Oxidized LDL induces the expression of ALBP/aP2 mRNA and protein in human THP-1 macrophages

Yuchang Fu,[1,*] Nanlan Luo,* and Maria F. Lopes-Virella[†]

Division of Endocrinology, Diabetes, and Medical Genetics,* Department of Medicine, Medical University of South Carolina, Charleston, SC 29403; and Ralph H. Johnson Veterans Administration Medical Center,[†] Charleston, SC 29401

Abstract The adipocyte lipid-binding protein (ALBP/aP2) belongs to a multigene family of fatty acid and retinoid transport proteins. This protein is abundantly expressed in the cytoplasm and nuclear region of adipocytes and is postulated to serve as a lipid shuttle, solubilizing hydrophobic fatty acids and delivering them to the appropriate metabolic system for utilization. This report demonstrates that human cholesterol-loaded THP-1 macrophages express ALBP/aP2 and that its expression can be stimulated by oxidized low density lipoprotein (oxLDL). The increase in mRNA expression was paralleled by a similar increase in ALBP/aP2 protein. The increase in ALBP/aP2 mRNA and protein in oxLDL-stimulated THP-1 macrophages is concentration and time dependent and is inhibited by treatment of the cells with an antioxidant inhibitor of nuclear factor-κB (NF-κB), pyrrolidine dithiocarbamate (PDTC), and with protein kinase C (PKC) inhibitors bisindolylmaleimide I and Ro-31-8220. These results suggest that activation of both NF-κB and PKC signaling pathways is necessary for oxLDL-induced ALBP/aP2 gene expression in THP-1 macrophages and that the upregulation of the fatty acid carrier may be a necessary event in foam cell formation.—Fu, Y., N. Luo, and M. F. Lopes-Virella. **Oxidized LDL induces the expression of ALBP/aP2 mRNA and protein in human THP-1 macrophages.** ***J. Lipid Res.*** **2000.** 41: **2017–2023.**

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The adipocyte lipid-binding protein (ALBP/aP2), a member of the intracellular fatty acid-binding protein (FABP) multigene family (1), selectively binds hydrophobic ligands such as long-chain fatty acids (2, 3). ALBP/aP2, the product of the FABP4 gene, exhibits significant sequence identity to other members of the FABP gene family such as myelin P2 protein (67%), the cardiac/skeletal muscle FABP (FABP3, 64%), and the keratinocyte fatty acid-binding protein (FABP5, 51%) (4). Whereas ALBP/aP2 expression was originally believed to be adipose specific, its expression has been reported in THP-1 cells and in certain bladder cell carcinomas (5). Although a specific physiological role has yet to be firmly established for any member of this family, gene disruption studies support the hypothesis that FABPs serve the cell as intracellular fatty acid chaperones (6–8).

The ALBP/aP2 gene is one of the cellular targets for the nuclear peroxisome proliferator-activated receptor γ (PPARγ) during adipocyte differentiation (9). Moreover, ALBP/aP2 is also upregulated in THP-1 cells and primary cell monocytes treated with PPARγ agonists (10). Consistent with the view of PPARγ as a primary determinant of ALBP/aP2 expression, mouse embryonic stem cells lacking both copies of the PPARγ gene lack expression of the ALBP/aP2 gene and are not able to develop into fat cells (11).

The expression of ALBP/aP2 in human monocytes raises the possibility that the intracellular trafficking of fatty acids may be critical in the etiology of foam cell formation and atherogenesis. Among monocyte/macrophage cell lines, THP-1 cells have been particularly useful to study the induction of scavenger receptor expression during their differentiation from monocytes to macrophages during exposure to phorbol myristate acetate (PMA) (12–14). Unlike the low density lipoprotein (LDL) receptor, macrophage scavenger receptors are not downregulated by an increase in the cellular cholesterol content and take up oxidized LDL (oxLDL) avidly (15–17), leading to the formation of foam cells and accelerated atherosclerosis (18). OxLDL not only induces accumulation of lipids in the arterial wall, leading to the transformation of macrophages into foam cells, but is also cytotoxic to vascular endothelial cells, where it acts as a chemoattractant, re-

Abbreviations: acLDL, acetylated low density lipoprotein; ALBP/aP2, adipocyte lipid-binding protein; EDTA, ethylenediaminetetraacetic acid; FABP, fatty acid-binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; glyLDL, glycated low density lipoprotein; IgG-IC, IgG immune complexes; I-κB, inhibitor of NF-κB; KLBP, keratinocyte lipid-binding protein; LDL, low density lipoprotein; NF-κB, nuclear factor κB; nLDL-IC, native LDL immune complexes; oxLDL, oxidized low density lipoprotein; oxLDL-IC, oxidized LDL immune complexes; PBS, phosphate-buffered saline; PDTC, pyrrolidine dithiocarbamate; PKC, protein kinase C; PMA, phorbol myristate acetate; PPARγ, peroxisome proliferator-activated receptor γ; SDS, sodium dodecyl sulfate.

[1] To whom correspondence should be addressed.

cruiting monocytes into the vessel wall. OxLDL also induces the expression of PPARγ in THP-1 cells (19–21); however, the molecular mechanisms involved in oxLDL-induced gene regulation in macrophages are still unclear.

In this article we demonstrate that one of the genes highly induced by oxLDL stimulation of THP-1 macrophages is ALBP/aP2. The expression of the ALBP/aP2 gene can also be upregulated in cholesterol-loaded THP-1 macrophages by Fcγ receptor-mediated uptake of native LDL immune complexes (nLDL-IC) or oxLDL immune complexes (oxLDL-IC). The upregulation of ALBP/aP2 gene expression in oxLDL-stimulated THP-1 macrophages is concentration and time dependent and is markedly inhibited by co-incubation with pyrrolidine dithiocarbamate (PDTC), an antioxidant inhibitor of the NF-κB signaling pathway, or with bisindolylmaleimide I, as well as 3-[1-[3-(amidinothio)propyl-1H-indol-3-yl]-3-(1-methyl-1H-indol-3-yl)] maleimide methanesulfonate (Ro-31-8220), two inhibitors of the protein kinase C (PKC) signaling pathway. These data suggest that activation of NF-κB and PKC signaling pathways in THP-1 macrophages is important for oxLDL-induced ALBP/aP2 gene expression. Upregulation of the ALBP/aP2 gene by oxLDL, nLDL-IC, or oxLDL-IC indicates that fatty acid transport in THP-1 macrophages may be involved in solubilization and delivery of lipid moieties from the LDL particle to lipid-signaling pathways.

MATERIALS AND METHODS

Human monocytic leukemia THP-1 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Tissue culture media were purchased from Life Technologies (Gaithersburg, MD). PMA and NF-κB inhibitor PDTC were purchased from Sigma (St. Louis, MO). PKC inhibitors bisindolylmaleimide I and Ro-31-8220 were purchased from Calbiochem (San Diego, CA). RNA isolation reagents were purchased from Biotecx Laboratory (Houston, TX). Nylon membranes and the transfer system were purchased from Schleicher & Schuell (Keene, NH). Radioisotopes were purchased from NEN Life Science Products (Boston, MA). A primer DNA-labeling kit was purchased from Roche Molecular Biochemicals (Indianapolis, IN). Spin columns for probe purification and hybridization solution were purchased from Clontech (Palo Alto, CA). Unless otherwise specified, all other reagents were purchased from Sigma.

Cell culture

Human monocytic leukemia THP-1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (Tissue Culture Biologicals, Tulare, CA), penicillin (100 U/ml) and streptomycin (100 mg/ml) at 37°C in 5% CO_2. THP-1 monocytes were treated with 100 nM PMA for 24 h to facilitate differentiation into macrophages. After treatment, the adherent macrophages were washed three times with phosphate-buffered saline (PBS) and incubated with cell culture medium for 24 h at 37°C until addition of oxLDL.

LDL isolation and modification

Human peripheral blood from healthy volunteers was collected in 10 μM ethylenediaminetetraacetic acid (EDTA). Native LDL (d = 1.019 to 1.063 g/ml) was isolated from the plasma by sequential ultracentrifugation at 60,000 rpm for 24 h at 10°C in a 60-Ti rotor (Beckman, Palo Alto, CA). Native LDL was washed and concentrated by ultracentrifugation at 40,000 rpm for 24 h at 10°C in an SW 41 rotor (Beckman). The concentrated LDL was dialyzed against 0.15 M NaCl and 300 μM EDTA (pH 7.4) at 4°C for 36 h. Acetylated LDL (acLDL) was prepared with acetic anhydride by the method of Basu et al. (22). Glycation of native LDL was performed with 150 mM glucose 6-phosphate at 37°C for 1 week followed by dialysis against 0.15 M NaCl and 300 μM EDTA (pH 7.4) at 4°C for 12 h. OxLDL was prepared by incubation of LDL (2 mg of LDL protein/ml) with 40 μM $CuCl_2$ in PBS for 24 h at 37°C followed by the addition of 300 μM EDTA, and dialysis against 0.15 M NaCl and 300 μM EDTA (pH 7.4) at 4°C for 36 h. The degree of LDL oxidation was determined by measurements of conjugated dienes (23) and fluorescent compounds (24). The formation of conjugated dienes and fluorescent compounds, as surrogates for oxidation of lipid and protein moieties, respectively, in oxLDL was 5.8- and 15-fold of those in native LDL, respectively. nLDL-IC, oxLDL-IC, or IgG immune complexes (IgG-IC) were prepared by incubating native LDL, oxLDL, or IgG overnight at 4°C with a rabbit anti-LDL or anti-IgG antiserum raised in our laboratory as previously described (25). The antigen-to-antibody ratio yielding the highest amount of precipitate was considered to correspond to the equivalence point and was used for the preparation of the insoluble immune complexes. Protein concentrations were determined by the Lowry protein assay, using bovine serum albumin as a standard (26). All lipoproteins and their immune complexes were sterilized by filtration through a 0.45-μm pore size filter (Gelman Sciences, Ann Arbor, MI) and stored at 4°C. Lipoproteins and their immune complexes were used within 2 weeks of preparation.

Stimulation analysis

All stimulation agents were added to the culture medium in a minimal volume (<10%) of PBS at a variety of concentrations. The incubation periods varied according to the experimental protocol. For each experiment, a minimum of three independent experiments was performed.

Inhibition analysis

A variety of inhibitors for the signaling pathways studied were added during the last 24 h of incubation to the culture medium, in a minimal volume (<0.1%) of dimethyl sulfoxide or ethanol and at several concentrations according to the experimental protocol. For each experiment, a minimum of three independent experiments was performed.

RNA isolation and Northern blot analysis

For each sample, about 5×10^6 cells were lysed in Ultraspec RNA isolation solution (Biotecx) and total cellular RNA was isolated according to the manufacturer protocol. Approximately 20 μg of each total RNA sample was separated on a 1% agarose-formaldehyde gel and transferred onto a nylon membrane with 20× SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0) solution. The RNA on the membrane was treated with a UV cross-linker. Probe corresponding to the partial coding region and 3′ region of the ALBP/aP2 gene was labeled to high specific activity ($>2 \times 10^9$ dpm/μg), using the random primer DNA labeling kit according to the manufacturer protocol. Membranes were prehybridized in ExpressHyb hybridization solution for 30 min and then hybridized with the probe for 1 h at 68°C. Hybridized membranes were washed once at room temperature for 30 min in 2× SSC containing 0.5% sodium dodecyl sulfate (SDS) and again for another 30 min at 55°C in 0.2× SSC containing 0.5% SDS. To control for equivalency of RNA loading and transfer, the membranes were stripped and rehybridized with a 1-kb human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA fragment purchased from the ATCC. Semiquantitative analysis

of autoradiograms was assessed by the Foto/UV21 imaging system, using NIH Image software.

Immunochemical analysis

THP-1 macrophages after incubation were washed twice with 1× PBS buffer and scraped off from culture dishes with cell lysis buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) containing freshly added protease inhibitor cocktail (Sigma). Twenty-five micrograms of protein per lane and known molecular weight markers (Bio-Rad, Hercules, CA) were separated by 10% SDS-polyacrylamide gel electrophoresis. Proteins were electrophoretically transferred onto polyvinylidene difluoride membranes and incubated overnight at 4°C with blocking solution (5% nonfat milk in PBS). The blocked membranes were incubated with immunopurified anti-murine ALBP/aP2 polyclonal antibody (kindly provided by D. A. Bernlohr, Department of Biochemistry, University of Minnesota, St. Paul, MN) (1:1000 dilution with 1% nonfat milk in PBS) for 1 h at room temperature, and washed three times with PBS buffer containing 0.1% Tween 20 for 15 min at room temperature with shaking. The membranes were then incubated with anti-rabbit antibody coupled to horseradish peroxidase (1:5000 dilution with 1% nonfat milk in PBS) for 1 h at room temperature, and washed four times with PBS buffer containing 0.1% Tween 20 for 15 min at room temperature with shaking. Immunodetection analyses were accomplished with the Enhance chemiluminescence kit (NEN Life Science Products). Semiquantitative analysis of film was performed with the Foto/UV21 imaging system, using NIH Image software.

RESULTS

Upregulation of ALBP/aP2 gene by oxLDL and LDL immune complexes in THP-1 macrophages

Because ALBP/aP2 plays an important role in lipid trafficking during differentiation of adipocytes, and is upregulated in monocytes treated with PPARγ agonists, we explored the possibility that expression of ALBP/aP2 might be stimulated in THP-1 cells by incubation of the cells with lipoproteins or lipoprotein immune complexes under conditions that lead to increased cellular lipid content. Human monocytic leukemia THP-1 cells were treated with 100 nM PMA for 24 h to induce their differentiation into macrophages. The PMA-treated THP-1 macrophages were subsequently washed and incubated with culture medium for an additional 24 h to ensure that PMA treatment would not directly contribute to, or affect, the results obtained. No upregulation of the ALBP/aP2 was detected after washing PMA away from culture medium. THP-1 macrophages were examined by flow cytometric analysis for the expression of CD14, which is a marker for macrophages. Our data indicated that 81.2% of THP-1 cells kept the differentiated status without PMA for up to 48 h, 63.9% of THP-1 cells up to 6 days (data not shown). After differentiation, THP-1 macrophages were treated with lipoprotein (native LDL), modified lipoproteins [acLDL, glycated LDL (glyLDL), and oxLDL], and lipoprotein-containing complexes (nLDL-IC and oxLDL-IC), with the general cell activator lipopolysaccharide (LPS), and with IgG anti-LDL (the IgG preparation used to prepare oxLDL-IC and nLDL-IC). IgG-IC were also used to stimulate the cells to determine whether nonspecific immune complexes or only those containing a lipoprotein moiety were able to induce stimulation of ALBP/aP2 (**Fig. 1**). Among these stimuli, oxLDL treatment led to the highest level of ALBP/aP2 mRNA (0.65 kb) in THP-1 macrophages compared with the controls (more than 3-fold). nLDL-IC and oxLDL-IC stimulation also led to high levels of ALBP/aP2 mRNA in THP-1 macrophages when compared with the controls, although native LDL itself did not induce the expression of ALBP/aP2 gene. The non-lipoprotein/stimuli (IgG-IC and IgG anti-LDL), acLDL, and glyLDL did not increase ALBP/aP2 mRNA.

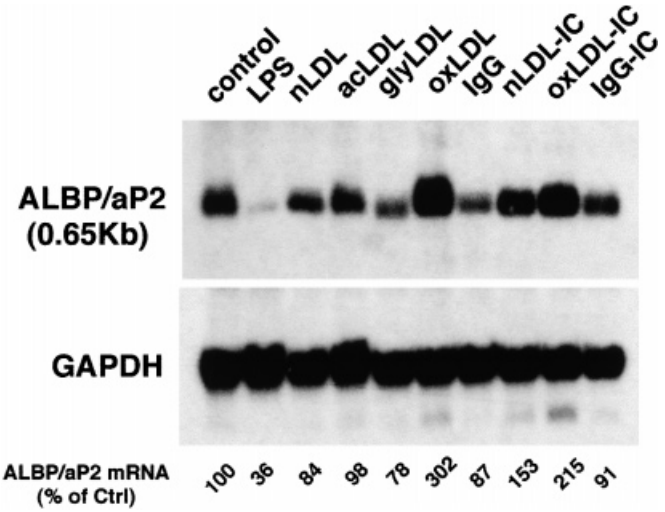

Fig. 1. ALBP/aP2 gene expression in THP-1 macrophages. THP-1 monocytes were treated with 100 nM PMA for 24 h, washed with PBS, and incubated for an additional 24 h at 37°C with medium alone. After the 24 h recovery, THP-1 macrophages were incubated for 24 h at 37°C with medium (lane control) or with medium containing LPS (100 μg/ml); native LDL (100 μg/ml); modified LDL (acLDL, glyLDL, and oxLDL; 100 μg/ml); IgG (100 μg/ml); or LDL immune complexes (nLDL-IC, oxLDL-IC, and IgG-IC; 150 μg/ml). After incubation, THP-1 macrophages were harvested for RNA isolation. Northern blot analysis, as described in Materials and Methods, was performed to examine the levels of ALBP/aP2 mRNA (0.65 kb), using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (1.4 kb) as control for RNA loading.

Upregulation of ALBP/aP2 by oxLDL is concentration and time dependent

We next examined whether the upregulation of ALBP/aP2 gene expression by oxLDL in THP-1 macrophages would be concentration and time dependent. After THP-1 monocytes were differentiated into macrophages by PMA treatment, the recovered THP-1 macrophages were incubated for 24 h with oxLDL in concentrations ranging from 5 to 100 μg/ml. Northern blot analysis showed that the levels of ALBP/aP2 mRNA in THP-1 macrophages gradually increased with increasing concentrations of oxLDL up to 100 μg/ml (**Fig. 2A**). To determine whether the increase in ALBP/aP2 mRNA was paralleled by a corresponding increase in protein, immunoblotting was performed. As shown in Fig. 2B, the greater than 3-fold upregulation of ALBP/aP2 mRNA was accompanied by a

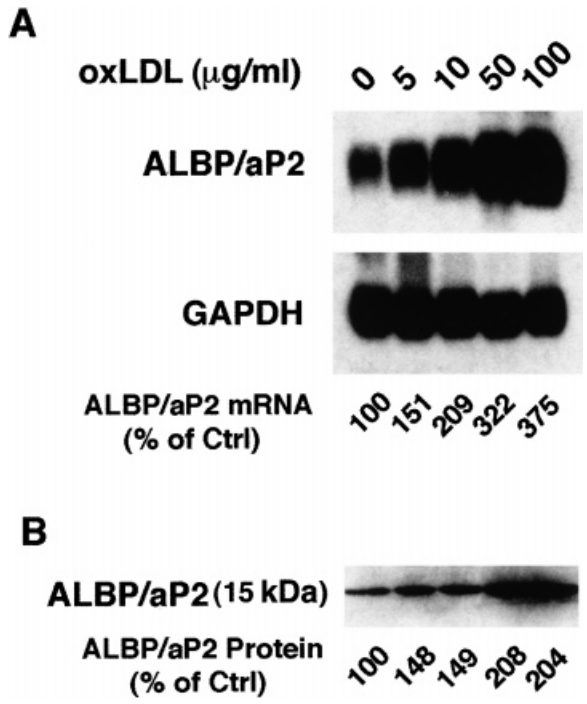

Fig. 2. Effect of oxLDL on the induction of ALBP/aP2 gene expression in THP-1 macrophages. THP-1 monocytes were treated with 100 nM PMA for 24 h to induce differentiation into macrophages. After recovering from the PMA treatment for an additional 24 h, the macrophages were incubated with various concentrations of oxLDL ranging from 5 to 100 μg/ml for 24 h. (A) Northern blot analysis was performed to examine the expression of ALBP/aP2. GAPDH mRNA was similarly examined on the same membrane to monitor RNA loading and transfer. (B) Western blot analysis for ALBP/aP2 protein (15 kDa) in THP-1 macrophages. The experimental design is the same as described in (A). After incubation, the cells were scraped and protein was extracted, and then used to examine the amounts of ALBP/aP2 protein by immunoblotting as described in Materials and Methods.

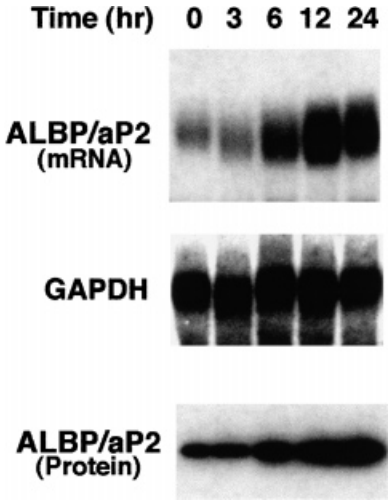

Fig. 3. Time course of ALBP/aP2 gene expression induced by oxLDL in THP-1 macrophages. THP-1 monocytes were treated with 100 nM PMA for 24 h to induce differentiation into macrophages. After recovering from the PMA treatment for 24 h, the cells were incubated with oxLDL (100 μg/ml) for 3 to 24 h. Total RNA isolation, Northern blot analysis, protein isolation, and Western blot analysis, as described in Materials and Methods, were performed to examine the expression of ALBP/aP2 gene. GAPDH cDNA was used to reprobe the same membrane to monitor RNA loading and transfer.

2-fold increase in protein expression (15 kDa). When the time course for upregulation was evaluated, using an oxLDL concentration of 100 μg/ml, maximal increases in ALBP/aP2 mRNA and protein levels were observed between 12 and 24 h of incubation (**Fig. 3**). During the incubation period, oxLDL-treated THP-1 macrophages showed no signs of cell apoptosis as observed by cell morphology and determined by Western blot for the caspase-dependent poly(ADP-ribose) polymerase (PARP) cleavage (data not shown). Thus, the expression of ALBP/aP2 gene induced by oxLDL in THP-1 macrophages is concentration and time dependent.

Effect of multiple signaling pathways on oxLDL-mediated upregulation of ALBP/aP2 in THP-1 macrophages

To understand the mechanisms involved in the upregulation of ALBP/aP2 gene expression by oxLDL, we examined the role of two different signaling pathways: nuclear factor κB (NF-κB) and PKC.

Previous studies have shown that activation of NF-κB, a transcription factor involved in immediate-early gene activation during inflammatory processes, is regulated by oxLDL in human macrophages (27). NF-κB activation involves phosphorylation of NF-κB inhibitor (I-κB) and proteolysis of this inhibitor by a large protease complex, the proteasome, followed by nuclear translocation of the dimer (28, 29). The antioxidant PDTC was found to selectively block the dissociation of I-κB from the cytoplasmic NF-κB, thus preventing the activation and nuclear translocation of NF-κB (30, 31). To evaluate whether the NF-κB signaling pathway would be involved in oxLDL-mediated upregulation of the ALBP/aP2 gene in THP-1 macrophages, THP-1 macrophages were co-incubated with oxLDL (100 μg/ml) and with different concentrations of PDTC ranging from 25 to 200 μM. **Figure 4** shows that the expression of ALBP/aP2 induced by oxLDL was inhibited by PDTC in a concentration-dependent manner. These results demonstrated that activation of the NF-κB signaling pathway is a key element in the regulation of ALBP/aP2 gene expression in THP-1 macrophages induced by oxLDL.

To investigate whether activation of the PKC signaling pathway would also be involved in oxLDL-mediated upregulation of the ALBP/aP2 gene in THP-1 macrophages, we co-incubated THP-1 macrophages for 24 h with oxLDL (100 μg/ml) and different concentrations of bisindolylmaleimide I (**Fig. 5**) or Ro-31-8220 (**Fig. 6**), two inhibitors of the PKC signaling pathway. Both PKC inhibitors inhibited oxLDL-induced ALBP/aP2 gene expression in THP-1 macrophages in a concentration-dependent manner.

DISCUSSION

The ALBP/aP2 was initially discovered in adipose tissue and is believed to function in the mobilization and traf-

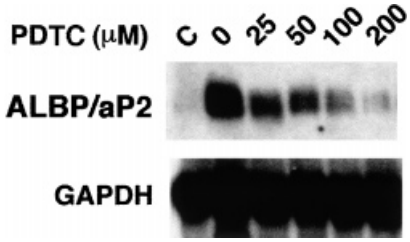

Fig. 4. Inhibition of the oxLDL-mediated upregulation of ALBP/aP2 expression by NF-κB inhibitor PDTC in THP-1 macrophages. THP-1 monocytes were treated with 100 nM PMA for 24 h to induce differentiate into macrophages. After recovering from the PMA treatment for 24 h, the cells were co-incubated with oxLDL (100 μg/ml) and increasing concentrations (25 to 200 μM) of PDTC, an inhibitor for the NF-κB signaling pathway, for 24 h. Lane C shows control macrophages without oxLDL and inhibitor treatment. Lane 0 indicates THP-1 macrophages treated only with oxLDL. After the co-incubation, the THP-1 macrophages were harvested and total cellular RNA was isolated. Northern blot analysis was performed as described in Materials and Methods. The same membrane was rehybridized with a GAPDH cDNA probe as a control for RNA loading and transfer.

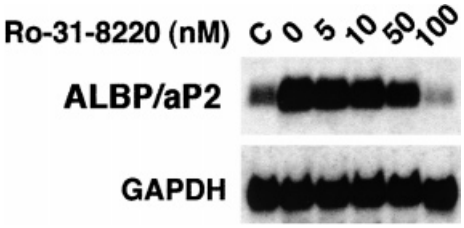

Fig. 6. Inhibition of oxLDL induced ALBP/aP2 gene expression by PKC inhibitor Ro-31-8220 in THP-1 macrophages. THP-1 monocytes were treated with 100 nM PMA for 24 h to induce the cells to differentiate into macrophages. After recovering from the PMA treatment for 24 h, the PKC inhibitor Ro-31-8220 was added at various concentrations (1 to 100 nM) with oxLDL (100 μg/ml) for 24 h of incubation. Lane C indicates control macrophages without oxLDL and inhibitor treatment. Lane 0 represents THP-1 macrophages treated only with oxLDL. After co-incubation, the cells were harvested, total cellular RNA was isolated, and Northern blot analysis was performed to examine the expression of ALBP/aP2. GAPDH cDNA was used to reprobe the same membrane to monitor RNA loading and transfer.

ficking of intracellular fatty acids. A second FABP, the keratinocyte lipid-binding protein (KLBP), is also expressed in adipocytes to a level about 1% that of ALBP/aP2. These two lipid-binding proteins are the only two lipid-binding proteins found in adipose tissue.

Pelton et al. (10) reported that ALBP/aP2 is upregulated in THP-1 cells after activation of PPARγ with its ligands. Our work extends their observation and demonstrates that the ALBP/aP2 gene is upregulated in THP-1 macrophages in response to stimulation with oxLDL, nLDL-IC, and oxLDL-IC. In human THP-1 macrophages, the uptake of oxLDL has been found to be related to the high expression of scavenger receptors in the cell, and

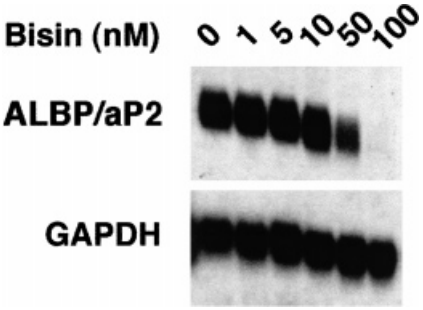

Fig. 5. Inhibition of oxLDL induced ALBP/aP2 gene expression by PKC inhibitor bisindolylmaleimide I in THP-1 macrophages. THP-1 cells were treated with 100 nM PMA for 24 h to induce the monocytes to differentiate into macrophages. After recovering from the PMA treatment for 24 h, the PKC inhibitor bisindolylmaleimide I was added to the THP-1 macrophages at various concentrations (1 to 100 nM) with oxLDL (100 μg/ml) for 24 h of incubation. Lane 0 represents THP-1 macrophages treated only with oxLDL. After the incubation, these macrophages were harvested, total cellular RNA was isolated, and then Northern blot analysis was performed to examine the expression of ALBP/aP2 as described in Materials and Methods. GAPDH cDNA was used to reprobe the same membrane to monitor RNA loading and transfer.

the uptake of nLDL-IC and oxLDL-IC is mediated by Fcγ receptors (32, 33). The presence of high concentrations of native LDL also stimulates the expression of either scavenger or Fcγ receptors (34, 35). The uptake of oxLDL, oxLDL-IC, and nLDL-IC promotes cholesterol accumulation in THP-1 macrophages and contributes to the formation of foam cells. Because of the high content of fatty acids in the LDL moiety, it is perhaps not surprising that its uptake induced upregulation of ALBP/aP2 gene expression. The increased expression of ALBP/aP2 in THP-1 macrophages exposed to oxLDL or to immune complexes containing native or oxLDL likely occurs to fulfill the increased lipid trafficking in the stimulated macrophages. Consistent with this hypothesis, LPS, which inhibits the expression of scavenger receptor in human macrophages (36), and native LDL, whose uptake is mainly mediated by the LDL receptor (15) and therefore will not lead to high cellular lipid accumulation, have little ability to upregulate the ALBP/aP2 gene in THP-1 macrophages. Interestingly, in the present study, exposure of macrophages to acLDL does not elicit a change in ABLP/aP2 levels, suggesting that induction of ALBP/aP2 gene is mediated either by different scavenger receptors that specifically bind to oxLDL, or by specific responses to oxidized components from oxLDL.

It has been reported that oxLDL stimulates the NF-κB and PKC signal transduction pathways in macrophage-like cells (27, 37). The NF-κB signaling pathway regulates the transcription of an exceptionally large number of genes, particularly those participating in immune and inflammatory responses (38, 39). The processes induced by oxLDL in atherosclerosis, such as endothelium injury, monocyte recruitment to the macrophage lineage, and foam cell formation, are generally considered inflammatory responses (40). On the other hand, stimulation of the PKC pathway is considered the most common activation mechanism in signal transduction systems. Furthermore, the uptake of

oxLDL by scavenger receptors I/II and the subsequent lysosomal degradation of LDL have been shown to be critical to the activation of the PKC signaling pathway (37). Our present study has shown that the activation of the NF-κB or PKC signal transduction pathway is directly or indirectly involved in oxLDL-induced ALBP/aP2 gene expression in human THP-1 macrophages.

The binding of fatty acids by ALBP/aP2 may affect their ability to be metabolized into endogenous second-messenger PPARγ ligands such as 9-hydroxyoctadecadienoic acid or 13-hydroxyoctadecadienoic acid (20, 21). Agonist binding by PPARγ allows for heterodimerized with the retinoid X receptor (41–44), targeting the expression of key genes involved in carbohydrate and lipid metabolism.

Findings suggest that PPARγ, originally found as a versatile regulator with an important role in adipocyte differentiation, may also be involved in oxLDL-mediated gene regulation in human macrophages and vascular smooth muscle cells (19–21, 45). Moreover, oxLDL can activate the expression of the PPARγ gene through a novel signaling pathway involving scavenger receptor-mediated particle uptake in macrophages. Consistent with this is the high expression levels of PPARγ in the foam cells of atherosclerotic lesions. The link between the scavenger receptor-mediated uptake of oxLDL and PPARγ expression suggests that PPARγ may be a key regulator for gene expression during atherogenesis and may be involved in monocyte differentiation. Troglitazone, one of the synthetic ligands for the activation of PPARγ, has been demonstrated to be able to induce ALBP/aP2 gene expression in human skeletal muscle cells and monocytes (10, 46).

Targeted disruption of ALBP/aP2 does not result in altered composition of the adipose fatty acid or triacylglycerol pools, changes in fatty acid uptake and re-esterification, or in differences in the morphology of the adipose tissue. Such null animals upregulate the expression of KLBP, potentially compensating for the loss of ALBP/aP2 (8, 47). However, ALBP/aP2 null animals, relative to wild-type littermates, do become obese when maintained on a high fat diet and exhibit reduced lipolytic capacity (8, 48). Moreover, Scheja et al. (49) have also shown a statistically significant decrease in circulating triglyceride, glucose, and insulin in ALBP/aP2-disrupted mice when compared with their wild-type littermates. Taken together, these results suggest that the intracellular FABP functions as a lipid chaperone, facilitating the movement of fatty acids out of the fat cells in adipose tissue. Clearly, in vivo studies, such as overexpression or inhibition of ALBP/aP2 gene expression in macrophages, will be required to determine the function of the ALBP/aP2 gene in lipid trafficking and foam cell formation.

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REFERENCES

1. Banaszak, L., N. Winter, Z. Xu, D. A. Bernlohr, S. Cowan, and T. A. Jones. 1994. Lipid-binding proteins: a family of fatty acid and retinoid transport proteins. *Adv. Protein Chem.* **45:** 89–131.
2. Matarese, V., and D. A. Bernlohr. 1988. Purification of murine adipocyte lipid-binding protein. Characterization as a fatty acid- and retinoic acid-binding protein. *J. Biol. Chem.* **263:** 14544–14551.
3. Baxa, C. A., R. S. Sha, M. K. Buelt, A. J. Smith, V. Matarese, L. L. Chinander, K. L. Boundy, and D. A. Bernlohr. 1989. Human adipocyte lipid-binding protein: purification of the protein and cloning of its complementary DNA. *Biochemistry.* **28:** 8683–8690.
4. Hunt, C. R., J. H-S. Ro, D. E. Dobson, H. Y. Min, and B. M. Spiegelman. 1986. Adipocyte P2 gene: developmental expression and homology of 5′-flanking sequences among fat cell-specific genes. *Proc. Natl. Acad. Sci. USA.* **83:** 3786–3790.
5. Celis, J. E., M. Østergaardk, B. Basse, A. Celis, J. B. Lauridsen, G. P. Ratz, I. Andersen, B. Hein, H. Wolf, R. F. Ørntoft, and H. H. Rasmussen. 1996. Loss of adipocyte-type fatty acid binding protein and other protein biomarkers is associated with progression of human bladder transitional cell carcinomas. *Cancer Res.* **56:** 4782–4790.
6. Coe, R. N., and D. A. Bernlohr. 1998. Physiological properties and functions of intracellular fatty acid-binding proteins. *Biochim. Biophys. Acta.* **1391:** 287–306.
7. Binas, B., H. Danneberg, J. McWhir, L. Mullins, and A. J. Clark. 1999. Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. *FASEB J.* **13:** 805–812.
8. Coe, N. R., M. A. Simpson, and D. A. Bernlohr. 1999. Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J. Lipid Res.* **40:** 967–972.
9. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* **8:** 1224–1234.
10. Pelton, P. D., L. Zhou, K. T. Demarest, and T. P. Burris. 1999. PPARγ activation induces the expression of the adipocyte fatty acid binding protein gene in human monocytes. *Biochem. Biophys. Res. Commun.* **261:** 456–458.
11. Rosen, E. D., P. Scarraf, A. E. Troy, G. Bradwin, K. Moore, D. S. Milstone, B. M. Spiegelman, and R. M. Mortensen. 1999. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. *Mol. Cell.* **4:** 611–617.
12. Auwerx, J. 1991. The human leukemia cell line, THP-1: a multifaceted model for the study of monocyte-macrophage differentiation. *Experientia.* **47:** 22–31.
13. Kodama, T., P. Reddy, C. Kishimoto, and M. Krieger. 1988. Purification and characterization of a bovine acetyl low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **85:** 9238–9242.
14. Via, D. P., L. Pons, D. K. Dennison, A. E. Fanslow, and F. Bernini. 1989. Induction of acetyl-LDL receptor activity by phorbol ester in human monocyte cell line THP-1. *J. Lipid Res.* **30:** 1515–1524.
15. Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown. 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA.* **76:** 333–337.
16. Henriksen, T., E. M. Mahoney, and D. Steinberg. 1982. Interactions of plasma lipoproteins with endothelial cells. *Ann. N.Y. Acad. Sci.* **401:** 102–116.
17. Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum. 1989. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320:** 915–924.
18. Steinberg, D. 1997. Low density lipoprotein oxidation and its pathobiological significance. *J. Biol. Chem.* **272:** 20963–20966.
19. Ricote, M., J. Huang, L. Fajas, A. Li, J. Welch, J. Najib, J. L. Witztum, J. Auwerx, W. Palinski, and C. K. Glass. 1998. Expression of the peroxisome proliferator-activated receptor gamma (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc. Natl. Acad. Sci. USA.* **95:** 7614–7619.
20. Nagy, L., P. Tontonoz, J. G. A. Alvarez, H. Chen, and R. M. Evans. 1998. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. *Cell.* **93:** 229–240.
21. Tontonoz, P., L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans. 1998. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell.* **93:** 241–252.

22. Busa, S. K., J. L. Goldstein, R. G. W. Anderson, and M. S. Brown. 1976. Degradation of cationized low density lipoprotein and regulation of cholesterol metabolism in homozygous familial hypercholesterolemia fibroblasts. *Proc. Natl. Acad. Sci. USA.* **73:** 3178–3182.
23. Esterbauer, H., G. Striegl, H. Puhl, and M. Rotheneder. 1989. Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radic. Res. Commun.* **6:** 67–75.
24. Cominacini, L., U. Garbin, A. Davoli, R. Micciolo, O. Bosello, G. Gaviraghi, L. A. Scuro, and A. M. Pastorino. 1991. A sample test for predisposition of LDL oxidation based on the fluorescence development during copper-catalyzed oxidative modification. *J. Lipid Res.* **32:** 349–358.
25. Griffith, R. L., G. T. Virella, H. C. Stevenson, and M. F. Lopes-Virella. 1988. Low density lipoprotein metabolism by human macrophages activated with low density lipoprotein immune complexes. A possible mechanism of foam cell formation. *J. Exp. Med.* **168:** 1041–1059.
26. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
27. Brand, K., T. Eisele, U. Kreusel, M. Page, S. Page, M. Haas, A. Gerling, C. Kaltschmidt, F. J. Neumann, N. Mackman, P. A. Baeurele, A. K. Walli, and D. Neumeier. 1997. Dysregulation of monocytic nuclear factor-κB by oxidized low-density lipoprotein. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1901–1909.
28. Thanos, D., and T. Maniatis. 1995. NF-κB: a lesson in family values. *Cell.* **80:** 529–532.
29. Baeuerle, P. A., and T. Henkel. 1994. Function and activation of NF-kappa B in the immune system. *Annu. Rev. Immunol.* **12:** 141–179.
30. Schreck, R., B. Meier, D. N. Mannel, W. Droge, and P. A. Baeuerle. 1992. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J. Exp. Med.* **175:** 1181–1194.
31. Ziegler-Heitbrock, H. W. L., T. Sternsdorf, J. Liese, B. Belohradsky, C. Weber, A. Wedel, R. Schreck, P. A. Baeverle, and M. Strobel. 1993. Pyrrolidine dithiocarbamate inhibits NF-kappa B mobilization and TNF production in human monocytes. *J. Immunol.* **151:** 6986–6993.
32. Gisinger, C., G. T. Virella, and M. F. Lopes-Virella. 1991. Erythrocyte-bound low-density lipoprotein immune complexes lead to cholesteryl ester accumulation in human monocyte-derived macrophages. *Clin. Immunol. Immunopathol.* **59:** 37–52.
33. Lopes-Virella, M. F., and G. T. Virella. 1994. Atherosclerosis and autoimmunity. *Clin. Immunol. Immunopathol.* **73:** 155–167.
34. Han, J., D. P. Hajjar, M. Febbraio, and A. C. Nicholson. 1997. Native and modified low density lipoproteins increase the functional expression of the macrophage class B scavenger receptor, CD36. *J. Biol. Chem.* **272:** 21654–21659.
35. Khoo, J. C., E. Miller, F. Pio, D. Steinberg, and J. L. Witztum. 1992. Monoclonal antibodies against LDL further enhance macrophage uptake of LDL aggregates. *Arterioscler. Thromb.* **12:** 1258–1266.
36. Van Lenten, B. J., A. M. Fogelman, J. Seager, E. Ribi, M. E. Haberland, and P. A. Edwards. 1985. Bacterial endotoxin selectively prevents the expression of scavenger-receptor activity on human monocyte-macrophages. *J. Immunol.* **134:** 3718–3721.
37. Claus, R., B. Fyrnys, H. P. Deigner, and G. Wolf. 1996. Oxidized low-density lipoprotein stimulates protein kinase C (PKC) and induces expression of PKC-isotypes via prostaglandin-H-synthase in P388D1 macrophage-like cells. *Biochemistry.* **35:** 4911–4922.
38. Baeuerle, P. A., and V. R. Baichwal. 1997. NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. *Adv. Immunol.* **65:** 111–137.
39. Barnes, P. J., and M. Karin. 1997. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.* **336:** 1066–1071.
40. Ross, R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature.* **362:** 801–809.
41. Kliewer, S. A., B. M. Forman, B. Blumberg, E. S. Ong, U. Borgmeyer, D. J. Mangelsdorf, K. Umesono, and R. M. Evans. 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA.* **91:** 7355–7359.
42. Lemberger, T., B. Desvergne, and W. Wahli. 1996. PPARs: a nuclear receptor signaling pathway in lipid metabolism. *Annu. Rev. Cell Dev. Biol.* **12:** 335–363.
43. Tontonoz, P., R. A. Graves, A. I. Budavari, H. Erdjument-Bromage, M. Lui, E. Hu, and B. Spiegelman. 1994. Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPARγ and RXRα. *Nucleic Acids Res.* **22:** 5628–5634.
44. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPARγ 2, a lipid-activated transcription factor. *Cell.* **79:** 1147–1156.
45. Marx, N., U. Schonbeck, M. A. Lazar, P. Libby, and J. Plutzky. 1998. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth cells. *Circ. Res.* **83:** 1097–1103.
46. Park, K. S., T. P. Ciaraldi, K. Lindgren, L. Abrams-Carter, S. Mudaliar, S. E. Nikoulina, S. R. Tufari, J. H. Veerkamp, A. Vidal-Puig, and R. R. Henry. 1998. Troglitazone effects on gene expression in human skeletal muscle of type II diabetes involve up-regulation of peroxisome proliferator-activated receptor-gamma. *J. Clin. Endocrinol. Metabol.* **83:** 2830–2835.
47. Bernlohr, D. A., N. R. Coe, M. A. Simpson, and A. V. Hertzel. 1997. Regulation of gene expression in adipose cells by polyunsaturated fatty acids. *Adv. Exp. Med. Biol.* **422:** 145–156.
48. Hotamisligil, G. S., R. S. Johnson, R. J. Distel, R. Ellis, V. E. Papaioannou, and B. M. Spiegelman. 1996. Uncoupling of obesity from insulin resistance through targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science.* **274:** 1377–1379.
49. Scheja, L., L. Makowski, K. T. Uysal, S. M. Wiesbrock, D. R. Shimshek, D. S. Meyers, M. Morgan, R. A. Parker, and G. S. Hotamisligil. 1999. Altered insulin secretion associated with reduced lipolytic efficiency in aP2−/− mice. *Diabetes.* **48:** 1987–1994.