Stimulation of mitogen activated protein kinase by LDL and oxLDL in human U-937 macrophage-like cells

Hans-Peter Deigner*, Ralf Claus

Pharmazeutisch-Chemisches Institut der Universität Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

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Abstract Mitogen activated protein kinase in extracts of U-937 macrophage-like cells was stimulated by LDL and oxLDL. A maximum value (161% of the basal phosphotransferase activity) was obtained after 6 min exposure to oxidized LDL (27 μg/ml) using APRTPGGRR peptide substrate. The activatory effect was more pronounced (LDL 181%, oxLDL 201%) when MAPK of stimulated cells was immunoprecipitated with anti-p42^{MAPK} antibodies and phosphotransferase activity was assayed in immune complexes. Stimulation produced by oxLDL was inhibited by poly I, fucoidan, dextran sulfate and by the MAPKK inhibitor PD 098059 but not by PMA-mediated depletion of PKC or by pre-treatment with chloroquine or with pertussis toxin. These results suggest a direct mitogenic effect of LDL which, in the case of oxLDL, is dependent on scavenger receptor ligation but not on G-protein mediated or PKC-dependent signal transduction.

Key words: Mitogen activated protein kinase; Lipoprotein; Oxidized LDL; U937 cells

1. Introduction

Mitogen activated protein kinase plays a key role in cellular regulation. The enzyme is a substrate for receptor protein tyrosine kinases and serves as a link between membrane bound tyrosine kinases and intracellular targets regulated by serine/threonine phosphorylation [1,2]. Treatment of cells with growth factors as well as with various other extracellular stimuli leads to activation of MAPKs (ERKs). Besides regulation of the activity of other serine/threonine kinases, MAP kinases are involved in the regulation of gene expression, cell growth, differentiation and cytoskeletal function [3-5].

Cellular proliferation has been reported to be stimulated by LDL in combination with serum [6,7] or with growth factors [8,9] and chemically modified LDLs, such as oxLDL and acLDL, induce macrophage growth [10]. Consistently, macrophage derived foam cells, generated by uptake of modified LDL through the scavenger receptor pathway [11], tend to proliferate in atherosclerotic lesions [12,13]. In the investigation of causes and consequences of atherosclerotic alterations, the role of oxidatively modified LDL is of particular interest [14]. However, although there are numerous investigations which deal with mitogenic properties of lipoproteins [9,15], whether mitogenic activity is an intrinsic property of LDL or oxLDL is still a matter of debate. In this respect, our study was performed to examine direct lipoprotein induced effects on mitogen activated protein kinase.

*Corresponding author. Fax: (49) (6221) 54 6430.

Abbreviations: BSA, bovine serum albumin; LysoPAF, 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine; MAP-kinase (MAPK), mitogen activated protein kinase; ERK, extracellular signal-regulated kinases; MAPKK, MAPK/ERK kinase; MEK, MAPK-activating enzyme; acLDL, acetylated low density lipoprotein; oxLDL, oxidized LDL; MBP, myelin basic protein; malBSA, maleylated bovine serum albumin; PAF, platelet activating factor, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; poly I, polyinosinic acid; PLA_2, phospholipase A_2; PKC, protein kinase C; PMA, phorbol-12-myristate-13-acetate; SR, scavenger receptor

2. Materials and methods

2.1. Materials

Human U-937 cells were from DSM (Braunschweig, Germany); glutamine, streptomycin, BSA (fatty acid free), sucrose, ethylenediaminetetraacetic acid (EDTA), ethyleneglycol-bis(β-aminomethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), dithiothreitol (DTT), aprotinin, leupeptin, benzamidine, phenylmethylsulfonyl fluoride (PMSF), polyinosinic acid, dextran sulfate, fucoidan, phorbol-12-myristate-13-acetate (PMA), E-Toxate, agarose immobilized phospholipase A_2 (10 U/200 μl) and protein A agarose were purchased from Sigma (Deisenhofen, Germany). Polyclonal anti-p42^{MAPK} antibodies and the MAP kinase substrate peptide (APRTPGGRR) were purchased from Biomol (Hamburg, Germany). Fetal calf serum (FCS), Triton X-100, *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES) were obtained from Boehringer Mannheim (Mannheim, Germany), RPMI 1640 medium was purchased from Gibco BRL (Eggenstein, Germany), penicillin from Gruenenthal (Stolberg, Germany). Silica gel (grade 60, 70–230 mesh) for column chromatography was from Machery-Nagel (Düren, Germany), all other reagents were purchased from Fluka (Neu-Ulm, Germany), Aldrich (Steinheim, Germany) and E. Merck (Darmstadt, Germany). Materials for cell culture were products of Nunc (Wiesbaden, Germany) and Greiner (Frickenhausen, Germany). γ-[^{32}P]ATP (3000 Ci/mM) was from Amersham (Braunschweig, Germany). Protein concentration was determined in a BioRad 450 microplate reader (Munich, Germany) using the BCA reagent from Pierce Chemical Co. (Rockford, IL, USA) with albumin as standard.

2.2. Cell culture

The human cell line U-937 [16] was cultured at 37°C in RPMI 1640 supplemented with glutamine (2 mM), 10% heat inactivated FCS, penicillin (50 U/ml) and streptomycin (50 μg/ml) in a humidified atmosphere (5% CO_2 in air). Monocytes were differentiated to resident macrophage-like cells by addition of 40 nM PMA (72 h) as described [17]. Cells were then cultured for another 48 h without PMA, plated at the indicated density, washed with serum free medium or buffer as indicated to remove non-adherent cells, then incubated with the respective stimuli for various time periods in serum free medium. PKC depletion prior experimentation was achieved by PMA treatment (18 h, 200 nM) of differentiated, macrophage-like cells and was confirmed by the absence of PKC α and β isozymes as determined by Western blot analysis (Western blotting of PKC isoforms was performed according to [18]). In some experiments, cells were cultured with lipoprotein-deficient serum (prepared by ultracentrifugation of FCS, ρ>1.21 according to Havel et al. [19]) for 48 h prior to experimentation.

2.3. Low density lipoprotein (LDL): preparation and oxidation

LDL was isolated from pooled human plasma containing EDTA (1 mM) by sequential flotation in an ultracentrifuge at preselected densities (between 1.019 and 1.063 g/ml) as described [19] and dialyzed

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extensively against Tris buffer (20 mM, pH 7.8, 150 mM NaCl, 0.2 mM EDTA). LDL samples were kept sterile under nitrogen in Tris buffer at 4°C and used within 2 weeks. Prior to oxidation, LDL was stored over polymyxin B agarose for at least 24 h, the polymyxin suspension removed by centrifugation and the supernatant passed through a polymyxin B agarose column (2 ml) using sterile, non-pyrogenic single-use material for intravenous application; the eluate then was dialyzed against Tris buffer (without EDTA). All preparations were then tested (also subsequent to oxidation) for absence of endotoxin. Endotoxin tests were carried out using E-Toxate. Oxidation of LDL was initiated by addition of $CuSO_4$ (20 μM), followed by incubation continued for up to 24 h at 37°C under sterile conditions; reactions were terminated by addition of EDTA (0.2 mM).

For determination of binding/uptake according to Tokuda et al. [20] [^{125}I]LDL (7.70 TBq/mM), prepared as described previously [21], was diluted with unlabelled LDL to attain a specific activity of 345 kBq/mg protein.

Treatment of oxidized LDL with phospholipase A_2 was carried out by incubation with 2 U immobilized phospholipase/80 μg lipoprotein (1 h, 37°C), the suspension then was removed by centrifugation (1500×*g*, 4 min).

2.4. Preparation of maleylated BSA

Albumin was modified with maleic anhydride as reported by Butler and Hartley [22]. Briefly, maleic anhydride in dioxane (80 μl, 1 M) was added to 20 mg BSA (10 mg/ml) in 0.1 M potassium pyrophosphate buffer (pH 8.5). The mixture was stirred on ice and the pH adjusted to 9.0 with NaOH (1 M). After 5 min, 2 ml of 0.5 M sodium phosphate buffer (pH 7.4) was added, the sample dialyzed against PBS or passed through a Bio-Rad P-6DGel column and checked for absence of endotoxin as described above. The modification was controlled by agarose gel electrophoresis (30 mA, 50 V, 1.5 h, 1.0% in Tris/CH_3COOH, pH 8.3, 8 mM supplemented with EDTA (0.2 mM)). Protein staining was carried out with Coomassie blue R250 or with the Silverstain kit from Bio-Rad.

2.5. MAP kinase assay

The cells (3×10^6/dish unless stated otherwise) were treated with the lipoproteins and other agents for various times in serum free RPMI 1640 medium as described above. Following incubation, the cells were washed twice and scraped into 1 ml medium, isolated by centrifugation at 1500×*g* for 0.5 min at 4°C, washed with 1 ml ice-cooled PBS, and resuspended in 50 μl homogenization buffer (20 mM HEPES, pH 7.4, containing 2 mM EDTA, 2 mM EGTA, 2 mM DTT, 300 mM sucrose, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml PMSF, 0.2 mM sodium vanadate and 10 μg/ml benzamidine). The homogenates were fast-frozen in liquid nitrogen and stored at −80°C for up to 2 weeks. After thawing, samples were sonicated at 0°C for 30 s, centrifuged at 20000×*g* and cytosolic MAP kinase activity determined in the supernatant by measuring the incorporation of γ-[^{32}P]ATP into a fragment of myelin basic protein, peptide (APRTPGGRR) [23]. The reaction was started by addition of 10 μl substrate peptide (250 μM) and 10 μl γ-[^{32}P]ATP (0.4 mM, 90000 kBq/sample) to a mixture of 20 μl cell lysate (80 μg protein) and 10 μl reaction buffer (100 mM Tris-HCl pH 7.0, 0.4 mM EGTA, 0.4 mM sodium vanadate, 40 mM $Mg(CH_3COOH)_2$). After incubation for 10 min at 25°C, phosphorylation was terminated by addition of trichloroacetic acid (10 μl, 40%) and an aliquot (40 μl) transferred to a Whatman phosphocellulose filter (P81, 4 cm^2). The filters were air-dried, washed four times in 75 mM orthophosphoric acid (5 ml/sample), dried again and Cerenkov radiation counted on a LKB-Wallac RACKBETA (Turku, Finland). Results were corrected by subtraction of the average value of three blank samples containing no cellular protein. Inhibition with PD 098059 [24] was achieved by 30 min treatment of cells with 60 μM PD 098059 (80 mM stock solution in dimethyl sulfoxide/ethanol, 1:1, v/v) prior to the addition of oxLDL.

2.6. Immune complex assay

Precipitation was carried out with cell lysate in homogenization buffer diluted with PBS (1 μg/μl cell protein) which was treated with the antibodies (5 μg/sample) for 12 h at 4°C. The immune complex was captured with 100 μl protein A agarose (2 h incubation, 4°C) and isolated by brief centrifugation at 14000×*g*. The pellet was washed three times with ice-cooled PBS (pH 7.4), resolubilized in 40 μl of reaction buffer (0.2% Triton X-100), centrifuged and phosphotransferase activity of the supernatant was measured as outlined above.

2.7. Synthesis of the MAPKK inhibitor PD 098059

3-(2-Amino-3-methoxy-phenyl)-chromen-4-one (PD 098059 [24]) was synthesized starting from 3-methoxy-2-nitrobenzoic acid chloride (obtained by reaction of 3-methoxy-2-nitrobenzoic acid, 1 g, 5 mM with PCl_5) and 2-hydroxy-acetophenone (495 mg, 3.6 mM) affording crude 2-(2-nitro-3-methoxy)-benzoyloxy-acetophenone; the latter intermediate was rearranged to 1-(2-nitro-3-methoxy-phenyl)-3-(2-hydroxy-phenyl)-propane-1,3-dione with potassium hydroxide and subsequently converted to 3-(2-nitro-3-methoxy-phenyl)-chromen-4-one with sulfuric acid/acetic acid at standard conditions [25]. Subsequent reduction of the nitro group to the corresponding amine 3-(2-amino-3-methoxy-phenyl)-chromen-4-one (PD 098059) was accomplished with raney-Ni/hydrazine in ethanol as described [26] and the product purified by silica gel chromatography using ethylacetate/chloroform/methanol 10/10/1, v/v as elution agent (total yield (not optimized, without purification of intermediates) 211 mg, 22% referring to 2-hydroxy-acetophenone). Identity and purity were confirmed by mass spectroscopy recorded on a MAT 311 A (Varian, Bremen, Germany) (70 eV, electron impact mode): m/z (%) = 267 (60.95) $[M]^+$, 250 (8.87), 224 (20.4), 147 (100), 57 (48.8) and by elemental analysis (carried out on a vario EL, Foss Heraeus, Hanau Germany): $C_{16}H_{13}NO_3$ (267.28) calculated (%): C 71.90, H 4.90, N 5.24; found: C 71.77, H 4.98, N 5.02.

3. Results

When differentiated resident U-937 cells were exposed to oxidized LDL (27 μg/ml) and the cell lysates were examined for MAPK activity as determined by phosphorylation of an MBP fragment (peptide APRTPGGRR), we found that the modified lipoprotein significantly stimulated phosphotransferase activity. As depicted in Fig. 1A, activation was maximal after 6 min of oxLDL treatment. Using a standard exposure time to oxLDL of 6 min, we then investigated concentration dependence and the effects of potentially inhibitory or stimulatory agents/procedures to get further insight into the stimulatory mechanisms involved. When increasing amounts of LDL or oxLDL were applied, a concomitant increase of MAPK activity was observed. Maximal stimulation (LDL 239%, oxLDL 176%) and apparent saturation was found in both cases at a concentration about 160 μg/ml (Fig. 1B), at concentrations >27 μg/ml, the LDL effect exceeded the oxLDL mediated one. The curve of the binding/uptake as determined with radioiodinated lipoproteins (Fig. 1B, inset) exhibited a shape similar to the dose-effect relationship and at a concentration >27 μg/ml, significantly more [^{125}I]LDL than [^{125}I]oxLDL was bound.

Treatment of LDL with phospholipase A_2 subsequent to oxidation further increased the stimulatory effect of oxLDL (27 μg/ml) (Fig. 2). A degree of activation similar to that obtained with oxLDL was achieved by administration of LysoPAF (200 nM) whereas plain PMA (200 nM) caused only little increase in phosphotransferase activity (not shown). When cells were cultured in lipoprotein deficient serum, stimulation provided by LDL was somewhat enhanced (189%, Fig. 2, in comparison to 164%, Fig. 1B) whereas the effect of oxLDL was unaltered. Pre-exposure to the inhibitor of lysosomal degradation chloroquine [27] (20 μM) could not prevent activation of MAPK by oxLDL. However, this result was achieved by competition with the scavenger receptor ligands poly I (40 μg/ml), fucoidan (10 μg/ml) (Fig. 2) and dextran sulfate (10 μg/ml, not shown). Furthermore, poly I somewhat reduced kinase activation by LDL. Control experi-

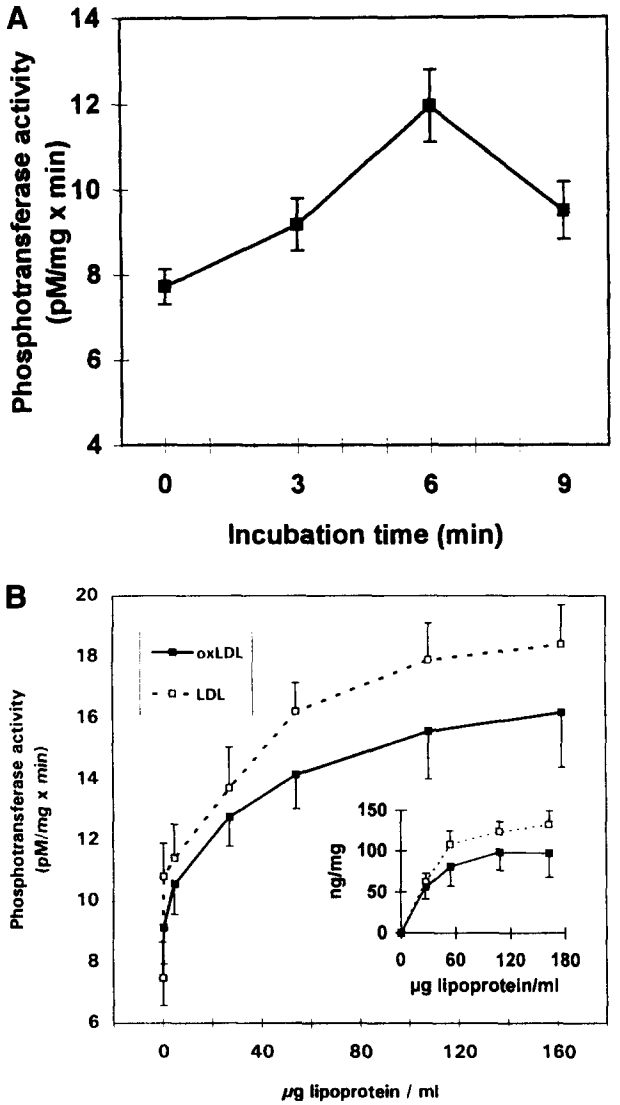

Fig. 1. (A) Differentiated U-937 cells (3×10^6) were treated with oxLDL (27 µg/ml) for the time indicated and the capacity of cell extracts to phosphorylate APRTPGGRR substrate peptide was measured as described in section 2; the data are representative of two separate experiments with triplicate determinations. (B) Increase of phosphotransferase activity with the lipoprotein concentration; macrophage-like U-937 cells were incubated (6 min) with increasing amounts of LDL or oxLDL and the capacity of cell extracts to catalyze APRTPGGRR peptide phosphorylation was assayed. Inset: the concentration-dependent binding/uptake of [^{125}I]oxLDL and [^{125}I]LDL was determined after 6 min incubation (displayed as ng/mg cell protein). Results are presented as the mean ± S.D. for triplicate determinations of a representative experiment.

ments confirmed that neither poly I, fucoidan nor dextran sulfate affected phosphorylation of the substrate peptide in the absence of lipoproteins (not shown). Consistent with an activation by mitogens, LPS (1 µg/ml) greatly increased the transfer of labeled phosphate to the peptide.

To confirm the activation of MAPK via MAPK kinase and to determine the relative contribution of this pathway to the observed oxLDL induced stimulation of peptide phosphorylation, the recently discovered inhibitor of MEK, PD 098059 [24], was synthesized and its effect on oxLDL induced MAPK activation examined. The agent (60 µM) completely blocked the effect of oxLDL, addition of the compound alone however did not alter the constitutive level of MAPK activity.

In order to eliminate the background of peptide phosphorylation probably caused by the contributions of other kinases and to further establish $p42^{MAPK}$ specificity of activation, experiments were carried out using immunoprecipitated enzyme. In this set of experiments the relative increase in phosphotransferase activity stimulated by oxLDL was greater than the enhancement induced in cellular extracts (averaging at approx. 200%) (Fig. 3). Pre-exposure of the cells to pertussis toxin (0.1 µg/ml, 24 h) did not affect this result and moreover, oxLDL induced stimulation was not abolished by PMA mediated down-regulation of PKC. MalBSA and native LDL (both 27 µg/ml) produced a stimulation (LDL 181%, malBSA 192%) similar to that caused by oxLDL. As observed with the total cell extracts, maximal activation (231%) was achieved with LPS (1 µg/ml).

4. Discussion

We found that LDL and oxLDL both can activate MAPK. The short exposure time required for activation as well as the absent effect of chloroquine, an inhibitor of lysosomal degradation [27], supports the suggestion of direct signal transduction subsequent to receptor occupation.

The effect of the inhibitor of MAPKK activation PD 098059 [28] on oxLDL induced MAP kinase stimulation and the reduction observed after administration of the SR ligands poly I, fucoidan and dextran sulfate provide evidence for the

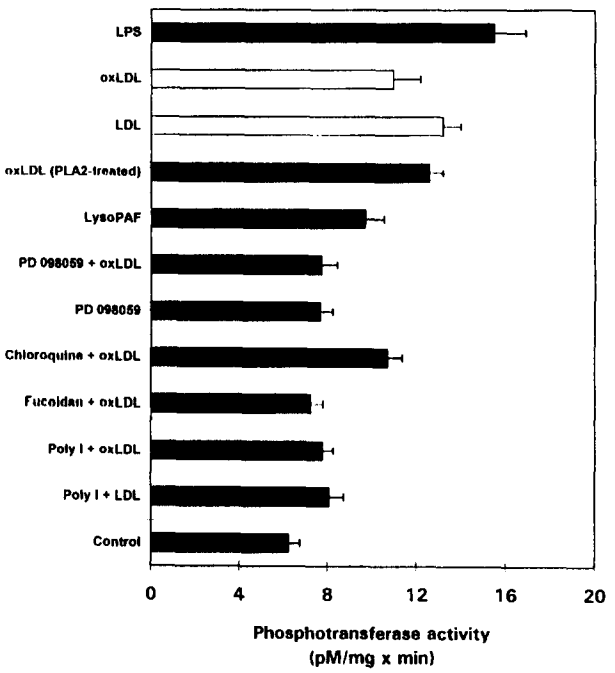

Fig. 2. Phosphotransferase activity (APRTPGGRR substrate peptide) of resident differentiated U-937 cell extracts was assayed after treatment with oxLDL (6 min, 27 µg (protein)/ml), PLA_2 treated oxLDL (2 U, 1 h) and/or with other stimuli/inhibitors; concentrations employed: LPS 1 µg/ml, lysoPAF 200 nM, poly I 40 µg/ml, fucoidan 10 µg/ml, chloroquine 20 µM, PD 098059 60 µM. Pretreatment of cells with selected agents (PD 098059, chloroquine, poly I, fucoidan, prior to the addition of oxLDL) was performed by incubation for 30 min. The open bars designate data obtained with cells cultured in lipoprotein deficient medium for 48 h. For LDL and oxLDL induced changes (without further additions) see Fig. 1b. The data are expressed as the means ± S.D. of two experiments with triplicate determinations.

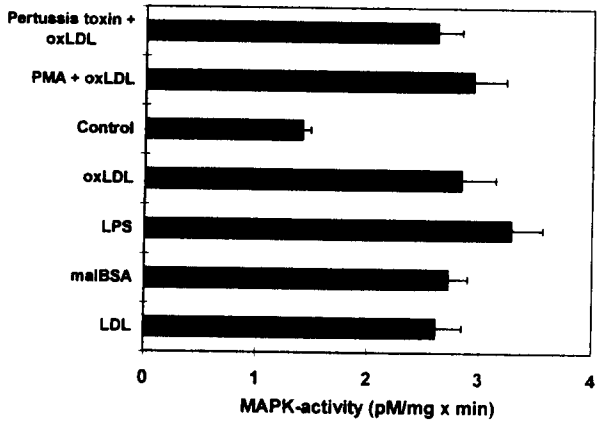

Fig. 3. Cells (1×10^7) were incubated with LDL, oxLDL, malBSA (all 27 μg/ml, 6 min), broken and MAPK precipitated with limiting amounts of polyclonal anti-$p42^{MAPK}$ antibodies. The isolated precipitates were washed, solubilized and MAPK activity was measured as described in section 2. As indicated, cells were pretreated with PMA (200 nM, 18 h) or pertussis toxin (0.1 μg, 24 h) for depletion of PKC or to block G-protein dependent processes; average values of three experiments ± S.D. are given.

transduction of signals starting with the occupation of scavenger receptors followed by subsequent steps involving MEK dependent activation of MAPK. Almost complete reduction of the phosphorylating capacity (close to control levels) by addition of PD 098059 prior to oxLDL further indicates that most of the (oxLDL produced) increase in phosphotransferase activity in cell extracts is due to stimulation of MAPK activity. Although these observations suggest that binding/uptake via scavenger receptors is mandatory, plain occupation of SR (as provided by polyanionic ligands like poly I) apparently is not sufficient. Consistently, growth induced by oxLDL has been described to be inhibitable by polyinosinic acid [10]. The partial blocking of the LDL induced effect by this agent might be explained by (potentially formed) poly I–LDL complexes and corresponding alterations in the receptor mediated uptake of these particles.

Results obtained with oxidized LDL and cell extracts could be confirmed by experiments carried out on immunoprecipitated kinase and we observed that malBSA also activates MAPK. In this context it is of note that maleylated albumin can replace the mitogen LPS in activating primed macrophages [29,30]. However, the assignment of such effects to specific receptors in this case requires further elucidation since Haberland and colleagues [31] have shown that maleylated albumin interacts with an additional binding site, distinct from the scavenger receptor in human monocytes/macrophages.

Resink and colleagues [15] have demonstrated that oxidized LDL as well as chemically modified LDL (acLDL, malondialdehyde modified LDL) were similar to LDL in stimulating DNA and protein synthesis in smooth muscle cells. These authors also suggested that the activation of mitogenic signals by lipoproteins does not depend on lipid peroxidation but may depend on direct activation of replication coupled signal transduction systems. Others [32], however, have stated that the growth stimulating effect of the chemically modified lipoprotein acLDL on murine macrophages was negligibly weak compared with that of oxLDL. Under our conditions, the addition of LDL provoked a similar (to equal amounts of oxLDL) stimulation of MAPK activity at concentrations ≤27 μg/ml and an even greater effect at >27 μg/ml, suggesting that both lipoproteins elicit similar (MAPK dependent) growth related effects. Contradictions are likely to be related to different numbers (and different types, apoB/E receptors or SR) of receptors available in distinct cell lines. We have shown that the shape of the dose-response curves for LDL and oxLDL induced MAPK activation was similar to the curve obtained for the binding/degradation of the radioiodinated lipoproteins. Although parameters obtained from degradation assays generally represent metabolism of the ligand at steady state rather than binding at equilibrium [33], the apparent dissociation constants determined at 37°C were found to be very similar to those determined for binding, uptake and degradation [34–36]. Hence, our data suggest a dose-response relationship of scavenger or apoB/E receptor binding/degradation and MAPK stimulation. Culturing of the cells in lipoprotein deficient serum produced only a small additional increase of MAPK activity in LDL stimulated cells indicating poor up-regulation of LDL receptors by this pretreatment. However, this result agrees with the previous observation of Hayashi and colleagues [17] who conversely found only sluggish down-regulation of LDL receptors in differentiated U-937 cells upon cholesterol accumulation. Among the components of LDL/oxLDL, there are several agents with potential mitogenic activity; e.g. oxidation of LDL is accompanied by the generation of lysophospholipids [37]. The presence of lysophospholipids in oxLDL is most probably due to the activity of the LDL associated PAF acetylhydrolase capable of hydrolyzing PAF-like compounds generated during oxidative fragmentation of LDL phospholipids [38,39]. In support of this interpretation, it has been demonstrated [32] that lysophosphatidylcholine is essential to the mitogenic effect of oxLDL on murine macrophages. These authors also described a treatment of acLDL with phospholipase A_2 which caused an increase in lysophosphatidylcholine concentration as well as an enhanced mitogenic activity of this lipoprotein. We found that incubation of oxLDL with phospholipase A_2 further stimulated phosphorylation of the substrate peptide which provides evidence of a stimulatory effect of lysophosphocholines. On the other hand, a recent report indicates that oxLDL contains phospholipids with PAF-like activity which are generated along with the oxidation of LDL and may account for mitogenic effects and stimulated growth of smooth muscle cells [40]. The fact that the mitogenic effect of oxLDL could be abolished by pretreatment with PAF acetylhydrolase supports this interpretation. Accordingly, LDL associated PAF-acetylhydrolase, just like the hydrolysis provided by exogenous phospholipases, may increase the mitogenic potential of oxidizing LDL by generation of lysophosphocholines. However, it may also cause the opposite effect, a reduction due to the hydrolysis of PAF-like compounds. Our data are compatible with the first mentioned possibility that LDL associated acetylhydrolase may increase the mitogenic potential of oxLDL. This assumption is also supported by the finding that PAF receptor mediated events (in murine macrophage-like cells) can be blocked by pertussis toxin [41], an observation which (unlike e.g. activation of MAPK by sphingophosphorylcholine [42]) we did not note for oxLDL induced activation of MAPK. Further analysis, however, will require (quantitative) consideration of the number/affinity of the respective receptors involved as well as a quantitation of

lysophosphocholines and PAF-like compounds of the respective particle. Furthermore, conclusions based on experimental data must account for the possibility that the presence of PAF-acetylhydrolase activity (during the oxidation of LDL) is likely to alter the modification as well as the binding/uptake of the resulting modified particle by scavenger receptors [43,44].

Lysophosphatidylcholine may modulate the effects induced by PKC activation [45] and we could demonstrate recently that oxLDL stimulates PKC activity in murine macrophage-like cells [46]. The results of this study, however, suggest that $p42^{MAPK}$ activation by oxidized LDL is independent of PKC activity (or activation) as shown by down-regulation of PKC following a prolonged treatment of the cells with phorbol ester.

In conclusion, our data support the notion that LDL and oxLDL both exert intrinsic primary mitogenic properties which in the case of the latter particle requires interactions with scavenger receptors.

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