LDLs stimulate p38 MAPKs and wound healing through SR-BI independently of Ras and PI3 kinase[S]

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Abstract **Intracellular signals elicited by LDLs are likely to play a role in the pathogenesis associated with increased LDL blood levels. We have previously determined that LDL stimulation of human skin fibroblasts, used as a model system for adventitial fibroblasts, activates p38 mitogen-activated protein kinases (MAPKs), followed by IL-8 production and increased wound-healing capacity of the cells. The proximal events triggering these responses had not been characterized, however. Here we show that MAPK kinases MKK3 and MKK6, but not MKK4, are the upstream kinases responsible for the activation of the p38 MAPKs and stimulation of wound closure in response to LDLs. Phosphoinositide 3 kinases (PI3Ks) and Ras have been suggested to participate in lipoprotein-induced MAPK activation. However, specific PI3K inhibitors or expression of a dominant-negative form of Ras failed to blunt LDL-induced p38 MAPK activation. The classical LDL receptor does not participate in LDL signaling, but the contribution of other candidate lipoprotein receptors has not been investigated. Using cells derived from scavenger receptor class B type I (SR-BI) knockout mice or the BLT-1 SR-BI inhibitor, we now show that this receptor is required for LDLs to stimulate p38 MAPKs and to promote wound healing. Identification of MKK3/6 and SR-BI as cellular relays in LDL-mediated p38 activation further defines the signaling events that could participate in LDL-mediated pathophysiological responses.—** Bulat, N., G. Waeber, and C. Widmann. **LDLs stimulate p38 MAPKs and wound healing through SR-BI independently of Ras and PI3 kinase. *J. Lipid Res.* 2009.** 50: **81–89.**

Supplementary key words scavenger receptor class B type I • mitogen-activated protein kinase • fibroblasts

A rise in LDL blood level increases the risk of developing atherosclerosis even in the absence of other risk factors (1). High LDL blood levels induce the formation of plaques (atheromas) in arteries that can ultimately rupture. Plaque detachment can induce thrombosis, leading to myocardial infarction and stroke (2).

Although extensively studied, the mechanism of atheroma formation is still incompletely characterized (3). In particular, how LDL particles modulate plaque formation and evolution is still debated (4, 5). It is likely, however, that LDLs regulate plaque formation by inducing specific signals in cells of the vessel wall. It is indeed known that lipoproteins in general, and LDLs in particular, can stimulate various signaling pathways, including mitogen-activated protein kinase (MAPK) pathways in such cells (6). Some MAPK pathways have been found to be activated in atherosclerotic plaques (7, 8). Additionally, the p38 MAPK pathway has recently been shown to be involved in the development of atheromas (9). Characterization of the way in which lipoproteins modulate these signaling pathways could therefore contribute to a better understanding of the development of atherosclerosis.

Adventitial fibroblasts are now recognized as being one of the players participating in vascular tissue remodeling and atheroma formation (10, 11). Previous work performed in our laboratory established that mouse embryonic fibroblasts (MEFs) and human primary fibroblasts, used as a model system for adventitial fibroblasts, activate the p38 MAPK pathway in response to LDL stimulation (12). This appears to be mediated by the cholesterol moiety of the LDL particles (13). LDL-stimulated fibroblasts secrete IL-8, which stimulates their wound-healing capacities in an autocrine fashion (14). This might be relevant for the development of atherosclerosis because IL-8 is detected in atheromas (15). Furthermore, elevated plasma levels of IL-8 are associated with an increased risk of coronary artery disease (16).

How LDL particles stimulate the p38 MAPK pathways is, however, mostly undefined. Here we show that LDLs require the scavenger receptor class B type I (SR-BI) to stimulate

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the MAPK kinases MKK3 and MKK6, which are responsible for the direct activation of the p38 MAPKs and the ensuing increased ability to close wounds. Other candidate proteins suggested to participate in lipoprotein-induced p38 MAPK stimulation, such as Ras and phosphoinositide 3 kinase (PI3K), were found to play no role in this response in fibroblasts.

MATERIALS AND METHODS

Materials

Wortmannin (catalog number W1628) and Ly294002 (catalog number L9908) were obtained from Sigma-Aldrich (Buchs, Switzerland) and were dissolved in DMSO as 1 mM and 10 mM stock, respectively, and stored at −20°C. BLT-1 was purchased from Chembridge (catalog number 5234221); this compound was prepared freshly as a 10 mM stock solution in DMSO (BLT-1 was found to lose its activity if stored in DMSO at 4°C). Anti-phospho-p38 (Thr180/Tyr182), anti-total p38, anti-phospho-cJun (Ser73), anti-cJun, anti-phospho-Akt (Ser473), anti-phospho-p44/42 (Thr202/Tyr204), and anti-actin antibodies were purchased from Cell Signaling Technology (Beverly, MA; catalog numbers 9211, 9212, 9251, 9252, 4051, 4376, and 4968 respectively). The anti-SR-BI antibody was purchased from Novus Biologicals (catalog number NB 400-104). The anti-Ras and anti-total p44/42 antibodies were purchased from Upstate (catalog numbers 05-516 and 06-182, respectively).

Plasmids

h-H-Ras(S17N).cmv encodes the S17N dominant-negative mutant of Harvey Ras (17). The .cmv extension indicates that the backbone plasmid is pCMV4. The h-H-Ras(S17N).lti plasmid encodes S17N Ras in a lentiviral vector. It was generated by subcloning the blunted *Hind*III fragment of h-H-Ras(S17N).cmv into the TRIP-PGK-ATGm-MCS-WHV lentiviral vector opened with *Sma*I. SR-BI.pCMV-SPORT6, which encodes the SR-BI receptor cDNA, was obtained from ImaGenes (catalog number IRAVp968E126D). Subcloning of the SR-BI cDNA in a lentiviral vector was performed by inserting the *Eco*RV-*Xho*I SR-BI.pCMV-SPORT6 fragment into the TRIP-PGK-ATGm-MCS-WHV lentiviral vector opened with *Not*I (blunted) and *Xho*I.

Lentivirus

Recombinant lentiviruses were produced as described previously (18). Briefly, 293T cells were transfected using the calcium-phosphate DNA precipitation method (19) with 10 μg of the lentiviral vector containing the cDNA of interest, 2.5 μg of the envelope protein-coding plasmid (pMD.G), and 7.5 μg of the packaging construct (pCMVΔ8.91). Two days after transfection, the medium containing the viruses was collected. The minimal amount of virus needed to express the protein of interest in 100% of the infected cells was determined by immunocytochemistry and corresponded to the dose used in subsequent experiments.

Cell culture

Wild-type, MKK3/6 double knockout, and MKK4 knockout MEFs were grown in DMEM (Gibco, catalog number 61965) supplemented with 10% decomplemented fetal calf serum (FCS) at 37°C and 5% CO_2. Prior to LDL stimulation, cells were washed twice in PBS and incubated in lipoprotein-free medium (DMEM complemented with 2.5 mg/ml of lipoprotein-free serum) for 24 h. SR-BI knockout MEFs were isolated from E14 embryos of the B6.129S2-Scarb1^{tm1Kri}/J strain (Jackson Laboratory, stock number 003379). Control MEFs were isolated from wild-type littermate embryos. Briefly, 14 day pregnant females were euthanized by cervical dislocation, and embryos were removed from placentas and placed in 10 cm plates in ice-cold PBS [116 mM NaCl, 10.4 mM Na_2HPO_4, 3.2 mM KH_2PO_4 (pH 7.25)]. Livers were dissected and discarded. Each embryo was minced and transferred, separately, in a falcon tube containing 50 ml of HBSS containing 0.05% Trypsin, 0.53 mM EDTA·4 Na (Gibco; catalog number 25300-062) for 1 h. Trypsinized cells were collected by centrifugation, washed in PBS twice, and resuspended in DMEM supplemented with 10% decomplemented FCS and with penicillin and streptomycin (100 U/ml and 100 μg/ml, respectively; Sigma, catalog number P0781) at 37°C and 5% CO_2. Human fibroblasts (GM01386; Coriell Cell Repositories) were grown in DMEM (Gibco, catalog number 61965) supplemented with 15% FCS at 37°C and 5% CO_2.

Preparation of lipoproteins and lipoprotein-free serum

LDLs were isolated from human plasma by sequential density ultracentrifugation as described previously (20, 21) and dialyzed for 48 h against PBS containing 100 μM EDTA. Human lipoprotein-free serum was prepared by removal of lipoproteins by ultracentrifugation at a density of 1.23 g/l (21). The protein concentration was measured by Bradford assay using BSA as a standard.

Western blot analysis

After the indicated treatments described in the figures, the cells were lysed on ice in mono Q-C buffer (17) supplemented with one tablet of EDTA-free protease inhibitor (Roche) per 50 ml. Protein concentration was measured by a Bradford assay using BSA as a standard. Lysates were mixed with sample buffer [62.4 mM Tris-HCl (pH 6.8), 10% glycerol, 5% β-mercaptoethanol (v:v), 2% sodium dodecyl sulfate (w:v), and 0.01% bromophenol blue] before loading on SDS-PAGE gels. Western blotting was performed and quantitated as described previously (22).

Wound-healing experiments

Human fibroblasts, wild-type, SR-BI$^{-/-}$, and MKK3$^{-/-}$/MKK6$^{-/-}$ MEFs were grown to confluence in 6-well plates. Cells were then starved in DMEM containing 0.1% FCS for 24 h. After starvation, the cells were wounded with a 1–200 μl yellow, nonbeveled tip (Starlabs catalog number S1111-0006) and treated as described in the figures. Quantitation of wound closure was performed as follows. Each wound was photographed at random locations in the well. The photographs were imported in Adobe Illustrator (CS3 edition). The wound borders were labeled with two lines. From each of these lines a perpendicular line was generated that crossed the center point of the picture. The distance measured on the perpendicular lines between the wound edges was then recorded and averaged. The wound width indicated in the figures corresponds to the average width calculated for five random pictures taken for each experimental condition.

Statistical analysis

All of the statistical analyses were performed with Microsoft Office Excel (2007 edition) using the two-tailed unpaired Student's *t*-test. Significance is indicated by an asterisk when $P < 0.05/n$, where P is the probability derived from the *t*-test analysis and n is the number of comparisons performed (Bonferonni correction).

RESULTS

MKK isoform requirement for LDL-induced p38 MAPK activation in fibroblasts

Depending on the cell type and stimulus used, p38 MAPKs can be activated by three members of the MKK family: MKK3, MKK6, and MKK4 (23, 24). To determine which MKK was involved in LDL-induced p38 MAPK activation, we used MEFs derived from wild-type, MKK3/MKK6 double knockout, and MKK4 knockout mice. These cells were treated with LDLs (200 μg/ml) for 15 min. Anisomycin (0.1 μg/ml; 30 min incubation) was used as a positive control. The levels of activated p38 MAPK were detected and quantitated by Western blot using a phospho-specific p38 MAPK antibody. LDLs induced an ~3-fold increase in the levels of phosphorylated p38 MAPKs in wild-type and MKK4$^{-/-}$ cells (**Fig. 1A**). No activation of p38 MAPKs was detected in MKK3$^{-/-}$/MKK6$^{-/-}$ cells (Fig. 1A). The MKK3$^{-/-}$/MKK6$^{-/-}$ cells were nevertheless able to activate the JNK MAPKs in response to anisomycin treatment (Fig. 1B), demonstrating that they did not have a general blockage in MAPK pathway activation. As described previously (24), MKK4$^{-/-}$ MEFs were strongly impaired in their capacity to stimulate the JNK MAPK pathway in response to anisomycin (Fig. 1B). Altogether, these results indicate that MKK4 ablation in MEFs abrogates their ability to activate the JNK MAPK pathway but does not impair their capacity to stimulate the p38 MAPK pathway in response to LDL stimulation. In fact, p38 MAPK activation levels were higher in cells lacking MKK4, compared with wild-type cells, suggesting that MKK4 can negatively modulate the p38 MAPK pathway.

PI3K involvement in p38 MAPK activation by LDLs

The proximal transduction machinery allowing LDLs to stimulate the p38 MAPK is still poorly understood. PI3Ks have been suggested to play a role in lipoprotein-induced p38 MAPK activation, but this is still controversial (6). Wortmannin and LY294002 are known to block all three classes of PI3Ks (25, 26). In MEFs, 1 μM wortmannin or 50 μM LY294002 blocked phosphorylation of Akt in response to serum (**Fig. 2A**). Because serum-induced Akt phosphorylation is known to depend on PI3Ks (27), this shows that PI3Ks in MEFs can be efficiently inhibited by wortmannin and LY294002 in our experimental system. However, these inhibitors had no effect on LDL-induced p38 MAPK activation in MEFs (Fig. 2B), indicating that PI3Ks are not involved in the transduction of the signal from LDLs to p38 MAPKs. Similar results were obtained in MKK4$^{-/-}$ MEFs (see supplementary Fig. I).

Role of Ras in LDL-induced p38 MAPK activation

Ras proteins have been suggested to participate in the activation of MAPK pathways in response to LDLs (6). To assess the role of Ras in LDL-induced p38 MAPK activation, MEFs were infected with lentiviruses encoding the dominant-negative S17N form of Ras. S17N Ras, as expected, efficiently blocked serum-induced extracellular signal-regulated kinase (ERK) stimulation (Fig. 2C; and

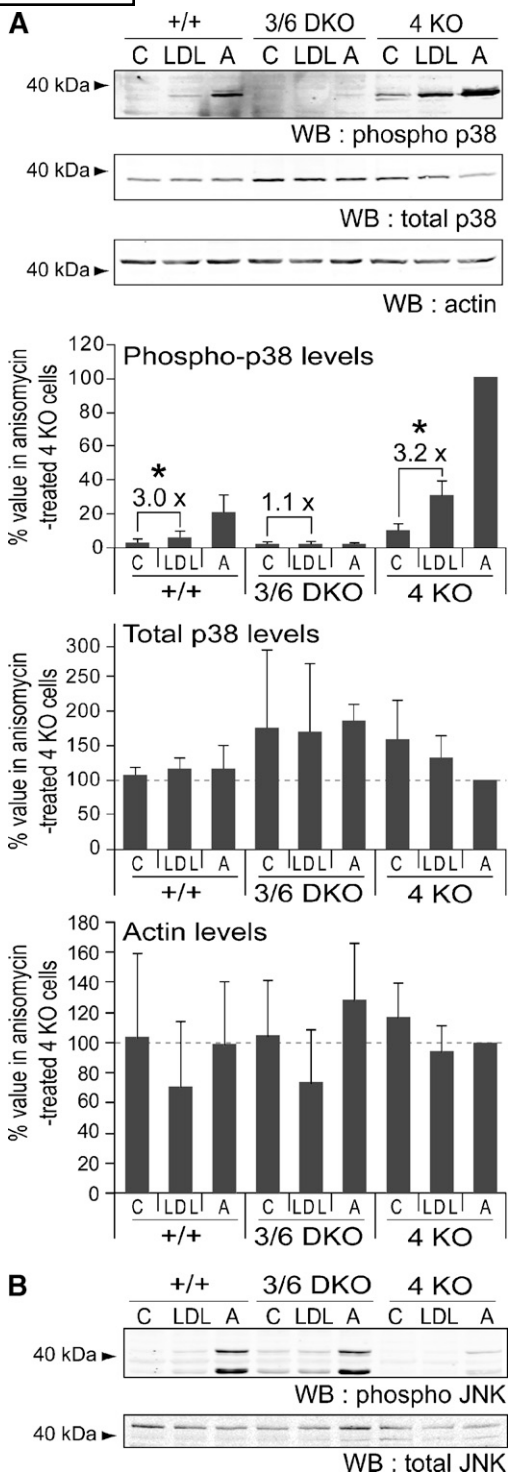

Fig. 1. Mitogen-activated protein kinase (MAPK) kinases MKK3 and MKK6, but not MKK4, are required for LDL-induced p38 MAPK activation. A: Wild-type (+/+), MKK3$^{-/-}$/MKK6$^{-/-}$ (3/6 DKO), and MKK4$^{-/-}$ (4KO) mouse embryonic fibroblasts (MEFs) were starved in lipoprotein-free medium as indicated in the Materials and Methods section and then stimulated with 0.1 μg/ml anisomycin (A) for 30 min or with 200 μg/ml of LDLs for 15 min. Total and activated p38 MAPK levels, as well as actin levels, were assessed by Western blot analysis. The bar graph represents the quantitation of the Western blot (WB) bands (mean ± SD of four separate experiments). B: Cells were treated as described in panel A. Activated and total JNK levels were assessed by Western blot analysis using phospho-specific and total anti-JNK antibody. * = significant difference.

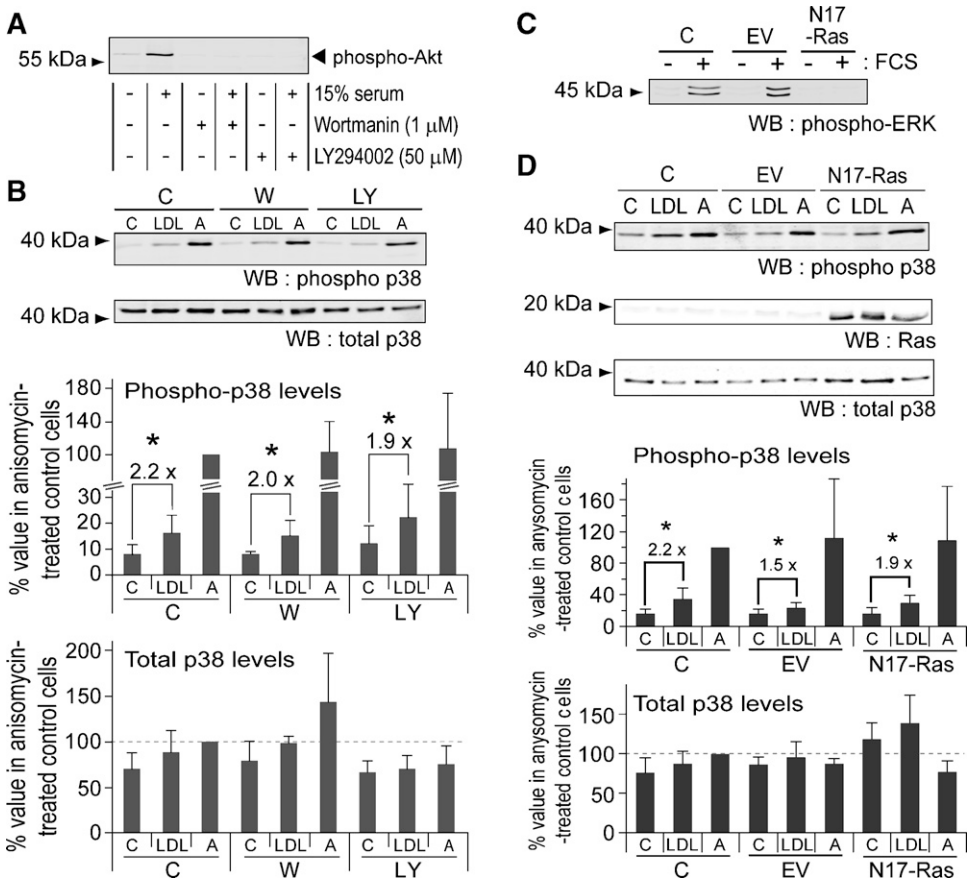

Fig. 2. Phosphoinositide 3 kinases and Ras are not required for LDL-induced p38 MAPK activation. A: Wild-type MEFs were starved in DMEM for 24 h. They were then preincubated or not for 1 h with wortmannin (1 μM) or LY294002 (50 μM) and stimulated or not for 15 min with 15% fetal calf serum (FCS). Activation of Akt was assessed by Western blot (WB) using an anti-phospho-Akt-specific antibody. B: Wild-type MEFs were starved in lipoprotein-free medium. Then they were either left untreated or were stimulated with 200 μg/ml LDLs for 15 min or with 0.1 μg/ml anisomycin (A) for 30 min. The levels of activated phospho-p38 MAPK and total p38 MAPK were visualized by Western blot analysis. The bar graph represents the quantitation of the phospho- and total p38 MAPK levels (mean ± SD of four separate experiments). C: Wild-type MEFs were infected or not with empty viruses or viruses expressing S17N Ras. They were then starved for 24 h in DMEM and stimulated or not with 15% FCS for 15 min. Levels of activated ERK MAPKs were assessed by Western blot analysis. D: Wild-type MEFs were infected or not as described in panel C. The cells were then starved in lipoprotein-free medium and stimulated or not with 200 μg/ml LDLs for 15 min or with 0.1 μg/ml anisomycin (A) for 30 min. The levels of activated p38 MAPK and total p38 MAPK were assessed by Western blot analysis. The results are expressed as in panel B (mean ± SD of five separate experiments). * = significant difference.

see supplementary Fig. IIA). S17N Ras was, however, unable to affect LDL-induced p38 MAPK activation (Fig. 2D; and see supplementary Fig. IIB). This indicates that Ras is not a component of the signaling transduction pathway leading to p38 MAPK activation in response to LDL stimulation.

SR-BI is required for p38 MAPK activation by LDLs and the increased wound-healing capacity that ensues

The LDL receptor is not used by LDLs to stimulate the p38 MAPK pathway (12), suggesting that other lipoprotein receptors mediate this response. SR-BI has been shown to mediate the activation of the ERK MKK by HDLs (28). SR-BI can also bind to LDL particles (29). To determine whether this receptor might also play a role in LDL-induced p38 MAPK activity, we generated MEFs from wild-type control and SR-BI knockout embryos and stimulated them with LDLs (**Fig. 3A**). In contrast to the control cells, MEFs lacking SR-BI were not able to stimulate the p38 MAPK pathway in response to LDLs (Fig. 3A). Infection of the SR-BI knockout MEFs with a lentivirus encoding SR-BI restored their ability to stimulate the p38 MAPKs when incubated with LDLs (Fig. 3B). BLT-1 is a chemical compound that inhibits SR-BI-mediated lipid transfer (30). This inhibitor blocked LDL-induced p38 MAPK activation (Fig. 3C). Altogether, these results indicate that in fibroblasts, LDL must interact with SR-BI and transfer lipids to cells in order to activate the p38 MAPK pathway.

LDL-induced p38 MAPK stimulation in fibroblasts promotes wound closure (14). **Figure 4A** shows that fibroblasts

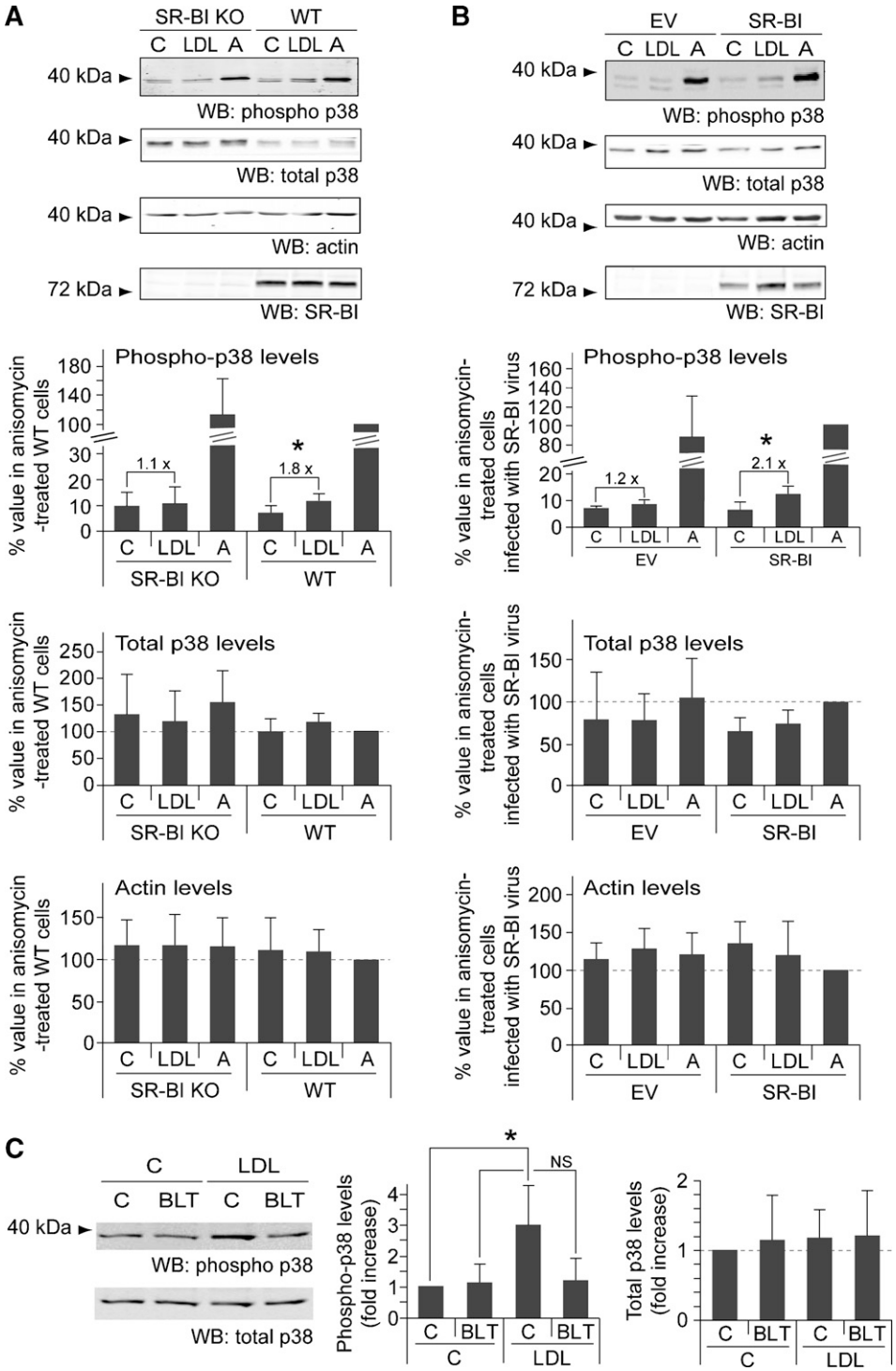

Fig. 3. LDLs stimulate p38 phosphorylation through the scavenger receptor class B type I (SR-BI) receptor. A: SR-BI receptor knockout (SR-BI KO) and wild-type (WT) MEFs were lipoprotein-starved for 24 h. The cells were then left untreated, stimulated with 200 μg/ml LDLs for 15 min, or incubated with 0.1 μg/ml anisomycin (A) for 30 min. The presence of phospho-p38 MAPKs, total p38 MAPK, actin, and SR-BI was assessed by Western blot (WB) analysis. The bar graphs represent the quantitation of the Western blot bands detected by the different antibodies (mean ± SD of four separate experiments). B: SR-BI KO cells were infected with either an empty virus (EV) or a virus expressing an SR-BI cDNA (SR-BI). After infection, the cells were lipoprotein-starved and treated as described in panel A (mean ± SD of three separate experiments). C: Wild-type MEFs were lipoprotein-starved for 24 h. Cells were then pretreated or not with 10 μM BLT-1 for 1 h and then stimulated or not with LDLs (200 μg/ml for 15 min). Total and phospho-p38 MAPK levels were assessed as in panel A (mean ± SD of 3 independent experiments). NS, not significant. * = significant difference.

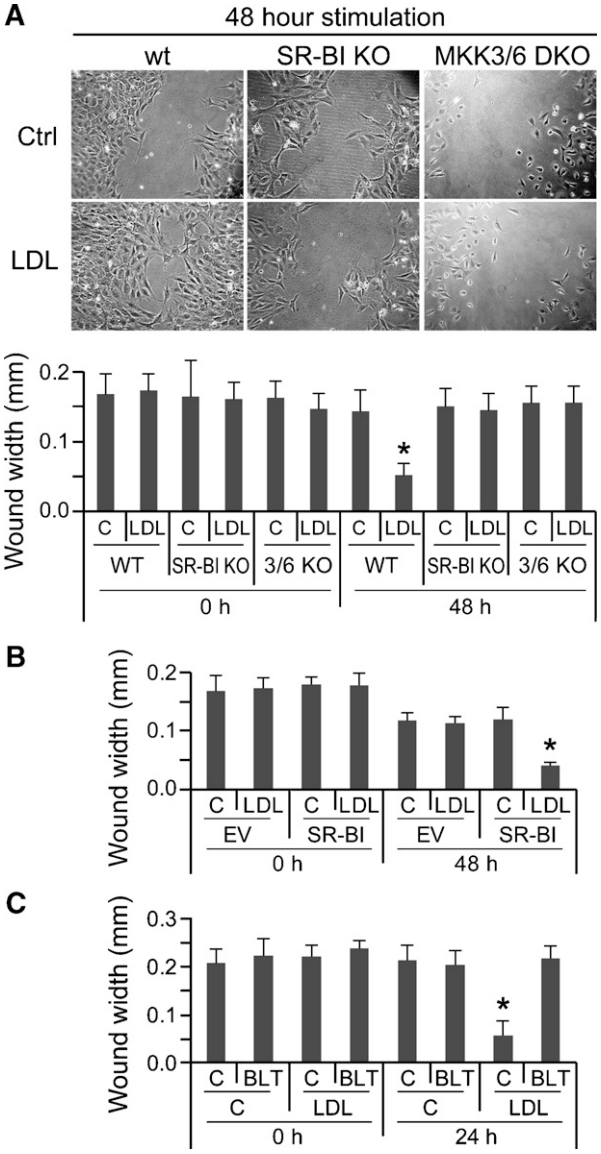

Fig. 4. SR-BI and MKK3/6 are required for LDLs to promote wound healing. A: Wild-type (wt), SR-BI receptor knockout (SR-BI KO), and MKK3$^{-/-}$/MKK6$^{-/-}$ double knockout (MKK3/6 KO) MEFs were starved in DMEM containing 0.1% FCS for 24 h. The cells were then wounded and either left untreated or treated with 200 μg/ml LDLs. Pictures were taken 48 h after wounding. The bar graphs represent wound width 0 h and 48 h after control or LDL stimulation (mean ± SD of three separate experiments). B: SR-BI receptor knockout MEFs were infected with either empty viruses (EV) or viruses expressing the SR-BI receptor cDNA (SR-BI). The cells were then starved and treated as in panel A (mean ± SD of two separate experiments performed in duplicate). C: Human fibroblasts were starved for 24 h in DMEM supplemented with 0.1% FCS. The cells were then preincubated or not with BLT-1 (10 μM for 1 h). They were then wounded and stimulated or not with LDLs (200 μg/ml). Wound width 0 h and 24 h after control or LDL stimulation is presented as in panel A (mean ± SD of three separate experiments). Statistical significance was assessed between the beginning and end time-points for each given condition. * = significant difference.

lacking either SR-BI or the MKK3/6 kinases were, as expected from their inability to stimulate the p38 MAPKs, greatly impaired in their capacity to favor wound healing in response to LDL stimulation. Re-expression of SR-BI in the SR-BI knockout MEFs rescued their ability to close wounds in response to LDL stimulation (Fig. 4B). These results were confirmed in human fibroblasts: BLT-1 blocked the ability of LDLs to stimulate wound closure (Fig. 4C).

The experiments described in Figs. 3, 4 demonstrate that SR-BI is a critical cell surface receptor that allows lipoproteins to activate p38 MAPK-dependent signaling events culminating in an increased capacity of the cells to heal wounds.

Role of SR-BI in lipoprotein-induced ERK phosphorylation

In fibroblasts, LDLs and HDLs can induce ERK phosphorylation (6). In the case of HDLs, activation of the Ras/Raf/MEK1/ERK MAPK pathway requires SR-BI (28). To assess whether LDLs would also rely on SR-BI to activate ERKs, wild-type and SR-BI knockout MEFs were stimulated with LDLs (and HDLs as control). Both types of lipoprotein particles induced ERK phosphorylation in wild-type cells, but this was not seen in cells lacking SR-BI (**Fig. 5**). This indicates that the SR-BI receptor is responsible for the activation of both the ERK and p38 MAPK pathways in response to lipoprotein stimulation.

DISCUSSION

There is ample evidence that LDLs can stimulate the p38 MAPK pathway in the cell types present in blood vessel walls, including vascular smooth muscle cells, endothelial cells, cells of the monocyte/macrophage lineage, and fibroblasts (6). The ensuing response varies, however, from one cell type to another. For example, the p38 MAPK pathway is required for LDLs to induce the expression of neuron-derived orphan receptor 1, a transcription factor mediating vascular smooth muscle cell proliferation (31). In contrast, LDLs do not induce proliferation of endothelial cells (32), but in this cell type, LDL-induced p38 MAPK activation modulates E-selectin expression (33) that could potentially contribute to cell migration (34). This exemplifies the fact that LDLs have a broad ability to stimulate the p38 MAPKs but that the resulting cell response will vary from one cell type to another, probably a reflection of different signaling events activated in the different cell types. Unfortunately, there is very little information on the signaling molecules allowing LDL to activate the p38 MAPKs.

In the present study, we have identified some of the molecular players involved in p38 MAPK activation by LDLs in fibroblasts. MAPKs are activated by phosphorylation on threonine and tyrosine residues by dual-activity kinases, the MKKs (35, 36). The p38 MAPKs can be stimulated by MKK3, 4, and 6, but they can also auto-activate after binding to TAB1 (37, 38). Using cells lacking MKK3/6 or 4, it was found that only MKK3 and 6 are required for LDL-

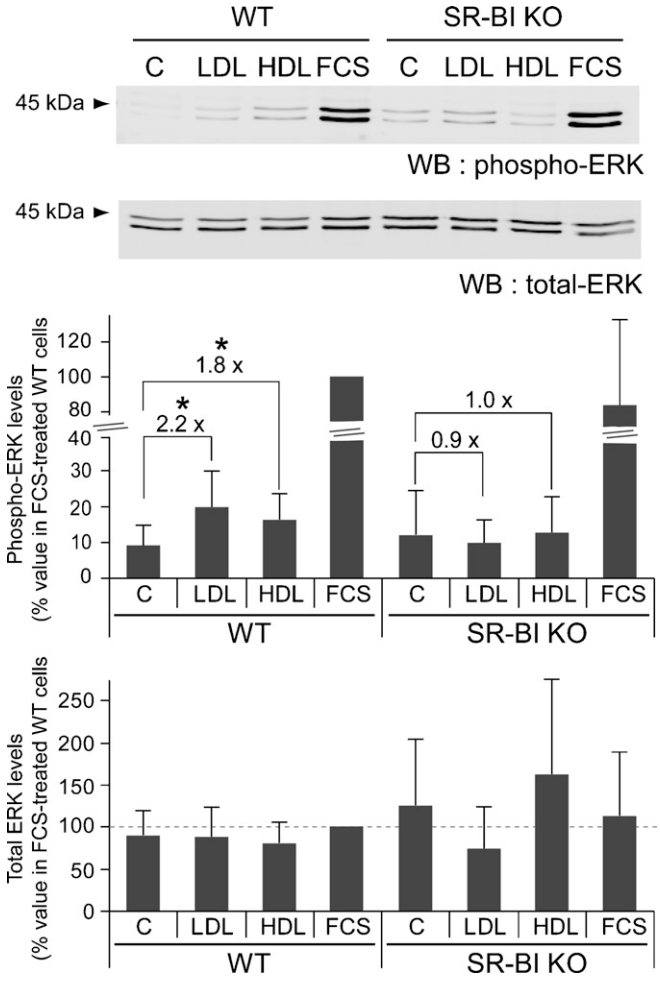

Fig. 5. LDLs and HDLs stimulate ERK phosphorylation in an SR-BI-dependent manner. Wild-type (WT) and SR-BI receptor knockout (SR-BI KO) MEFs were lipoprotein-starved for 24 h. The cells were then either left untreated, stimulated with 200 μg/ml LDLs or HDLs for 15 min, or incubated with 15% FCS for 20 min. The levels of total and activated ERK were assessed by Western blot (WB) analysis and quantitated as shown in the bar graphs below the blots (mean ± SD of four separate experiments). * = significant difference.

induced p38 MAPK activation. Other potential activators such as MKK4 or TAB1 seem therefore dispensable for this response. In fact, the implication of MKK4 in p38 MAPK stimulation is tenuous (23). Most studies indeed indicate that MKK4 is restricted to the activation of the JNK MAPK pathway (24, 38, 39). The manner in which LDLs activate p38 MAPKs appears therefore to follow the "classical" MKK3/6 pathway.

It was previously shown that it is the cholesterol moiety of LDLs that mediates the activation of the p38 MAPKs in fibroblasts, endothelial cells, and smooth muscle cells (13). Interestingly, this LDL cholesterol-mediated MKK3/6-p38 MAPK signaling event is also induced in free cholesterol-loaded macrophages (40). Activation of the p38 MAPKs leads to IL-8 secretion in fibroblasts (14) and to TNFα secretion in macrophages (13). Cholesterol delivered to different cell types within vessel walls can therefore lead to p38 MAPK activation and secretion of cytokines that are found in atheromas (15) and suspected to play an important role in their formation and evolution (16).

The upstream activators of MKK3 and -6 in response to LDL stimulation are not yet well defined. The role of a few signaling proteins has been investigated, however. For example, Ras appears to be activated in endothelial cells and CHO cells in response to LDL stimulation (33, 28). Moreover, on the basis of experiments performed in endothelial cells with a dominant-negative mutant of Ras, it was suggested that this small GTP binding protein is required for LDL-induced p38 MAPK activation (33). However, using the same approach, we ruled out the involvement of Ras in LDL-induced p38 MAPK stimulation in fibroblasts. Consistent with this notion is our observation that PI3Ks, which are Ras effectors (41), are not implicated in the LDL-mediated p38 MAPK response. This is in line with previous results obtained in smooth muscle cells showing that LDLs did not stimulate PI3Ks (42). LDLs can therefore activate the p38 MAPK in a variety of cell types, but the mechanisms employed may not be identical. Actually, targeting cell-specific upstream activators of p38 MAPK could represent an advantage if one needs to develop therapeutic strategies to inhibit the activation of p38 MAPK in selected cell populations involved in atheroma formation.

LDLs stimulate p38 MAPK activation independently of the classical LDL receptor (12). We now show that it is the SR-BI scavenger receptor that mediates LDL-induced p38 MAPK activation and the resulting increase in wound closure capacity of fibroblasts. SR-BI was first discovered as a native and modified LDL receptor (43) but can also interact with other lipoproteins (29). The manner in which lipoproteins stimulate the p38 MAPKs via SR-BI appears to rely on the transfer of lipids from lipoproteins to cells. This was inferred from the use of the BLT-1 inhibitor that efficiently prevented LDLs from activating the p38 MAPK pathway. This inhibitor does not prevent lipoprotein binding to SR-BI, but blocks the transfer of lipids from SR-BI-bound lipoproteins to cells (30). We have shown previously that cholesterol is the major component of LDLs and HDLs activating the p38 MAPKs (13). Moreover, both HDL and LDL particles transfer cholesterol to cells via SR-BI with similar efficacies (44). These observations support the notion that cholesterol is the bioactive lipid, transferred from lipoproteins to fibroblasts, that mediates the stimulation of the p38 MAPK pathway.

The role of SR-BI in the development of atherosclerosis is not fully understood. In mice, liver SR-BI receptors have anti-atherogenic properties (45, 46) as long as their expression is close to physiological levels (47). In contrast, if mice express high levels of SR-BI in the liver, they are more susceptible to atherosclerosis development (47). The SR-BI receptor can also be found in human atherosclerotic plaques (48, 49). Conceptually, an increase in LDL levels can stimulate the SR-BI receptors in blood vessel- and atheroma-associated cells to mediate intracellular cholesterol transfer. This in turn can activate intracellular signals, including the stimulation of the p38 MAPK pathway and the ensuing cellular responses (e.g., IL-8 secretion). A

series of evidence indicates that the LDL-induced signaling and cellular events we have defined play a role in the development of atherosclerosis. For example, the p38 MAPK pathway is involved in blood vessel wall hyperplasia (7, 9), and IL-8, which is produced in atheromas (15), is a cardiovascular risk factor (16). The detailed characterization of the signaling and cellular events elicited by LDLs in blood vessel cells therefore contributes to a better understanding of the mechanisms underlying atheroma formation. This might lead to the development of new strategies to fight cardiovascular diseases.

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