TGF-β1 Up-Regulates Expression of ABCA1, ABCG1 and SR-BI through Liver X Receptor α Signaling Pathway in THP-1 Macrophage-Derived Foam Cells

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Aim: High density lipoprotein (HDL) and its apolipoproteins can promote cholesterol efflux from macrophage foam cells via the ATP-binding cassette transporter A1 (ABCA1), ABCG1, and scavenger receptor class B type I (SR-BI). Liver X receptors (LXRs) operate as cholesterol sensors which may protect from cholesterol overload by stimulating cholesterol efflux from cells to HDL through ABCA1, ABCG1 and SR-BI. The regulation of ABCA1, ABCG1 and SR-BI expression by cytokines present within the microenvironment of the atheroma may play an important role in determining the impact of reverse cholesterol transport on the atherosclerotic lesion. In the current study, we examined the effect of transforming growth factor-β1 (TGF-β1) on expressions of ABCA1, ABCG1 and SR-BI and explored the role of LXR α in the regulation of ABCA1, ABCG1 and SR-BI in THP-1 macrophage-derived foam cells.

Methods and Results: TGF-β1 significantly increased expressions of ABCA1, ABCG1 and SR-BI at both transcriptional and translational levels in a dose-dependent and time-dependent manner. Cellular cholesterol content was decreased while cholesterol efflux was increased by TGF-β1 treatment. Moreover, LXR α was up-regulated by TGF-β1 treatment. In addition, LXR α small interfering RNA completely abolished the promotion effect induced by TGF-β1.

Conclusion: These results provide evidence that TGF-β1 up-regulates expressions of ABCA1, ABCG1 and SR-BI through the LXR α pathway in THP-1 macrophage-derived foam cells.


Key words: ABCA1, ABCG1, SR-BI, TGF-β1, LXR α, Atherosclerosis

Introduction

The accumulation of lipoprotein cholesterol in the artery wall is thought to be an important factor in the development of atherosclerosis. After retention and modification in arteries, atherogenic lipoproteins are taken up by macrophages, bringing about macrophage-derived foam cells15. In past decades, high density lipoprotein (HDL) has been proposed to decrease atherosclerosis mainly by reverse cholesterol transport (RCT), a process by which HDL carries excess cholesterol from peripheral tissues and cells, including foam cells, back to the liver for removal from the body. The amount of lipid accumulation in macrophages reflects the balance between the rate of cholesterol accumulation/uptake and its removal via the reverse
cholesterol transport pathway). Mobilization to the plasma membrane and efflux to extracellular acceptors of intracellular cholesterol are important mechanisms of cells in regulating the cholesterol level, which is the first step in reverse cholesterol transport.

It has been demonstrated that efflux of cholesterol from cells can occur by several mechanisms, including unmediated aqueous diffusion or specific receptor-mediated processes. ABCA1 is a member of the ATP-binding cassette superfamily, which couples the energy provided by ATP hydrolysis to the transport of a wide variety of molecules across membranes. ABCA1 is thought to mediate the active efflux of cholesterol and phospholipids to apolipoprotein (apo) acceptors, most importantly apoA-I, the major apo of HDL. In addition to ABCA1, ABCG1, another member of the ABC transporter superfamily, was also shown to be capable of mediating the active efflux of cholesterol and phospholipids mainly to lipid-rich acceptor particles in macrophages. Moreover, scavenger receptor class B type I (SR-BI) facilitates the bidirectional flux of free cholesterol (FC) between cells and lipoprotein, and the flux of FC between lipoproteins and cells expressing SR-BI is closely linked to the phospholipid content and composition of the lipoprotein.

Liver X receptors α and β (LXR α and LXR β) are ligand-activated transcription factors involved in the control of lipid metabolism and inflammation. Recently, studies from our laboratory reported that synthetic LXR agonists could inhibit the progression of atherosclerosis in apoE−/− mice fed a high-fat/high-cholesterol diet. The endogenous activators of these receptors are oxysterols and intermediates in the cholesterol biosynthetic pathway. LXR α and LXR β serve as cholesterol sensors that regulate the expression of multiple genes involved in the efflux, transport, and excretion of cholesterol. Recent studies have outlined the importance of LXR signaling pathways in the development of metabolic disorders such as hyperlipidemia and atherosclerosis. It is reported that ABCA1 and ABCG1 are both ubiquitously expressed and are subject to regulation by cholesterol loading and by treatment with agents that activate the nuclear hormone receptor LXR. In addition to this, previous research revealed that both promoter activity and protein levels of SR-BI were positively regulated by LXR/RXR activated by 22-R-OH cholesterol and a functional LXR response element (LXRE) in the human SR-BI promoter that binds both LXR α and LXR β.

Atherosclerosis is a chronic inflammatory condition, involving enhanced monocyte/endothelial cell interactions. Increasing evidence suggests a pivotal role for inflammatory processes in all phases of atherosclerosis from fatty streak lesions to plaque rupture. Local and systemic soluble inflammatory mediators are critical players in regulating atherosclerotic plaque development. Foam cell formation can be mainly modulated through the regulation of lipoprotein uptake and cholesterol efflux. TGF-β has been shown to regulate proteins involved in lipoprotein uptake, including inhibition of SRAI/II and CD36 expression. In addition, TGF-β was demonstrated to enhance macrophage cholesterol efflux by up-regulating mRNA expression of ABCA1 and ABCG1. However, although TGF-β has the potential to modulate macrophage lipoprotein uptake and cholesterol efflux, the detailed mechanism of effect on ABCA1, ABCG1 and SR-BI induced by TGF-β in foam cells has not been fully elucidated.

In the present study, we attempt to further explore TGF-β1 on expression of ABCA1, ABCG1 and SR-BI at both mRNA and protein levels, and to further determine the possible mechanism through which TGF-β1 affects expressions of ABCA1, ABCG1 and SR-BI, which is associated with intracellular cholesterol efflux. We demonstrated that TGF-β1 could up-regulate expressions of ABCA1, ABCG1 and SR-BI in THP-1 macrophage-derived foam cells and also found that LXR α expression was increased when cells were treated with TGF-β1. Furthermore, LXR α small interfering RNA completely abolished the effects of TGF-β1 on the expressions of ABCA1, ABCG1 and SR-BI. Taken together, our data suggested that TGF-β1 might increase the expressions of ABCA1, ABCG1 and SR-BI by up-regulating the LXR α expression in THP-1 macrophage-derived foam cells.

Materials and Methods

TGF-β1 (R&D Systems Inc., Minneapolis, MN, USA), TRIzol Reagent (Invitrogen, Carlsbad, USA), BCA Protein Assay Kit (Pierce Chemical, Rockford, IL, USA), ReverAid™ First Strand cDNA Synthesis Kit (#k1622) (Fermentas, Burlington, Ontario, Canada), DyNAmoTM SYBR® Green qPCR Kits (Finnzymes, Espoo, Finland) and immobilon-P transfer membranes (Millipore, MA, USA) were obtained as indicated. All other chemicals were of the best grade available from commercial sources.

Preparation of Ox-LDL

Native LDL was purchased from Sigma (city, state, country). Native LDL (200 μg protein/mL) was oxidized by exposure to CuSO4 (5 μmol/L free Cu2+...
in phosphate-buffered saline at 37°C for 18–20 h. Control incubations were performed in the presence of 200 μmol/L EDTA without CuSO₄. Oxidation was terminated by refrigeration. Oxidation of LDL was confirmed by measuring thiobarbituric acid-reactive substances (TBARS) with malonaldehyde bis (dimethyl acetal) (MDA) as the standard. The TBARS content of ox-LDL was 1.12±0.08 versus 0.24±0.06 nmol/100 μg protein in the native LDL preparation (p<0.01). Protein content was determined by a bicinchoninic acid (BCA) protein assay kit using bovine serum albumin as the standard.

**Cell Culture and Foam Cell Formation Evaluated by Oil Red O staining**

Human THP-1 cells were seeded in six-well plates at 1.0×10⁶ cells per well in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 20 IU/mL penicillin, and 20 μg/mL streptomycin and maintained at 37°C in an humidified atmosphere of 5% CO₂. The cells were differentiated into macrophages by the addition of 100 ng/mL phorbol 12-myristate 13-acetate (PMA) for 72 h. Macrophages were transformed into foam cells by incubation in the presence or absence of 50 mg/mL ox-LDL in serum-free RPMI 1640 medium containing 0.3% bovine serum albumin (BSA) for 48 h. Cultured macrophages were then washed once with PBS and fixed in 4% paraformaldehyde-PBS for 10 min. After rinsing with 60% isopropanol again; thereafter, macrophages were stained with 0.3% Oil Red O in 60% isopropanol for 10 min and then washed with 60% isopropanol again; thereafter, macrophages were counterstained with hematoxylin for 3 min. After copious washing with water, macrophages were photographed with a microscope at 400× magnification.

**RNA Isolation and Real-Time Quantitative PCR Analysis**

Total RNA from cells was extracted using TRIzol reagent in accordance with the manufacturer’s instructions. Real-time quantitative PCR, using SYBR Green detection chemistry, was performed on Roche Light Cycler Run 5.32 Real-Time PCR System. Melt curve analyses of all real-time PCR products were performed and shown to produce a single DNA duplex. Quantitative measurements were determined using the ΔΔ Ct method and the expression of β-actin was used as the internal control.

**Western Blot Analyses**

Cells were harvested and protein extracts prepared as previously described. They were then subjected to Western blot analyses [10% SDS-polyacrylamide (SDS-PAGE); 30 μg protein per lane] using rabbit anti-ABCA1, anti-ABCG1 and anti-SR-BI- (Novus Biologicals Littleton, CO, USA), mouse anti-LXR α (PPMX, Tokyo, Japan) and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) -specific antibodies. The proteins were visualized using a chemiluminescence method (ECL Plus Western Blotting Detection System; Amersham Biosciences, Foster City, CA, USA).

**Transfection for LXR α Silencing**

Short-interfering RNA (siRNA) specific for human LXR α (Santa Cruz Biotechnology) and non-silencing control siRNA were synthesized by the Biology Engineering Corporation (Shanghai, China). THP-1 macrophage-derived foam cells (2×10⁶ cells/well) were transfected using Lipofectamine 2000 (Invitrogen). Real-time RT-PCR was performed 48 h after transfection. In comparison to control siRNA, the siRNA of LXR α suppressed the expression of LXR α proteins by 84% according to Western blot analysis.

**Cellular Cholesterol Efflux Experiments**

Cells were cultured as indicated above and then labeled with 0.2 μCi/mL [³H]cholesterol. After 72 h, cells were washed with phosphate-buffered saline (PBS) and incubated overnight in RPMI 1640 medium containing 0.1% (w/v) bovine serum albumin (BSA) to allow equilibration of [³H]cholesterol in all cellular pools. Equilibrated [³H]cholesterol-labeled cells were washed with PBS and incubated in 2 mL efflux medium containing RPMI 1640 medium and 0.1% BSA with or without 25 μg/mL human plasma apoA-I. A 150 μL sample of efflux medium was obtained at the times designated and passed through a 0.45 μm filter to remove any floating cells. Monolayers were washed twice in PBS, and cellular lipids were extracted with isopropanol. Medium and cell-associated [³H]cholesterol was then measured by liquid scintillation counting. Percent efflux was calculated by the following equation: [total media counts/(total cellular counts + total media counts)]×100%.

**Phospholipid Efflux Experiments**

Cells were cultured as indicated above, and then incubated with 2 μCi/mL of [³H]choline chloride to label the phospholipids. After 72 h, cells were subsequently washed with PBS and incubated overnight in RPMI 1640 medium containing 0.1% (w/v) BSA. After 6 hours of incubation with medium and 10 mg/mL apoA-I, efflux medium was collected, centrifuged to remove cell debris as above, and aliquots were
taken for extraction and separated by thin-layer chromatography using silica G plates developed in chloroform/methanol/ammonia [25% (w/v)]/water [50:65:5:4 (vol/vol)]. Phospholipid spots were visualized by I2 vapors and identified by co-migration with standards. Relative radioactivity was measured by Phosphoscreen and quantified by PhosphorImager (Molecular Dynamics Inc.). Phospholipid efflux was expressed as percent counts in the supernatant versus the total for each individual lipid.

**High Performance Liquid Chromatography Assays**
High performance liquid chromatography (HPLC) analysis was conducted as described previously. Briefly, cells were washed with PBS three times. The appropriate volume (usually 1 mL) of 0.5% NaCl was added to about 50–200 µg cellular proteins/mL. Cells were sonicated using an ultrasonic processor for 2 min. The protein concentration in the cell solution was measured using a BCA kit. A 0.1 mL aliquot cell solution (containing 5–20 µg protein) was used to measure free cholesterol, and another aliquot for total cholesterol detection. Free cholesterol was dissolved in isopropanol (1 mg cholesterol/mL) and stored at −20°C as stock solution. Cholesterol standard calibration solution ranging from 0 to 40 µg cholesterol/mL was obtained by diluting the cholesterol stock solution in the same cell-lysed buffer.

A 0.1 mL sample (cholesterol standard calibration solution or cell solution) was supplemented with 10 µL reaction mixture including 500 mM MgCl2, 500 mM Tris–HCl (pH 7.4), 10 mM dithiothreitol, and 5% NaCl. Then, 0.4 U cholesterol oxidase in 10 µL 0.5% NaCl was added to each tube for free cholesterol determination, or 0.4 U cholesterol oxidase plus 0.4 U cholesterol esterase for total cholesterol measurement. The total reaction solution in each tube was incubated at 37°C for 30 min, and 100 µL methanol: ethanol (1:1) was added to stop the reaction. Each solution was kept cold for 30 min to allow protein precipitation, and then centrifuged at 1,500 rpm for 10 min at 15°C. A 10 µL sample of supernatant was applied to a System Chromatographer (PerkinElmer Inc.) including a PerkinElmer series 200 vacuum degasser, a pump, a PerkinElmer series 600 LINK, a PerkinElmer series 200 UV/vis detector, and a Discovery C-18 HLPC column (Supelco Inc.). The column was eluted using isopropanol:n-heptane:acetonitrile (35:13:52) at a flow rate of 1 mL/min for 8 min. Absorbance at 216 nm was monitored. Data were analyzed with TotalChrom software from PerkinElmer.

**Statistical Analysis**
Data are expressed as the means ± S.D. Results were analyzed by one-way ANOVA and Student’s t test, using SPSS 13.0 software. Statistical significance was obtained when p values were less than 0.05.

**Results**
**TGF-β1 Up-Regulates ABCA1, ABCG1 and SR-BI Expression in THP-1 Macrophage-Derived Foam Cells**
Previous researches revealed that macrophage cholesteryl ester accumulation decreased and cholesterol efflux increased in macrophages and macrophage-derived foam cells after being treated by TGF-β[26, 27]. In the present study, we first examined the effect of TGF-β1 on expressions of ABCA1, ABCG1 and SR-BI in THP-1 macrophage-derived foam cells by real-time quantitative PCR and Western immunoblotting assays. As shown, TGF-β1 increased their expression at both transcriptional (Fig. 1A, 1B) and translational (Fig. 1C, 1D) levels in a dose-dependent and time-dependent manner.

ABCA1, ABCG1 and SR-BI are key players in reverse cholesterol transport and are critical for regulating cellular cholesterol homeostasis[31]. As ABCA1, ABCG1 and SR-BI were up-regulated by TGF-β1, we next examined the effect of TGF-β1 on cholesterol content and apoAI-specific cholesterol efflux in THP-1 macrophage-derived foam cells by high performance liquid chromatography and liquid scintillation counting assays. Cellular cholesterol content was decreased (Table 1, 2) while cholesterol efflux to apoAI was increased (Fig. 1E, 1F, 1G) by TGF-β1. This suggests that TGF-β1 could increase apoAI-mediated cholesterol efflux, possibly through up-regulating the expressions of the ABCA1, ABCG1 and SR-BI pathways in THP-1 macrophage-derived foam cells.

Because ABCA1, ABCG1 and SR-BI are both involved in cholesterol and phospholipid homeostasis in the cell[11, 12, 32], we then examined the possibility that TGF-β1 up-regulates phospholipid efflux or decreases the synthesis of phospholipids; foam cells were incubated with [3H]choline chloride to label choline-containing phospholipids. As demonstrated, TGF-β1 resulted in a significant increase of apoAI-mediated phospholipid efflux (Fig. 1H, 1I).

**LXR α Is Involved in TGF-β1-Induced Up-Regulation of ABCA1, ABCG1 and SR-BI in THP-1 Macrophage-Derived Foam Cells**
LXRs have been shown to regulate the expres-
Fig. 1. Dose-dependent and time-dependent effects of TGF-β1 on ABCA1, ABCG1 and SR-BI expression and ABCA1/ABCG1/SR-BI-dependent cholesterol and phospholipid efflux from THP-1 macrophage-derived foam cells.

A. C, E and H, Cells were treated with 10 mg/mL apoAI and with TGF-β1 at 0 ng/mL, 0.01 ng/mL, 0.1 ng/mL and 1.0 ng/mL for 24 h, respectively. B, D, F and I, Cells were treated with 10 mg/mL apoAI and 5 mg/mL BSA for 24 h or treated with 10 mg/mL apoAI and with 1.0 ng/mL TGF-β1 for 0 h, 6 h, 12 h, 24 h, respectively. G, Cells were treated with 0 ng/mL TGF-β1 and 0 mg/mL BSA or with 1.0 ng/mL TGF-β1 and 0 mg/mL apoAI for 24 h. A and B, ABCA1, ABCG1 and SR-BI genes were measured by real-time quantitative PCR. C and D, ABCA1, ABCG1 and SR-BI protein expressions were measured by Western immunoblotting assays. E, F and G, ApoAI-specific cholesterol efflux was analyzed by liquid scintillation counting assays as shown above. H and I, apoA-I-mediated phospholipid efflux was calculated by subtracting the efflux to medium and expressed as the percentage of total cellular and medium phospholipids. All results are expressed as the mean ± S.D. from three independent experiments, each performed in triplicate. *p < 0.05 vs vehicle.
Table 1. Effect of TGF-β1 on cholesterol content at different concentrations in THP-1 macrophage-derived foam cells

<table>
<thead>
<tr>
<th>TGF-β1 (ng/mL)</th>
<th>Control</th>
<th>0.01 ng/mL</th>
<th>0.1 ng/mL</th>
<th>1.0 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>496 ± 44</td>
<td>487 ± 36</td>
<td>345 ± 30*</td>
<td>296 ± 29*</td>
</tr>
<tr>
<td>FC (mg/dL)</td>
<td>196 ± 21</td>
<td>195 ± 21</td>
<td>142 ± 21*</td>
<td>122 ± 18*</td>
</tr>
<tr>
<td>CE (mg/dL)</td>
<td>300 ± 30</td>
<td>292 ± 19</td>
<td>203 ± 15*</td>
<td>174 ± 16*</td>
</tr>
<tr>
<td>CE/TC (%)</td>
<td>60.4</td>
<td>60.0</td>
<td>58.9</td>
<td>58.8</td>
</tr>
</tbody>
</table>

THP-1 macrophage-derived foam cells were divided into four groups and cultured in medium containing 0 ng/mL, 0.01 ng/mL, 0.1 ng/mL and 1.0 ng/mL TGF-β1 for 24h, respectively. Cellular cholesterol and cholesterol ester were extracted as described above. HPLC was performed to determine the cellular total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE). The results are expressed as the mean ± S.D. from three independent experiments, each performed in triplicate. *p < 0.05, vs control group.

Table 2. Effect of TGF-β1 on cholesterol content at different time in THP-1 macrophage-derived foam cells

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>24 h (BSA)</th>
<th>0 h (TGF-β1)</th>
<th>6 h (TGF-β1)</th>
<th>12 h (TGF-β1)</th>
<th>24 h (TGF-β1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>510 ± 46</td>
<td>520 ± 50</td>
<td>493 ± 36</td>
<td>368 ± 32*</td>
<td>341 ± 36*</td>
</tr>
<tr>
<td>FC (mg/dL)</td>
<td>195 ± 22</td>
<td>201 ± 27</td>
<td>192 ± 21</td>
<td>142 ± 14*</td>
<td>135 ± 17*</td>
</tr>
<tr>
<td>CE (mg/dL)</td>
<td>315 ± 26</td>
<td>319 ± 29</td>
<td>301 ± 16</td>
<td>226 ± 20*</td>
<td>206 ± 23*</td>
</tr>
<tr>
<td>CE/TC (%)</td>
<td>61.7</td>
<td>61.3</td>
<td>61.1</td>
<td>61.5</td>
<td>60.5</td>
</tr>
</tbody>
</table>

THP-1 macrophage-derived foam cells were divided into five groups and cultured in medium containing 5 mg/mL bovine serum albumin (BSA) for 24 h and then cultured in medium at 37°C containing 1.0 ng/mL TGF-β1 for 0 h, 6 h, 12 h, 24h respectively. Cellular cholesterol and cholesterol ester were extracted as described above. HPLC was performed to determine the cellular total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE). The results are expressed as the mean ± S.D. from three independent experiments, each performed in triplicate. *p < 0.05, vs 0 h group.

sions of ABCA1, ABCG1 and SR-BI, which serve as free-cholesterol and phospholipid translocators enabling cholesterol efflux from macrophages to various acceptors, including nascent cholesterol-poor HDL, and thus have a central role in the regulation of reverse cholesterol transport [17-20]. To confirm whether the LXR α expression can be affected by TGF-β1, real-time quantitative PCR and Western immunoblotting analysis were performed. As shown, the expression of LXR α mRNA (Fig. 2A, 2B) and protein (Fig. 2C, 2D) was increased when cells were treated with TGF-β1.

We then examined the effect of LXR α siRNA on the up-regulation of ABCA1 and ABCG1, and SR-BI induced by TGF-β1. Treatment with siRNA for LXR α down-regulated LXR α protein expression by 84% (Fig. 2E) and completely reversed the up-regulation of TGF-β1 on ABCA1, ABCG1 and SR-BI expressions (Fig. 2F, 2G). At the same time, cellular cholesterol efflux (Fig. 2H) and phospholipid efflux (Fig. 2I) in cells treated by the combination of LXR α siRNA and TGF-β1 were completely abolished as compared with those treated by TGF-β1 alone.

**Discussion**

Cellular cholesterol efflux, the first step in RCT, plays an important role in reducing the accumulation of lipids in the arterial wall and preventing the development of atherosclerosis. Cholesterol efflux occurs by different pathways, including transport mediated by specific proteins, and cholesterol efflux from mouse peritoneal macrophages has recently been shown to be mainly mediated by ABCA1, ABCG1 and SR-BI [11]. Liver X receptor can up-regulate the expressions of ABCA1, ABCG1 and SR-BI by forming heterodimers with retinoid X receptor [17-20]. TGF-β has been demonstrated to have a positive role in cholesterol trafficking by promoting cellular processes associated with reverse cholesterol transport. Here, we provide evidence that TGF-β1 may up-regulate the expression of these three major cholesterol transporters through the LXR α signaling pathway in THP-1 macrophage-derived foam cells.
A and B, TGF-β1 increased the expression of LXR α at both mRNA and protein levels. Cells were treated with TGF-β1 at 0 ng/mL, 0.01 ng/mL, 0.1 ng/mL, and 1.0 ng/mL; respectively, and cells were treated with 1.0 ng/mL for 0 h, 6 h, 12 h, 24 h, respectively, or with 5 mg/mL BSA for 24 h. A and B, total RNA was extracted and real-time quantitative PCR was performed to determine the expression of LXR α mRNA. C and D, Western immunoblotting assays using antibody against human LXR α and β-actin were conducted. Similar results were obtained in three independent experiments. Data are the mean ± S.D. *P<0.05 vs baseline. E, F, G, H and I, THP-1 macrophage-derived foam cells were transfected with control or LXR α siRNA, and then incubated with TGF-β1 (1.0 ng/mL) for 24 h. E, Protein samples were immunoblotted with anti-LXR α or anti-β-actin antibodies. Data represent three experiments with different cell preparations. F and G, mRNA and protein expression of ABCA1, ABCG1 and SR-BI were determined using real-time quantitative PCR and Western immunoblotting assays. H and I, Cellular cholesterol efflux and phospholipid efflux from THP-1 macrophage-derived foam cells were analyzed by liquid scintillation counting assays as shown above. Similar results were obtained in three independent experiments. Data are the mean ± S.D. *p<0.05 vs baseline.
TGF-\(\beta\) is known to have anti-inflammatory properties, as evidenced by the profound systemic inflammatory response reported for TGF-\(\beta^{-/-}\) mice\(^\text{30}\). In atherosclerosis, TGF-\(\beta\) could contribute to plaque stability through the inhibition of metalloproteinase activity and increased matrix deposition\(^\text{31}\). TGF-\(\beta\) might significantly increase cholesterol efflux in macrophage-derived foam cells from apoE\(^{-/-}\) mice\(^\text{30}\), and cholesterol, alone or complex in lipoproteins, could suppress TGF-\(\beta\) responsiveness by increasing lipid raft and/or caveolae accumulation of TGF-\(\beta\) receptors and facilitating rapid degradation of TGF-\(\beta\) and thus suppressing TGF-\(\beta\)-induced signaling\(^\text{30}\). Here we showed that TGF-\(\beta1\)-treated macrophages exhibited a significant increase in cholesterol efflux and phospholipid efflux mediated by apoA-I or HDL. The increase in apoA-I-mediated efflux is consistent with an increase at both the transcriptional and translational levels in ABCA1, ABCG1 and SR-BI expressions in TGF-\(\beta1\)-treated foam cells. These findings support the notion that TGF-\(\beta1\) may have atheroprotective properties by increasing cholesterol efflux and phospholipid efflux, and reducing macrophage foam cell formation.

Several studies have demonstrated that proinflammatory cytokines, such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interleukin-1\(\beta\) (IL-1\(\beta\)), reduce the expression of LXR \(\alpha\) and its heterodimeric partner. In contrast, interleukin-10 (IL-10), an anti-inflammatory and atheroprotective cytokine, stimulates LXR \(\alpha\) in cultured THP-1 macrophages\(^\text{30}\). We therefore tested the effect of TGF-\(\beta1\) on the expression of LXR \(\alpha\) to identify whether the expression of LXR \(\alpha\) changed during the course of up-regulating the expression of ABCA1, ABCG1 and SR-BI induced by TGF-\(\beta1\). TGF-\(\beta1\) markedly stimulated the expression of LXR \(\alpha\) at both mRNA and protein levels in THP-1 macrophage-derived foam cells. LXR \(\alpha\) siRNA was then used to assess whether LXR \(\alpha\) is involved in the up-regulation of these three major cholesterol transporters induced by TGF-\(\beta1\). The results showed that the up-regulation of mRNA and protein expression of ABCA1, ABCG1 and SR-BI were completely reversed by LXR \(\alpha\) siRNA. At the same time, cellular cholesterol efflux and phospholipid efflux were also completely reversed by LXR \(\alpha\) siRNA. These results reveal that LXR \(\alpha\) is involved in the up-regulation of ABCA1, ABCG1 and SR-BI induced by TGF-\(\beta1\) in THP-1 macrophage-derived foam cells.

The inhibition of macrophage foam cell formation by TGF-\(\beta1\) involves alterations in gene expression, likely mediated through a signal transduction pathway. After the binding of TGF-\(\beta1\) to its receptor, the primary downstream signaling events are mediated by Smads, a novel family of signaling molecules. Heteromeric complexes of Smads enter the nucleus and, along with other transcription factors, regulate the expression of TGF-\(\beta1\)-responsive genes\(^\text{35}\). Recent study showed cross-talk between TGF-\(\beta1\) and LXR signaling pathways and stimulation of cells with TGF-\(\beta1\) and LXR agonists has a synergistic effect on the expression of the LXR target gene ABCG1\(^\text{30}\).

Whether Smad complexes mediate TGF-\(\beta1\)-induced changes in macrophage gene expression observed in the present study remains to be determined. TGF-\(\beta1\) can also activate mitogen-activated protein (MAP) kinase (MAPK) cascades, thereby mediating some TGF-\(\beta1\)-induced downstream signaling events\(^\text{37}\); however, whether the role of MAPK is involved in the up-regulation of LXR \(\alpha\) induced by TGF-\(\beta1\) has not been fully elucidated.

In conclusion, this study addresses the missing link between TGF-\(\beta1\) treatment and increasing cholesterol efflux and phospholipid efflux by providing evidence that up-regulating TGF-\(\beta1\) is positively associated with expressions of ABCA1, ABCG1 and SR-BI, possibly by TGF-\(\beta1\) promoting LXR \(\alpha\) expression. These findings raise the possibility that future drugs able to selectively modulate TGF-\(\beta1\) expression might provide a novel form of therapy that enhances the activity of this cholesterol-removal pathway and prevents atherosclerosis.

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References


15) Xiaoyan Dai, Xiang Ou, Xinrui Hao, Dongli Cao, Yaling Tang, Yanwei Hu, Xiaoxu Li, Chaoke Tang: Effect of T0901317 on hepatic proinflammatory gene expression in apoE−/− mice fed a high-fat/high-cholesterol diet. Inflammation, 2007; 30: 105-117


