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Constitutive activation of LXR in macrophages regulates metabolic and inflammatory gene expression: identification of ARL7 as a direct target

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Abstract **Ligand activation of liver X receptors (LXRs) has been shown to impact both lipid metabolism and inflammation. One complicating factor in studies utilizing synthetic LXR agonists is the potential for pharmacologic and receptor-independent effects. Here, we describe an LXR gain-of-function system that does not depend on the addition of exogenous ligand. We generated transgenic mice expressing a constitutively active VP16-LXRα protein from the aP2 promoter. These mice exhibit increased LXR signaling selectively in adipose and macrophages. Analysis of gene expression in primary macrophages derived from two independent VP16-LXRα transgenic lines confirmed the ability of LXR to drive expression of genes involved in cholesterol efflux and fatty acid synthesis. Moreover, VP16-LXRα expression also suppressed the induction of inflammatory genes by lipopolysaccharide to a comparable degree as synthetic agonist. We further utilized VP16-LXRα-expressing macrophages to identify and validate new targets for LXRs, including the gene encoding ADP-ribosylation factor-like 7 (ARL7). ARL7 has previously been shown to transport cholesterol to the membrane for ABCA1-associated removal and thus may be integral to the LXR-dependent efflux pathway. We show that the ARL7 promoter contains a functional LXRE and can be transactivated by LXRs in a sequence-specific manner, indicating that ARL7 is a direct target of LXR. These findings provide further support for an important role of LXRs in the coordinated regulation of lipid metabolic and inflammatory gene programs in macrophages.**—Hong, C., R. Walczak, H. Dhamko, M. N. Bradley, C. Marathe, R. Boyadjian, J. V. Salazar, and P. Tontonoz. **Constitutive activation of LXR in macrophages regulates metabolic and inflammatory gene expression: identification of ARL7 as a direct target.** ***J. Lipid Res.*** **2011.** 52: **531–539.**

Supplementary key words ADP-ribosylation factor-like 7 • liver X receptor • nuclear receptor • macrophage • inflammation • lipid metabolism

Coronary heart disease remains one of the leading causes of death in the industrialized world. Early atherosclerotic plaques are composed largely of macrophages that have been converted to foam cells following uptake of modified lipoproteins and other cellular debris (1). The inability of macrophages to efflux sufficient amounts of engorged cholesterol to the reverse cholesterol transport (RCT) pathway contributes significantly to foam cell formation (1, 2). Understanding the molecular mechanisms governing lesion development and macrophage cholesterol efflux will be essential to unraveling the pathogenesis of atherosclerosis.

Previous studies have established that the RCT pathway is regulated by members of the nuclear receptor superfamily, specifically liver X receptor/retinoid X receptor (LXR/RXR) heterodimers (3–6). LXRs are commonly recognized as "cholesterol sensors." Early studies identified their natural ligands as various oxysterols and intermediates of the cholesterol biosynthetic pathway, such as 22(R)-hydroxycholesterol, 27-hydroxycholesterol, 24,25(S)-epoxycholesterol, and desmosterol (5, 7, 8). The two LXR isotypes (a and b) share a high degree of sequence homology in spite of a diverging expression pattern. LXRβ is found ubiquitously, whereas LXRα is expressed primarily in the liver, intestine, adipose, and macrophages (9).

Analysis of mice lacking one or both LXR isotypes have revealed a key role for LXR signaling in bile acid synthesis, cellular cholesterol efflux and the movement of cholesterol through high density lipoproteins to the liver for excretion

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Abbreviations: ARF, ADP riboyslation factor; ARL, ADP-ribosylation factor-like; LXR, liver X receptor; QPCR, quantitative PCR; RCT, reverse cholesterol transport; RXR, retinoid X receptor; SREBP, sterol-regulatory element binding protein.

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via the RCT pathway (4, 10–12). In macrophages, LXRs directly regulate the expression of a battery of genes involved in cholesterol transport, including ABCA1, ABCG1, apolipoprotein (apo)E, LPL, and phospholipid transfer protein (13–16). LXRs also regulate the absorption of cholesterol from the intestine and its excretion from the liver through regulation of ABCG5 and ABCG8 expression (14, 17–19). Targeted activation of LXR by synthetic agonists promotes cholesterol efflux from macrophages and protects against the development of atherosclerosis in mice (11, 20).

In addition to their functions in cholesterol homeostasis, LXRs have also emerged as regulators of inflammatory gene expression and innate immunity (11, 21). Ligand-activated LXR inhibits the induction of inflammatory genes such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and monocyte chemotactic protein-1 (MCP-1) in response to inflammatory stimuli (22–24). Additional studies have demonstrated that activation of TLR3 and TLR4 by bacterial and viral components inhibits LXR signaling, suggesting that LXR functions to regulate cross-talk between inflammatory and metabolic pathways. Finally, LXR ligands ameliorate inflammation in several in vivo assays, including murine models of contact dermatitis and atherosclerosis (10, 20, 21, 23).

Here, we describe an LXR gain-of-function system that does not depend on the addition of exogenous ligand. We generated transgenic mice expressing a constitutively active VP16-LXRα protein from the aP2 promoter (25, 26). These mice exhibit increased LXR signaling selectively in adipose tissue and macrophages, and confirm the ability of LXR to regulate pathways for lipid metabolism and inflammation independently of exogenous ligands. To further increase our understanding of the role of LXR in metabolism and inflammation, we employed this transgenic model to identify new LXR target genes. ADP riboyslation factor-like 7 (ARL7) was identified as a novel target gene. Members of the ARL family have between 40% and 60% sequence homology. Members of the ADP riboyslation factor (ARF) GTP-binding protein family have been shown to be involved in intracellular trafficking (27–31). Specifically, ARL7, a member of this family, has recently been implicated in macrophage cholesterol efflux (28, 32). We identified ARL7 as a direct LXR target gene using both gain- and loss-of-function models. Together with earlier studies, the work presented demonstrates that ARL7 is involved in the LXR-dependent cholesterol efflux pathway.

METHODS

Reagents and plasmids

The specific LXR agonists GW3965 and T0901317 were provided by Tim Willson and Jon Collins (GlaxoSmithKline). RXR

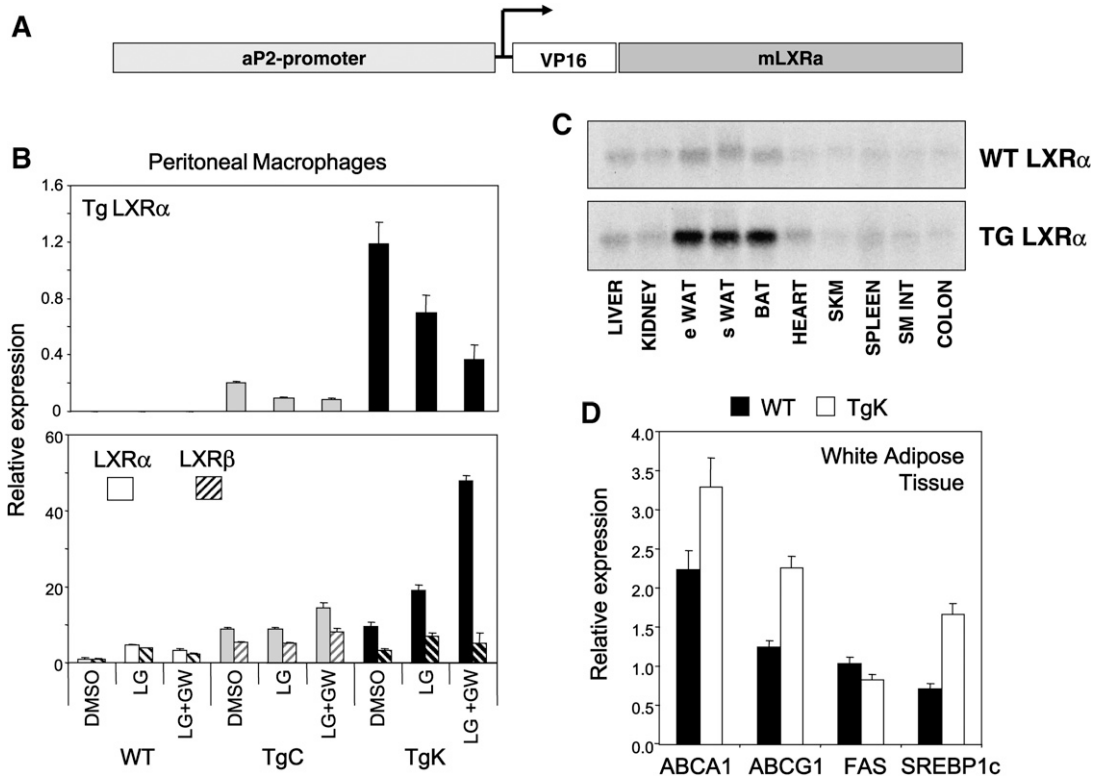

Fig. 1. Generation of aP2-VP16-LXRα transgenic mice. A: VP-16 LXRα transgenic mice were generated using an aP2 enhancer/promoter-driven construct. The VP16 coactivator was fused to the murine human LXRα gene to confer constitutive activity. B: Thioglycollate-elicited WT and transgenic mouse peritoneal macrophages were treated with DMSO or GW3965 (1 mM) and LG 268 (100 nM). Real-time PCR was performed examining mRNA expression levels of the Transgene, LXRa, and LXRb. C: Northern blot analysis was performed to examine the transgene expression pattern. The aP2 promoter restricts transgene expression to macrophages and adipose tissue. D: White adipose tissue was harvested from WT and transgenic mice (n = 5). Real-time PCR was performed demonstrating an induction of known LXR target genes in the adipose tissue of the transgenic mice.

agonist, LG268, was provided by Rich Heyman (Ligand Pharmaceuticals). Ligands were dissolved in dimethyl sulfoxide before use in cell culture. LXR ligands were used at 1 μM, whereas RXR ligand was used at 100 nM. 22(R)-hydroxycholesterol was purchased from Sigma and used at 2.5 μM. Lipolysaccarides (33) from *Salmonella typhimurium* were purchased from Sigma. Plasmids expressing full-length murine LXRα were subcloned into the pBABE retroviral vector and packaged into retrovirus by transfection into Phoenix A cells. RAW 264.7 cells were infected with retrovirus to produce stably transfected cell lines. Cells were selected using 6 μg/ml puromycin for 1 week.

Generation of transgenic mice

Using an aP2 Promotor construct, LXRa was fused to VP16 creating a constitutively active receptor (25, 26). Transgenic mice were generated by microinjection of the ap2-LXRa construct into a C57Bl/6 embryo at the University of California, Irvine Transgenic Core. Two founder lines were identified and bred.

RNA analysis

Total RNA was isolated from tissues using TRIzol (Invitrogen). One microgram of total RNA was reverse transcribed with random hexamers using the Taqman Reverse Transcription Reagents Kit (Applied Biosystems). Sybergreen (Diagenode) real-time quantitative PCR assays were performed using an Applied Biosystems 7900HT sequence detector. Results show averages of duplicate experiments normalized to 36B4. The primer sequences are available upon request.

Animals and diets

All animals were housed in a temperature-controlled room under a 12 h light/12 h dark cycle and under pathogen-free conditions. LXRα$^{-/-}$, *LXRb*$^{-/-}$, and *LXRab*$^{-/-}$ mice on a C57Bl/6 background were originally provided by David Mangelsdorf, University of Texas Southwestern Medical Center, Dallas, TX. The transgenic *aP2-LXRa* mice on C57Bl/6 background were generated by our laboratory. Two independent founders were chosen for expansion based on level of transgene expression and activation of LXR target genes. Males were used in all experiments and were fed standard chow. For ligand treatment studies, mice were gavaged with either vehicle or 20 mg/kg of GW3965 once a day for 3 days. Tissues were harvested 4 h after the last gavage. All animal experiments were approved by the Institutional Animal Care and Research

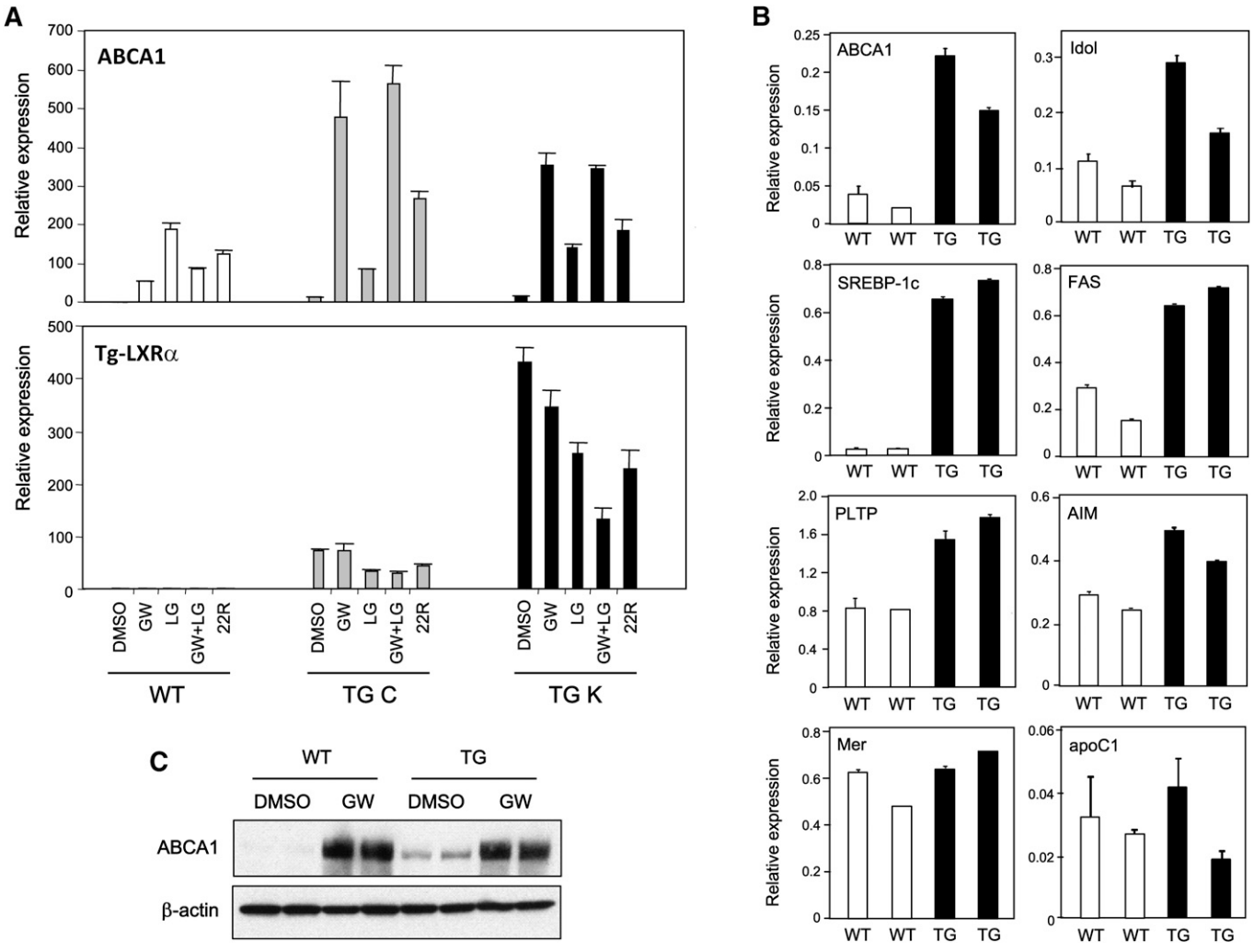

Fig. 2. VP16-LXRα transgene enhances basal expression of metabolic target genes. A: Thioglycollate-elicited wild-type and transgenic mouse peritoneal macrophages were treated with DMSO, GW3965 (1 μM), or 22(R)-hydroxycholesterol (2.5 μM) and LG 268 (100 nM) (10 ng/ml). Real-time PCR was performed examining mRNA expression of ABCA1 and the transgene. B: Basal expression of LXR target genes from two independent macrophage samples from WT and Tg K mice was determined by real-time PCR. C: Immunoblot analysis of ABCA1 protein expression in WT and Tg K macrophages treated with vehicle or GW3965 (1 μM).

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Cell culture

Macrophage cell lines RAW264.7, RAW-vector, RAW-LXRα, and RAW-LXRβ cells were cultured in DMEM containing 10% FBS. Primary peritoneal macrophages were obtained from thioglycollate-treated mice 4 days after injection. For gene expression studies, cells were placed in DMEM plus 0.5% FBS plus 5 μM simvastatin plus 100 μM mevalonic acid overnight. Cells were then treated with DMSO or ligand for LXR as indicated for 24 h. Total RNA was extracted and analyzed by real-time PCR. For macrophage inflammatory responses, peritoneal cells were placed in DMEM plus 0.5% FBS plus 5 μM simvastatin plus 100 μM mevalonic acid overnight. Cells were then stimulated with DMSO or ligand for LXR (1 μM GW3965) for 24 h. Cells were then treated with or without LPS (10 or 100 ng/ml) for 5 h. Total RNA was extracted and analyzed by real-time PCR. Human peripheral blood monocytes cells were harvested using standard procedures then cultured in DMEM and treated for 24 h with DMSO and ligand for LXR (1 μM GW3965). Protein levels were determined by Western blot analysis from whole cell lysates. Antibodies were obtained from Santa Cruz Biotechnology for COX-2 (Clone C-20), for ABCA1 from Novus Biologicals, and β-actin from Sigma (Clone A2066).

RESULTS

To further our understanding of the function of LXR we developed a ligand-independent gain-of-function mouse model. A transgenic expression vector was engineered to generate an N-terminal-VP16 activation domain-LXRα-fusion protein under the control of the aP2 promoter (aP2-LXRα; **Fig. 1A**). The expression of aP2 is specific to adipose tissue and macrophages, therefore transgene expression, as expected, is limited to these tissues. Moreover, the addition of the VP16 transcriptional activation domain was designed to generate a constitutively active receptor. Real-time PCR analysis confirmed transgene expression as well as increased total LXRα expression in primary macrophages derived from two independent founder lines (Fig. 1B). In addition, Northern blot analysis verified that the transgene was expressed selectively in adipose tissue and macrophages (Fig. 1C and data not shown).

We and others have previously shown that LXRs contribute to the regulation of genes involved in lipid metabolism in adipose tissue. aP2-LXRα transgenic mice were initially generated to address the potential contribution of adipose tissue LXR signaling to systemic lipid metabolism. Consistent with prior studies employing synthetic LXR

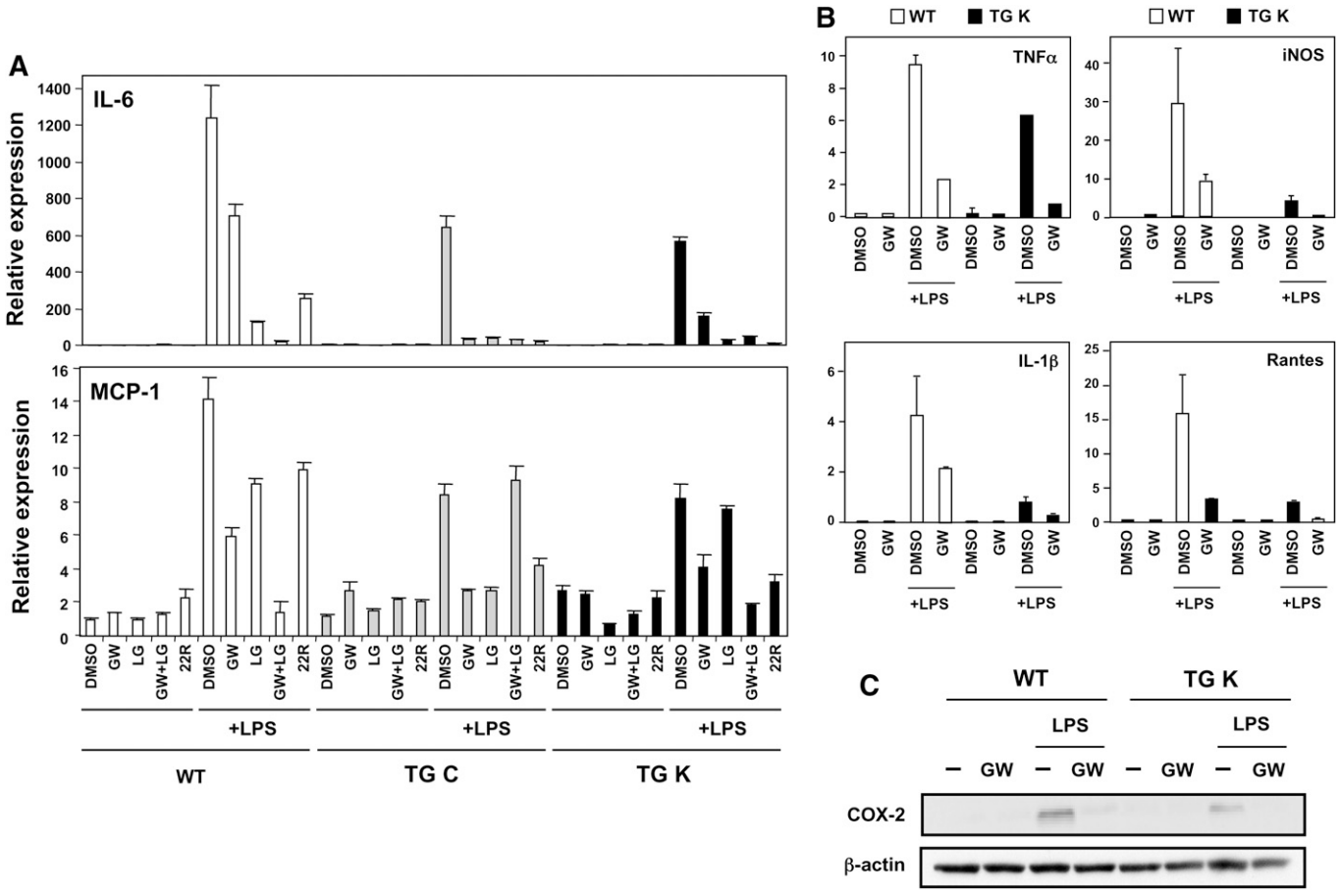

Fig. 3. VP16-LXRα transgene enhances repression of inflammatory genes. A: Thioglycollate-elicited wild-type and transgenic mouse peritoneal macrophages were treated with DMSO, GW3965 (1 μM), or 22(R)-hydroxycholesterol and LG 268 (100 nM) in absence or presence of LPS (10 ng/ml). QPCR was performed examining mRNA expression of MCP-1 and IL-6. B. Repression of inflammatory gene target genes in two independent macrophage samples from WT and Tg K mice was determined by real-time PCR. C: Immunoblot analysis of COX-2 protein expression in WT and TgK macrophages treated with GW3965 and/or LPS as indicated.

ligands, aP2-LXRα transgenic mice exhibited increased expression of the LXR target genes ABCA1 and sterol-regulatory element binding protein (SREBP)-1c in both white and brown adipose tissue. This observation confirms that the LXRα-VP16 fusion protein functions as a constitutively active receptor in vivo (Fig. 1D). Contrary to our initial hypotheses, however, we have not observed consistent alterations in systemic lipid or glucose metabolism in the aP2-LXRα transgenic lines (data not shown).

Multiples groups have established that in macrophages, LXR plays an integral role in modulating both lipid metabolism and inflammatory pathways. LXRs regulate the transcription of genes directly involved in the cholesterol efflux pathway. The macrophages derived from two different lines of aP2-LXRα transgenic mice maintain an increased basal expression of known LXR target genes, including ABCA1, Idol, SREBP-1c, FAS, AIM, and PLTP (**Fig. 2A**, B and data not shown). However, not all LXR target genes were consistently induced in transgenic macrophages. For example, basal expression of Mer and ApoC1 was not different (Fig. 2B). Further examination demonstrated the engineered LXRα-VP16 fusion protein retains the ability to be activated by ligand potentiating the induction of LXR target genes (Fig. 2A and data not shown). Finally, immunoblot analysis confirmed markedly enhanced basal expression of ABCA1 protein levels in LXRα-VP16 transgenic macrophages (Fig. 2C).

Next, we characterized the impact of the VP-16-LXRα on inflammatory gene expression in macrophages. Previous studies have employed potent synthetic LXR agonists as tools to show that ligand activation of LXR antagonizes the induction of inflammatory genes such as iNOS and tumor necrosis factor (TNF)α in response to LPS and other inflammatory stimuli (10, 23). One limitation of this approach, however, is the potential for pharmacologic and receptor-independent effects. We therefore addressed whether constitutively active LXR would also repress inflammatory gene expression. As shown in **Fig. 3A**, induction of interleukin (IL)-6 and MCP-1 gene expression in response to LPS was significantly blunted in macrophages derived from both aP2-LXRα transgenic lines. Furthermore, in line with the gene activation results, the transgene also potentiated the repressive effects of exogenous LXR agonists (Fig. 3A). Similar enhanced repression of inflammatory target genes was also observed for TNF α, IL-1β, iNOS, and RANTES (Fig. 3B). We also confirmed that LPS-induced levels of COX-2 protein were reduced in aP2-LXRα transgenic macrophages (Fig. 3C).

Microscopic examination of wild-type and aP2-LXRα transgenic macrophages provided additional evidence for an effect of constitutive LXR expression on macrophage activation state. Treatment of macrophages with LPS is known to stimulate macrophage activation. Activation is associated with characteristic morphological changes, including increased attachment and spreading. Activation of the LXR pathway in WT macrophages strongly repressed these morphological changes (**Fig. 4**). Remarkably, aP2-LXRα transgenic macrophages were refractory to these activation-associated morphological changes even in the absence of exogenous LXR agonist (Fig. 4). Collectively, the data from our constitutive LXR transgenic model provides strong confirmation of the ability of LXR to reciprocally regulate gene expression linked to lipid metabolism and inflammation in macrophages.

We further used primary macrophages from aP2-LXRα transgenic mice in combination with RAW cells overexpressing LXRα to search for previously unknown LXR responsive genes. As expected, our transcriptional profiling studies using Affymetrix arrays detected known target genes such as ABCA1, ABCG1, and AIM (not shown). In addition, these studies also led to the identification of ARL7 as a gene responsive to LXR expression and synthetic LXR ligand (**Fig. 5**). Real-time PCR analysis verified that ARL7 expression was expressed at enhanced basal levels in macrophages derived from VP-16-LXRα transgenic mice (Fig. 5A). Furthermore, ARL7 expression was induced by

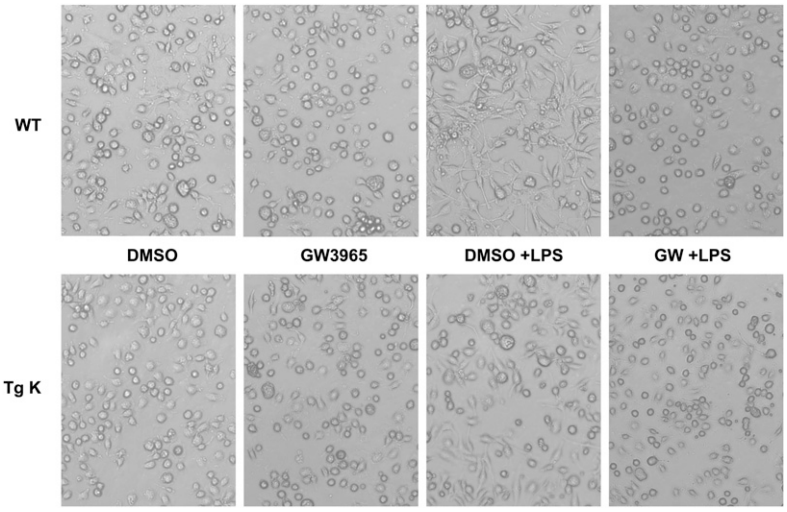

Fig. 4. VP16-LXRα antagonizes macrophage activation by LPS. Thioglycollate-elicited wild-type and transgenic mice wild-type and transgenic peritoneal macrophages were pretreated with DMSO or GW 3965 (1 μM) for 18 h. The cells were then treated with LPS (10 ng/ml) for 36 h and analyzed by light microscopy.

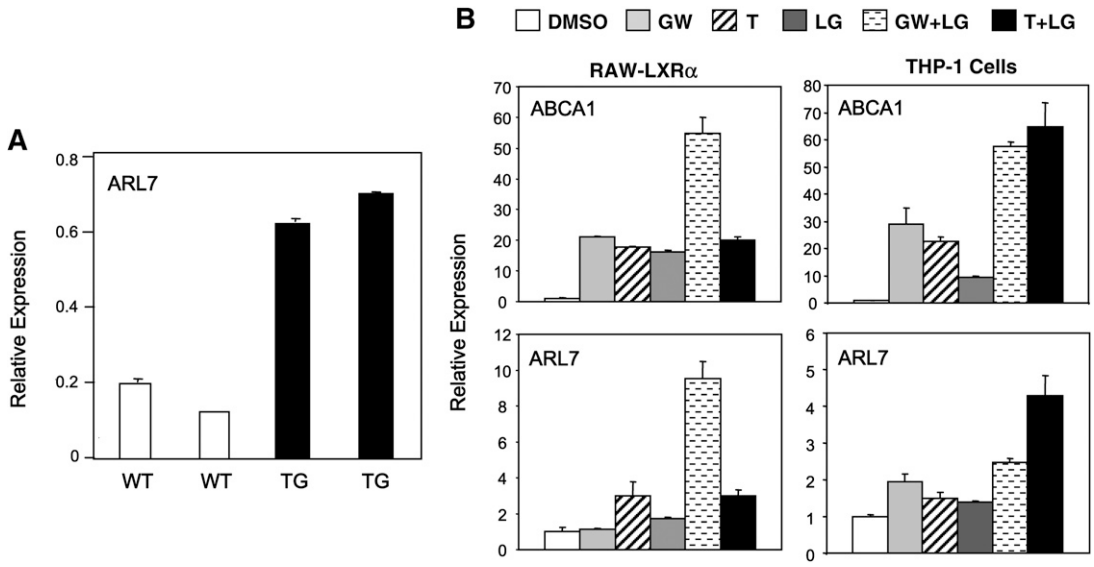

Fig. 5. LXR-responsive expression of ARL7 in murine and human macrophage cell lines. A: Basal expression of ARL7 in two independent macrophage samples from WT and Tg K mice was determined by real-time PCR. B: Murine (RAW 264.7) and human (THP-1) macrophage cell lines were treated with DMSO or GW3965 or T01317 (1 μM) and LG268 (100 nM). Real-time PCRwas performed examining mRNA expression levels of ABCA1 and ARL7.

the LXR ligands, T1317 and GW3965, and/or the RXR ligand, LG268, in LXRα-expressing RAW cells (Fig. 5B). Expression of ABCA1 was monitored as a control. A similar response to LXR/RXR ligands was observed in the human macrophage cell line THP-1, indicating that ARL7 regulation by LXR is conserved across species (Fig. 5B).

To complement the studies using synthetic agonists, we also studied ARL7 regulation in macrophages genetically lacking LXR expression. Peritoneal macrophages and bone marrow-derived macrophages were harvested from WT, LXRα$^{-/-}$, LXRβ$^{-/-}$, and LXRαβ$^{-/-}$ mice. As shown in **Fig. 6A**, expression of ARL7 was induced in response to LXR agonist in WT, LXRα$^{-/-}$, and LXRβ$^{-/-}$ macrophages, indicating that each LXR isotype is capable of regulating ARL7. However, genetic ablation of both LXRs resulted in the clear loss of ARL7 regulation, transcriptionally phenocopying classic LXR target genes such as ABCA1 (Fig. 6A). Importantly, similar regulation of ARL7 mRNA expression was observed in human peripheral blood-derived monocytes.

To further extend these findings, studies were performed to test the ability of LXR ligands to induce ARL7 expression in vivo. C57/B6 mice were gavaged with vehicle or GW3965 (20 mg/kg/day) for three days and various tissues harvested for gene expression analysis. ARL7 mRNA levels were increased significantly in the livers and spleens of ligand treated mice, consistent with in vivo regulation by LXRs (Fig. 6C).

To determine if ARL7 was a direct target for LXR regulation, RAW cells ectopically expressing LXRa treated with LXR ligand in the presence or absence of cycloheximide (CHX), an inhibitor of protein synthesis. As shown in Fig. 6D, induction of ARL7 mRNA expression by LXR agonist was preserved in the presence of cycloheximide, indicating that new protein synthesis is not required for the effect of LXR on ARL7.

Sequence analysis of the ARL7 5′-flanking region identified two DR-4 LXR response elements (LXREs; Direct Repeats with a 4 nucleotide spacer) at –1405 bp and −5215 bp relative to the transcription start site (**Fig. 7A**). Electrophoretic mobility shift assay were performed to test the ability of LXR/RXR heterodimers to bind the putative ARL7 LXREs. As shown in Fig. 7B, in vitro translated LXRα and RXRα protein bound to a radiolabeled oligonucleotide spanning the ARL7 LXRE sequence. By contrast, a mutated version of this sequence was unable to bind LXR/RXR. Addition of an LXR produced a supershifted complex, confirming the presence of LXR in the protein-DNA complex In addition, an excess of unlabeled wild-type but not mutant ARL7 LXRE was able to compete for complex formation (Fig. 7C).

Finally, we examined the ability of LXR to transactivate the ARL7 promoter in transient transfection assays. Two luciferase reporter constructs were generated. The first contained sequence from −1 to −1408 bp to (ARL7 WT LUC, containing the –1405 LXRE) and the second contained sequence from –1 bp to −1395 bp (ARL7 del LUC, deleting just the LXRE). Cotransfection of LXR and RXR expression vectors stimulated activity of the wild-type promoter construct but not the deletion construct lacking the LXRE (Fig. 7D). As expected, addition of LXR agonist produced a further increase in reporter activity in an LXRE-dependent manner. Collectively, these data identify ARL7 as a direct transcriptional target of LXR in macrophages.

DISCUSSION

The nuclear receptors LXRα and β have been shown to play important roles in both inflammatory and metabolic pathways. Prior studies have relied on synthetic LXR ligands and in vitro overexpression systems. Though these

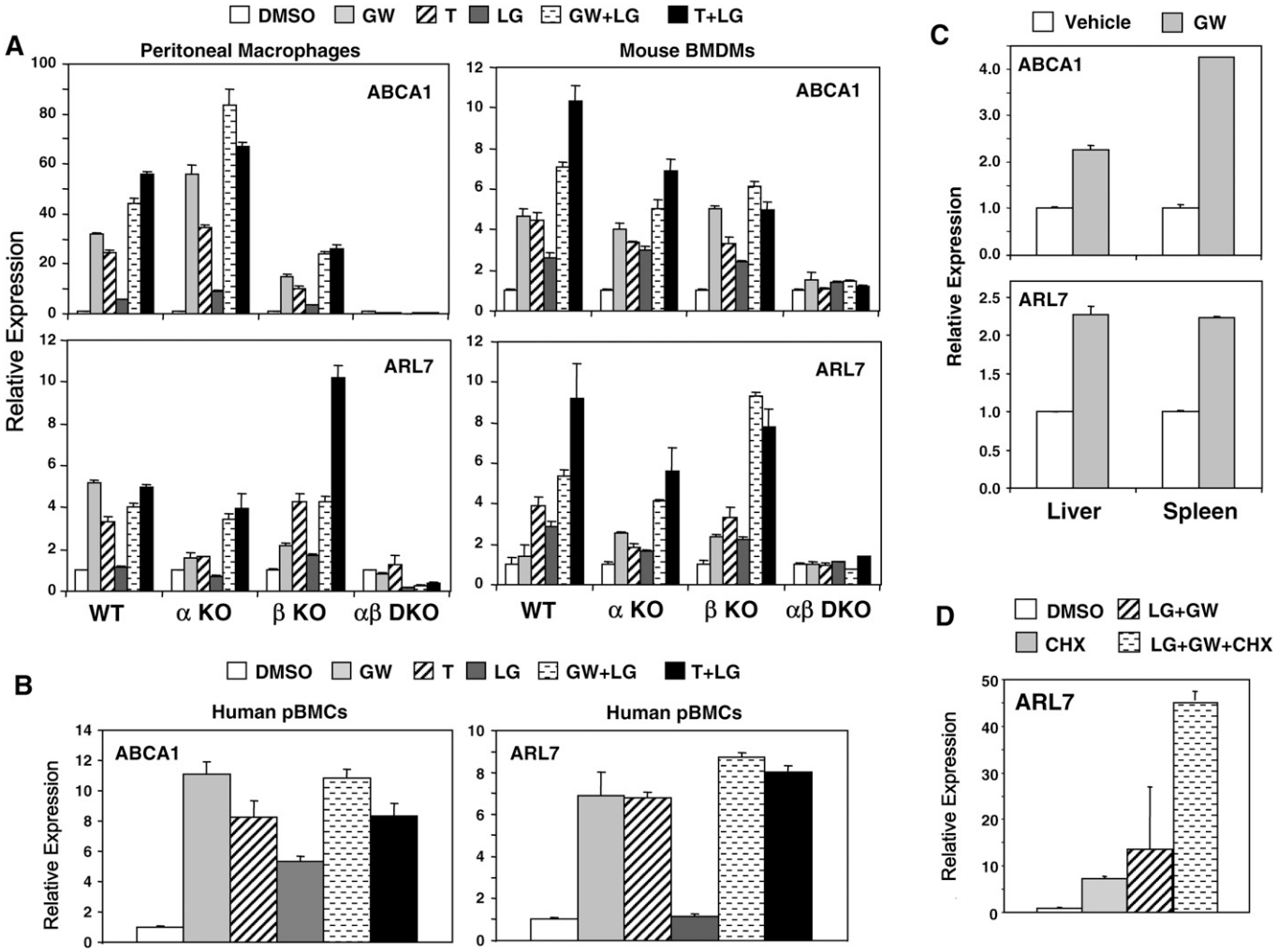

Fig. 6. LXR-dependent expression of ARL7 in vitro and in vivo. A: Bone marrow derived macrophages as well as thioglycollate-elicited wild-type, *Lxr*αβ$^{-/-}$, Lxrα$^{-/-}$, and *Lxr*β$^{-/-}$ mouse peritoneal macrophages were treated with DMSO or GW3965 or T01317 (1 μM) and LG268 (100 nM). B: Peripheral blood mononuclear cells were collected from human blood samples and treated with DMSO or GW3965 or T01317 (1 μM) and LG268 (100 nM). Real-time PCR was performed to examine mRNA expression levels of ABCA1 and ARL7. C: C57Bl/6 mice (n = 3) were gavaged daily for 3 days with GW3965 (20 mg/kg) or vehicle. On the final day of gavage, tissue samples were harvested and total RNA from the liver and spleen were isolated. Real-time PCR was performed to examine mRNA expression levels. D: Murine macrophages ectopically expressing LXRα were treated with DMSO or GW3965 (1 μM) and cycloheximide. mRNA expression levels of ARL7 were determined by real-time PCR.

systems have advanced our understanding of LXR inflammatory biology, the potential for nonspecific effects of the ligand remains a concern in some cases. Here, we describe a novel in vivo overexpression model in which LXRα is constitutively expressed in macrophages and adipose tissue yet remains responsive to synthetic and endogenous ligands. In two different lines of transgenic mice, the constitutively active LXR positively regulated known target genes typically repressed in the basal state of wild-type mice. Furthermore, in this overexpression system, the macrophages were basally protected against inflammatory stimuli such as LPS, as demonstrated by the repression of known inflammatory genes such as iNOS, COX-2, IL-6, and MMP-9. Ligand activation of LXR conveyed an increase in repression of the inflammatory genes. Moreover, VP16-LXRa transgenic macrophages were refractory to LPS-activated morphological changes. These studies provide additional in vivo evidence for the pro-metabolic and the anti-inflammatory roles of LXR.

The ADP riboylation factor (ARF) GTP-binding protein family consists of Arl (Arf-like), Arp (Arf-related proteins) and the SAR (Secretion-associated and Ras-related) proteins. ARL7 together with ARL4 and ARL6 form a small subfamily that are related to one another by a common C terminus, which induces nuclear localization (28). Published research by Engel et al. (32) demonstrated that ARL7 is involved in the movement of cholesterol from the perinuclear compartment to ABCA1 for export through the RCT pathway. Mass spectrometry studies have shown that members of this growing family interact with a-tubulin (31, 34), and prior work has pointed to a role for ARL7 in microtubule-dependent intracellular vesicular transport process (34, 35).

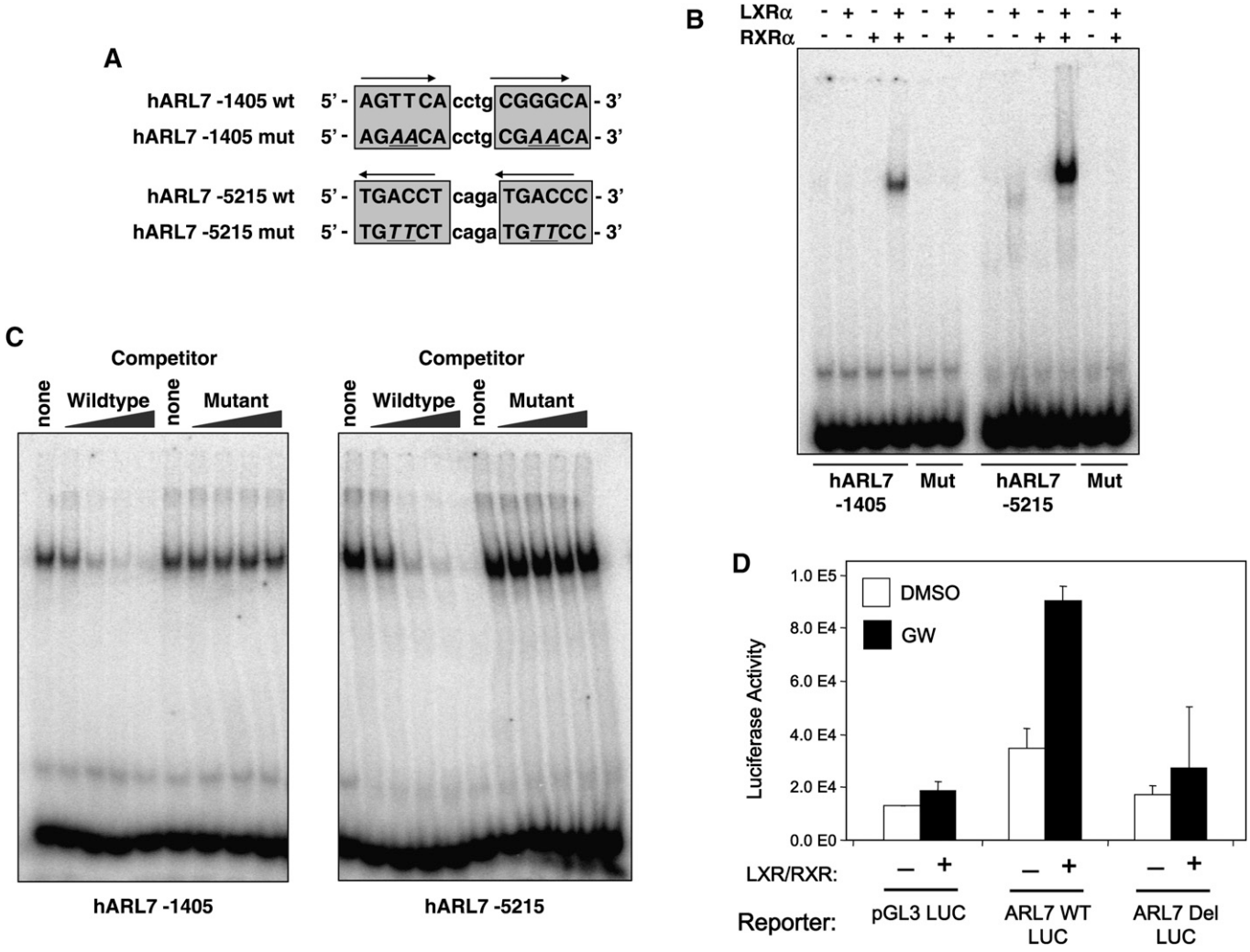

Fig. 7. Identification of functional LXREs in the ARL7 promoter. A: Two LXRE DR4 binding sites were identified 1405 bp and 5215 bp upstream of the ARL7 transcriptional start site. B, C: Electromobility shift assays were performed using labeled oligonucleotides corresponding to either the wild-type or mutant LXRE DR4 sites, purified LXRα protein and in vitro translated RXRα protein. An antibody to LXRα was used to verify identity of the band, resulting in a supershift. Unlabeled wild-type or mutant LXR DR4 oligonucleotides were used as competitors. D: LXR and RXR heterodimerize to activate the ARL7 promoter. Wild-type and mutant promoter-luciferase constructs were generated and transiently transfected with LXR and RXR expression plasmids into HEK 293T cells. Cells were treated with DMSO and GW3965 (1 uM) for 24 h. Luciferase measurements were normalized to β-galactosidase activity. Experiments were done in triplicate and results were averaged for each point.

Using transcriptional profiling techniques concurrently with traditional molecular assays, we demonstrated that ARL7 is a bona fide LXR target gene. ARL7 was first identified as LXR-responsive through analysis of RAW cells and the aP2-LXRα transgenic macrophages (21, 36). Transcriptional studies of macrophages from single and double knockout LXR mice treated with LXR ligand revealed that both receptors independently regulate ARL7 and induction was abolished only in the absence of both receptors. Additionally, a perfect LXR response element was identified in the ARL7 promoter region that efficiently bound the LXR/RXR heterodimer and regulated transcription from the promoter.

Numerous studies have characterized the activation of complementary pathways in lipid metabolism by LXR as well as repression of inflammatory pathways. These data definitely demonstrate ARL7 is a bona fide LXR target gene though the exact molecular mechanism by which ARL7 modulates cholesterol metabolism remains unclear. Future mechanistic studies using overexpression or knockdown techniques may uncover the downstream effects of ARL7 activation and how it functions differently than its family members. The encompassing position of LXR as a regulator of cholesterol metabolism strongly suggests that ARL7 might be a conduit to undiscovered regulatory pathways in lipid metabolism.

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