injury both in experimental animals and humans (1 and references therein). The evidence linking the role of atherogenic lipoproteins and glomerular injury was primarily obtained from experimental animals, in which either cholesterol-feeding or endogenous hyperlipidemia provoked the development of glomerular injury and glomerulosclerosis (1 and references therein and reference 2). In addition, specific lipid-lowering drugs or a modified lipid diet could prevent or reverse the development of glomerulosclerosis (1 and references therein). Although increased systemic concentrations of atherogenic lipoproteins (e.g., LDL) have long been recognized as one of the metabolic abnormalities associated with renal disease, recent studies have indicated a pathobiological accumulation of lipids and apolipoprotein B-containing lipoproteins (e.g., LDL) within the glomerulus of diverse renal diseases (3,4). Furthermore, recognizing the in vitro ability of mesangial cells to oxidatively modify LDL, it has been suggested that the LDL that accumulated within the mesangium may undergo in vivo oxidative modification (5,6) and may initiate multiple cellular and molecular events associated with glomerular injury (7). Because few cells accumulate in the mesangium during the early stages of glomerular injury, LDL entrapped in the mesangium would generally undergo minimal oxidative modification. This minimal oxidative modification of LDL (mm-LDL) may occur initially and serve as a more pathophysiological modulator of vascular and glomerular responses compared with highly oxidized LDL (ox-LDL) (8). Although no in vivo accumulation of mm-LDL compared with ox-LDL has been reported in vascular or glomerular tissues, recent studies have shown that the mm-LDL (prepared in vitro) could induce the expression of monocyte chemoattractant peptides in various tissues, including kidney of mice and in cultured vascular and glomerular cells (8–10). Recent studies have shown the accumulation of ox-LDL within the rat glomeruli, with focal glomerulosclerosis induced by both puromycin aminonucleoside and high dietary cholesterol (11). Increased circulating concentrations of lipid peroxidation products associated with decreased levels of endogenous anti-oxidative factors were also observed in patients with chronic renal failure (12,13), thus providing a favorable environment for oxidation-related events.

Recently, we and others have provided evidence of cellular and molecular mechanisms by which atherogenic lipoproteins may induce the development of glomerular injury. These studies showed that the activation of glomerular endothelial and
mesangial cells with LDL and, with greater activity, oxidatively modified variants (mm-LDL and ox-LDL) stimulated the synthesis of adhesion molecules and monocyte chemotactic peptides associated with monocyte adhesion to the glomerular capillary wall and infusion in the mesangium, characteristic early features of glomerulosclerosis (14–17). Furthermore, LDL has been shown to stimulate the proliferation of mesangial cells (6,18), suggesting that atherogenic lipoproteins, retained within the mesangium, may serve as endogenous pathobiological activators inducing cellular events associated with glomerular injury.

The ability of active components of LDL or, more importantly, its oxidatively modified forms to stimulate various pathobiological processes is not clearly understood. However, increased formation of lyosphosphatidylcholine (lysoPC) from phosphatidylcholine (PC) of LDL is consistently seen in either cellular- or free radical-mediated generated ox-LDL (19–24). The lysoPC content is increased by approximately 10- to 15-fold in ox-LDL compared with native LDL. The elevated levels of lysoPC in ox-LDL were shown to modulate endothelium-dependent relaxation, vascular endothelin-1 synthesis, and endothelial cell motility, characteristic features of endothelial dysfunction associated with vascular diseases (20–23,25). On the basis of these studies, lysoPC, at least in part, has been implicated as one of the known active components of ox-LDL that induces cellular responses. However, other yet unidentified component(s) of ox-LDL may perhaps have a greater role in multiple effects of ox-LDL.

Although the major signal transduction events associated with lipoproteins have been attributed to the stimulation of protein kinase C (PKC) and increased generation of cAMP (26–28), intracellular signaling processes by atherogenic lipoproteins in mesangial cells are unknown. Furthermore, the involvement of lipoprotein-induced intracellular phosphorylation signaling mechanisms associated with cell proliferation has not been proven. Activation of the mitogen-activated protein kinase (MAP kinase) cascade has been considered one of the major intracellular signaling pathways that integrate and transmit mitogenic stimuli-mediated transmembrane signals from cytoplasm to nucleus involved in cell proliferation (29 and references therein). MAP kinases are serine threonine cytoplasmic kinases and are activated by mitogenic stimuli through the phosphorylation of tyrosine and threonine residues (29). Activated cytoplasmic MAP kinases have been shown to translocate into the nucleus and activate various transcription factors and proto-oncogenes associated with cell growth and proliferation. In this study, we examined the role of LDL, oxidatively modified variants of LDL, and lysoPC (an active component of oxidized LDL) in the activation of mesangial cell MAP kinase.

Materials and Methods

Materials

Goat polyclonal anti-mouse p42 MAP kinase was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). [32P]ATP and [3H]thymidine were obtained either from Amersham (Arlington Heights, IL) or DuPont New England Nuclear Research Products (Boston, MA). Protein A-Sepharose, myelin basic protein (MBP), fetal bovine serum, lysoPC, PC (palmitoyl and oleyl), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Lipoprotein Isolation and Modification

Blood samples were collected from healthy human volunteers (pooled from four to five subjects at each time of isolation). During the study, LDL was isolated six to eight times, and a total of 10 volunteers donated blood for LDL isolation used in this study. Serum was isolated by centrifugation; a preservative mixture containing antibiotics, antioxidents, and protease inhibitors was added to the serum to avoid lipoprotein modification; and LDL was isolated as described earlier (9,15). mm-LDL was prepared by dialyzing native LDL (without preservatives) against 10 μM FeSO4 for 92 h at 4°C. ox-LDL was prepared by incubating native LDL with 100 μM CuSO4 in Ham’s F-10 media for 24 h at 37°C. The modification in mm-LDL was examined spectrophotometrically by measuring the conjugated diene formation at 232 nm. The extent of LDL oxidative modification and the purity of LDL were assessed by examining its migration using agarose gel electrophoresis. Protein content of lipoprotein was measured by Lowry’s method, using bovine serum albumin as a standard (30). During the isolation of LDL and its modification, standard precautions were taken to minimize the contamination of endotoxin in lipoprotein preparations. For example, unused pyrogen-free tubes, glasswares that were oven-dried at 180°C, and autoclaved salt solutions or phosphate-buffered saline were used to minimize endotoxin contamination during the preparation of lipoproteins. The endotoxin concentrations (determined by limulus amebocyte lysate assay kit; BioWhittaker, Walkersville, MD) in LDL preparations were undetectable. However, using 100- to 200-fold dilution of endotoxin standard, negligible amounts of endotoxins in the range of 0.05 to 0.1 pg/μg LDL were seen in LDL samples. The minimum concentration of endotoxin required to stimulate mesangial cells and other cells was in nanogram quantities, and hence the lipoprotein effects would be independent of endotoxin-mediated responses (data not shown).

Cell Culture and Treatment

The murine mesangial cells used in these studies (simian virus 40 transformed, mes-13) were obtained from American Type Culture Collection (Rockville, MD). Mesangial cells (5 × 10³/well in 6-well plates) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% fetal bovine serum for 24 h to attain approximately 80% confluence. After 24 h of incubation of cells with serum-free DMEM, the medium was replaced by fresh serum-free DMEM, and cells were incubated with either LDL, ox-LDL, or mm-LDL (5 to 50 μg/ml) for 15 min to 24 h. Similarly, quiescent mesangial cells were incubated with either lysoPC or PC (10 μM) for 15 min to 24 h. After the incubation, the medium was removed and the cells were washed with 1 ml of cold Hanks’ balanced salt solution and scraped into 0.5 ml of MAP kinase lysis buffer (50 mM Hepes, pH 7.5, 100 mM NaCl, 2 mM ethylenediamine tetra-acetic acid, 1 mM vanadate, 40 mM paranitrophenyl phosphate, 1 μM pepstatin, 1 μg/ml leupeptin, 2 μg/ml aprotonin, and 1% NP-40). After a brief centrifugation, the supernatant (cell lysate) was aliquoted and stored at −80°C for not more than 3 d before assaying MAP kinase.

Partial Purification of MAP Kinase and Assay

MAP kinase activity in the cell lysate was determined by an assay, using partially purified MAP kinase prepared by a phenyl-Sepharose resin method (31). Partially purified cellular MAP kinase was incubated with MBP and [32P]ATP at 37°C for 20 min. Phosphorylated
MBP was separated by its adsorption to phosphocellulose discs, and the extent of phosphorylation was determined by measuring the radioactivity contained in the discs.

**Immunoprecipitation and Assay of p42 MAP Kinase**

MAP kinase in cell lysate (20 to 30 μg) was immunoprecipitated using goat polyclonal anti-p42 MAP kinase antibody (0.4 μg) and protein A-Sepharose (50%). After washing, the kinase activity of the immune complex was assayed with MBP as a substrate in a reaction mixture containing 7.5 mM Hepes, pH 7.5, 10 mM magnesium acetate, 50 μM ATP, and 4 μCi [32P]ATP in a total volume of 40 μl (32). The reaction was performed at 30°C for 20 min, and the phosphorylated MBP was either separated by a phosphocellulose disc method or taken in 20 μl of 3X Laemmli sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For SDS-PAGE, the contents were heated at 80°C for 5 min and were resolved by PAGE (10%). The gel was stained with Coomassie blue, dried, and exposed to x-ray film for 1 to 4 h. The MBP bands were then cut out, and the radioactivity was measured by liquid scintillation spectrometry. MAP kinase activity was expressed as the incorporation of picomoles of phosphate into MBP per milligram of cell lysate protein.

**Mesangial Cell Proliferation**

Quiescent mesangial cells were incubated with various concentrations of LDL, mm-LDL, or ox-LDL (0 to 20 μg/ml) for 24 h. Six hours before harvesting, cells were pulsed with 1.5 μCi of [methyl-3H]thymidine. Cells were washed (3 times) with phosphate-buffered saline and digested with 0.5N NaOH. An aliquot of cell digest was used to measure radioactivity as an index of cell DNA synthesis and proliferation.

**Statistical Analyses**

Results are presented as mean values ± SEM for three to four separate experiments. A t test was used to compare the means, and a P value of less than 0.05 was considered significant.

**Results**

Previous studies have shown similar morphologic and functional characteristics between transformed murine mesangial cells, used in this study, and primary cultures of murine mesangial cells (9,15,33,34). In this study, we examined the effect of native LDL, mm-LDL, ox-LDL, and lysoPC (an active component of ox-LDL) on the activation of MAP kinase by mesangial cells at various doses and time points. Both partial purification and immune complex kinase assays were used as standard methods to measure MAP kinase activities. These assays were validated by showing increased MAP kinase activity in cells treated with platelet-derived growth factor (PDGF) and epidermal growth factor over control by 103 and 370%, respectively. The activities of both p44 and p42 MAP kinase isoforms were initially determined by immune precipitation and in vitro kinase assay. However, in these cells, basal levels of p44 MAP kinase activity were found to be severalfold lower than basal levels of p42 MAP kinase (data not shown), and p44 MAP kinase assay would not be sensitive enough to assess alterations in activity. Because of these findings, we limited our studies to p42 MAP kinase.

The incubation of mesangial cells with either LDL, mm-LDL, or ox-LDL (10 μg/ml) for various time points (15 min to 24 h) indicated a biphasic effect to stimulate MAP kinase activity (Figure 1). The stimulation of cells with native and oxidatively modified variants of LDL for 15 min induced the activation of MAP kinase by 125 to 150% when compared with respective controls (Figure 1). At 15 min of stimulation, the effect of mm-LDL to induce MAP kinase was greater than that of either LDL or ox-LDL. The incubation of mesangial cells with lipoproteins for 30 min to 6 h did not induce MAP kinase activity (Figure 1, data not shown for time points 1, 1.5, and 6 h). However, the stimulation of cells with lipoproteins for 8 and 12 h markedly induced the activation of MAP kinase (Figure 1). At 24 h of incubation, mm-LDL, but not LDL and ox-LDL, retained stimulatory effects on MAP kinase. In early (15 min) and late (8 to 12 h) time points, the effect of mm-LDL to induce MAP kinase activity was higher than that of LDL. ox-LDL showed greater induction of MAP kinase when compared with LDL at late time points of 8 to 24 h. LDL isolated from different healthy individuals (pooled from four to five subjects, and six to eight different LDL preparations of a total of 10 subjects) gave a similar induction of MAP kinase activity (data not shown). Additional studies were performed to examine the possibility of mild LDL oxidation by mesangial cells in culture and effects on LDL-induced MAP kinase activity. The incubation of mesangial cells with LDL (10 μg/ml) in the presence of butylated hydroxytoluene (20 μM, a known inhibitor of cell-mediated LDL oxidation; references 19 and 21) for 15 min or 8 h did not alter LDL-induced MAP kinase activation, suggesting the specific effects of LDL rather than from oxidative modification (data not shown). A dose–response study showed that the incubation of mesangial cells with either LDL, mm-LDL or ox-LDL induced MAP kinase activity dose dependently (10 to 25 μg/ml), and the degree of induction of MAP kinase at a higher dose of lipoprotein (50 μg/ml) was lower than that of 25 μg/ml concentration (Figure 2). The MAP kinase activity at 100 μg/ml of mm-LDL was similar to the 50 μg/ml concentration, and higher than 100 μg/ml mm-LDL showed moderately decreased effects (data not shown). The induction of MAP kinase by both mm-LDL and ox-LDL (10 to 25 μg/ml) was approximately 1.5-fold higher than that of LDL.

In additional experiments, immunoprecipitation of p42 MAP kinase and kinase assay were done to measure MAP kinase activity to confirm results obtained by the partial purification method. The incubation of mesangial cells with LDL, mm-LDL, and ox-LDL increased the phosphorylation of MBP as an index of increased MAP kinase activity (Figure 3). The relative quantitative analysis by densitometric scanning of autoradiogram indicated the following arbitrary values: for control and LDL doses of 5 and 10 μg/ml: 0.30, 0.37, and 0.4; for control and mm-LDL doses of 5 and 10 μg/ml: 0.30, 0.40, and 0.60; and for control and ox-LDL doses of 5 and 10 μg/ml: 0.61, 0.91, and 0.90, respectively. To determine the association of lipoprotein-induced MAP kinase activation with cell proliferation, we examined the effect of LDL, mm-LDL, and ox-LDL on 3H-thymidine incorporation into mesangial cell DNA as an index of cell proliferation. The results indicated that the incu-
Figure 1. Incubation time response of the effect of LDL, mildly oxidatively modified LDL (mm-LDL), and oxidatively modified LDL (ox-LDL) on mitogen-activated protein kinase (MAP kinase) activity. Quiescent mesangial cells were incubated with lipoproteins (10 μg/ml) for various time points (15 min to 24 h). After the incubation, cell monolayers were washed and lysed in a lysis buffer. MAP kinase activity in cell lysate was determined by a kinase assay, using myelin basic protein (MBP) (as a substrate) and partially purified MAP kinase as described in Materials and Methods. The data are presented as percent induction of MAP kinase activity over control. Data are shown as the mean ± SEM of four experiments.
Figure 2. Dose–response of the effect of LDL, mm-LDL, and ox-LDL on MAP kinase activity. Quiescent mesangial cells were incubated with various concentrations of lipoproteins (10 to 50 μg/ml). After the incubation, cells were washed, lysed, and MAP kinase activity was determined by the partial purification method, as described in Figure 1 and in Materials and Methods. MAP kinase activity is shown as picomoles of phosphate incorporation into MBP per milligram of cell lysate protein. Data are shown as the mean ± SEM of three experiments.

Figure 3. Representative autoradiogram showing the effect of atherogenic lipoproteins on MBP phosphorylation as a measure of mesangial cell MAP kinase activity. Quiescent mesangial cells were incubated with either LDL, mm-LDL, or ox-LDL (5 and 10 μg/ml) for 24 h. The cells were washed and extracted with MAP kinase lysis buffer. p42 MAP kinase was immunoprecipitated using polyclonal anti-p42 MAP kinase antibody and protein A-Sepharose. The kinase activity of the immune complex was assayed with MBP and [32P]ATP as described in Materials and Methods. The phosphorylated MBP was separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the gel was stained, dried, and exposed to x-ray film for 1 to 4 h. The blot is representative of three separate experiments.

not clearly understood. Using murine mesangial cells as an in vitro model system, we examined the effect of human plasma LDL and its oxidatively modified forms (mm-LDL and ox-LDL, exhibiting greater atherogenicity) on the activation of p42 MAP kinase, a key intracellular signal that converges growth regulatory transmembrane signals from cytoplasm to nucleus involved in cell proliferation.

Although the use of a homologous model system (i.e., LDL and target cells from the same species) would be a better experimental model to understand the pathobiological role of LDL, the use of human plasma LDL in in vitro cell cultures derived from mammalian species, including mouse cells has been widely referenced in the current biomedical literature. Human and other mammalian (e.g., mouse) LDL and apo B (the major protein of LDL) share extensive homology with respect to chemical and physical characteristics, apo B DNA sequences, and LDL receptor binding domain (35–37). Additional studies indicated that administration of human plasma LDL (its modified forms) to mice caused an increase in serum colony-stimulating activity and monocyte chemotactic protein-1 expression in various murine tissues (including kidney), and an increase in the adhesion of leukocytes to the endothelium of postcapillary venules and arterioles, suggesting the biological activity of human LDL in other mammalian species.
Figure 4. Effect of lysophosphatidylcholine (lysoPC) and phosphatidylcholine (PC) on mesangial cell MAP kinase. Quiescent mesangial cells were incubated with either lysoPC or PC (10 µg/ml) for various time points (5 to 180 min). After incubation, cells were lysed, and MAP kinase activity was measured by the partial purification method as described in Figure 1 and Materials and Methods. MAP kinase activity is shown as picomoles of phosphate incorporation into MBP per milligram of cell lysate protein. Data are shown as the mean ± SEM of three experiments.

We and others have reported that human LDL can stimulate glomerular mesangial cells from various species, including mice, rat, and human (5,18,39,40). Because of these similarities in biological responses of human LDL, the findings of the present investigation regarding the effect of human LDL on murine mesangial cell intracellular signaling events associated with mesangial cell proliferation are relevant to renal disease in man.

In this study, we examined the role of native LDL and oxidatively modified variants of LDL (mm-LDL and ox-LDL, both more atherogenic forms of LDL) on the activation of MAP kinase. Incubation of mesangial cells with LDL, and with greater potency, mm-LDL and ox-LDL, stimulated the activation of MAP kinase, as assessed by using both partial purification and immunoprecipitation of p42 MAP kinase assay. Although the effects of native LDL may be anticipated from some degree of LDL oxidation in the presence of mesangial cells, the use of antioxidants and protease inhibitors during LDL isolation and incubation with mesangial cells should have prevented oxidative modification of LDL during the experimental conditions. Furthermore, LDL samples, obtained after incubation with mesangial cells, failed to show evidence of either minimal or oxidative modification as assessed by conjugated diene formation or electrophoretic mobility on agarose gels, suggesting the inability of LDL to oxidatively modify in cultures of mesangial cells. Additional studies indicated that the incubation of mesangial cells with LDL (10 µg/ml) in the presence of butylated hydroxytoluene (20 µM, a known inhibitor of cell-mediated LDL oxidation; references 19 and 21) for 15 min or 8 h did not alter LDL-induced MAP kinase activation, suggesting that the observed effects of LDL on MAP kinase are specific for LDL rather than from mild oxidative modification of LDL. The increased MAP kinase activity in lipoprotein-treated mesangial cells correlated to the increased cell proliferation, suggesting the association of MAP kinase cascade activation in lipoprotein-mediated mesangial cell proliferation. However, ox-LDL showed a lesser degree of mesangial cell proliferation compared with LDL and mm-LDL; ox-LDL, at >20 µg/ml concentration, exhibited inhibitory effects on cell proliferation. Using human and rat mesangial cells, previous studies showed inhibitory effects of ox-LDL on 3H-thymidine incorporation (5,6,18,41). However, both LDL and ox-LDL were shown to stimulate human aortic smooth muscle cell proliferation and PDGF expression (42). This variance in cell proliferation in response to ox-LDL in transformed murine mesangial cells and other cells may be multifactorial, including differences in species, the extent of oxidation of LDL, and doses of ox-LDL used in different studies.

Although these studies suggest the role of atherogenic lipoproteins in signaling events associated with mesangial cell proliferation, the specific component(s) of mm-LDL or ox-LDL to modulate MAP kinase is not clearly known. Because the dose–response of exogenous lysoPC on MAP kinase correlates with increased lysoPC content in ox-LDL, it is possible...
that the increased lysoPC abundance in ox-LDL may, at least in part, be associated with ox-LDL-induced MAP kinase activation. Because lysoPC at longer time incubation (3 to 24 h) had no stimulatory effects on MAP kinase activation, other yet unidentified components of ox-LDL may be involved in ox-LDL-mediated prolonged time activation of MAP kinase. Similarly, the effect of mm-LDL to stimulate MAP kinase could involve other yet unidentified components. Previous studies have shown that mm-LDL exhibits a moderate increase in thiobarbituric acid-reactive substances (TBARS), hydroperoxides, and lysoPC, and a marked increase in cholesterol-α-epoxides (8). These authors have shown that the polar lipid fraction of mm-LDL (e.g., oxidized phospholipids) and cholesterol-epoxides are major active components of mm-LDL that stimulate monocyte adhesion to endothelial cells and monocyte chemotaxis. Thus, it is likely that these and other unidentified components of mm-LDL may be involved in MAP kinase activation. Furthermore, because the time kinetics of the generation of specific components of mm-LDL or ox-LDL may vary and are not clearly characterized, it would be difficult to demonstrate a dose-response relationship between the oxidation of LDL and its effects. In this regard, Kusuhara et al. (43) recently examined the correlation between the extent of LDL oxidative products and MAP kinase activity in vascular smooth muscle cells. These authors used ox-LDL prepared by different techniques (including hypochlorite-mediated oxidation [that preferentially modifies protein component] and CuSO₄-induced and soybean lipoxygenase-mediated oxidation [which preferentially oxidize lipid components]), which generated differential amounts of oxidative products implicated in multiple pathobiological responses. Comparative correlation tests indicated that there was not a good correlation between lipid peroxidation, including TBARS and lipid peroxide content, and MAP kinase activation in smooth muscle cells. Thus, it was suggested that the increased levels of aldehydes (TBARS) or lipid peroxides may not be likely primary candidates for alterations in MAP kinase. Rather, other yet unidentified component(s) of LDL that increase during their oxidative modification are more likely to be involved in MAP kinase and related events. Additional studies are required to characterize specific components of mm-LDL or ox-LDL that are involved in the activation of MAP kinase.

The cellular and molecular processes involved in atherogenic lipoprotein-mediated induction of MAP kinase may be multifactorial and may involve multiple mechanisms. One possibility is that the ability of these atherogenic lipoproteins to induce PDGF in mesangial cells may contribute to the autophosphorylation of PDGF receptors and subsequent downstream cytoplasmic activation of the MAP kinase cascade. Previously, we have shown that the increased expression of mesangial cell PDGF by atherogenic lipoproteins occurred between 1 and 4 h and that the PDGF message was at a basal level between the early time points of 15 to 30 min and 24 h of incubation (39). Because both LDL and oxidized variants of LDL biphassically stimulated MAP kinase (at 15 min and 8 to 24 h) and because exogenous PDGF can activate mesangial cell MAP kinase within minutes (5 to 30 min; by 12 to 24 h, activity returned to basal levels; reference 44), it is unlikely that the effects of lipoproteins on MAP kinase would be mediated through the increased expression of PDGF. Another possibility is that atherogenic lipoproteins, or their active components, may by some mechanism induce transmembrane protein tyrosine kinase activity and/or activate PKC pathways, thus stimulating the MAP kinase cascade. In this regard, we have shown that lysoPC, an active component of oxidatively modified LDL (e.g., mm-LDL and ox-LDL), markedly stimulated mesangial cell MAP kinase activity. The time sequence of generation of lysoPC within the LDL molecule during its oxidative modification and release is not clearly understood. The observed biphasic effects of ox-LDL or mm-LDL could be mediated by either lysoPC and/or other oxidative lipid or protein products generated at later time points. In our previous studies and studies that are currently under way, we have found that both LDL and, to a higher degree, mm-LDL stimulated mesangial cell transmembrane protein tyrosine phosphorylation (40 to 200 kD) and increased total protein tyrosine kinase activity (45). Furthermore, apo B-100, the major apolipoprotein in LDL, has Src-homology (SH1) domains that are kinase-activating regions for protein tyrosine kinase (46). Thus, the effect of LDL and its modified forms to stimulate MAP kinase may be dependent on upstream protein tyrosine kinase-mediated signaling processes. Because LDL and its oxidized forms are known to stimulate phosphoinositide turnover and PKC activation, it is conceivable that the activation of PKC, through Raf phosphorylation and activation, may stimulate LDL-mediated MAP kinase. Recent studies indicated that ox-LDL-induced MAP kinase activation in vascular smooth muscle is dependent on the activation of PKC (43). Although we do not have data for the involvement of PKC in LDL or ox-LDL-induced MAP kinase in mesangial cells, our previous studies showed that both protein tyrosine kinase and PKC are involved in lysoPC-induced MAP kinase activation in mesangial cells (47). The increased activation of MAP kinase by atherogenic lipoproteins may provoke downstream cytoplasmic and nuclear intracellular signaling events leading to nuclear proto-oncogene expression and mesangial cell proliferation.

In summary, we have provided direct evidence that both LDL and, with greater potency, oxidatively modified variants of LDL and their active component lysoPC stimulated the activation of mesangial cell MAP kinase. This observation strongly suggests a key signal transduction event involved in lipoprotein-induced mesangial cell proliferation. Additional studies are warranted to identify active components of lipoproteins, their involvement in events occurring upstream of MAP kinase, and the nuclear processes associated with atherogenic lipoprotein-mediated cell proliferation.

Acknowledgments

This study was supported by a Merit Review from the Department of Veterans Affairs and an Institutional Fellowship from the National Kidney Foundation of Southern California. We thank Dr. Rama Pai for technical assistance in proliferation assays.
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