Liver X Receptor β (LXRβ) Interacts Directly with ATP-binding Cassette A1 (ABCA1) to Promote High Density Lipoprotein Formation during Acute Cholesterol Accumulation

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Cells have evolved multiple mechanisms for maintaining cholesterol homeostasis, and, among these, ATP-binding cassette protein A1 (ABCA1)-mediated cholesterol efflux is highly regulated at the transcriptional level through the activity of the nuclear receptor liver X receptor (LXR). Here, we show that in addition to its well defined role in transcription, LXR directly binds to the C-terminal region (2247LTSFL2251) of ABCA1 to mediate its post-translational regulation. In the absence of cholesterol accumulation in the macrophage-like cell line THP-1, the ABCA1-LXRβ complex stably localizes to the plasma membrane, but apolipoprotein A-I (apoA-I) binding or cholesterol efflux does not occur. Exogenously added LXR ligands, which mimic cholesterol accumulation, cause LXRβ to dissociate from ABCA1, thus freeing ABCA1 for apoA-I binding and subsequent cholesterol efflux. Photoaffinity labeling experiments with 8-azido-[α-32P]ATP showed that the interaction of LXRβ with ABCA1 inhibits ATP binding by ABCA1. This is the first study to show that a protein-protein interaction with the endogenous protein suppresses the function of ABC proteins by inhibiting ATP binding. LXRβ can cause a post-translational response by binding directly to ABCA1, as well as a transcriptional response, to maintain cholesterol homeostasis.

Disruption of cellular cholesterol homeostasis can lead to a variety of pathological conditions, including cardiovascular disease (1). ATP-binding cassette protein A1 (ABCA1), an important regulator of cholesterol homeostasis, mediates the release of cellular excess free cholesterol and phospholipids to apolipoprotein A-I (apoA-I), an extracellular acceptor circulating in plasma, to form high density lipoprotein (HDL) (2–5). HDL formation is the only pathway through which excess cholesterol can be eliminated from nonhepatic cells. Defects in ABCA1 cause Tangier disease (6–8), a condition in which patients have a near absence of circulating HDL, prominent cholesterol-ester accumulation in tissue macrophages, and premature atherosclerotic vascular disease (1, 9).

The ABCA1-mediated release of cholesterol is highly regulated at the transcriptional level. When excess cholesterol accumulates in cells, intracellular concentrations of oxysterols increase and activate liver X receptor (LXR), which, in turn, stimulates ABCA1 gene transcription and increased expression of ABCA1 with associated elimination of excess cholesterol (10–12). However, cholesterol is required for cell function and proliferation, and the intracellular cholesterol concentration must be maintained within a narrow range. Consequently, ABCA1-mediated cholesterol release is also regulated at the post-translational level. Several proteins, including syntrophins (13, 14), JAK2 (15), and LXRβ (16), have been reported to interact with ABCA1 and modulate its degradation and function. However, the precise mechanism(s) by which ABCA1 activity is regulated post-translationally remains unclear.

We previously reported (16) that a fraction of cytosolically localized LXRβ may interact with ABCA1 on the plasma membrane and modulate the function of ABCA1. In WI-38 and THP-1 cells, endogenous LXRβ interacts with ABCA1 under conditions in which LXR ligands do not accumulate, i.e. when cholesterol is not in excess. LXRβ suppresses ABCA1-mediated cholesterol efflux. However, the mechanism by which LXRβ suppresses ABCA1 functions was not clear. In this study, we identified two leucine residues in the C-terminal region of ABCA1 responsible for the interaction with LXRβ and showed that LXRβ interaction suppresses ATP binding to ABCA1 and thereby keeps ABCA1 standby on the plasma membrane for acute cholesterol accumulation.

EXPERIMENTAL PROCEDURES

Materials—The LXR ligand TO901317, 22(R)-hydroxycholesterol, 25-hydroxycholesterol, and Alexa Fluor 546 succinimidyl esters were purchased from Cayman Co., Sigma, Stela-roids, and Molecular Probes, respectively. A cholesterol oxidase kit for measurement of free cholesterol and 9-cis-retinoic acid was purchased from Wako.

Antibodies—Rabbit anti-human ABCA1 polyclonal antisemur was generated against the linker region (amino acids 1134–1345) of human ABCA1 as described previously (16). The rat anti-human ABCA1 monoclonal antibody KM3073 was
generated against the first extracellular domain (amino acids 45–639) of human ABCA1 as described previously (13). Anti-LXRβ (PP-K8917-00) antibody was purchased from Perseus Proteomics, anti-hemagglutinin (HA) f7 antibodies (sc-7392) were purchased from Santa Cruz Biotechnology, and anti-FLAG rabbit polyclonal antiserum (F7425) was purchased from Sigma. Fluorescently labeled secondary antibodies were purchased from Molecular Probes.

**Cell Culture and Transfection**—THP-1 cells were maintained in RPMI 1640 medium (Sigma) containing 10% FBS under a humidified atmosphere of 5% CO2 and 95% air at 37 °C. THP-1 cells were differentiated into macrophages by exposure to 50 ng/ml phorbol 12-myristate 13-acetate for 72 h. Macrophages were incubated in 10 nM retinoic acid for 12 h to induce the expression of ABCA1 to facilitate detection. HEK293 cells were maintained in DMEM (Nacalai Tesque) containing 10% FBS. Cells in 100-mm culture dishes were transfected with 10 μg of DNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

**Constructs**—Site-directed mutations were introduced into ABCA1 using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA) with the appropriate synthetic oligonucleotides. The integrity of the mutated DNA was confirmed by sequencing. C-terminal fragments of aHA-ABCA1 were generated by PCR using KOD-plus (Toyobo) fused with Gal4-DNA binding domain (DBD), and inserted into the pCMX vector. hLXRβ was fused with VP16 and inserted into the pCMX vector. The reporter plasmid and β-galactosidase plasmid were kindly provided by Dr. Makoto Makishima.

**Biotinylation of Cell Surface Proteins**—HEK293 cells were co-transfected with ABCA1 and LXRβ. Cells were biotinylated as reported previously (17). The procedure was validated by confirming that ABCG5, which does not reach the plasma membrane when expressed without ABCG8 (18), was not biotinylated (data not shown).

**Cellular Lipid Release Assay**—HEK293 cells were subcultured in 100-mm dishes at a density of 3 × 10^6 cells/dish in DMEM with 10% FBS. After incubation for 24 h, cells were transfected with ABCA1 and FLAG-tagged LXRβ using Lipofectamine 2000. At 12 h after transfection, cells were resuspended and transplanted to 60-mm dishes at a density of 1.5 × 10^5 cells in DMEM with 10% FBS. At 24 h after transfection, cells were washed with DMEM containing 0.2% bovine serum albumin (BSA) and then incubated with DMEM containing 0.02% BSA and 10 μg/ml apo-A-I, with or without 100 nM TO901317, for 2 h at 37°C. The lipid content of the medium was determined as described previously (19). THP-1 cells were subcultured in 60-mm dishes at a density of 3 × 10^6 cells/dish in RPMI 1640 medium containing 10% FBS and prepared as described above. Cells were then washed with RPMI 1640 medium containing 0.02% BSA and incubated in RPMI 1640 medium containing 0.02% BSA and 10 μg/ml apoA-I, with or without 100 nM TO901317 for 2 h at 37°C.

**ApoA-I Binding Assay**—Cells were subcultured on collagen-coated glass coverslips (IWAKI) in 12-well dishes, and apoA-I binding was analyzed as described previously (17).

**Immunostaining and Fluorescence Microscopy**—Cells were grown on glass coverslips, transfected, and fixed with 4% para-formaldehyde in PBS (PBS with 0.87 mM CaCl2 and 0.49 mM MgCl2) for 30 min at room temperature. In some experiments, permeabilization was performed with 0.4% Triton X-100. Cells were blocked with 10% goat serum diluted with PBS for 30 min, incubated with primary antibodies, and incubated with the fluorescently labeled secondary antibodies. The cells were directly viewed with a Zeiss confocal microscope LSM510.

**Membrane Preparation and Immunoprecipitation**—HEK293 cells were transiently transfected with ABCA1 and LXRβ. Cells were lysed by nitrogen cavitation in isotonc buffer containing protease inhibitors (100 μg/ml 4-aminophenylmethanesulfonyl fluoride hydrochloride, 10 μg/ml leupeptin, and 2 μg/ml aprotinin). After nuclei were removed by centrifugation (2,800 × g), membranes were prepared by centrifugation (20,000 × g) and lysed with 1% Nonidet P-40. Proteins were precipitated from the membrane lysate (300 μg) with 2.5 μg of anti-ABCA1 linker antibody. ABCA1 and LXRβ were detected with the anti-ABCA1 antibody KM3073 and anti-LXRβ antibody.

**RNA Interference**—Small interfering RNAs (siRNAs) specific for ABCA1 and a scrambled control were obtained from Invitrogen. THP-1 cells were transfected for 72 h with 120 nM siRNAs using RNAiMAX (Invitrogen) according to the manufacturer’s instructions.

**ATP Binding Analysis**—The cellular membrane fraction was prepared from HEK293 cells and THP-1 cells. Membranes were incubated with 5 μM 8-azido-[α-32P]ATP, 2 mM ouabain, 0.1 mM EGTA, 3 mM MgCl2, and 40 mM Tris-Cl, pH 7.5, in a total volume of 10 μl for 10 min on ice or 37°C. To analyze the high affinity ATP binding, the reaction was done at 37°C and stopped by the addition of 500 μl of ice-cold 40 mM Tris-HCl buffer containing 0.1 mM EGTA and 1 mM MgSO4 or MgCl2. TO901317 was added to the membrane fraction just before the addition of 8-azido-[α-32P]ATP. The supernatant containing unbound ATP was removed from the membrane pellet by centrifugation (15,000 × g, 5 min, 2°C), and this procedure was repeated three times. The pellets were resuspended in 8 μl of wash buffer and irradiated for 5 min (at 254 nm, 8.2 mW/cm2) on ice. To analyze the initial low affinity ATP binding, the reaction was performed on ice and immediately irradiated for 5 min on ice. The membrane pellets were lysed with PBS containing 1% Nonidet P-40 and protease inhibitors. Proteins were precipitated from the membrane lysate (100 μg) with 1 μg of anti-ABCA1 antibody. Samples were analyzed by autoradiography after electrophoresis in a 7% SDS-polyacrylamide gel. ABCA1 was detected with the anti-ABCA1 antibody KM3073. Experiments were done in triplicate.

**Mammalian Two-hybrid Assay**—Cells were transfected using calcium phosphate buffer as described (20). Cells were harvested, and luciferase and β-galactosidase activities were measured using a luminometer (Molecular Devices). DNA cotransfection experiments were done with 0.7 μg of reporter plasmid, 0.1 μg of pCMX-β-galactosidase, 0.1 μg of VP16-tagged LXRβ or VP16, 0.1 μg of ABCA1 fused with Gal4-DBD, and 1 μg of pcDNA3.1 per 12 wells of a 96-well plate. Luciferase data were normalized to an internal β-galactosidase control and represent the mean (± S.D.) of triplicate assays.
RESULTS

Leucine Residues of ABCA1 Mediate Its Interaction with LXRβ—To characterize the interaction between ABCA1 and LXRβ in more detail, we first wished to identify the region of ABCA1 that binds LXRβ. We fused the C-terminal region of ABCA1 to GAL4-DBD, and its interaction with VP16-tagged LXRβ was examined using a mammalian two-hybrid interaction system (supplemental Fig. 1). LXRβ was able to bind a fragment containing the C-terminal 120 amino acid (2142–2261), but a fragment encompassing residues 2142–2229 lacking the C-terminal 21 amino acids did not interact with LXRβ, suggesting that this region is responsible for the interaction of LXRβ with ABCA1.

The C-terminal 21 amino acids of ABCA1 contain nine periodically aligned hydrophobic residues (Fig. 1A), and we examined the potential role of these residues in LXRβ binding. The hydrophobic amino acids were individually replaced with alanine, and each mutant ABCA1 construct was co-expressed with WT and mutant ABCA1, we examined the localization of LXRβ on the plasma membrane. HEK293 cells stably expressing FLAG-LXRβ were transiently transfected with ABCA1(207HA) (Fig. 2), but when cells were transfected with HA-LXRβ and ABCA1 on the plasma membrane. HEK293 cells stably expressing FLAG-LXRβ were transfected with HA-tagged WT ABCA1 L2247A mutant or L2251A mutant. Cells were incubated with 100 nM TO901317 for 30 min at 28 h after transfection. Cells were fixed with 4% paraformaldehyde and immunostained with anti-FLAG rabbit polyclonal antiserum or anti-HA (F7) antibody. Experiments were performed at least twice, and we got similar results.

Leucine residues previously implicated in ABCA1 function (21), did not affect the observed interaction.

Leucine residues of ABCA1 mediate its interaction with LXRβ. A, the amino acid sequence of the C-terminal 50 residues of ABCA1 is shown. The VFVNFA and LTSFL motifs and the PDZ binding motif are underlined. The mutated residues are indicated by dots, and the identified essential leucine residues are indicated by asterisks. B and C, HEK293 cells were co-transfected with LXRβ and WT or mutant ABCA1. At 28 h after transfection, membranes prepared by nitrogen cavitation were subjected to immunoprecipitation (IP) with anti-ABCA1 polyclonal antiserum (16). Cell lysates (10%) (B) and precipitated proteins (C) were subjected to immunoblotting with the anti-ABCA1 monoclonal antibody KM3073 or anti-LXRβ monoclonal antibody. Mutants were analyzed at least twice, and we got similar results.

FIGURE 1. Leucine residues of ABCA1 mediate its interaction with LXRβ. A, the amino acid sequence of the C-terminal 50 residues of ABCA1 is shown. The VFVNFA and LTSFL motifs and the PDZ binding motif are underlined. The mutated residues are indicated by dots, and the identified essential leucine residues are indicated by asterisks. B and C, HEK293 cells were co-transfected with LXRβ and WT or mutant ABCA1. At 28 h after transfection, membranes prepared by nitrogen cavitation were subjected to immunoprecipitation (IP) with anti-ABCA1 polyclonal antiserum (16). Cell lysates (10%) (B) and precipitated proteins (C) were subjected to immunoblotting with the anti-ABCA1 monoclonal antibody KM3073 or anti-LXRβ monoclonal antibody. Mutants were analyzed at least twice, and we got similar results.

Statistical Analysis—Statistical significance was determined by Student’s t test. Unless indicated otherwise, results are given as the means ± S.E. (n = 3).

Co-localization of LXRβ and ABCA1 on the Plasma Membrane—To better characterize the interaction of LXRβ with WT and mutant ABCA1, we examined the localization of ABCA1(207HA), a construct bearing an HA tag between residues 207 and 208 within the first extracellular domain, by confocal microscopy. Insertion of this HA tag does not affect apoA-I binding (22, 23), cholesterol efflux (22, 23), internalization (22, 23), or the interaction with LXRβ (data not shown). HEK293 cells stably expressing FLAG-LXRβ were transiently transfected with ABCA1(207HA) (Fig. 2), but when cells were observed by immunofluorescence microscopy, the intense nuclear staining of FLAG-LXRβ obscured any extranuclear localization of LXRβ (supplemental Fig. 2), and this phenomenon was previously reported for LXRβ (24). To overcome this difficulty and detect FLAG-LXRβ localized to the plasma membrane, we performed immunostaining of cells fixed with paraformaldehyde without the permeabilization treatment. As shown in supplemental Fig. 3, this allowed for the detection of the FLAG peptide fused to the cytosolic C terminus of ABCA1 expressed in HEK293 cells, suggesting that a portion of the antibody (IgG) passed through the plasma membrane and interacted with FLAG peptide in the vicinity of the plasma membrane. However, probably because the amount of the antibody passed into through the plasma membrane is limited, and because the antibody does not enter the nucleus without the...
permeabilization treatment, LXRβ in the nucleus does not react with the antibody. This allowed for the detection of FLAG-LXRβ co-localized with ABCA1(207HA) on the plasma membrane (Fig. 2). When the mutant constructs were expressed in HEK293 cells, L2247A or L2251A substitution did not affect the surface expression of ABCA1, but LXRβ no longer co-localized with ABCA1 (Fig. 2). These results suggest that a fraction of cytosolically localized LXRβ directly interacts with ABCA1 on the plasma membrane, and the two leucine residues (Leu2247 and Leu2251) are required for the interaction. The co-localization was abrogated following the addition of TO901317. We previously reported that the addition of an LXR agonist, TO901317, to the lysate impairs the co-precipitation of ABCA1 with or without LXRβ. Cells were reseeded into 60-mm dishes 12 h after transfection, and at 28 h after transfection apoA-I-dependent cholesterol efflux (for 2 h) was analyzed in the presence or absence of 100 nM TO901317. Values represent the means ± S.E. (error bars) of three samples. *, p < 0.01. C, effect of LXRβ on the surface expression of ABCA1. Cell surface proteins were biotinylated immediately after performance of the cholesterol efflux assay, and biotinylated proteins were precipitated with avidin-agarose. The total (10%) and precipitated surface ABCA1 were detected with the anti-ABCA1 monoclonal antibody KM3073 (13). The amount of vinculin was analyzed as a loading control. Experiments were performed at least twice, and we got similar results.

Effects of LXRβ on ApoA-I Binding and Lipid Release by ABCA1—We next examined the effects of the Leu2247 or Leu2251 mutations on ABCA1 function. When LXRβ was co-expressed with WT ABCA1, no apo-A-I was found associated with cell membranes (Fig. 3A), but when TO901317 was added to cells, apo-A-I binding was clearly observed within 20 min. Interestingly, apo-A-I bound to cells expressing L2247A or L2251A mutant ABCA1 even when LXRβ was co-expressed. Although it is difficult to quantify apo-A-I binding by confocal fluorescence microscopy, these results suggest that the interaction of LXRβ with ABCA1 through residues Leu2247 and Leu2251 represses apo-A-I binding to ABCA1 and that apo-A-I binding activity of ABCA1 is restored soon after LXRβ dissociation.

When ABCA1-L2247A, ABCA1-L2251A, or ABCA1-L2247A/L2251A was expressed alone, cholesterol efflux to apo-A-I was comparable with cells expressing WT ABCA1 in the absence or presence of TO901317 (black bars and empty bars, respectively, in Fig. 3B). Co-expression of LXRβ greatly reduced cholesterol efflux by cells expressing WT ABCA1, but there was no effect of LXRβ expression on cholesterol efflux by cells expressing the mutant constructs (gray bars) despite similar levels of exogenous protein expression (Fig. 3C). Following addition of TO901317 to cells co-expressing WT ABCA1 and LXRβ, cholesterol efflux was not only restored within 2 h, but the amount of secreted cholesterol increased by 2-fold (hatched bars) compared with that in the absence of LXRβ. Cholesterol efflux by cells expressing mutant ABCA1 was not affected by the addition of TO901317 either in the presence or absence of LXRβ. Co-expression of LXRβ doubled the plasma membrane levels of WT ABCA1 as shown previously (16), but surface levels of the mutants (L2247A, L2251A, and L2247/L2251A) were not affected by LXRβ expression (Fig. 3C). These results suggest that the interaction of ABCA1 with LXRβ through residues Leu2247 and Leu2251 stabilizes ABCA1 on the plasma membrane, leading to its increased surface expression. However, the plasma membrane-localized ABCA1-LXRβ complex does not bind apo-A-I or mediate cholesterol efflux. Exogenously added LXR ligand, which mimics cholesterol accumulation, caused LXRβ to dissociate from ABCA1, thus freeing ABCA1 for apo-A-I binding and subsequent cholesterol efflux.
LXR Binding to ABCA1 Inhibits ATP Binding

Effect of LXRβ on ABCA1 ATP Binding—Both apoA-1 binding and lipid efflux are ATP-dependent processes (25), and we next examined the effects of the LXRβ interaction on the ATP binding activity of ABCA1. When membranes were prepared from HEK293 cells expressing ABCA1, incubated with 8-azido-[α-32P]ATP at 37 °C for 10 min and irradiated after the removal of free ATP, WT ABCA1 was specifically photoaffinity-labeled (Fig. 4, lane 5) as reported previously (2). However, ABCA1 was not photoaffinity-labeled in membranes isolated from cells expressing ABCA1-MM, a mutant in which two lysine residues (Lys939 and Lys1952) critical for ATP hydrolysis were replaced by methionine, suggesting that ATP tightly binds to the nucleotide binding domain of ABCA1 and lysine-to-methionine mutations prevent tight ATP binding (lane 6). Co-expression of LXRβ abrogated the photoaffinity labeling of ABCA1, despite similar levels of ABCA1 contained in the membrane fraction (Fig. 4A, lane 7). However, addition of TO901317 to the membrane fraction of cells co-expressing ABCA1 and LXRβ led to photoaffinity labeling of ABCA1 (lane 8) comparable with that seen in the absence of LXRβ (lane 6). Under the same conditions, the co-precipitation of ABCA1 with LXRβ was abrogated (16). ABCA1-L2247A/L2251A double mutant was labeled as efficiently as WT ABCA1 (lane 9), but this was not inhibited by LXRβ co-expression (lanes 11 and 12), suggesting that the interaction of LXRβ with ABCA1 via Leu2247 and Leu2251 prevents tight ATP binding. When membranes were incubated with 8-azido-[α-32P]ATP on ice for 10 min and irradiated without removing free ATP, the immunoprecipitated ABCA1 was photoaffinity-labeled (Fig. 4B). However, ABCA1 was not photoaffinity-labeled when LXRβ was co-expressed, suggesting that ATP cannot access to the nucleotide binding site of ABCA1-LXRβ complex.

ATP Binding of ABCA1 in THP-1 Cells—We analyzed ATP binding of endogenously expressed ABCA1 in THP-1 cells. Membranes were prepared from THP-1 cells cultured under control conditions, incubated with 8-azido-[α-32P]ATP at 37 °C for 10 min, and irradiated after the removal of free ATP. Under these conditions, no photoaffinity labeled band of the appropriate size was detected (Fig. 5A); however, when membranes were treated with TO901317 for 2 h, a band of 260 kDa was observed. When ABCA1 expression was knocked down using siRNA, the intensity of this band was substantially reduced (Fig. 5B). Considered together, these results suggest that endogenous LXRβ interacts with ABCA1 on the plasma membrane in THP-1 cells, and this interaction impairs ATP binding by ABCA1.

Cholesterol Efflux and ApoA-I Binding by THP-1 Cells—No ATP binding by ABCA1 in THP-1 cells under the normal conditions (Fig. 5) suggests that ABCA1 is nonfunctional in THP-1 cells under those conditions. Indeed, no cholesterol efflux was observed when apoA-I was added to the medium of THP-1 cells under the normal conditions despite significant expression of ABCA1 on the plasma membrane (Fig. 6, A).
Additionally, there was no apoA-I binding to the cell surface observed (Fig. 6C). However, addition of the LXR agonist TO901317 to the medium led to substantial cell binding by apoA-I (Fig. 6C), and apoA-I-dependent cholesterol efflux was detected within 2 h (Fig. 6A). Total and cell surface levels of ABCA1 did not change over this time course (Fig. 6B), thus, these effects were not mediated via changes in the subcellular localization of ABCA1 or transcriptional activation by LXR. Additionally, neither apoA-I binding (Fig. 6C) nor cholesterol efflux (Fig. 6A) was detected when expression of ABCA1 was blocked by siRNA (Fig. 6B), arguing that apoA-I specifically bound to ABCA1, leading to cholesterol efflux. Finally, the addition of the endogenous LXR ligands 22(R)- or 25-hydroxycholesterol stimulated cholesterol efflux to an extent similar to that seen with TO901317, suggesting that these observations are physiologically relevant (supplemental Fig. 4). Taken together, these results indicate that ABCA1 on the plasma membrane of THP-1
cells is nonfunctional under normal conditions because LXRβ interaction with ABCA1 suppressed ATP binding to ABCA1, and the increase of LXR ligands immediately triggers apoA-I binding to ABCA1 and subsequent cholesterol efflux before de novo ABCA1 transcription in THP-1 cells.

**DISCUSSION**

In this study, we showed that LXRβ interacts directly with ABCA1 and that the interaction with ABCA1 recruits LXRβ in the vicinity of the plasma membrane in the absence of LXR ligands, i.e. when cholesterol is not in excess in cells. By the addition of LXR ligands, LXR dissociates from ABCA1 and disappears from the vicinity of the plasma membrane. LXRβ interaction blocks apoA-I binding to ABCA1 and cholesterol loading by ABCA1 onto apoA-I, thus keeping ABCA1 standby on the plasma membrane.

We first identified two leucine residues, Leu\(^{2247}\) and Leu\(^{2251}\), responsible for the interaction with LXRβ. These are contained within a motif \((2247\text{LTSFL}\^{2251})\) with similarities to the \(\phi\text{XX\phi}\) (26) motif found in nuclear receptor co-activators and co-repressors, and the co-activator/co-repressor site of LXRβ may mediate its interaction with ABCA1. Because apoA-I binding to ABCA1 and cholesterol efflux by ABCA1, blocked by LXRβ interaction, are both ATP-dependent processes (25), we examined the effects of the LXRβ on the ATP binding/hydrolysis activity of ABCA1. LXRβ interaction blocked ATP binding at 0 °C or the formation of stable complex with ATP at 37 °C.

When multidrug transporters, MDR1 (ABCB1), MRP1 (ABCC1), and MRP2 (ABCC2) hydrolyze ATP, γ-phosphate dissociates from the catalytic site before ADP does, and they trap Mg-ADP in the presence of orthovanadate, an analog of phosphate, and form a stable inhibitory intermediate during the ATP hydrolysis cycle. Therefore, these proteins can be specifically photoaffinity-labeled when these proteins in crude membranes were reacted with 8-azido-\([\alpha\text{-}32\text{P}]\)ATP at 37 °C in the presence of orthovanadate (27–30). However, ABCA1 forms a stable complex with nucleotide even in the absence of orthovanadate when incubated at 37 °C (Fig. 4A). ABCA1 was originally proposed not to be an active transporter but a regulator in apoA-I-dependent cholesterol release based on this unique ATP-binding feature (31). Now it was reported that ABCA1 functions as a receptor for apoA-I as well as an active lipid transporter (25). This unique ATP-binding feature of ABCA1 could be related to the unique functions of ABCA1. LXRβ interaction with two leucine residues, Leu\(^{2247}\) and Leu\(^{2251}\), of ABCC1 probably causes some drastic conformational changes in ABCA1, which block ATP binding to two ATP binding domains of ABCA1 and thereby block apoA-I binding to ABCA1 and cholesterol loading by ABCA1 onto apoA-I.

Current models predict that ABCA1 expression on the cell surface should promote cholesterol efflux, but when THP-1 cells were cultured under control conditions, no apoA-I binding or no cholesterol efflux was observed despite significant expression of ABCA1 on the plasma membrane. Furthermore, no ATP binding by ABCA1 was observed, suggesting that ABCA1 expressed on the cell surface is nonfunctional in THP-1 cells under these conditions. Addition of LXR agonists led to ATP binding by ABCA1, substantial cell binding by apoA-I, and apoA-I-dependent cholesterol efflux by ABCA1. We previously reported that endogenous cytosolically localized LXRβ in THP-1 cells interacted with ABCA1 on the plasma membrane under conditions in which cholesterol does not accumulate. Taken together, these results indicate that ABCA1 on the plasma membrane of THP-1 cells is nonfunctional under normal conditions because LXRβ interaction with ABCA1 suppressed ATP binding to ABCA1 and that the increase of LXR ligands immediately triggers apoA-I binding to ABCA1 and subsequent cholesterol efflux before de novo ABCA1 transcription in THP-1 cells.

The half-life of ABCA1 is 1–2 h (13, 32–34), but the transcription, splicing, translation, and maturation of ABCA1, at >2,000 amino acid residues, take more than 4 h after transcriptional activation (35). Thus, if cells relied solely on the transcriptional regulation of ABCA1 to modulate cholesterol efflux, there could be a substantial delay between the detection of increased cellular cholesterol levels and increased protein expression of ABCA1. This delay could be detrimental for macrophages during acute cholesterol accumulation following the phagocytosis of apoptotic cells. We propose that LXRβ, whose expression does not change during cholesterol accumulation (26, 36), has two distinct important roles in cholesterol homeostasis in resting macrophages: (i) LXRβ acts as a trigger to activate the transcription of LXRα thereby promoting the vigorous transcriptional activation of genes leading to the elimination of free cholesterol, and (ii) LXRβ maintains ABCA1 at the plasma membrane as a stable but inert ABCA1-LXRβ/RXR complex, allowing it to facilitate cholesterol efflux rapidly. As illustrated in Fig. 7, in the absence of excess cholesterol, LXRβ, probably as a heterodimer with RXR as reported (16), binds to the C-terminal region \((2247\text{LTSFL}\^{2251})\) of ABCA1. ABCA1-LXRβ/RXR complex stably localizes to the plasma membrane, but ATP binding to the nucleotide binding domain
of ABCA1 is blocked by LXRβ/RXR, and consequently apoA-I binding or cholesterol efflux does not occur. When cholesterol accumulates, oxysterols bind to LXRβ leading to its dissociation from ABCA1. This allows ABCA1 immediately to become functional, leading apoA-I binding and the release of excess cholesterol. Thus, LXRβ exerts an immediate effect to rid the cell of excess cholesterol, and it also promotes a slower transcriptional response leading to changes in gene expression that allow for a robust cellular response to cholesterol accumulation. It has been reported that a protein, US6, of the human cytomegalovirus, prevents ATP binding to the ABC transporter associated with antigen processing (TAP) and inhibits the function of TAP to evade immune surveillance (37). This is the first study to show that a protein-protein interaction of the endogenous protein suppresses the function of ABCA1 by inhibiting ATP binding, and LXRβ acts as both a transcriptional and post-translational regulator of ABCA1 activity.

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