Bacterial lipopolysaccharide induces expression of ABCA1 but not ABCG1 via an LXR-independent pathway

Rebecca Kaplan,* Xiaodong Gan,* John G. Menke,† Samuel D. Wright,* and Tian-Quan Cai[1],*

Departments of Atherosclerosis and Endocrinology,* and Molecular Endocrinology,† Merck Research Laboratories, Rahway, New Jersey 07065

Abstract Two ATP-binding cassette transporter proteins, ABCA1 and ABCG1, may mediate an active efflux of cellular cholesterol and phospholipids. They are ubiquitously expressed and are subject to regulation by cholesterol loading or by treatment with agents that activate the nuclear hormone receptor LXR. Earlier studies in both primates and non-primates reported that treatment with endotoxin (bacterial lipopolysaccharide, LPS) reduces plasma levels of HDL cholesterol. To determine if such HDL reduction correlates with a change in ABCA1 or ABCG1 expression, their expressions were measured in THP-1 monocytes and mice treated with LPS. LPS treatment leads to a rapid, dose-dependent increase of ABCA1 but not ABCG1 mRNA expression. Analysis of mouse livers showed that LPS treatment decreases expression of CYP7A, another target gene of LXR. When THP-1 cells were transfected with the ABCA1 promoter construct (−928 to +101 bp), promoter activity was significantly increased by treatment of 22(*R*)-hydroxycholesterol but not by LPS. Together, these studies show that LPS regulates ABCA1 expression through an LXR-independent mechanism. Further studies showed that treatment with *Rhodobacter sphaeroiders* LPS, an LPS antagonist, or PD169316, a specific p38 MAP kinase inhibitor, prevented the induction of ABCA1 by LPS. Therefore, this suggests that both transport of LPS from the plasma membrane to an intracellular site and activation of p38 MAP kinase are involved in the LPS-mediated induction of ABCA1.—Kaplan, R., X. Gan, J. G. Menke, S. D Wright, and T-Q. Cai. **Bacterial lipopolysaccharide induces expression of ABCA1 but not ABCG1 via an LXR-independent pathway.** ***J. Lipid Res.*** **2002.** 43: **952–959.**

Supplementary key words LPS • HDL • lipid transporter • regulation • nuclear hormone receptor • CYP7A • MAP kinase

The risk of coronary heart disease is inversely correlated with the plasma concentration of HDL cholesterol (HDL-C) (1–2). A major mechanism postulated to contribute to the anti-atherosclerosis effect of high plasma HDL is reverse cholesterol transport. In this process, HDL facilitates the transfer of cholesterol from peripheral cells to the liver for biliary excretion (3). Our understanding of the mechanisms that regulate HDL-C has received a major advance with the elucidation of the cause of Tangier disease (TD). Patients with TD are characterized by near or complete absence of circulating HDL and by the accumulation of cholesteryl esters in many peripheral tissues (4–5). Three groups have now independently identified the ATP-binding cassette transporter 1 (ABCA1) as the defective gene responsible for TD (6–8).

ABCA1 is a member of the ATP-binding cassette superfamily. These proteins couple the energy provided by ATP hydrolysis to the transport of a wide variety of molecules across membranes (9–12). 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 (13-14). Due to mutations, however, the function of ABCA1 in patients with TD is impaired. Therefore, cellular cholesterol efflux in TD patients is defective, which leads to accumulation of excess cellular cholesterol and defective formation of HDL (15–17).

In addition to ABCA1, another member of the ABC transporter superfamily, ABCG1, was recently shown to also be capable of mediating an active efflux of cholesterol and phospholipids in macrophages (18). In fact, these two genes appear to share some other similarities. For example, 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 (18–21). Therefore, understanding the means of ABCA1 and ABCG1 regulation is a critical step toward a better understanding of their role in lipid metabolism.

Numerous earlier studies in both primates and non-primates have reported that administration of bacterial LPS (endotoxin) decreases plasma levels of HDL-C (22). A number of possibilities have been postulated as mechanisms that underline the reduction of HDL upon LPS treatment, including the reduction of apoA-I, apoE, LCAT,

Abbreviations: ABCA1, ATP-binding cassette transporter 1; LPS, bacterial lipopolysaccharide; MMP-9, matrix metalloproteinase 9; OH Ch, hydroxycholesterol.

[1] To whom correspondence should be addressed.
e-mail: tianquan_cai@merck.com

Copyright © 2002 by Lipid Research, Inc.
This paper is available online at http://www.jlr.org

Downloaded from www.jlr.org by guest, on June 14, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

hepatic lipase, or cholesteryl ester transfer protein (CETP) (22). Recently, Beigneux et al. (23) reported that treatment of hamsters with LPS resulted in a reduction of mRNA expression of several nuclear receptors, including RXR and LXR, in liver. We thus hypothesized that treatment of LPS may reduce the expression of ABCA1. Surprisingly, we found that treatment of cells or mice with LPS resulted in a significant increase of ABCA1 but not ABCG1 mRNA expression. Furthermore, we found that LPS regulates ABCA1 expression through an LXR-independent mechanism.

MATERIALS AND METHODS

Cell culture

THP-1 cells, a human monocytic leukemia cell line, HepG2 cells, a human hepatocellular carcinoma cell line, and CaCO-2 cells, a human intestinal epithelial cell line, were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in their respective media (THP-1 cells: RPM1 1640 with 10% heat inactivated FCS, 50 U/ml penicillin G, 50 μg/ml streptomycin sulfate; HepG2 cells: MEM with 10% heat inactivated FCS, non-essential amino acids, sodium pyruvate; CaCO-2 cells: OPTI-MEM with 10% heat inactivated FCS, non-essential amino acids, vitamins) in an atmosphere containing 5% CO_2 and 95% air. Before assay, cultured cells were harvested and washed once with PBS, and then resuspended in the assay medium (same as that of complete medium but with only 0.5% of FCS). All reagents used in the experiments were diluted in the same assay medium. LPS from *Salmonella minnesota* R595 (LPS) and LPS from *Rhodobacter sphaeroides* (Rs-LPS) were purchased from List Biological Laboratories (Campbell, CA).

RNA isolation

Total RNA was extracted from the cultured cells using TRIZOL reagent according to the protocol provided by the manufacturer (Life Technologies, Grand Island, NY). The RNA was treated with DNase (Ambion Inc., Austin, TX) before analysis by real-time quantitative RT-PCR.

Primers and fluorogenic probes

Oligonucleotide primers and TaqMan probes were designed using Primer Express software (Applied Biosystems) and were synthesized by Applied Biosystems. Sequences of probes and primers were listed in **Table 1**. Primers and probes for GAPDH were purchased from Applied Biosystems.

Real-time quantitative PCR

Real-time quantitative TaqMan PCR analysis was used to determine the relative levels of ABCA1, ABCG1, CYP7A, and MMP-9 mRNA. RT-PCR and TaqMan PCR reactions were performed according to the manufacturer's instructions (PE Biosystems, TaqMan Gold RT-PCR protocol and TaqMan Universal PCR Master Mix). Sequence-specific amplification was detected with an increased fluorescent signal of FAM (reporter dye) during the amplification cycle. Amplification of the human GAPDH gene was performed in the same reaction on all samples tested as an internal control for variations in RNA amounts. Levels of the different mRNAs were subsequently normalized to GAPDH mRNA levels, and were presented as fold difference of treated cells against untreated cells.

Plasmid construction and mutagenesis

A firefly luciferase reporter construct was generated with nucleotides –928 to +101 of human ABCA1 (20). Human genomic DNA (Clontech, Palo Alto, CA) was PCR-amplified with ABCA1 specific primers containing a *Bgl*II restriction site on the 5′ end of the forward primer (Dexter-3F GATCGATCAGATCTTAAGTTGGAGGTCTGGAGTG) and a *Hind*III restriction site on the 5′ end of the reverse primer (Dexter-3R GATCGATCAAGCTTGCTCTGTTGGTGCGCGGA). This fragment was cloned into the *Bgl*II-*Hind*III site of pGL3 Basic vector (Promega Inc., Madison, WI) and confirmed by sequencing (ACGT Inc., Northbrook, IL) to create the ABCA1-Luc construct.

A similar construct with a mutation of the DR4 element of the ABCA1 promoter (DR4Mut1-Luc) (20, 24) was created by site directed mutagenesis of the 5′ half-site of the ABCA1-Luc DR4 element. The mutagenesis was performed using the GeneEditor system (Promega Inc.) and mutagenic oligonucleotides according to the manufacturer's instructions. The mutagenic oligonucleotide, Mut-1, was designed to introduce a *Pst*I site into the 5′ half-site of the ABCA1 DR4 element as described (24). A restriction digestion with *Pst*I (Life Technologies Inc., Bethesda, MD) was used to screen for mutants and the full length sequence was confirmed (ACGT Inc.) to lack any additional mutations in the ABCA1 sequence.

DNA transfection and reporter gene assays

Transfections were performed in 24-well plates with FuGENE 6 reagent according to the manufacturer's instructions (Roche Diagnostics Corp., Indianapolis, IN). THP-1 cells were maintained in complete RPMI containing 10% heat inactivated FCS, 50 U/ml penicillin G and 50 μg/ml streptomycin sulfate. Immediately prior to transfection, the complete media was replaced by serum and antibiotic free media to a concentration of 5×10^5

TABLE 1. Sequences of primers and probes for real-time quantitative PCR

Gene	Species	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)	Probe (5′ to 3′)
ABCA1	Human	TGTCCAGTCCAGTAA TGGTTCTGT	AAGCGAGATATGGTC CGGATT	ACACCTGGAGAGAAGCTT TCAACGAGACTAACC
ABCG1	Human	TGCAATCTTGTGCCA TATTTGA	CCAGCCGACTGTTCT GATCA	TACCACAACCCAGCA GATTTTGTCATGGA
MMP-9	Human	CTGAGAACCAATCTC ACCGACA	AGAGATTTCGACTCT CCACGCA	AACCATAGCGGTACAGGT ATTCCTCTGCCAG
ABCA1	Mouse	AGTGATAATCAAAGT CAAAGGCACAC	AGCAACTTGGCACTA GTAACTCTG	TTCCCGGTGACACAT CCATTGCTG
ABCG1	Mouse	TTCATCGTCCTGGGC ATCTT	CGGATTTTGTATCTG AGGACGAA	ATCTCCCTGCGGCTC ATCGCCT
CYP7A	Mouse	CAAAACCTCCAATCT GTCATGAGA	GCGTTAGATATCCGG CTTCAAA	AGGGATGTATGCCTT CTGCTACCGAGTGAT

Downloaded from www.jlr.org by guest, on June 14, 2012

ASBMB JOURNAL OF LIPID RESEARCH

cells/ml and 500 μl were added per well of 24-well plates. The constructs, ABCA1-Luc or DR4Mut1-Luc, were co-transfected with pSV-β-Galactosidase (Promega Inc.) in the concentrations recommended by the manufacturer to maintain a FuGENE to DNA ratio of 3:1. Five hours after transfection, cell treatments with compound were initiated, and treatments were stopped at 48 h from the beginning of transfection. Cells were harvested using Reporter Lysis Buffer and assayed for β-galactosidase and luciferase activities with assay systems purchased from Promega. All transfections were performed in triplicate.

Immunoblot analysis

Protein extracts were prepared from THP-1 cells (2×10^6 cells). Samples (20 μg each) were subjected to SDS-PAGE, and electrophoretically separated proteins were transferred to nitrocellulose membrane. Membranes were blocked in PBS buffer containing 10% dry milk, and probed with a rabbit anti-human ABCA1 antibody (kindly provided by Dr. Mason W. Freeman) (25). Bound antibody was detected with an anti-rabbit IgG-horseradish peroxidase antibody and enhanced chemiluminescence plus (Pierce).

Animal procedures

C57BL/6N mice were purchased from Taconic (Germantown, NY). These animals were maintained in normal light cycle and were provided with normal chow diet and water. Mice were injected intraperitoneally with either bacterial LPS (at the indicated doses in 0.5 ml of 0.9% saline) or saline alone. Five hours after LPS administration, the mice were euthanized. Liver samples were collected for RNA analyses. All procedures were approved by the Institutional Animal Care and Research Advisory Committee at Merck.

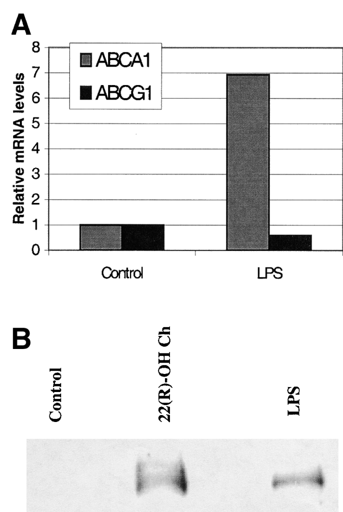

Fig. 1. Bacterial lipopolysaccharide (LPS) induces expression of ABCA1 mRNA and protein levels in THP-1 cells. THP-1 cells (5×10^5 cells/well) were plated in 6-well plates, and mixed with buffer, LPS (1 μg/ml), or 22(*R*)-hydroxycholesterol (5 μM). After ~24 h (for protein) or ~16 h (for RNA extracts) of incubation at 37°C, protein or RNA extracts were prepared from the cultured cells. A: mRNAs for ABCA1 and ABCG1 were measured via TaqMan PCR analysis as described in Materials and Methods. Results are described as fold of control (untreated cells), and data are shown as the means of duplicate determinations. B: ABCA1 proteins were detected via immunoblot with antibodies specific to human ABCA1 as described.

RESULTS

Bacterial LPS induces expression of ABCA1 but not ABCG1

To ask if bacterial LPS would influence the expression of ABCA1, THP-1 monocytes were treated with LPS, and ABCA1 mRNA levels were determined. THP-1 cells expressed readily detectable levels of ABCA1 mRNA. TaqMan PCR analysis of ABCA1 mRNA yielded an average Ct of 25.4 ± 1.5 ($n = 18$). The Ct is the cycle number at which amplification becomes logarithmic. Each Ct unit increase represents a 2-fold increase in mRNA expression. We found that treatment of THP-1 cells with LPS resulted in a marked increase of ABCA1 mRNA (**Fig. 1A**) and protein levels (Fig. 1B). Overnight incubation of THP-1 cells with 1 μg/ml of LPS resulted in a greater than 6-fold induction of ABCA1 mRNA. Additional studies showed that induction of ABCA1 mRNA was dependent on both concentration (**Fig. 2A**) and duration of LPS treatments (Fig. 2B). A similar concentration- and time-dependent induction of ABCA1 by LPS was also observed in cultured HepG2 cells (data not shown).

ABCG1, another member of the ABC transporter superfamily, was recently shown to also be capable of mediating an active efflux of cholesterol and phospholipids (18).

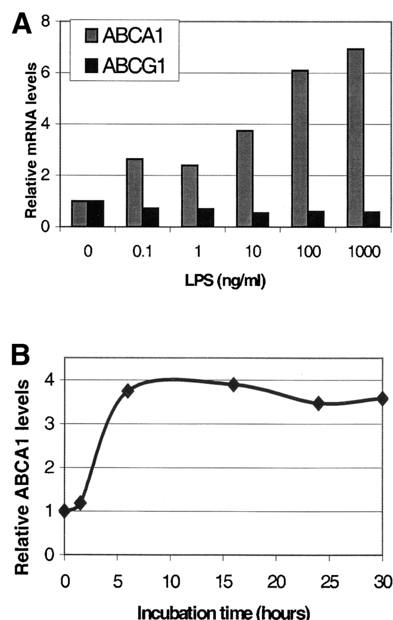

Fig. 2. Time and concentration-dependent induction of ABCA1 expression by LPS. THP-1 cells (5×10^5 cells/well) were plated in 6-well plates, and mixed with increasing concentrations of LPS (A) or buffer (B) or 1 μg/ml of LPS. Following different times of incubation at 37°C, RNA samples were prepared from the cultured cells, and ABCG1 and/or ABCA1 mRNA were measured as described in Materials and Methods. A: Relative mRNA expressions of ABCA1 versus ABCG1 following ~16 h of stimulation with indicated concentrations of LPS. B: Expression of ABCA1 mRNA following indicated times of stimulation with 1 μg/ml of LPS. Results are described as fold of control (untreated cells done in parallel with each time point), and data are shown as the means of duplicate determinations.

Downloaded from www.jlr.org by guest, on June 14, 2012

ABCA1 and ABCG1 appear to share a similar mechanism of regulation, since they were both upregulated upon treatment with LXR agonists (18–21). We thus decided to make a parallel measurement of ABCG1 mRNA. Similar to that of ABCA1, THP-1 cells also expressed readily detectable levels of ABCG1 mRNA. TaqMan PCR analysis of ABCG1 mRNA yielded an average Ct of 31.2 ± 2.3 (n = 18), indicating that basal levels of ABCG1 expression were significantly lower than that of ABCA1 expression. To our great surprise, we found treatment of LPS failed to significantly increase the expression of ABCG1 (Fig. 1A, and 2A), suggesting that mechanism of ABCA1 and ABCG1 regulation could potentially be different.

Regulation of ABCA1 and ABCG1 mRNA expression by LXR ligands

Expression of ABCA1 and ABCG1 are both upregulated by the treatment of agents that activate LXR (18–20). Prior studies, however, often measure the expression of these two genes under separate conditions. We decided to measure changes of ABCA1 and ABCG1 expression in parallel upon treatment of LXR agonists as we did for the aforementioned treatment of LPS. Consistent with prior reports (18–20), treatment of THP-1 or HepG2 cells with agents that activate LXR, including 22(*R*)- and 25-hydroxycholesterol, resulted in a marked increase of ABCA1 and ABCG1 mRNA (**Fig. 3**). In contrast, addition of 22(*S*)-hydroxycholesterol, which does not activate LXR, failed to significantly change the expression of ABCA1 or ABCG1. Interestingly, the degree of ABCG1 induction appears always to be larger than that of ABCA1. For example, treatment of THP-1 cells with 22(*R*)-hydroxycholesterol increases ABCA1 mRNA expression by 12-fold, but it increases ABCG1 by 25-fold (Fig. 3A). Similar induction patterns were observed in a cultured hepatocyte cell line, HepG2 cells (Fig. 3B), and an intestinal epithelial cell line, CaCO-2 cells (data not shown).

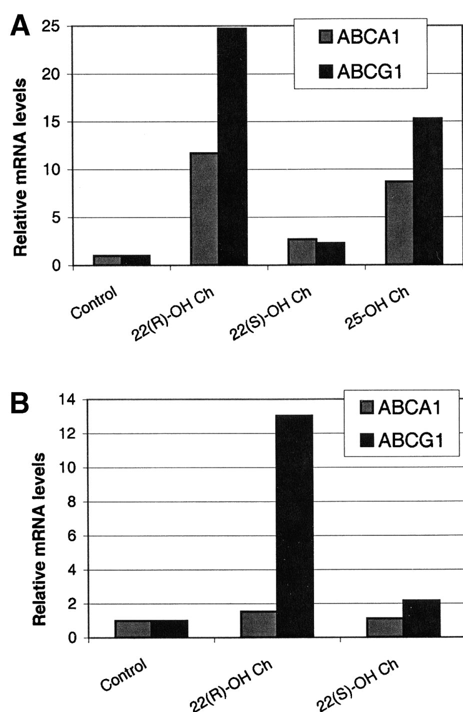

Fig. 3. Induction of ABCA1 and ABCG1 expression by LXR agonist. THP-1 cells (A) or HepG2 cells (B) were plated in 6-well plates (5×10^5 cells/well), and mixed with 22(*R*)-, 22(*S*)-, or 25-hydroxycholesterol (5 μM each). After overnight incubation (∼16 h) at 37°C, RNA samples were prepared from the cultured cells, and mRNAs for ABCA1 and ABCG1 were measured as described in Materials and Methods.

Administration of LPS increases ABCA1 but decreases CYP7A expression in mice

To ask if induction of ABCA1 by LPS would also occur in vivo, we injected mice with various amount of LPS. Five hours after LPS challenge, mice were euthanized, and mRNA levels of ABCA1 in the liver were analyzed. As shown in **Fig. 4A**, LPS treatment resulted in a dose-dependent increase of ABCA1 mRNA in mice. For example, administration of LPS at 250 μg/mouse produced an over 3-fold induction of ABCA1 mRNA ($P < 0.01$, n = 5). Similar to that of THP-1 cells, no significant increase of ABCG1 mRNA was observed in LPS treated mice. In fact, at higher doses, a trend of decreased levels of ABCG1 was detected.

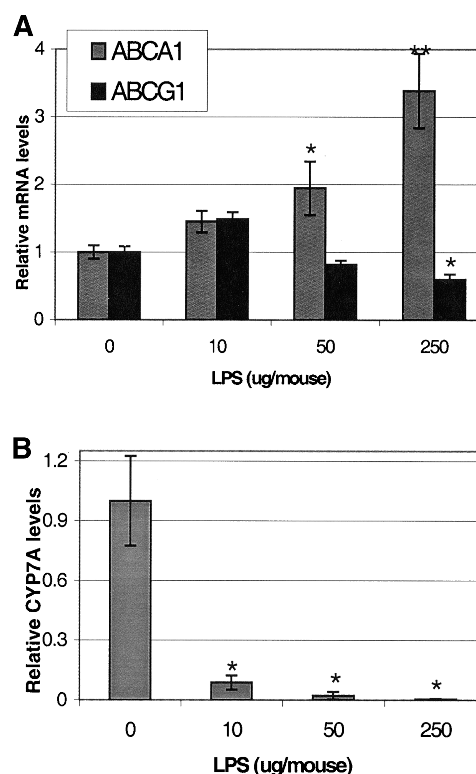

Fig. 4. In vivo effect of LPS on the expression of ABCA1, ABCG1, and CYP7A. Mice were intraperitoneally injected with varying doses of LPS. After 5 h, mice were euthanized and liver samples were collected. RNAs were prepared from the liver samples and mRNAs for ABCA1 and ABCG1(A), and CYP7A (B) were measured as described in Materials and Methods. Results are described as fold of control mice (treated with saline), and data are shown as the means ± SE of each group (n = 5). The * and ** indicate statistical significance of the treated samples versus controls at $P < 0.05$ and 0.01, respectively.

Downloaded from www.jlr.org by guest, on June 14, 2012

Earlier studies have demonstrated that as with ABCA1, the mouse CYP7A gene, (critical for bile acid biosynthesis) also contains an LXR response element in its promoter region (26). Therefore, activation of LXR would increase the expression of ABCA1 and CYP7A in mouse. To ask if LPS-mediated induction of ABCA1 requires an activation of LXR, we made a parallel measurement of ABCA1 and CYP7A in these mice treated with LPS. We found that in complete opposition to that of ABCA1, administration of LPS resulted in a dose-dependent reduction of CYP7A (Fig. 4B). These data further support an LXR-independent regulation of ABCA1 by LPS.

LPS fails to increase promoter activity of ABCA1

To further verify if LPS-mediated induction of ABCA1 expression involves activation of LXR, we prepared a human ABCA1 promoter-luciferase construct (−928 to +101 bp), and transfected it into THP-1 cells. Consistent with earlier reports (20, 24), addition of 22(*R*)-hydroxycholesterol significantly increased promoter activity of ABCA1 (**Fig. 5**). Deletion of the DR4 element from the promoter of ABCA1 completely eliminates the inducible activity of 22(*R*)-hydroxycholesterol, thus confirming an LXR-dependent regulation of ABCA1 transcription by 22(*R*)-hydroxycholesterol (data not shown). Using this identical assay system, we next studied a role of LPS on promoter activities of ABCA1. We found that addition of LPS fails to mediate a significant change of the ABCA1 promoter activity (Fig. 5), though parallel mRNA analysis showed a strong induction of ABCA1 (data not shown). These data further strengthen our conclusion that LPS-mediated induction of ABCA1 must be LXR-independent.

Role of LPS uptake and intracellular transport in LPS-mediated induction of ABCA1

To ask if induction of ABCA1 mRNA requires an uptake of LPS, we tested the role of CD14, a membrane protein necessary for cellular binding and response to LPS (27), and found that coincubation of LPS with monoclonal antibodies against CD14 significantly reduced LPS-mediated induction of ABCA1 mRNA (data not shown). To ask if binding of LPS would be sufficient to mediate an ABCA1 mRNA induction, we studied ABCA1 expression upon treatment of *Rhodobacter sphaeroiders* LPS (Rs-LPS). Unlike that of active LPS, cells may bind to Rs-LPS, but are unable to transport it from plasma membrane to an intracellular site (28). Thus, Rs-LPS is unable to mediate a pro-inflammatory response, and may be used as an antagonist of LPS (29, 30). Consistant with previous studies (31), we found that treatment of cells with LPS but not with Rs-LPS led to a strong induction of matrix metalloproteinase 9 (MMP-9), a well-defined proinflammatory gene (data not shown). Parallel analysis of ABCA1 mRNA revealed an essentially identical response pattern (**Fig. 6**). ABCA1 mRNA was dose-dependently induced by LPS but not by Rs-LPS (Fig. 6A). As with MMP-9, addition of Rs-LPS suppressed LPS-mediated induction of ABCA1 (Fig. 6B). Similar to our earlier findings, no significant changes of ABCG1 mRNA expression were detected in the presence of LPS or Rs-LPS. These data demonstrated a role of LPS uptake and intracellular transport in LPS-mediated induction of ABCA1 mRNA expression.

Involvement of p38 MAP kinase in LPS-mediated induction of ABCA1 expression

Responses of cells to LPS stimulation, such as production of MMP-9, were previously shown to involve activation of p38 mitogen-activated protein (MAP) kinase (32). We thus questioned if LPS-mediated ABCA1 induction would

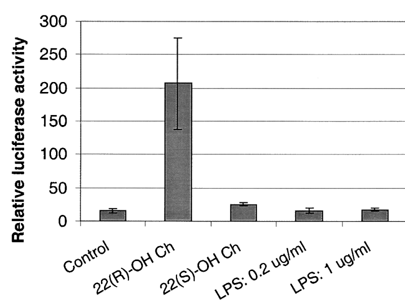

Fig. 5. Effect of LPS and LXR agonist on promoter activity of ABCA1 in THP-1 cells. A fragment of the human ABCA1 promoter (from –928 to + 101 bp) was linked to the firefly luciferase reporter gene. The resulting plasmid was cotransfected with a control reporter plasmid (pSV-β-galactosidase) in THP-1 cells. Cells were treated with LPS (0.2 or 1 μg/ml), 22(*R*)-, or 22(*S*)-hydroxycholesterol (5 μM). After 48 h, cells were lysed, and activities for the firefly luciferase and β-galactosidase were measured. Results are expressed as a ratio between the firefly luciferase and β-galactosidase activities, and data are shown as the means ± SD of triplicates of a representative experiment. Three to four independent experiments were performed.

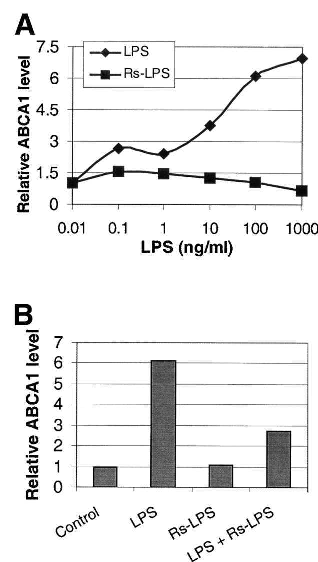

Fig. 6. Effect of LPS and *Rhodobacter sphaeroiders* LPS (Rs-LPS) on expression of ABCA1 in THP-1 cells. THP-1 cells (5×10^5 cells/well) were plated in 6-well plates, and mixed with increasing concentrations of LPS (u) (A), or Rs-LPS (n) and buffer (B), LPS (100 ng/ml), Rs-LPS (100 ng/ml), or combination LPS and Rs-LPS (100 ng/ml each). After overnight incubation (∼16 h) at 37°C, RNA samples were prepared from the cultured cells, and mRNAs for ABCA1 were measured as described in Materials and Methods.

Downloaded from www.jlr.org by guest, on June 14, 2012

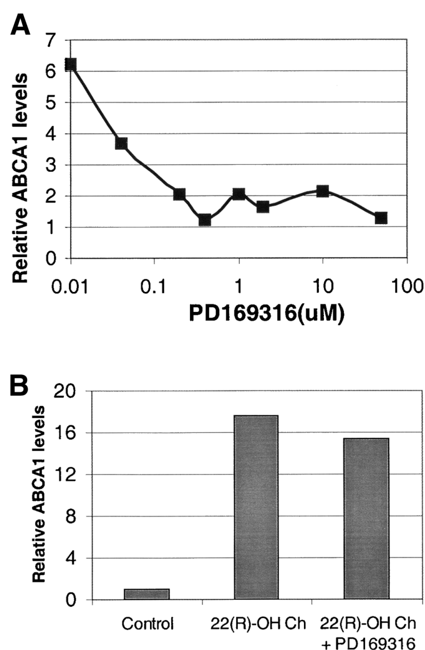

Fig. 7. Effect of p38 MAP kinase inhibitor on ABCA1 expression in THP-1 cells. THP-1 cells (5×10^5 cells/well) were plated in 6-well plates, and mixed with LPS (1 μg/ml) (A) and increasing concentrations of PD1396316 or 22(*R*)-hydroxycholesterol (5 μM) (B) in the presence or absence of PD169316 (10 μM). After overnight incubation (~16 h) at 37°C, RNA samples were prepared from the cultured cells, and mRNAs for ABCA1 were measured as described in Materials and Methods.

also require an activation of p38 MAP kinase. Before addition of LPS stimulation, THP-1 cells were pretreated for 30 min with increasing concentrations of a well-defined specific p38 MAP kinase inhibitor, PD169316 (33). As shown in **Fig. 7A**, addition of PD169316 significantly reduced LPS-mediated induction of ABCA1 mRNA. At 0.4 μM, PD169316 completely eliminated the LPS-mediated increase of ABCA1 mRNA. In a parallel experiment, we found that treatment with PD169316 failed to significantly affect the ABCA1 mRNA expression induced by LXR agonist 22(*R*)-hydroxycholesterol (Fig. 7B).

DISCUSSION

LXR-independent regulation of ABCA1 expression

Expression of ABCA1 may be affected by loading cells with cholesterol (21), or by the treatment of cells with specific oxysterols (20), geranylgeranyl pyrophosphate (34), cAMP (35), transforming growth factor (TGF)-β (36), or interferon γ (37). While it is clear that effects of specific oxysterols or geranylgeranyl pyrophosphate on ABCA1 expression are mediated through LXR (20, 24, 34), mechanisms of other mediators used to influence the expression of ABCA1 remain unknown. Here, we report for the first time that bacterial LPS induces the expression of ABCA1. Furthermore, we provide strong evidence indicating that induction of ABCA1 by LPS treatment is mediated through an LXR-independent mechanism. We have demonstrated this through three independent studies. First, we showed that treatment of cells with LPS or LXR agonists resulted in a differential regulatory pattern of ABCA1 and ABCG1. While ABCG1 mRNA is more effectively up-regulated by the treatment of agents that activate LXR, we found that ABCA1 but not ABCG1 mRNA was increased upon the treatment of bacterial LPS. Second, treatment of mice with LPS resulted in an opposite expression pattern of ABCA1 and CYP7A. If LPS-mediated induction of ABCA1 is driven via an increased activity of LXR, both ABCA1 and CYP7A should be increased when mice were treated with LPS, since in mice both of these genes have a strong LXR responsive element in their promoter region (20, 24, 26). In fact, a reduction of CYP7A demonstrated here (Fig. 4B) and previously by others (38) is consistent with a potentially reduced activity of LXR, as the expression of nuclear receptor RXR and LXR may be reduced upon LPS treatment (23). Finally, treatment of cells with LPS or LXR agonist resulted in a differential effect on promoter activities of ABCA1 that contains a well-defined LXR response element. While 22(*R*)-hydroxycholesterol and LPS both increased expression of ABCA1, only 22(*R*)-hydroxycholesterol increased the promoter activity of ABCA1. As reported by others (20, 24), we found deletion of the LXR-responsive element in the ABCA1 promoter abolished the stimulatory activity of 22(*R*)-hydroxycholesterol. Together, these studies ruled out the possibility that LPS would increase the expression of ABCA1 by either directly acting as an LXR agonist, by affecting generation of endogenous LXR ligand, or by any other means to influence the activation of LXR.

While the exact mechanism(s) used by LPS to regulate ABCA1 expression remain unknown, results from our studies clearly demonstrated the involvement of LPS uptake, transportation of LPS from plasma membrane to an intracellular site, and activation of p38 MAP kinase (Figs. 6, 7). Furthermore, results from our preliminary studies suggested that LPS did not change the stability of ABCA1 mRNA (data not shown). It should be noted that the ABCA1 promoter used in our study for constructing an ABCA1-luciferase reporter assay (−928 to +101 bp) by no means is a complete ABCA1 promoter. The element required for LPS response could be located in the upstream area that was not present in our construct. Further studies are ongoing to elucidate the mechanism(s) of LPS-mediated regulation of ABCA1.

Differential regulation of ABCA1 and ABCG1

ABCA1 and ABCG1 both are members of the ATP-binding cassette (ABC) transporter superfamily. ABCA1 is a full transporter ABC protein, containing two symmetrical halves with each half having six transmembrane domains and one ABC, whereas ABCG1 is half transporter, containing six transmembrane domains and a single ABC (9–12). ABCA1 and ABCG1 both are ubiquitously expressed and subject to regulation (18–21). Our current understandings of the regulation of these two genes have led us to believe that ABCA1 and ABCG1 may share similar mechanisms of regulation. Expression of ABCA1 and

ASBMB JOURNAL OF LIPID RESEARCH JLR

Downloaded from www.jlr.org by guest, on June 14, 2012

ABCG1 both may be suppressed by the zinc finger protein 202 (ZNF202)(39) and upregulated by cholesterol loading or by the treatment of specific oxysterols including 25-, 20(*S*)-, and 22(*R*)-hydroxycholesterol (18–21). Induction of both ABCA1 or ABCG1 by these specific oxysterols depended on activation of LXR (18–20, 24). Indeed, ABCA1 and ABCG1 appear to share some functional similarities. They are both capable of mediating the active efflux of cholesterol and phospholipids in macrophages (8, 18).

A number of in vivo observations, however, have led us to believe that the function of these two genes should be different. A defect in ABCA1 alone is sufficient to prevent HDL formation, as seen in patients with TD (6–8). In fact, a recent study reported that the expression of ABCG1 in TD patients is higher than that of normal patients (40). While over expression of ABCA1 in mice may result in an increase of plasma levels of HDL-C (41–42), over expression of ABCG1 resulted in a significant reduction of HDL-C (43). It is unclear what the physiological relevance could be for an increased expression of ABCA1 in mice treated with LPS or during sepsis. As HDL may neutralize a proinflammatory effect of LPS (44–45), it is tempting to speculate that in addition to cholesterol and phospholipid efflux, ABCA1 may also transfer out LPS. If so, induction of ABCA1 by LPS could potentially serve as protective machinery.

A functional difference of these two genes underlies the importance for a mechanism that would differentially regulate the expression of ABCA1 and ABCG1. Here, we report for the first time a unique mechanism that differentiates the regulation of ABCA1 and ABCG1 expression. As we discussed above, such a mechanism is LXR-independent and requires activation of p38 MAP kinase. A further understanding of the mechanisms that differentiate the expression of ABCA1 and ABCG1 may facilitate our understanding toward the functional differences of these two genes.

Manuscript received 6 November 2001 and in revised form 8 February 2002.

REFERENCES

1. Kwiterovich, P. O., Jr. 1998. The antiatherogenic role of high-density lipoprotein cholesterol. *Am. J. Cardiol.* **82:** 13Q–21Q.
2. Genest, J. J., J. R. McNamara, D. N. Salem, and E. J. Schaefer. 1991. Prevalence of risk factors in men with premature coronary artery disease. *Am. J. Cardiol.* **67:** 1185–1189.
3. Fielding, C. J., and P. E. Fielding. 1997. Intracellular cholesterol transport. *J. Lipid Res.* **38:** 1503–1521.
4. Fredrickson, D. S. 1964. The inheritance of high density lipoprotein deficiency (Tangier Disease). *J. Clin. Invest.* **43:** 22–236.
5. Assmann, G., A. von Eckardstein, and H. B. Brewer. 1995. Familiar HDL deficieny: Tangier disease. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, editors. McGraw-Hill, New York. 2053–2072.
6. Rust, S., M. Rosier, H. Funke, J. Real, Z. Amoura, J. C. Piette, J. F. Deleuze, H. B. Brewer, N. Duverger, P. Denefle, and G. Assmann. 1999. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat. Genet.* **22:** 352–355.
7. Bodzioch, M., E. Orso, J. Klucken, T. Langmann, A. Bottcher, W. Diederich, W. Drobnik, S. Barlage, C. Buchler, M. Porsch-Ozcurumez, W. E. Kaminski, H. W. Hahmann, K. Oette, G. Rothe, C. Aslanidis, K. J. Lackner, and G. Schmitz. 1999. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat. Genet.* **22:** 347–351.
8. Brooks-Wilson A, M. Marcil, S. M. Clee, L. H. Zhang, K. Roomp, M. van Dam, L. Yu, C. Brewer, J. A. Collins, H. O. Molhuizen, O. Loubser, B. F. Ouelette, K. Fichter, K. J. Ashbourne-Excoffon, C. W. Sensen, S. Scherer, S. Mott, M. Denis, D. Martindale, J. Frohlich, K. Morgan, B. Koop, S. Pimstone, J. J. Kastelein, M. R. Hayden, et al. 1999. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat. Genet.* **22**: 336–345.
9. Dean, M., and R. Allikmets. 1995. Evolution of ATP-binding cassette transporter genes. *Curr. Opin. Genet. Dev.* **5:** 779–785.
10. Decottignies, A., and A. Goffeau. 1997. Complete inventory of the yeast ABC proteins. *Nat. Genet.* **15:** 137–145.
11. Allikmets, R., B. Gerrard, A. Hutchinson, and M. Dean. 1996. Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. *Hum. Mol. Genet.* **5:** 1649–1655.
12. Schwiebert, E. M. 1999. ABC transporter-facilitated ATP conductive transport. *Am. J. Physiol.* **276:** C1–C8.
13. Bortnick, A. E., G. H. Rothblat, G. Stoudt, K. L. Hoppe, L. J. Royer, J. McNeish, and O. L. Francone. 2000. The correlation of ABC1 mRNA levels with cholesterol efflux from various cell lines. *J. Biol. Chem.* **275:** 2863–28640.
14. Wang, N., D. L. Silver, P. Costet, and A. R. Tall. 2000. Specific binding of apoA-1, enhanced cholesterol efflux and altered plasma membrane morphology in cells expressing ABC1. *J. Biol. Chem.* **275:** 33053–33058.
15. Lawn, R. M., D. P. Wade, M. R. Garvin, X. Wang, K. Schwartz, J. G. Porter, J. J. Seilhamer, A. M. Vaughan, and J. F. Oram. 1999. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J. Clin. Invest.* **104:** R25–R31.
16. Francis, G. A., R. H. Knopp, and J. F. Oram. 1995. Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier Disease. *J. Clin. Invest.* **96:** 78–87.
17. Rogler, G., B. Trumbach, B. Klima, K. J. Lackner, and G. Schmitz. 1995. HDL-mediated efflux of intracellular cholesterol is impaired in fibroblasts from Tangier disease patients. *Arterioscler. Thromb. Vasc. Biol.* **15:** 683–690.
18. Klucken, J., C. Buchler, E. Orso, W. E. Kaminski, M. Porsch-Ozcurumez, G. Liebisch, M. Kapinsky, W. Diederich, W. Drobnik, M. Dean, R. Allikmets, and G. Schmitz. 2000. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. *Proc. Natl. Acad. Sci. USA.* **97:** 817–822.
19. Venkateswaran, A., J. J. Repa, J. M. Lobaccaro, A. Bronson, D. J. Mangelsdorf, and P. A. Edwards. 2000. Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols. *J. Biol. Chem.* **275:** 14700–14707.
20. Costet, P., Y. Luo, N. Wang, and A. R. Tall. 2000. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J. Biol. Chem.* **275:** 28240–28245.
21. Langmann, T., J. Klucken, M. Reil, G. Liebisch, M. F. Luciani, G. Chimini, W. E. Kaminski, and G. Schmitz. 1999. Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. *Biochem. Biophys. Res. Commun.* **257:** 29–33.
22. Hardardottir, I., C. Grunfeld, and K. R. Feingold. 1995. Effects of endotoxin on lipid metabolism. *Biochem. Soc. Trans.* **23:** 1013–1018.
23. Beigneux, A. P., A. H. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold. 2000. The acute phase response is associated with retinoid X receptor repression in rodent liver. *J. Biol. Chem.* **275:** 16390–16399.
24. Schwartz, K., R. M. Lawn, and D. P. Wade. 2000. ABC1 gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR. *Biochem. Biophys. Res. Commun.* **274:** 794–802.
25. Fitzgerald, M. L., A. J. Mendez, K. J. Moore, L. P. Andersson, H. A. Panjeton, and M. W. Freeman. 2001. ATP-binding cassette transporter A1 contains an NH2-terminal signal anchor sequence that translocates the protein's first hydrophilic domain to the exoplasmic space. *J. Biol. Chem.* **276:** 15137–15145.
26. Chiang, J. Y., R. Kimmel, and D. Stroup. 2001. Regulation of cholesterol 7α-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha). *Gene.* **262:** 257–265.
27. Wright, S. D. 1995. CD14 and innate recognition of bacteria. *J. Immunol.* **155:** 6–8.
28. Thieblemont, N., R. Thieringer, and S. D. Wright. 1998. Innate immune recognition of bacterial lipopolysaccharide: dependence on interactions with membranelipids and endocytic movement. *Immunity.* **8:** 771–777.

Downloaded from www.jlr.org by guest, on June 14, 2012

29. Kovach, N. L., E. Yee, R. S. Munford, C. R. Raetz, and J. M. Harlan. 1990. Lipid IVA inhibits synthesis and release of tumor necrosis factor induced by lipopolysaccharide in human whole blood ex vivo. *J. Exp. Med.* **172:** 77–84.
30. Lynn, W. A., and D. T. Golenbock. 1992. Lipopolysaccharide antagonists. *Immunol. Today.* **13:** 271–276.
31. Shu, H., B. Wong, G. Zhou, Y. Li, J. Berger, J. W. Woods, S. D. Wright, and T. Q. Cai. 2000. Activation of PPARalpha or γ reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. *Biochem. Biophys. Res. Commun.* **267:** 345–349.
32. Underwood, D. C., R. R. Osborn, S. Bochnowicz, E. F. Webb, D. J. Rieman, J. C. Lee, A. M. Romanic, J. L. Adams, D. W. Hay, and D. E. Griswold. 2000. SB 239063, a p38 MAPK inhibitor, reduces neutrophilia, inflammatory cytokines, MMP-9, and fibrosis in lung. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **279:** L895–L902.
33. Kummer, J. L., P. K. Rao, and K. A. Heidenreich. 1997. Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. *J. Biol. Chem.* **272:** 20490–20494.
34. Gan, X., R. Kaplan, J. G. Menke, K. MacNaul, Y. Chen, C. P. Sparrow, G. Zhou, S. D. Wright, and T. Q. Cai. 2001. Dual mechanisms of ABCA1 regulation by geranylgeranyl pyrophosphate. *J. Biol. Chem.* **276:** 48702–48708.
35. Takahashi, Y., M. Miyata, P. Zheng, T. Imazato, A. Horwitz, and J. D. Smith. 2000. Identification of cAMP analog inducible genes in RAW264 macrophages. *Biochim. Biophys. Acta.* **1492:** 385–394.
36. Panousis, C. G., G. Evans, and S. H. Zuckerman. 2001. TGF-β increases cholesterol efflux and ABC-1 expression in macrophage-derived foam cells. Opposing the effects of ifn-γ. *J. Lipid Res.* **42:** 856–863.
37. Panousis, C. G., and S. H. Zuckerman. 2000. Interferon-γ induces downregulation of Tangier disease gene (ATP-binding-cassette transporter 1) in macrophage-derived foam cells. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1565–1571.
38. Feingold, K. R., D. K. Spady, A. S. Pollock, A. H. Moser, and C. Grunfeld. 1996. Endotoxin, TNF, and IL-1 decrease cholesterol 7 α-hydroxylase mRNA levels and activity. *J. Lipid Res.* **37:** 223–228.
39. Porsch-Ozcurumez, M., T. Langmann, S. Heimerl, H. Borsukova, W. E. Kaminski, W. Drobnik, C. Honer, C. Schumacher, and G. Schmitz. 2001. The zinc finger protein 202 (ZNF202) is a transcriptional repressor of ATP binding cassette transporter A1 (ABCA1) and ABCG1 gene expression and a modulator of cellular lipid efflux. *J. Biol. Chem.* **276:** 12427–12433.
40. Lorkowski, S., M. Kratz, C. Wenner, R. Schmidt, B. Weitkamp, M. Fobker, J. Reinhardt, J. Rauterberg, E. A. Galinski, and P. Cullen. 2001. Expression of the ATP-binding cassette transporter gene ABCG1 (ABC8) in Tangier disease. *Biochem. Biophys. Res. Commun.* **283:** 821–830.
41. Vaisman, B. L., G. Lambert, M. Amar, C. Joyce, T. Ito, R. D. Shamburek, W. J. Cain, J. Fruchart-Najib, E. D. Neufeld, A. T. Remaley, H. B. Brewer, Jr., and S. Santamarina-Fojo. 2001. ABCA1 overexpression leads to hyperalphalipoproteinemia and increased biliary cholesterol excretion in transgenic mice. *J. Clin. Invest.* **108:** 303–309.
42. Singaraja R. R., V. Bocher, E. R. James, S. M. Clee, L. H. Zhang, B. R. Leavitt, B. Tan, A. Brooks-Wilson, A. Kwok, N. Bissada, Y. Y. Yang, G. Liu, S. R. Tafuri, C. Fievet, C. L. Wellington, B. Staels, M. R. Hayden. 2001. Human ABCA1 BAC transgenic mice show increased HDL-C and ApoAI dependant efflux stimulated by an internal promoter containing LXREs in intron 1. *J. Biol. Chem.* **276**: 33969–33979.
43. Ito T, S. L. Sabol, M. Amar, C. Knapper, C. Duarte, R. D. Shamburek, S. Meyn, S. Santamarina-Fojo, H. B. Brewer. 2000. Adenovirus-mediated expression establishes an in vivo role for human ABCG1 (ABC8) in lipoprotein metabolism. *Circulation.* **102 (Suppl. II):** 311.
44. Levine, D. M., T. S. Parker, T. M. Donnelly, A. Walsh, and A. L. Rubin. 1993. In vivo protection against endotoxin by plasma high density lipoprotein. *Proc. Natl. Acad. Sci. USA.* **90:** 12040–12044.
45. Wurfel, M. M., E. Hailman, and S. D. Wright. 1995. Soluble CD14 acts as a shuttle in the neutralization of lipopolysaccharide (LPS) by LPS-binding protein and reconstituted high density lipoprotein. *J. Exp. Med.* **181:** 1743–1754.

Downloaded from www.jlr.org by guest, on June 14, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR