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Activation of the p38 MAP kinase pathway is required for foam cell formation from macrophages exposed to oxidized LDL

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Endocytosis of oxidized low density lipoproteins (oxLDL) by macrophages, mediated by scavenger receptors, is thought to play a central role in foam cell formation and, thus, in the pathogenesis of atherosclerosis. OxLDL activates several MAP kinases, including the ERK, JNK and p38 MAP kinases, but the role of these activations in oxLDL uptake has not been studied. In the present investigation, we find that SB203580, a specific inhibitor of p38, blocks oxLDL-exposed J774 cells from becoming foam cells. Inhibition of foam cell formation by blockade of the p38 pathway is, at least in part, due to inhibition of oxLDL-induced up-regulation of the scavenger receptor CD36. Using pharmaceutical inhibitors and dominant active MAP kinase kinases, we demonstrated that activation of the p38 pathway, but not the ERK or JNK pathways, is necessary and sufficient to transactivate PPARγ, a nuclear receptor that has recently been shown to play a pivotal role in oxLDL-induced CD36 expression. Our results for the first time demonstrate a regulation of CD36 by p38, and the importance of the p38 pathway in regulation of foam cell formation.

Key words: Foam cell; oxidized LDL; p38 mitogen-activated protein kinase.

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Abbreviations:
ATF2, activating transcription factor 2; CD36, scavenger receptor B; DR-1, direct repeat with one nucleotide spacer; ERK, extracellular signal-regulated protein kinase; GST, glutathione S-transferase, JNK, c-Jun amino-terminal kinase; LBD, ligand-binding domain; MAP, mitogen-activated protein; MKK, MAP kinase kinase; mPPARγ, mouse peroxisome proliferator-activated receptor gamma; nLDL, native low-density lipoprotein, oxLDL, oxidized low-density lipoprotein; SR, scavenger receptor; TK, thymidine kinase.

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Atherosclerosis is a leading cause of morbidity and mortality in developed countries. The formation of atherosclerotic lesions involves endothelial cells, monocytes/macrophages, smooth muscle cells, and a regulatory network of growth factors and cytokines (1). Deposition of cholesterol esters within artery cells, especially in macrophages, converting them into foam cells, is fundamental to the pathogenesis of atherosclerosis (2, 3). A better understanding of the mechanisms by which monocytes/macrophages accumulate low-density lipoprotein (LDL)-derived cholesterol is urgently needed. Oxidation of LDL is believed to play a signifi-

cant role in foam cell formation. Oxidized LDL (oxLDL) is involved in the induction of adhesion molecules, chemokines, and many other inflammatory molecules. OxLDL is also a potent chemoattractant for monocytes, which are drawn to, and accumulate in, the subendothelium where monocyte-derived macrophages subsequently develop into lipid-laden foam cells (4). The uptake and deposition of oxLDL within cells are believed to be the causes of foam cell formation. Cultured macrophages can take up chemically modified LDL, such as oxLDL and acetyl LDL (acLDL), and develop foam cell-like morphology. This cell system is widely used to study the molecular mechanisms of foam cell formation (2, 3).

It is well known that scavenger receptors (SR) are responsible for the uptake of oxLDL (5). A number of these, which specifically bind oxLDL, have been identified on the surface of macrophages (6–11). Recent data suggest important roles for the SR-A and CD36 (SR-B) receptors in the pathogenesis of atherosclerosis, since monocytes derived from mice deficient in SR-A, or from humans lacking functional CD36, exhibit significantly decreased uptake of oxLDL (12, 13). Resting macrophages do not express a significant amount of CD36 and, therefore, induction of CD36 may be a very important event for oxLDL uptake.

Multiple intracellular signal pathways have been reported to be activated by oxLDL. Intracellular Ca^{2+} and NF-κB activity is altered during oxLDL treatment. Recent investigations, using smooth muscle cells, have demonstrated that the ERK and p38 MAP kinases are activated by oxLDL stimulation (14, 15). However, the consequences of these signaling events have not been fully elucidated. Recent studies by Evans' group showed that the peroxisome proliferator-activated receptor γ (PPARγ) is involved in oxLDL-induced cellular changes, and oxLDL-induced CD36 expression is mediated by PPARγ-dependent transcription (16). Whether this transcriptional event is linked with kinase cascades has not been addressed.

PPARγ is a transcription factor which belongs to the steroid hormone receptor superfamily. Initially, PPAR were discovered as mediators of peroxisome proliferation induced by hypolipidemic drugs of the fibrate class (17). Later, natural fatty acids were also found to activate these receptors. This suggested a novel concept for the regulation of cellular lipid metabolism (18). PPARα and PPARγ regulate two separate, yet linked, arms of lipid homeostasis, namely the catabolism of lipids (PPARα) and their storage (PPARγ). PPARγ is expressed by both monocytes/macrophages and foam cells in atherosclerotic plaques (16). Through inherent peroxisome proliferator-activated response elements (PPAREs), PPARγ downregulates the transcription of proinflammatory cytokines, induces the expression of proatherogenic proteins, such as the metalloproteinase MMP-9 (gelatinase B) and the scavenger receptor CD36 (16, 19–21). It also downregulates the activity of inducible nitric oxide synthetase (iNOS), a proinflammatory enzyme (16, 22). Moreover, PPARγ is also activated by oxLDL and hydroperoxy fatty acids (16).

Mitogen-activated protein (MAP) kinases play an important role in many cellular processes, such as proliferation, apoptosis, and adaptation to changes in the extracellular environment (23–26). At least three major groups of MAP kinases have been identified in mammalian cells so far: (i) extracellular signal-regulated kinase (ERK), (ii) c-Jun N-terminal kinase (JNK) or stress-activated protein kinase (SAPK), and (iii) p38 MAP kinase. The ERK pathway is preferentially activated by growth-related stimuli, while the JNK and p38 pathways are often linked with cellular stress. All of these MAP kinases can, however, be activated by oxidative stress in a variety of cells.

In recent years we have been interested in the biological consequences of p38 activation. It has been demonstrated that p38 can directly or indirectly target various proteins to control transcription and translation in a cell-specific manner (27). Using SB203580, a highly specific inhibitor of the p38 MAP kinase which is effective both in vitro and in vivo (IC_{50} for J774 cells is 0.5 1.0 μM), many different genes were found to be regulated through the p38 signaling pathway (28–32). Given the established role of p38 in gene regulation, we explored whether p38 might serve a similar function in the oxLDL-stimulated transformation of macrophages to foam cells. We found that p38 is activated by oxLDL, and that such activation is required for oxLDL-induced CD36 expression and foam cell formation. A mechanism underlying this process

is that p38 can, either directly or indirectly, regulate PPAR-dependent transcription. Thus, our study demonstrated an interaction between a nuclear receptor and the MAP kinase pathway.

MATERIALS AND METHODS

Materials

Chemicals were purchased from Sigma (St. Louis, MO, USA), unless otherwise indicated. Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 medium were from GibcoBRL (Grand Island, NY, USA). PD98059 and SB203580 were from Calbiochem (La Jolla, CA, USA). Luria broth was purchased from BIO 101 (La Jolla, CA, USA). FITC-conjugated monoclonal antibodies against CD36 were from Pharmingen (San Diego, CA, USA), while DOTAP was from Boehringer Mannheim (Indianapolis, IN, USA). Recombinant protein G agarose was from GibcoBRL (Grand Island, NY, USA). Anti-ERK1 polyclonal antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and glutathione-sepharose 4B beads for glutathione S-transferase (GST) fusion protein purification were from Amersham Pharmacia Biotech (Stockholm, Sweden). *Escherichia coli* BL21 (DE3) were from Novagen (Madison, WI, USA). The plasmid −273/luc, −263/luc, UAS-TK-luc, GAL4-mPPARγ-LBD, and CMXβ-gal were kind gifts from Dr. M. Evans (the Salk Institute for Biological Studies, La Jolla, CA, USA). The −273 to +47 and −263 to +47 of the CD36 promoter were cloned into the luciferase reporter vector pGL-BASIC (Fig. 4A). The UAS-TK-luc is a reporter gene containing four copies of a GAL4-binding site. GAL4-mPPARγ-LBD is a chimeric receptor expression plasmid in which the LBD of mPPARγ is fused to the DNA-binding domain of the yeast transcription factor GAL4 (Fig. 5A). CMXβ-gal, used as a control for transfection efficiency, is an expression plasmid of β-galactosidase (16, 33). The plasmids pcDNA3-MKK6b(E), pcDNA3-MKK7(D), and pcDNA3-MKK1(E), GAL4-ELK1, GAL4-MEF2A, GAL4-ATF2 were described previously (34). The GAL4-responsive plasmid pG5E1bLuc contains five GAL4-binding sites cloned upstream of a minimal promoter driving a luciferase gene (35).

Cell cultures

THP-1 and J774 cells, both from the American Type Culture Collection (ATCC, Manassas, VA, USA), were maintained in RPMI 1640 medium, while CV-1 cells (ATCC) were grown in DMEM culture medium. Both media were supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin, and 1% non-essential amino acids.

Preparation of recombinant protein

The GST fusion protein of activating transcription factor 2 (ATF2), used as a substrate for p38 and JNK kinases, was prepared as described previously (36). *Escherichia coli* BL21(DE3) were transformed with a pGEX vector containing cDNA encoding GST-ATF2. The bacteria were grown in Luria broth at 37°C until A_{600}=1.0, at which time isopropyl-D-thiogalactopyranoside (final concentration 1 mM) was added for another 2 h. *E. coli* from 100 ml growth medium were collected by centrifugation (8,000 x *g*, 10 min), the pellet was resuspended in 5 ml phosphate-buffered saline (PBS, pH 7.4), and the bacteria were lysed by sonication using an Ultrasonics cell disrupter (W-375). Insoluble material was then removed by centrifugation (10,000 x *g*, 30 min), and the supernatant was applied to a glutathione sepharose 4B column (0.5 ml). The column was washed with 10 ml PBS, pH 7.4, and the bound protein was then eluted with 10 mM glutathione in 50 mM Tris-HCl, pH 8.0. The expressed GST-ATF2 was soluble, when analyzed by SDS-PAGE, and the protein was >90% pure.

Preparation of oxLDL

Human LDL (1.025 g/ml<d<1.050 g/ml) was isolated by sequential ultracentrifugation (37). It was dialyzed against PBS and exposed to UV-C (254 nm) light for 8 h (38). The degree of oxidation was then measured by assessing its electrophoretic mobility on a 1% agrose gel. Lipoprotein protein was measured according to Lowry et al. (39).

Immunoprecipitation and kinase assays

The ERK1, JNK2, and p38 MAP kinases were immunoprecipitated from THP-1 cells in immunoprecipitation buffer (20 mM Tris-HCl, 120 mM NaCl, 10% glycerol, 1 mM Na_3VO_4, 2 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, pH 7.5). The cells had been grown in the presence or absence of oxLDL for different numbers of days. To isolate the antibody-kinase complex, recombinant protein G agarose was added. The in vitro kinase assays were performed essentially as described previously (40). Briefly, the G-protein-agarose immunoprecipitates were washed six times in immunoprecipitation buffer followed by two washes in kinase buffer (25 mM Hepes, pH 7.4, 25 mM β-glycerophosphate, 25 mM $MgCl_2$, 1 mM dithiothreitol, and 0.1 mM Na_3VO_4). The kinase reactions were initiated by addition of MAP kinase substrates (GST-ATF2 for p38 and JNK and MBP for ERK) and 50 μM γ-^{32}P-ATP (15 μCi) in a final volume of 40 μl kinase buffer. The kinase reactions were run at 30°C for 30 min, and the phosphorylated substrates were then separated on a 10% polyacrylamide gel and visualized by autoradiography. Cell lysate protein was measured according to Lowry et al. (39).

Light microscopy

J774 cells, grown on coverslips, were exposed (or not) to oxLDL only, or to oxLDL together with

either SB203580 or PD98059 (a specific inhibitor of MEK1/2). The cells were then examined and photographed using a Leica RS232C photo microscope.

Flow cytometry

Cells were washed with ice-cold PBS containing 0.5% BSA, then incubated on ice with FITC-conjugated anti-CD36 Mabs for 30 min and, finally, analyzed on a Becton-Dickinson FACScan machine using CellQuest software.

Transfection assays

CV-1 cells were subcultured into 12-well plates (5×10^5 cells/well) and grown under standard culture conditions in DMEM supplemented with 10% fetal bovine serum. The cells were transfected (using liposomal delivery by DOTAP according to the manufacturer's directions) with one of the reporter genes, −273/luc, −263/luc, pG5E1bLuc, or UAS-TK-luc in the presence of a chimeric receptor (GAL4-mPPARγ-LBD), together with one of three activated MAP kinase expression vectors. A β-galactosidase expression plasmid (CMXβ-gal) was used as a control for transfection efficiency. When necessary, the expression plasmid was diluted with an empty carrier plasmid (pcDNA3) to ensure equal amounts of DNA during each transfection. After another 6 h, the medium was changed to a complete medium, and cell extracts were prepared 48 h following the transfection.

Statistical analysis

Experiments were repeated three times, and the values are given as arithmetic means±SD. Statistical significance was determined using Student's *t* test. A p value ≤0.05 was considered significant. (*: p≤0.05, **: p≤0.01).

RESULTS

OxLDL activates MAP kinases

Because MAP kinases are involved in many cellular changes in response to stressful extracellular stimuli, and because oxLDL was reported to activate p38 and ERK MAP kinases in smooth muscle cells (14, 41, 42), we explored whether oxLDL can also activate MAP kinases in a human monocytic cell line THP-1. THP-1 cells were treated with oxLDL (100 μg/ml) for different periods of time, and p38, JNK1, and ERK2 were then immunoprecipitated and subsequently used to measure the enzyme activity by an in vitro kinase assay. The recombinant protein GST-ATF2 (1–109) was used as substrate for p38 and JNK, while myelin basic protein (MBP) was used for ERK. As shown in Fig. 1, oxLDL induced activation of the p38, JNK and ERK MAP kinases. p38 and ERK had similar kinetics of activation, which reached a maximum at 24 h and then declined to a basal level of activity at 48 h. JNK had slower kinetics, and the activity was maintained for up to 72 h. Analyses of several independent experiments revealed that the degree of activation for the three kinases is very close (~3- to 5-fold). The different kinetics, however, of the three MAP kinases suggest that their role may be different in oxLDL-induced cellular changes.

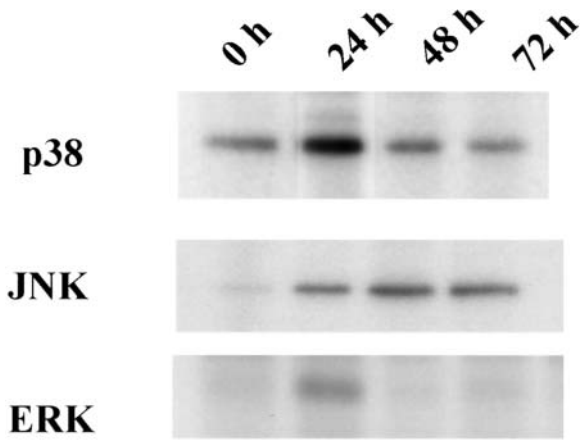

Fig. 1. OxLDL induces MAP kinase activation
The figure shows an autoradiographic estimation of the activity of three different MAP kinases. OxLDL (100 μg/ml) was added to THP-1 cells in complete medium for the indicated periods of time. Cell lysates were then immunoprecipitated with antibodies against p38, JNK, and ERK. The activity of these MAP kinases was finally assayed as described in the Materials and Methods section. The recombinant protein GST-ATF2 was used as a substrate for the p38, and JNK MAP kinases, while MBP was used as a substrate for the ERK MAP kinase. Equal amounts of protein were used.

p38 activation is required for oxLDL-induced foam cell formation

To determine the function of p38 in oxLDL-induced monocytes/macrophages, we evaluated the effect by inhibition of p38 on oxLDL-induced foam cell formation. J774 is a murine macrophage cell line that it was shown can be induced to differentiate to foam cells (43). J774 cells were treated with oxLDL in the presence of different doses of SB203580 and cell morphology was examined at different times. OxLDL induced J774 cells to differentiate to

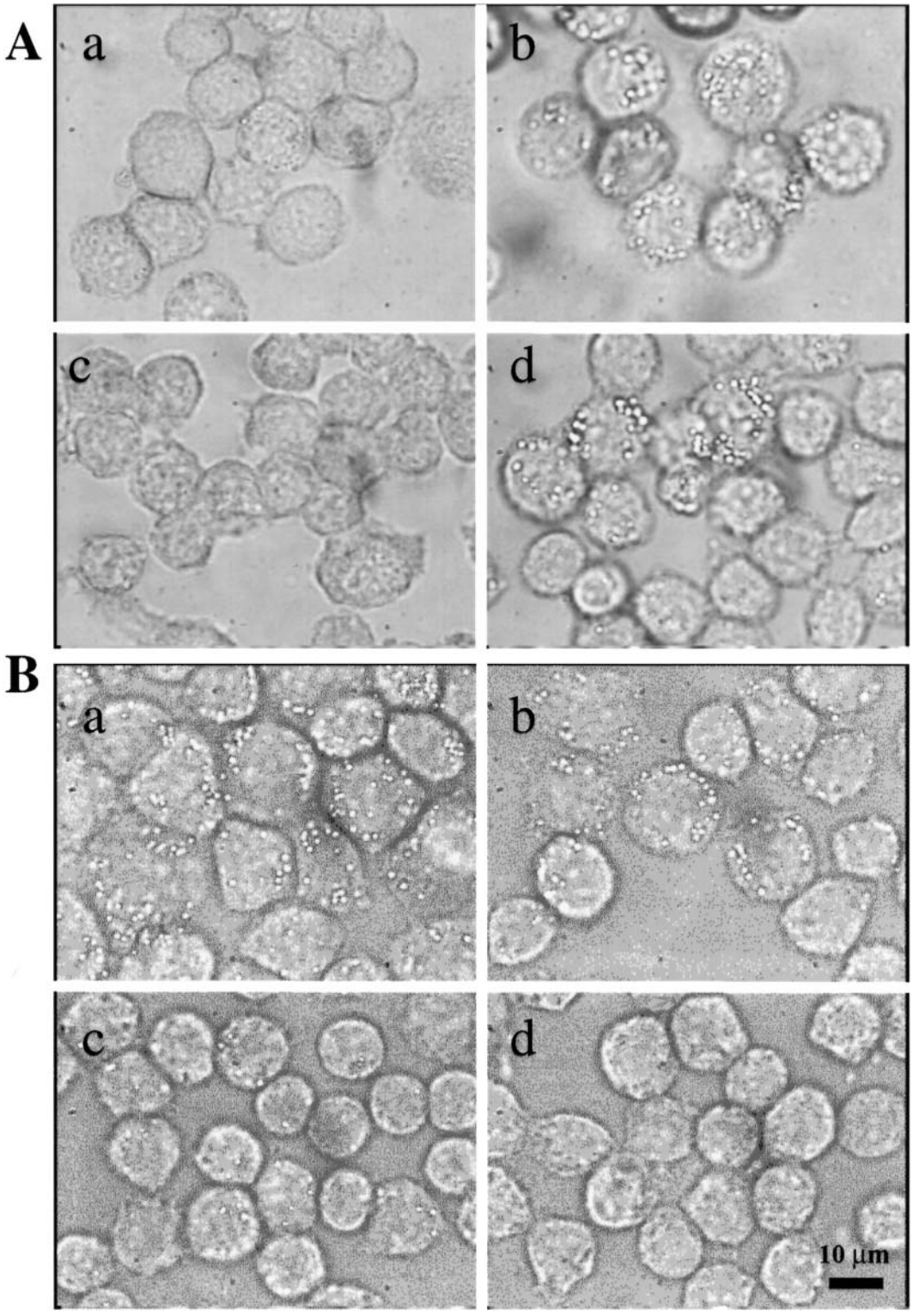

Fig. 2. SB203580 blocks oxLDL-induced formation of foam cells
A, J774 cells were exposed for 8 days to: (a) no additive; (b) oxLDL (100 μg/ml); (c) oxLDL (100 μg/ml) and SB203580 (20 μM); (d) oxLDL (100 μg/ml) and PD98059 (20 μM). **B**, J774 cells were exposed for 8 days to oxLDL (100 μg/ml) and SB203580 in concentrations of: (a) 0.02 μM, (b) 0.2 μM, (c) 2 μM, and (d) 20 μM. The experiments were performed thrice, and representative micrographs are shown.

foam cells in about 8 days (Fig. 2A). Interestingly, the p38 inhibitor SB203580 dose dependently inhibited this differentiation process with a complete inhibition at 20 μM (Fig. 2B), which correlated with a dose-dependent inhibition of p38 in this cell line (data not shown). In contrast, inhibition of the ERK pathway by PD98059 had no effect. These data suggested that the p38 pathway, but not the ERK pathway, is involved in lipid deposition in J774 cells, suggesting an important role of the p38 pathway in foam cell formation.

p38 upregulates CD36 expression and promotes lipid uptake

Since p38 was found to regulate many inducible genes (27), we thought a similar mechanism might be involved in the regulation of lipid accumulation in macrophages. CD36 is a SR which plays an important role in oxLDL uptake. The induction of this SR by oxLDL is crucial for foam cell formation (16, 33). We therefore tested whether p38 activation is required for CD36 induction by oxLDL. THP-1 cells were treated with oxLDL for 3 days in the presence or absence of SB203580 (20 μM) or PD98059 (20 μM). Cell surface CD36 was measured by FACS analysis using an anti-CD36 antibody. As reported by Nakagawa et al., oxLDL stimulation leads to an about 60% increase in CD36 on the cell surface (44). SB203580, at 20 μM, almost completely blocked CD36 induction by oxLDL, while PD98059 had no inhibitory effect but rather enhanced CD36 expression (Fig. 3). Thus, the p38 pathway is involved in CD36 induction, which is at least a part of the mechanism by which the p38 pathway regulates foam cell formation.

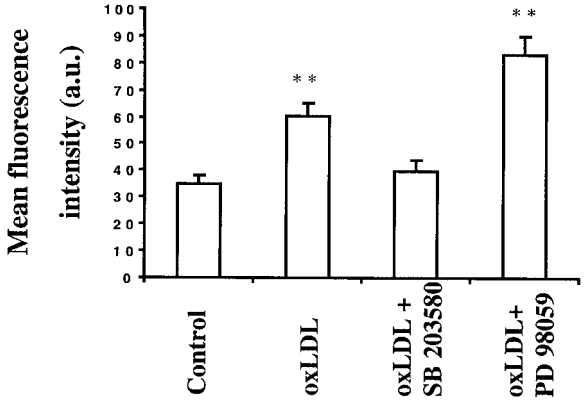

Fig. 3. SB203580 inhibits oxLDL-induced CD36 expression

THP-1 cells were exposed to oxLDL (100 μg/ml) for 3 days. Simultaneously, some cells were also exposed to SB203580 (20 μM) or PD98059 (20 μM). Nothing was added to the control cells. Flow cytometry was used to detect the expression of CD36 using FITC-conjugated anti-CD36 antibodies, as described in the Materials and Methods section. SB203580 blocks the expression of CD36, while PD98059 does not. The histogram is a summary showing arithmetic means±SD, n=3, * $p \leq 0.05$, ** $p \leq 0.01$.

The p38-mediated CD36 upregulation is DR-1 element dependent

DR-1 (a direct repeat with one nucleotide spacer, and a hormone response element, found in the CD36 promoter) was reported to be important for oxLDL-induced CD36 expression (16, 33). We then thought it necessary to test whether DR-1 is also important for p38-mediated CD36 expression. To do so, we adapted a reporter gene system that has been used by Evans' group for evaluation of nuclear receptor - mediated DR-1 - dependent gene expression (Fig. 4A) (16). Two CD36 reporters, one (−273/luc) with DR-1 and another (−263/luc) without DR-1, and CV-1 cells which lack many nuclear receptors were used in the experiments. The reporter genes were transfected into the respective CV-1 cells and the cells were treated with or without oxLDL. As expected, oxLDL induced −273/luc expression while −263/luc was not affected (Fig. 4B), which supports the notion that DR-1 has a primary role in CD36 induction (16). The induction of −273/luc was almost completely blocked by SB203580, but not by PD98059 (Fig. 4B), suggesting a requirement of p38 activation in oxLDL-induced CD36 expression. To evaluate whether the p38 pathways alone can influence CD36 expression, we co-expressed −273/luc or −263/luc with dominant active MKK1, MKK6b, or MKK7 in CV-1 cells. Each of these dominant active molecules has previously been shown to specifically activate a given MAP kinase pathway. As shown in Fig. 4C, activation of the p38 MAP kinase pathway by expression of MKK6b(E) led to a significant upregulation of –273/luc reporter gene expression, while it had no effect on the –263/luc reporter gene which has no DR-1 element. Activation of the

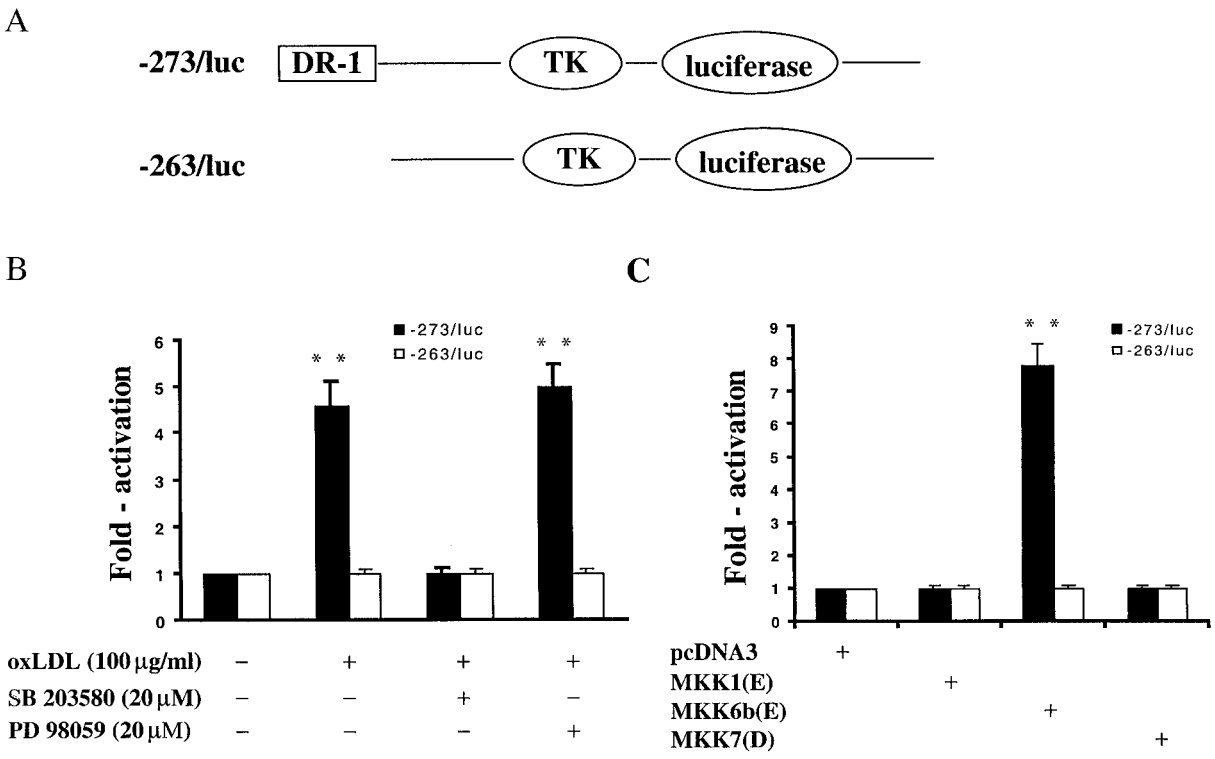

Fig. 4. The p38 MAP kinase pathway regulates expression of CD36 through interference with the DR-1 element of the CD36 promotor

A, −273/luc is a luciferase reporter gene with a DR-1 element of the CD36 promotor from −273 to +47. −263/luc is a corresponding reporter gene without the DR-1 element (−263 to +47). **B,** CV-1 cells were co-transfected with (i) CMXβ-gal and (ii) either the −273/luc or the −263/luc reporter genes, and then exposed to oxLDL (100 μg/ml) alone, or together with SB203580 (20 μM) or PD98059 (20 μM). **C,** CV-1 cells were co-transfected with (i) CMXβ-gal and (ii) either the −273/luc or the −263/luc reporter genes, and (iii) one of the constitutively active p38, JNK and ERK activators MKK6b(E), MKK7(D), or MKK1(E), as described in the Materials and Methods section. The ratio of luciferase activity to β-galactosidase activity is presented as arithmetic means±SD, n=3, * $p \leq 0.05$, ** $p \leq 0.01$.

ERK or JNK pathways by MKK1(E) or MKK7(D), respectively, had no effect on the CD36 reporter-gene expression. As we noticed before (45), dominant active MKK6b induced higher reporter gene expression than stimulus such as oxLDL. This is most likely due to MKK6b(E) being constitutively active while oxLDL-induced p38 activation is transient. Collectively, our data demonstrated that a specific MAP kinase pathway, the p38 pathway, regulates CD36 expression via either a direct or an indirect regulation of the DR-1 element located in the CD36 promoter.

The p38 MAP kinase pathway upregulates the transactivation activity of PPARγ through its ligand-binding domain

It was recently reported that PPARγ forms a heterodimeric DNA-binding complex with the 9-cis retinoic acid receptor (RXR) (16, 33). This complex activates transcription of target genes by binding to the DR-1 element. Since activation of CD36 transcription by the p38 MAP kinase pathway is abolished by deletion of the DR-1 element, it is possible that PPARγ is involved in p38-mediated CD36 induction.

The ligand-binding domain (LBD) of PPARγ is the regulatory domain for transcriptional activation in response to ligands. Here we again used a previously established method (33), as illustrated in Fig. 5A, to test whether a LBD of PPARγ is important for p38-mediated gene activation. A chimeric transcription factor with the LBD of mPPARγ fused to the DNA-binding domain of the yeast transcription factor GAL4 was used in the experiments. The chimeric transcription factor is able to bind to the GAL4-DNA binding motif, but its tran-

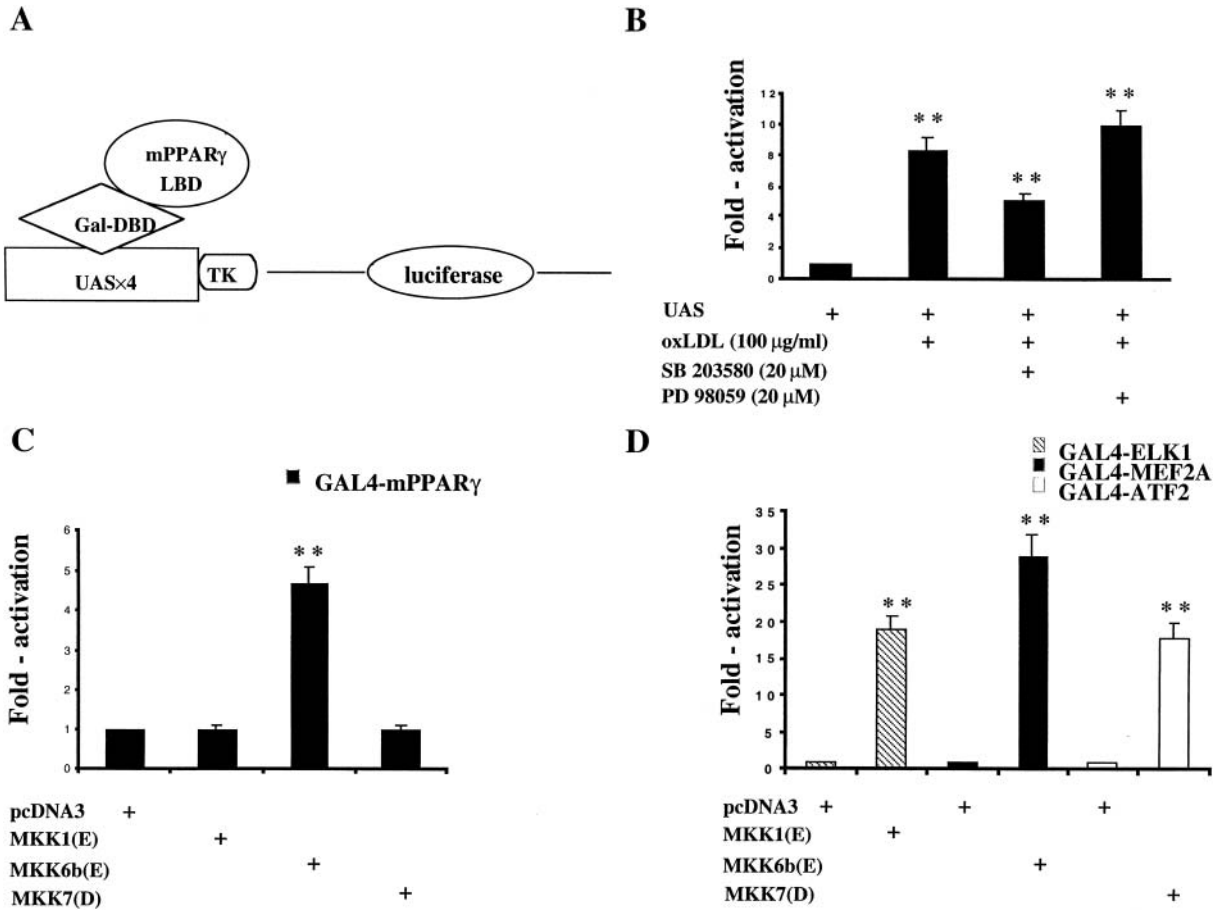

Fig. 5. MKK6b(E) regulates the PPARγ through post-translational activation
A, The construct GAL4-mPPARγ contains a GAL4-DNA-binding domain and a mPPARγ LBD. A TK promoter, having four GAL4-binding sites in front of it, drives the luciferase gene. **B,** CV-1 cells were co-transfected with either (i) CMXβ-gal, (ii) UAS-TK-luc, or (iii) GAL4-mPPARγ, and exposed to oxLDL (100 μg/ml) alone or together with SB203580 (20 μM) or PD98059 (20 μM) for another 24 h. **C,** CV-1 cells were co-transfected with either (i) CMXβ-gal, (ii) UAS-TK-luc, or (iii) GAL4-mPPARγ and one of the MAP kinase kinase (MKK) vectors MKK6b(E), MKK7(D), MKK1(E), or the empty vector pcDNA3, as described in the Materials and Methods section. **D,** CV-1 cells were co-transfected with CMXβ-gal and pG5E1bLuc, and (i) GAL4-ELK1 and MKK1(E), or (ii) GAL4-MEF2A and MKK6b(E), or (iii) GAL4-ATF2 and MKK7(D), as described in the Materials and Methods section. The ratio of luciferase activity to β-galactosidase activity is given as arithmetic means±SD, n=3, * p≤0.05, ** p≤0.01.

scription is regulated by modification of LBD of PPARγ. The system is independent of endogenous PPARγ and RXRα and it can be induced by ligand binding to the PPARγ LBD (16, 33). The readout of this system is a reporter that is controlled by GAL4-binding sites. By using this system, we found that oxLDL-induced upregulation of the transactivation activity of PPARγ can be partially blocked by SB203580 (Fig. 5B). PD98059 did not have an inhibitory effect, but rather enhanced the reporter gene expression. These results are similar to the result we obtained using the CD36 reporter gene (Fig. 4); however, the inhibitory effect of SB203580 on the PPARγ-dependent reporter gene expression was not as dramatic as on the CD36 promoter-driven reporter. This may suggest that other nuclear receptor(s) are also involved in DR-1-dependent CD36 expression and the results in Fig. 4 reflect an inhibition of multiple transcription factors by SB203580. Although an incomplete inhibition of the PPARγ reporter expression by SB203580, activation of the p38 pathway by the expression of MKK6b(E) significantly up-regulates the PPARγ-dependent gene expression (Fig. 5C). Since the fused transcription factor

had only PPARγ LBD, the increased transactivation activity of the chimeric transcription factor is through PPARγ LBD. This activation appears to be specific for the p38 pathway, because activation of the ERK or JNK pathways, by MKK1(E) or MKK7(D), respectively, had no effect (Fig. 5C). The activation of each MAP kinase pathway was confirmed by the other reporter genes (Fig. 5D). MKK1(E), MKK6b(E) and MKK7(D) activated the GAL4-ELK1, GAL4-MEF2A, and GAL4-ATF2 reporters, respectively. Thus, the p38 MAP kinase pathway can directly, or indirectly, regulate PPARγ activity via its LBD, which is likely to be a part of the mechanism by which p38 regulates CD36 upregulation.

DISCUSSION

OxLDL is believed to cause foam cell formation and, thus, the initiation or acceleration of the atherosclerotic process. In oxLDL, both the lipids and the apo-B protein are oxidized. Their oxidation products are potent stress stimuli that initiate many cellular responses. It is well known that oxidative stress, such as exposure to H_2O_2, activates MAP kinases in vivo (46). In this report we show that oxLDL also triggers stress responses, such as p38 activation. We have found that activation of the p38 pathway is required for foam cell formation. It appears that the p38 pathway is involved in a PPAR-mediated transcription of CD36, which enhances lipid uptake and thereby facilitates foam cell formation.

The p38 pathway was found to play a very important role in inflammation, because almost all microbial pathogens, as well as many proinflammatory cytokines, can activate p38 (27). The finding that the p38 pathway also plays a role in oxLDL uptake is very interesting, because a possible link between infection and atherosclerosis was recently proposed (47). Activation of p38 by pathogens could be an additive factor in the development of atherosclerosis. Newly published results of antibiotic treatment of vascular diseases appear to provide further support for the infection hypothesis, as well as suggesting a new, and quite different, therapeutic approach to atheromatous cardiovascular diseases (48). The finding of an involvement of the p38 pathway in foam cell formation may provide an additional clue to the development or improvement of new treatments for this fatal disease.

It was recently reported that the oxidized lipid components of oxLDL, 9-HODE and 13-HODE, act as endogenous activators and ligands of the nuclear receptor PPARγ to upregulate CD36 expression. We show here that activation of p38, but not of JNK or ERK, is also involved in CD36 induction. Interestingly, the p38-mediated CD36 induction is DR-1-dependent, because deletion of the DR-1 element in the CD36 promoter completely abolished the induction (Fig. 4). Because a reporter gene with a GAL4 DNA-binding domain and a PPARγ LBD is regulated by p38 activation (Fig. 5), the ligand-binding domain of PPARγ appears to be targeted by the p38 pathway. We do not know at present whether activation of the p38 pathway leads to direct modification of PPARγ, or if p38 activation leads to other cellular change(s), such as generating oxidized lipid, which in turn may activate PPARγ. It is clear that a post-translational regulation of PPARγ by the p38 pathway exists because the GAL4 reporter gene system used in our study is independent of endogenous PPARγ and, therefore, has no link to PPARγ induction. The p38 pathway may, on the other hand, be involved in the regulation of PPARγ expression, because p38 may promote PPARγ expression through phosphorylating C/EBPβ, a transcription factor which controls PPARγ expression (49). Thus, p38 may influence PPARγ activity at multiple levels.

The level of scavenger receptors is a key factor that controls oxLDL uptake. At present, several scavenger receptors have been identified, including the class A scavenger receptor SR-A; the class B scavenger receptors SR-BI, and CD36; the class C scavenger receptor CD68; and the Fc receptor CD32 (6–11). Simply blocking CD36 upregulation may not fully explain the significant effect of SB203580 on foam cell formation. It is possible that the p38 pathway may influence other cellular event(s) that are required for foam cell development as well. Whether the p38 pathway regulates any of the other types of scavenger receptors awaits further investigation. The finding that a MAP kinase pathway is active in the regulation of

lipid uptake and foam cell formation has provided new insight into the mechanism of atherosclerosis development. A future challenge will be to define the mechanism by which the MAP kinase interacts, or coordinates, with nuclear receptor(s) in order to develop new strategies for the prevention and treatment of atherogenesis.

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