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Effect of Apolipoprotein A-I on ATP Binding Cassette Transporter A1 Degradation and Cholesterol Efflux in THP-1 Macrophage-derived Foam Cells

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Abstract Cholesterol-loaded macrophage foam cells are a central component of atherosclerotic lesions. ATP binding cassette transporter A1 (ABCA1), the defective molecule in Tangier disease, mediates the efflux of phospholipid and cholesterol from cells to apolipoprotein A-I (apoA-I), reversing foam cell formation. This study investigated the effect of apoA-I on ABCA1 degradation and cholesterol efflux in THP-1 macrophage-derived foam cells. After exposure of the cultured THP-1 macrophage-derived foam cells to apoA-I for different time, cholesterol efflux, ABCA1 mRNA and protein levels were determined by FJ-2107P type liquid scintillator, RT-PCR and Western blot, respectively. The mean ABCA1 fluorescence intensity on THP-1 macrophage-derived foam cells was detected by flow cytometry. Results showed that apoA-I markedly increased ABCA1-mediated cholesterol efflux from THP-1 macrophage-derived foam cells. This was accompanied by an increase in the content of ABCA1. ApoA-I did not alter ABCA1 mRNA abundance. Significantly, thiol protease inhibitors increased the level of ABCA1 protein and slowed its decay in THP-1 macrophage-derived foam cells, whereas none of the proteosome-specific inhibitor lactacystin, other protease inhibitors, or the lysosomal inhibitor NH_4Cl showed such effects. The apoA-I-mediated cellular cholesterol efflux was enhanced by thiol protease inhibitors. Our results suggested that thiol protease inhibitors might provide an alternative way to upregulate ABCA1 protein. This strategy is especially appealing since it may mimic the stabilizing effect of the natural ligands apoA-I.

Key words ATP binding cassette transporter A1; apoA-I; flow cytometry; cholesterol; lipoproteins

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 cells. High-density lipoprotein (HDL) plays a role in transporting cholesterol from peripheral tissues to the liver. The elevated level of HDL is associated with a decrease in atherosclerosis and the apolipoproteins to remove cholesterols from foam cells. Transgenic overexpression of the HDL-associated apoA-I or apoE decreases atherosclerosis in animals [1]. This has led to human clinical trials in which apoA-I is infused intravenously as a treatment for atherosclerosis [2]. However, there is uncertainty as to the underlying mechanisms of protection, and it is debated whether HDL is acting to promote the reverse cholesterol transport, by anti-inflammatory or antithrombotic effect [3].

Tangier disease, a condition characterized by the low level of HDL and cholesterol accumulation in macrophages, is caused by mutations in the ATP-binding cassette transporter A1 (ABCA1) [4]. The identification of mutations in ABCA1 in patients with Tangier disease and familial HDL deficiency demonstrated that inadequate transport of phospholipid and cholesterol to the extracellular space results in the hypercatabolism of lipid-poor nascent HDL particles [4]. ABCA1 in macrophage, hepatocyte, or other cells mediates phospholipid and cholesterol efflux to extracellu-

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lar apoA-I-associated HDL, which absorbs further lipid during its maturation in the bloodstream and eventually returns its cholesterol to the liver [5]. Thus, the upregulation of ABCA1 expression may play an important role in HDL formation and promotion of cholesterol efflux in foam cell [6].

The cellular expression of ABCA1 is highly regulated by small molecule agonists of LXR. In macrophages, the level of ABCA1 proteins is markedly increased with cholesterol loading [7]. In human monocyte-derived macrophages loaded with acetyl-LDL cholesterol esters, the increase of ABCA1 proteins appears disproportionate to the induction of ABCA1 mRNA [8], and the turnover of ABCA1 proteins in macrophages is rapid [9]. These observations suggest that the ABCA1 protein turnover could play a role in regulation of ABCA1 function. ABCA1 binds and cross-links to apoA-I [5], raising the possibility that apoA-I modulates the turnover of ABCA1. Indeed, a recent study has revealed that degradation of ABCA1 protein is the factor of the regulation at cellular ABCA1 protein level [10].

In this study, we investigated the effect of apoA-I on the degradation of ABCA1 proteins and cholesterol efflux in THP-1 macrophage-derived foam cells.

Materials and Methods

Materials

The human monocyte line THP-1 was purchased from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. A kit of reverse transcription-polymerase chain reaction was provided by Promega Inc. Trizol and the specific primers for *ABCA1* and *GAPDH* gene sequences were provided by Shanghai BioTechnologies Co., Ltd.. Goat anti-human ABCA1 antibody and fluorescein isothiocyanate (FITC)-labeled affinity-purified rabbit anti-goat IgG were purchased from Santa Crusz Biotechnology, Inc. [^{3}H]-cholesterol, cholesterol oxidase and esterase, bovine serum albumin (BSA), apoA-I, phorbol myristate acetate (PMA), acetonitrile, cholesterol, leupeptin, ALLN, pepstatin A, aprotinin, phosphoramiddon and lactacytin were from Sigma. Other chemicals were commercially available.

LDL isolation and oxidization

Low density lipoprotein (LDL) was isolated from nonfrozen human plasma as described [11]. The lower section of human plasma after isolation of VLDL/IDL was divided equally into two Quick-Seal tubes. The protein solution in each tube was adjusted to 1.063 g/ml in a total volume of 35 ml using 38% NaBr and 0.15 M NaCl. After being sealed, the tube was centrifuged at 42,000 rpm for 20 h at 8 °C. About 5 ml upper section of the tube solution was collected. The isolated LDL was oxidized with $CuSO_4$ (10 μM) for 18 h at 37 °C. The oxidation state of LDL was measured by the thiobarbituric acid-reactive substances assay. HDL was isolated as described [12].

Cell culture

THP-1 cells were maintained in RPMI 1640 containing 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 and 95% air. Differentiation of THP-1 monocytes into macrophages was induced by plating the cells at a density of 1.0×10^6 cells/well in a six-well plate in the presence of 160 nM PMA for 24 h and then used for the experiments.

Assessment of cholesterol efflux

Assessment of cholesterol efflux was performed as described previously [13]. THP-1 macrophages were incubated in fresh growth medium containing [^{3}H]-cholesterol (0.2 μCi/ml) for 48 h, and cholesterol-loaded using 50 μg/ml oxidized LDL. Labeled cells were washed, and incubated in the serum-free medium containing 2 mg/ml BSA for additional 24 h to equilibrate the [^{3}H]-cholesterol between intracellular and extracellular. Then the cells were washed with PBS and subjected to different treatments:
(1) control: fresh medium alone for 24 h;
(2) BSA: fresh medium containing 2 g/L fatty acid-free BSA for 24 h
(3) apoA-I0: fresh medium containing 10 μg/ml apoA-I, with incubation for 0 h;
(3) apoA-I1: fresh medium containing 10 μg/ml apoA-I, with incubation for 6 h;
(4) apoA-I2: fresh medium containing 10 μg/ml apoA-I, with incubation for 12 h;
(5) apoA-I3: fresh medium containing 10 μg/ml apoA-I, with incubation for 24 h.

After different treatments, incubation media were collected and centrifuged. Cells in precipitate were lysed in 1 mM HEPES (pH 7.5) containing 0.5% Triton X-100. Then, aliquots of the supernatant and the cell lysate were subjected to FJ-2107P type liquid scintillator to determine radioactivity. Cholesterol efflux is expressed as a percentage in Formula (1), in which $Efflux_{cholesterol}$ is the cholesterol efflux, $([^3H]\text{-cholesterol})_o$ is the concentration in culture medium and $([^3H]\text{-cholesterol})_i$ is the one in cell.

$$\text{Efflux}_{\text{cholesterol}} = \frac{([^3\text{H}]\text{-cholesterol})_o}{([^3\text{H}]\text{-cholesterol})_o + ([^3\text{H}]\text{-cholesterol})_i} \times 100\% \quad (1)$$

Oil red O (ORO) staining procedure

Cells were washed with PBS for 3 times and fixed in 50% isopropanol for 1 min. 0.6% oil red O (ORO) stock solution was mixed with equal volume distilled H_2O as a staining solution, and filtered using a 5 μm filter. The fixed cells were incubated with the ORO staining solution for 10 min, then washed with distilled H_2O. Haematoxylin was added to stain cell nucleoli for 5 min followed by several washes with distilled H_2O. THP-1 macrophage-derived foam cells were observed and photos were taken using an Olympus microscope.

Semi-quantitative analysis of cell lipid ORO staining was performed according to the lipid droplet accumulation in the cytoplasm as described previously with minor modification [14].

(1) cells with no lipid droplets were designated "ORO^-";
(2) cells with a lipid droplet area equal to the nucleus were designated "ORO^+";
(3) cells with lipid droplet area twice of the nucleus were designated "ORO^{++}".

ORO^+ and ORO^{++} indicated two levels of cell oxygen tension, and these two kinds foam cells were together counted as ORO^+ cells for statistical convenience [14].

HPLC analysis

HPLC analysis was conducted as described previously [15]. Briefly, cells were washed with PBS for three times. The appropriate volume (usually 1 ml) of 0.5% NaCl was added to about 50–200 μg cellular proteins per ml. Cells were sonicated using an ultrasonic processor for 2 min. The protein concentration in cell solution was measured using BCA kit. 0.1 ml aliquot cell solution (containing 5–20 μg protein) was used to measure the 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 of cholesterol per ml was obtained by diluting the cholesterol stock solution in the same cell lysed buffer.

0.1 ml of each sample (cholesterol standard calibration solutions, or cell solutions) was supplemented with 10 μl reaction mixture including 500 mM $MgC1_2$, 500 mM Tris-HCl (pH 7.4), 10 mM dithiothreitol, and 5% NaCl. 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 of 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 1500 rpm for 10 min at 15 °C. 10 μl of supernatant was applied onto a System Chromatographer (PerkinElmer Inc.) including a PerkinElmer series 200 vacuum degasser, a pump, a PerkinElmer series 600 LINK, and a PerkinElmer series 200 UV/vis detector and a Disovery 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.

Flow cytometry analysis

Cells were washed with PBS twice, fixed, and permeated using the paraformaldehyde-Triton method [16]. The permeated cells were washed and incubated with 1.5% goat anti-human ABCA1 antibody at 37 °C for 60 min. Cells were then washed and incubated with FITC-labeled rabbit anti-goat IgG at 37 °C for 30 min. After incubation, cells were washed with PBS twice, suspended in PBS, and then analyzed using a flow cytometer (Coulter epics altra HyperSort™ system, USA). At least 10,000 cells were gated by light scatter and collected in a list mode manner. Cells incubated only with FITC-labeled rabbit anti-mouse IgG were run in parallel and served as negative control.

RT-PCR

Total RNA was isolated using Trizol reagent, and the concentration was determined after the sample was dissolved in diethylpirocarbonate-treated water. RNA was stored at –70 °C, and treated with DNase I before use.

RT-PCR was performed in a total volume of 20 μl at 42 °C for 60 min, using 2 μg of total RNA, 0.5 μg of oligo (dT) (15 nt), 4 μl of 25 mM $MgC1_2$, 2 μl of RT-PCR buffer (10×), 2 μl of 10 mM dNTP, 0.5 μl of recombinant RNasin ribonuclease inhibitor, and 15 U of AMV reverse transcriptase (High Concentration). The DNA fragment encoding human ABCA1 was amplified using 5 μl RT-PCR product, 1.8 μl of 10 mM dNTP mixture, 4 μl of 25 mM

$MgC1_2$, 9.8 μl of reverse transcription buffer (10×), 50 pM of ABCA1 primer 1 (forward primer) and primer 2 (reverse primer), and 2.5 U *Taq* DNA polymerase in a total volume of 100 μl. The PCR condition was as follows: denatured at 95 °C for 1 min, annealed at 60 °C for 1 min, and polymerized at 72 °C for 1 min for 34 cycles; followed by extension for 10 min at 72 °C. For GAPDH, the primers used in PCR were GAPDH primer 1 (forward primer) and primer 2 (reverse primer).

PCR amplification was performed using the specific primers (forward: 5′-GCTGCTGAAGCCAGGGCATGG-G-3′, and reverse: 5′-GTGGGGCAGTGGCCATACTCC-3′) to get the ABCA1 fragment (306 bp), or the specific primers (forward: 5′-TCACCATCTTCCAGGAGCGAG-3′ and reverse: 5′-TGTCGCTGTTGAAGTCAGAG-3′) to obtain the GAPDH fragment (697 bp).

PCR products were separated on 1.5% agarose gels. Densitometric quantification (EB staining) of the intensity of GAPDH and ABCA1 cDNA products was determined using the Labwords analysis software. The relative abundance of ABCA1 cDNA was expressed as the ratio of ABCA1 versus GAPDH cDNA density.

Protein sample preparation and Western blot

Total cell lysates were prepared using PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS supplemented with protease inhibitors. The protein concentration in cellular supernatant was determined by the BCA assay. Equal amounts of proteins (typically 80 μg) were separated on 6% SDS-PAGE gel and electrophoretically transferred to PVDF membrane. The transferred PVDF membranes were probed with ABCA1 antibody. Immunoreactivity was detected by ECL test. Protein content was calculated by densitometry using Labwords analysis software.

Statistical analysis

Quantitative data were expressed as mean ± SD. Statistical significance of the data was evaluated by analysis of variance and the 2-tailed Student's *t*-test. $P<0.05$ was considered significant difference. For nonquantitative data, results showed the typical one from at least 3 independent experiments.

Results

Formation of THP-1 macrophage-derived foam cells

THP-1 macrophages were incubated with 50 μg/ml oxidized LDL for 48 h to induce foaming. Cells were then stained with ORO and observed using a microscope. Lipid droplets appeared in cytoplasm (Fig. 1). The results of HPLC analysis showed that the total cholesterol, free cholesterol and cholesterol ester in THP-1 microphage-derived foam cells were significantly higher compared with that of the normal macrophages. Cholesterol ester in foam cells increased to about 596% of that of the normal macrophages (316 mg/g protein vs. 53 mg/g protein), strongly suggesting that cells accumulated lipids in their cytoplasm. Of the total cholesterol, the cholesterol ester content was higher than 60% (Fig. 2).

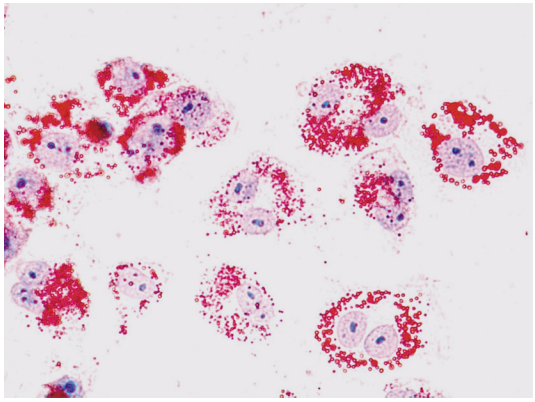

Fig. 1 THP-1 macrophage-derived foam cells double-stained by ORO and hematoxylin

The red color indicated the lipid droplets (both free cholesterol and cholesterol ester) stained by the ORO. The blue color indicated the nucleus stained by hematoxylin. THP-1 macrophate-derived foam cells were full of the cholesterol (×40).

Effect of apoA-I on cholesterol level and efflux in

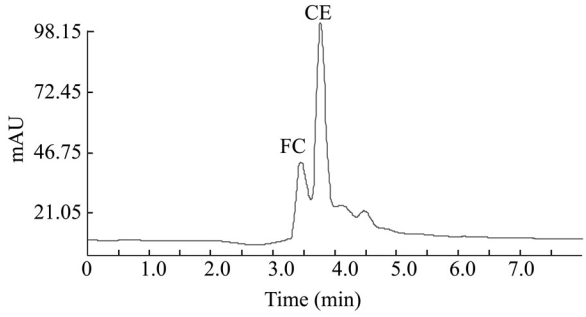

Fig. 2 Chromatography profile of free cholesterol (FC) and cholesterol ester (CE) in THP-1 macrophage-derived foam cells

The content of free and esterified cholesterols in THP-1 macrophage-derived foam cells was identified by absorbance peak at 216 nm in HPLC analysis.

THP-1 macrophage-derived foam cells

THP-1 macrophage-derived foam cells derived from oxidized LDL were treated with 10 μg/ml apoA-I for 0, 6, 12, and 24 h, then stained with ORO. The lipid droplet contents in cells treated with apoA-I decreased significantly at 12 h and 24 h (data not shown). The total cholesterol, free cholesterol and cholesterol ester in THP-1 macrophage-derived foam cells were all significantly lower after treated with 10 μg/ml apoA-I for 12 h and 24 h compared with that of control (Table 1).

Cholesterol efflux was determined as described in "Materials and Methods". As shown in Fig. 3, the cholesterol efflux of THP-1 macrophages-derived foam cells significantly increase after being treated by 10 μg/ml apoA-I for 12 h and 24 h (P<0.05)

Effect of apoA-I on expression of *ABCA1* in THP-1 macrophage-derived foam cells

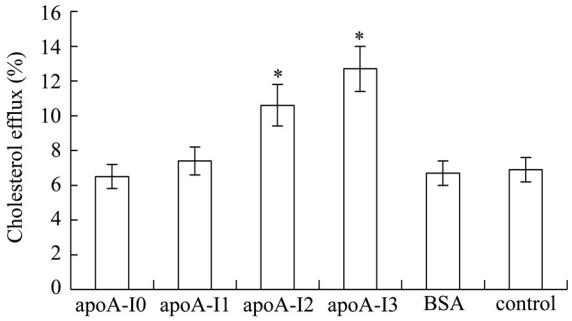

Fig. 3 The effect of apoA-I on cholesterol efflux in THP-1 macrophage-derived foam cells

Data are represented as mean ± SD of triplicate wells from the 3 independent experiments. *P<0.05 vs. group apoA-I0, BSA, or control.

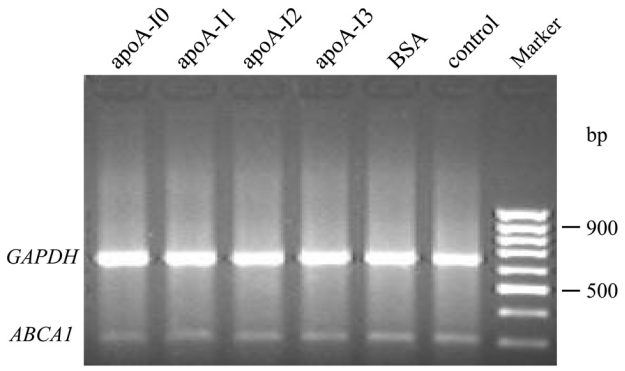

Fig. 4 Effect of apoA-I on ABCA1 mRNA expression in THP-1 macrophage-derived foam cells

RT-PCR products from apoA-I treated THP-1 macrophage-derived foam cells were analyzed by using 1.5% agarose gel. After the treatment of apoA-I, no significant changes were shown in transcription level of cell *ABCA1* among different groups.

The effect of apoA-I on ABCA1 mRNA expression in THP-1 macrophage-derived foam cells was detected. As shown in Fig. 4, the level of ABCA1 mRNA in THP-1 macrophage-derived foam cells was not significantly changed by the treatment of 10 μg/ml apoA-I.

We also measured the ABCA1 protein change in THP-1 macrophage-derived foam cells. 10 μg/ml apoA-I significantly increased the protein level of ABCA1 after 12 or 24 h treatment as shown by Western blot result [Fig. 5(A)], which was also confirmed by the quantitative analysis [Fig. 5(B)]. These data demonstrated that ABCA1 was not only expressed in THP-1 macrophage-derived foam cells but also increased by apoA-I.

The expression of ABCA1 in THP-1 macrophage-derived foam cells was also measured by flow cytometry. As shown in Fig. 6, the mean ABCA1 fluorescent intensity

Table 1 Effect of apoA-I on the levels of the total, free and esterified cholesterols in THP-1 macrophage-derived foam cells (mean ± SD, n=3, mg/g protein)

Groups	Incubation condition		Total cholesterol (TC)	Free cholesterol (FC)	Cholesterol ester (CE)	CE/TC (%)
	Treatment	Time (h)				
Control	Free medium	24	494 ± 45	186 ± 19	308 ± 38	62.3
BSA	2 g/L fatty acid-free BSA	24	484 ± 46	183 ± 17	301 ± 31	62.1
apoA-I0	10 μg/ml apoA-I	0	504 ± 53	188 ± 19	316 ± 34	62.7
apoA-I1	10 μg/ml apoA-I	6	473 ± 56	177 ± 23	296 ± 37	62.5
apoA-I2	10 μg/ml apoA-I	12	353 ± 37*	142 ± 15*	211 ± 22*	59.8
apoA-I3	10 μg/ml apoA-I	24	222 ± 24*	98 ± 11*	124 ± 14*	55.9

*P<0.05 vs. group control, BSA, or apoA-I0.

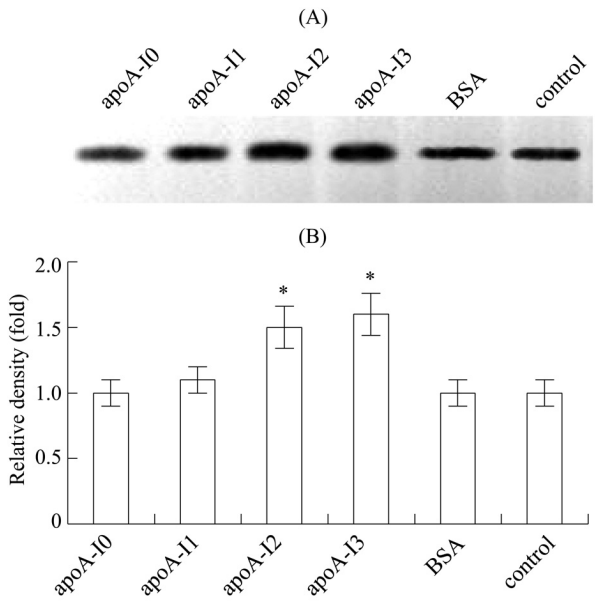

Fig. 5 Effect of apoA-I on ABCA1 protein level in THP-1 macrophage-derived foam cells

(A) Western blot. (B) Quantitative analysis of (A) using densitometry from 3 independent experiments. *P<0.05 vs. the group apoA-I0, BSA, and control.

in THP-1 macrophage-derived foam cells was markedly higher compared with that of group apoA-I0, BSA, or control.

Effect of protease inhibitors on degradation of ABCA1 protein in THP-1 macrophage-derived foam cells

Various protease inhibitors were incubated with THP-1 macrophage-derived foam cells for 2 h to detect their effects on degradation of ABCA1 protein in THP-1 macrophage-derived foam cells. Results showed that protease inhibitors have different effect. Thiol protease inhibitors, including leupeptin and ALLN, increased the level of ABCA1; whereas other inhibitors, including pepstatin A, aprotinin, phosphoramidon, lactacystin (a proteosome-specific inhibitor), and NH_4Cl (a lysosomal inhibitor), did not influence the level of ABCA1 (Fig. 7).

Effect of protease inhibitors on cholesterol efflux in THP-1 macrophage-derived foam cells

THP-1 macrophage-derived foam cells were incubated with thiol protein inhibitor (ALLN), apoA-I or both for 4 h. Results showed that both apoA-I and ALLN increased the cholesterol efflux significantly in comparison with

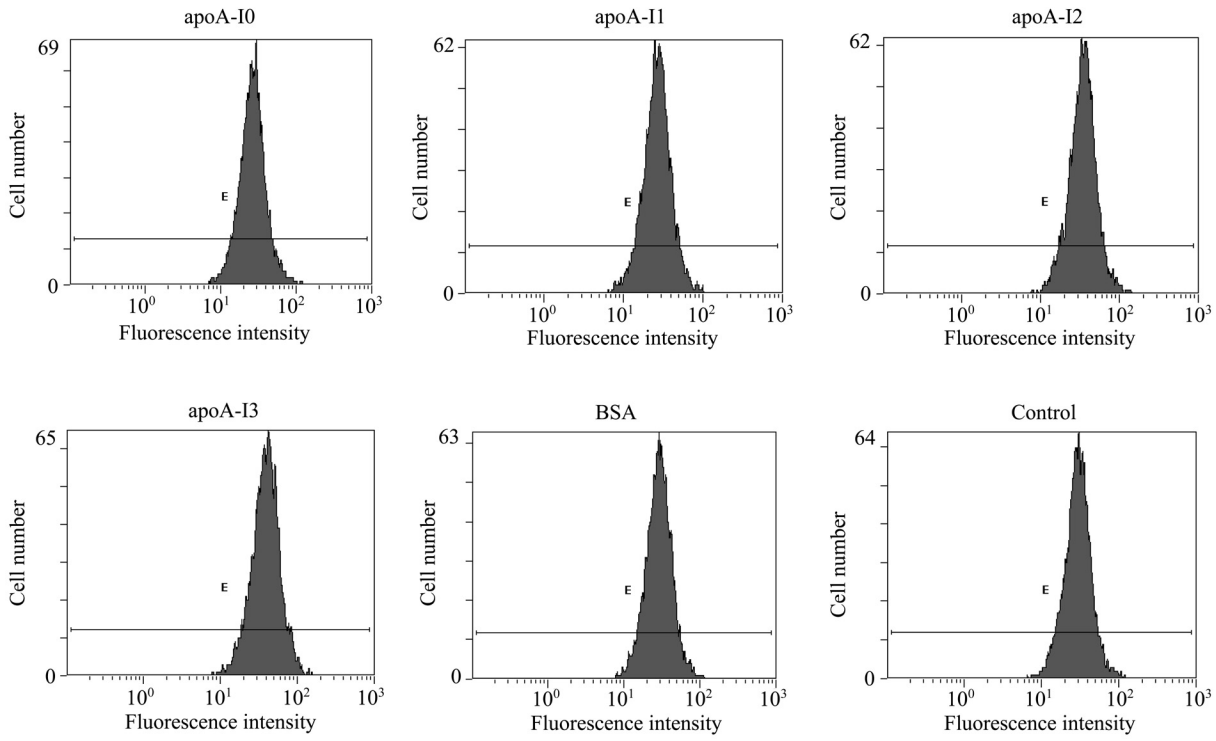

Fig. 6 Effect of apoA-I on the mean ABCA1 fluorescence intensity in THP-1 macrophage-derived foam cells

The relative fluorescence intensity of ABCA1 was 31.2, 32.4, 38.7, 42.6, 30.9, and 31.4 for different treatment as apoA-I0, apoA-I1, apoA-I2, apoA-I3, BSA, and control, respectively.

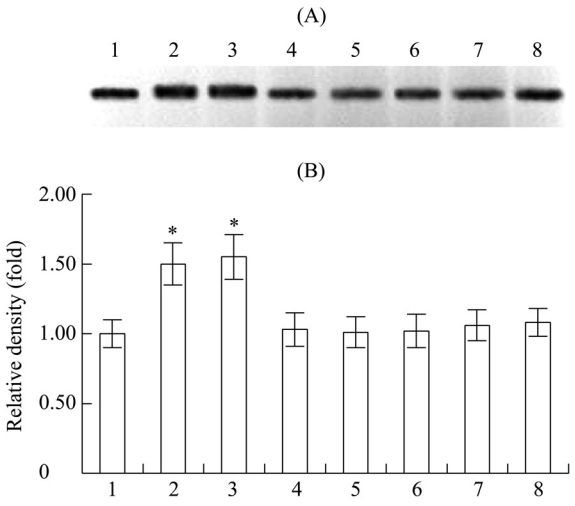

Fig. 7 Effect of protease inhibitors on level of ABCA1 protein in THP-1 macrophage-derived foam cells

(A) Western blot. (B) Quantitative analysis of (A) using densitometry. Data in (B) were represented as mean ± SD, n=3. 1, 10 μg/ml apoA-I; 2, 10 μg/ml apoA-I + 50 μM ALLN; 3, 10 μg/ml apoA-I + 500 μg/ml leupeptin; 4, 10 μg/ml apoA-I + 20 μM pepstatin A; 5, 10 μg/ml apoA-I + 10 μM of aprotinin; 6, 10 μg/ml apoA-I + 250 μg/ml of phosphoramidon; 7, 10 μg/ml apoA-I + 5 μM lactacytin; 8, 10 μg/ml apoA-I + 5 mM NH_4C1. *P<0.05 vs. group 1.

the control group, and the apoA-Imediated cholesterol efflux was significantly improved by the addition of apoA-I (Fig. 8).

THP-1 macrophage-derived foam cells were incubated with various protein inhibitors in the presence of 10 μg/ml apoA-I for 4 h, respectively. The apoA-I-mediated cholesterol efflux from THP-1 macrophage-derived foam cells was increased by ALLN and leupeptin, but other protein inhibitors did not increase the apoA-I-mediated cholesterol efflux from THP-1 macrophage-derived foam cells (Fig. 9). The apoA-I-mediated cholesterol efflux increased by ALLN was demonstrated in a dose-dependent manner (Fig. 10).

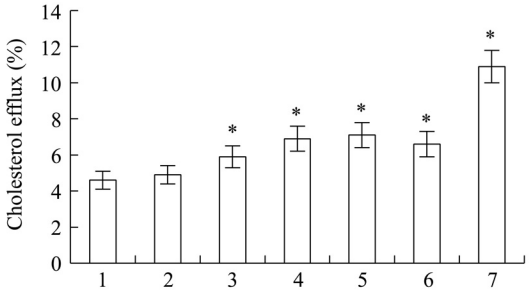

Fig. 8 Effect of thiol protease inhibitors on cholesterol efflux in THP-1 macrophage-derived foam cells

Data were represented as mean ± SD, n = 3. 1, control group; 2, 2.5 μg/ml of apoA-I group; 3, 5 μg/ml of apoA-I group; 4, 10 μg/ml of apoA-I group; 5, 20 μg/ml of apoA-I group; 6, 50 μM of ALLN group; 7, 10 μg/ml of apoA-I + 50 μM of ALLN group. *P<0.05 compared with the control group.

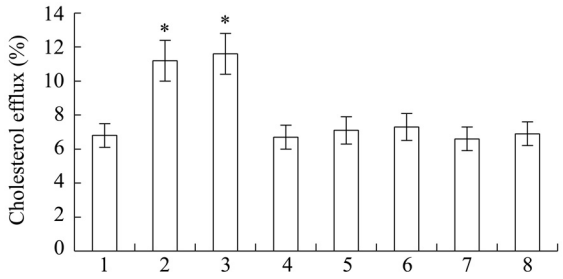

Fig. 9 Effect of protease inhibitor on cholesterol efflux in THP-1 macrophage-derived foam cells

Data were represented as mean ± SD, n = 3. 1, 10 μg/ml apoA-I; 2, 10 μg/ml apoA-I + 50 μM ALLN; 3, 10 μg/ml apoA-I + 500 μg/ml leupeptin; 4, 10 μg/ml apoA-I + 20 μM pepstatin A; 5, 10 μg/ml apoA-I + 10 μM aprotinin; 6, 10 μg/ml apoA-I + 250 μg/ml phosphoramidon; 7, 10 μg/ml apoA-I + 5 μM lactacytin; 8, 10 μg/ml apoA-I + 5 mM NH_4C1. *P<0.05 vs. group 1.

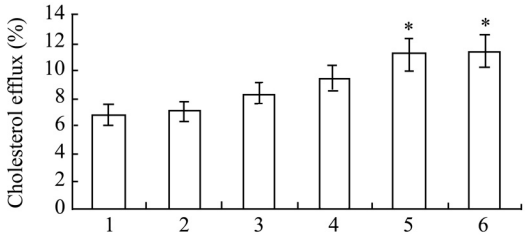

Fig. 10 Effect of ALLN on cholesterol efflux in THP-1 macrophage-derived foam cells

Data were represented as mean ± SD, n = 3. 1, 10 μg/ml apoA-I; 2, 10 μg/ml apoA-I + 10 μM ALLN; 3, 10 μg/ml apoA-I + 20 μM ALLN; 4, 10 μg/ml apoA-I + 30 μM ALLN; 5, 10 μg/ml apoA-I + 40 μM ALLN; 6, 10 μg/ml apoA-I + 50 μM ALLN. *P<0.05 vs. group 1.

Effect of HDL on the level of ABCA1 protein in THP-1 macrophage-derived foam cells

After exposure of THP-1 macrophage-derived foam cells to 30 μg/ml of HDL for 0 h, 6 h, 12 h, 24 h, cellular proteins were prepared for Western blot. Results showed that HDL did not increase the level of ABCA1 protein in THP-1 macrophage-derived foam cells (data not shown).

Discussion

ABCA1 plays a major role in cholesterol homeostasis and HDL metabolism [17–19]. ABCA1 mediates cellular

cholesterol and phospholipid efflux to lipid-poor apolipoproteins, and upregulation of ABCA1 activity is antiatherogenic [20–23]. Hypercholesterolemia is a risk factor for the development of atherosclerosis. Both lipoproteins and the macrophages play important roles in the development of atherosclerosis. ABCA1 is expressed in a variety of human tissues with highest expression levels found in placenta, liver, lung, adrenal glands, fetal tissues [8] and various cell lines [24], and within atherosclerotic tissues [25]. Cholesterol efflux, an important mechanism by which high HDL protects against atherosclerosis, is initiated by docking of apoA-I, a major HDL protein, to specific binding sites followed by activation of ABCA1 and translocation of cholesterol from intracellular compartments to the exofacial monolayer of the plasma membrane where it is accessible to HDL [26–28].

In the present study, we investigated effect of apoA-I on the ABCA1 expression, regulation, degradation, and the role in cholesterol efflux in THP-1 macrophage-derived foam cells. Our findings demonstrate that ABCA1 is expressed in THP-1 macrophage-derived foam cells. ApoA-I decreases the lipid droplets in THP-1 macrophage-derived foam cells. The total cholesterol, free cholesterol and cholesterol ester in THP-1 macrophage-derived foam cells significantly decrease. ApoA-I significantly increases cholesterol efflux and the level of ABCA1 protein in THP-1 macrophages-derived foam cells, but not changes the ABCA1 mRNA level in these cells. Degradation of ABCA1 is strongly blocked by the thiol protease inhibitors leupeptin and ALLN [29] so that this protein is very likely catabolized by a thiol protease-mediated pathway such as calpain or cathepsin. Because lactacystin did not influence the level of ABCA1, proteosome may be excluded from the potential pathways responsible for catabolism of ABCA1 [30].

It is also unlikely that lysosomal degradation is primarily responsible because the effect of NH_4Cl was negative. Interestingly, apoA-I increased ABCA1 in THP-1 macrophage-derived foam cells by retarding its degradation. Thus, apoA-I in its free form apparently stabilizes ABCA1 by protecting it from this protease-mediated catabolic pathway and leads to the increase of this protein level in the cells. Inhibition of thiol protease further increases ABCA1 and the apoA-I-mediated cellular cholesterol efflux accordingly.

When apoA-I was given as HDL, its effect on ABCA1 stabilization was negligible. This result indicates that lipid-free HDL apolipoproteins are mainly responsible for the ABCA1-related events. A certain small proportion of apolipoproteins should always remain in a free form because of the reversible nature of their interaction with lipid surface in the interstitial fluid to which most cells in the body are directly exposed [31]. In addition, an active mechanism by lipid transfer proteins may be involved in the cycling of HDL apolipoproteins between lipid-bound and lipid-free forms [32]. However, it is difficult to estimate what proportion of apolipoproteins is actually in a lipid-free form because the parameters that regulate the equilibrium are mostly undetermined. Nevertheless, free apolipoprotein concentration is a regulatory factor for stability of ABCA1 and HDL assembly by the apolipoprotein-cell interaction.

ABCA1 expression is highly regulated, on both transcriptional and posttranscriptional levels. The interaction of ABCA1 with apoA-I modulates ABCA1 expression on posttranscriptional levels. Cholesterol efflux promoted by ABCA1 leads to decrease the activation of LXR/RXR by oxysterols and ultimately decreases ABCA1 transcription and protein levels, but only after cells are no longer cholesterol-loaded [33–35]. In contrast, the present study indicates that apoA-I has a positive effect on ABCA1 protein expression, and this is likely to be important in macrophage foam cells. Intense interest has recently centered on the possibility that increasing macrophage cholesterol efflux could represent a novel approach to treatment of atherosclerosis [3]. LXR/RXR targets a battery of genes that mediate cholesterol efflux, transport, and excretion, and LXR activators are antiatherogenic [36]. However, LXR/RXR also increases transcription of SREBP1c and its target genes, causing fatty liver and hypertriglyceridemia [37, 38]. Our results suggest that thiol protease inhibitors might provide an alternative way to upregulate ABCA1 protein. This strategy is especially appealing since it may mimic the stabilizing effect of the natural ligands apoA-I.

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