National Cholesterol Awareness Month Article

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Group X Secretory Phospholipase A_2 Negatively Regulates ABCA1 and ABCG1 Expression and Cholesterol Efflux in Macrophages

Preetha Shridas, William M. Bailey, Florence Gizard, Rob C. Oslund, Michael H. Gelb, Dennis Bruemmer, Nancy R. Webb

Objective—GX $sPLA_2$ potently hydrolyzes plasma membranes to generate lysophospholipids and free fatty acids; it has been implicated in inflammatory diseases, including atherosclerosis. To identify a novel role for group X (GX) secretory phospholipase A_2 ($sPLA_2$) in modulating ATP binding casette transporter A1 (ABCA1) and ATP binding casette transporter G1 (ABCG1) expression and, therefore, macrophage cholesterol efflux.

Methods and Results—The overexpression or exogenous addition of GX $sPLA_2$ significantly reduced ABCA1 and ABCG1 expression in J774 macrophage-like cells, whereas GX $sPLA_2$ deficiency in mouse peritoneal macrophages was associated with enhanced expression. Altered ABC transporter expression led to reduced cholesterol efflux in GX $sPLA_2$-overexpressing J774 cells and increased efflux in GX $sPLA_2$-deficient mouse peritoneal macrophages. Gene regulation was dependent on GX $sPLA_2$ catalytic activity, mimicked by arachidonic acid and abrogated when liver X receptor (LXR)α/β expression was suppressed, and partially reversed by the LXR agonist T0901317. Reporter assays indicated that GX $sPLA_2$ suppresses the ability of LXR to transactivate its promoters through a mechanism involving the C-terminal portion of LXR spanning the ligand-binding domain.

Conclusion—GX $sPLA_2$ modulates gene expression in macrophages by generating lipolytic products that suppress LXR activation. GX $sPLA_2$ may play a previously unrecognized role in atherosclerotic lipid accumulation by negatively regulating the genes critical for cellular cholesterol efflux. **(*Arterioscler Thromb Vasc Biol*. 2010;30:2014-2021.)**

Key Words: ABC transporter ■ fatty acids ■ lipases ■ lipoproteins ■ macrophages ■ prostaglandins ■ LXR ■ cholesterol efflux ■ secretory phospholipase A_2

The secretory phospholipase A_2 ($sPLA_2$) family represents a group of structurally related calcium-dependent enzymes that hydrolyze glycerophospholipids at the *sn*-2 position to liberate lysophospholipids and free fatty acids. Ten enzymatically active $sPLA_2$s have been identified in mammals, of which group X (GX) $sPLA_2$ exhibits several unique characteristics. This enzyme has the highest affinity for zwitterionic phospholipids, including phosphatidylcholine, and, therefore, is the most potent $sPLA_2$ in hydrolyzing the outer leaflet of intact mammalian membranes in vitro.[1] GX $sPLA_2$ is synthesized as an inactive zymogen that is processed to an active form through the proteolytic removal of an N-terminal propeptide.[2] Based on recent studies in genetically manipulated mice, GX $sPLA_2$ appears to play an important role in inflammatory diseases of the lung[3,4] and in the pathogenesis of myocardial ischemia/reperfusion injury.[5] Interestingly, some of the biological effects attributed to GX $sPLA_2$ in vitro are independent of its catalytic activity.[6]

GX $sPLA_2$ is present in human and mouse atherosclerotic lesions, where it may promote atherosclerotic processes through its ability to hydrolyze lipoprotein particles. In the case of low-density lipoprotein, GX $sPLA_2$ hydrolysis generates an altered particle that is avidly absorbed by macrophages to form foam cells.[7] In this study, we report a novel mechanism by which GX $sPLA_2$ may promote cholesterol accumulation in macrophages. Our data indicate that GX $sPLA_2$ suppresses macrophage expression of ABCA1 and ABCG1, transport proteins that reduce cellular lipid content by effluxing excess cholesterol to extracellular acceptors.

Methods

The supplemental information (available online at http://atvb.ahajournals.org) provides a description of RNA extractions, quanti-

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From the Graduate Center for Nutritional Sciences (P.S., W.M.B., F.G., D.B., and N.R.W.), Saha Cardiovascular Research Center, University of Kentucky Medical Center, Lexington; the Department of Internal Medicine, Division of Endocrinology, (P.S., W.M.B., F.G., D.B., and N.R.W.), University of Kentucky Medical Center, Lexington; the Department of Veterans Affairs Medical Center (P.S., W.M.B., and N.R.W.), Lexington, KY; the Department of Chemistry (R.C.O. and M.H.G.), University of Washington, Seattle; and the Department of Biochemistry (M.H.G.), University of Washington, Seattle.

Correspondence to Nancy R. Webb, PhD, the Department of Internal Medicine, University of Kentucky Medical Center, 535 CT Wethington Bldg, 900 S Limestone St, Lexington, KY 40536-0200. E-mail nrwebb1@uky.edu

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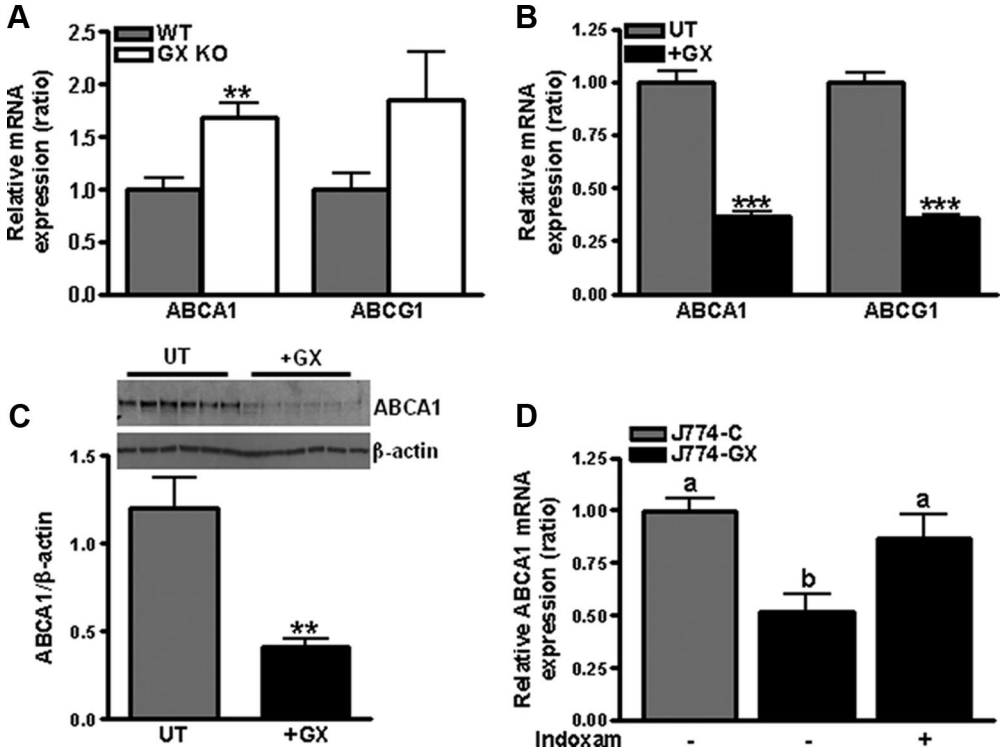

Figure 1. A, Relative expression of ABCA1 and ABCG1 mRNAs in MPMs from WT and GX knockout mice. Data are relative to WT MPM values. $^{**}P<0.01$ vs WT MPMs. B, J774 cells were treated with or without 0.1-μg/mL GX $sPLA_2$ for 8 hours before RT-PCR (n=6). $^{***}P<0.001$ vs untreated cells. C, Western blot analysis of lysates (10-μg protein) from control J774 cells (UT) and cells treated with 0.1-μg/mL recombinant GX $sPLA_2$ (+GX). Results from the densitometric analysis are shown below the blot. $^{**}P<0.01$. D, Relative expression of ABCA1 mRNA was quantified in untreated J774-C and J774-GX cells and in J774-GX cells treated with 20-μmol/L indoxam for 20 hours (n=4); data are representative of 2 experiments. Data that are not significantly different ($P>0.05$) are indicated with the same letter.

tative PCR (qPCR), immunoblotting, mouse peritoneal macrophage (MPM) isolation, production of gene-targeted mice, and the generation of J774 cells stably overexpressing FLAG-tagged GX $sPLA_2$ and the catalytically inactive mutant H46Q. More detailed methods for cholesterol efflux, the arachidonic acid release assay, small and interfering RNA (siRNA) suppression, and reporter assays are also provided in the supplemental information section.

Cell Treatments

For some experiments, J774 cells were incubated with 0.1-μg/mL mouse GX $sPLA_2$[8] in complete medium (DMEM medium supplemented with 10% heat-inactivated FBS, 100-U/mL penicillin, and 100-μg/mL streptomycin) for 8 hours before analyses. For other experiments, J774 cells were incubated with 10-μmol/L lysophosphatidylcholine (Sigma, St Louis, Mo) or vehicle (ethanol) in DMEM medium containing 1% fatty acid–free BSA (Sigma) for 20 hours. Alternatively, J774 cells were incubated with 10-μmol/L arachidonic acid (AA) (Sigma) or vehicle (dimethyl sulfoxide) in DMEM medium containing 1% fatty acid–free BSA for 8 hours. For indomethacin, 1 μmol/L (Sigma), treatments, the drug or vehicle (ethanol) was added to J774 cells either with or without lipopolysaccharide (LPS), 100 ng/mL (Cat No. L2654; Sigma) in complete medium for 6 hours. At the end of the incubation time, prostaglandin E_2 (PGE_2) in cell culture media was quantified by enzyme immunoassay (Cayman Chemical Company, Ann Arbor, Mich), following the manufacturer's protocol.

Description of Assays and Statistical Analyses

The AA release assay was performed as previously described.[9] For the cholesterol efflux assay, cholesterol efflux to apolipoprotein AI (Biodesign International, Saco, Me) and high-density lipoprotein was performed as previously described.[10] For the reporter assay, luciferase activities were analyzed using a commercially available system (Dual-Luciferase Reporter Assay; Promega, Madison, Wis).

Data are expressed as mean±SEM. Results were analyzed by the *t* test or 1-way ANOVA, followed by the Bonferroni posttest. $P<0.05$ was considered statistically significant. Data were tested for normality and equal variance before analysis.

Results

GX $sPLA_2$ Reduces Macrophage Expression of ABCA1 and ABCG1

In studies investigating the role of GX $sPLA_2$ in macrophage lipid metabolism, we unexpectedly found that MPMs deficient in GX $sPLA_2$ had significantly increased ABCA1 mRNA, and a trend for increased ABCG1, compared with wild-type (WT) MPMs (Figure 1A). When MPMs from WT mice were treated with 20-μmol/L indoxam, an $sPLA_2$ inhibitor,[11] ABCA1 and ABCG1 expression increased by 2.4- and 1.7-fold, respectively, an effect that did not reach statistical significance (supplemental Figure IA). Incubations with recombinant mGX $sPLA_2$ significantly reduced ABCA1 and ABCG1 expression in J774 macrophage-like cells (Figure 1B) in a manner that was dose dependent (supplemental Figure IB). Cells treated with GX $sPLA_2$ expressed significantly reduced amounts of ABCA1 protein (Figure 1C). Consistent with results produced by exogenous addition, forced overexpression of GX $sPLA_2$ resulted in significantly reduced ABCA1 and ABCG1 expression in J774 cells (J774-GX cells) compared with control (J774-C cells) (Figure

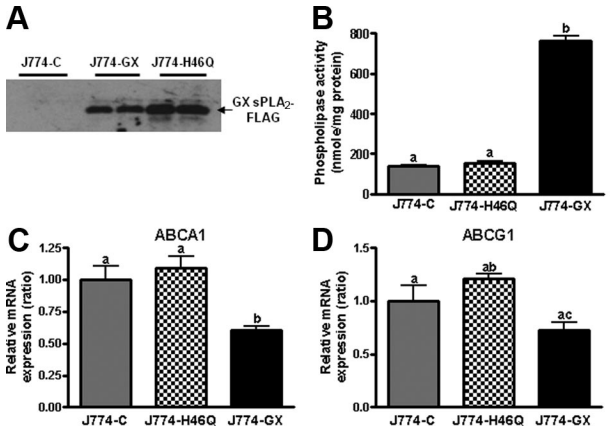

Figure 2. A, Conditioned media (after 20 hours) from J774-C, J774-GX, or J774–H46Q were immunoblotted with anti–FLAG antibody. B, Phospholipase activity in conditioned media from J774-C, J774–H46Q, and J774-GX cells. C and D, Relative expression of ABCA1 and ABCG1 in J774-C, J774–H46Q, and J774-GX cells by RT-PCR (n=4); data are representative of 3 experiments. Data that are not significantly different ($P>0.05$) are indicated with the same letter.

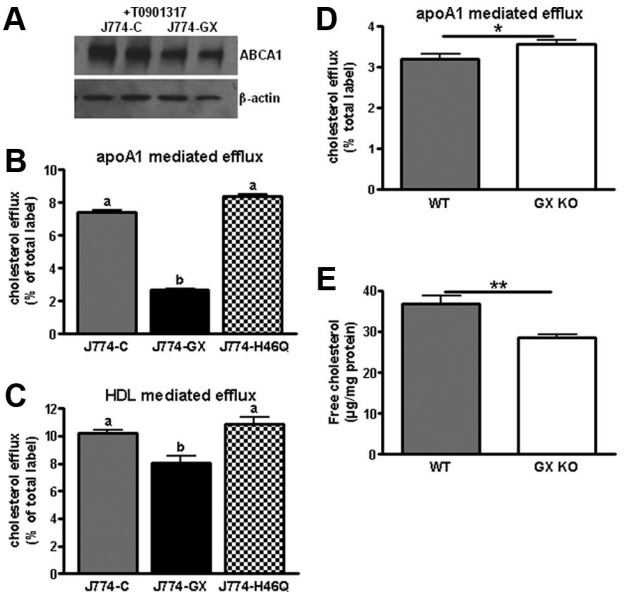

Figure 3. A, Western blot analysis of cell lysates (10-μg protein) after treatment with 5-μmol/L T0901317 for 12 hours. B, Cellular cholesterol efflux stimulated by 5-hour incubations with 10-μg/mL apoA1. C, Cellular cholesterol efflux stimulated by 5-hour incubations with 50-μg/mL high-density lipoprotein. Data that are not significantly different ($P>0.05$) are indicated with the same letter. D, Cellular cholesterol efflux by MPMs, stimulated by 5-hour incubation with 10-μg/mL apoA1. E, FC content of MPMs isolated from WT and GX knockout mice. $^{*}P<0.05$ and $^{**}P<0.01$.

1D; ABCG1 data not shown). Treatments with indoxam restored gene expression to normal levels in J774-GX cells, indicating a specific effect of the transgene for modulating gene expression (Figure 1D).

To directly show that GX $sPLA_2$ hydrolytic activity is required for its regulatory effect, we generated J774 cells stably overexpressing a catalytically inactive mutant (J774-H46Q cells). J774-GX and J774-H46Q cells secreted comparable levels of recombinant protein (Figure 2A). Culture medium from J774-GX cells exhibited significantly more (4- to 5-fold) phospholipase activity compared with J774-C or J774-H46Q cells (Figure 2B). Compared with J774-C cells, the expression of ABCA1 and ABCG1 mRNA was significantly reduced in J774-GX cells, but not J774-H46Q cells (Figure 2C and D). Taken together, our data demonstrate that GX $sPLA_2$ catalytic activity suppresses the expression of ABCA1 and ABCG1 in macrophages.

GX $sPLA_2$ Reduces Macrophage Cholesterol Efflux

Cholesterol efflux, stimulated by apoA1 and high-density lipoprotein, was assessed in J774-C, J774-GX, and J774-H46Q cells and MPMs isolated from WT and GX knockout mice. For these experiments, cells were preincubated with the LXR agonist T0901317 to increase the expression of ABCA1 and ABCG1. Such treatments did not abolish the difference in ABCA1 protein expression between J774-C and J774-GX cells (Figure 3A). J774-GX cells exhibited significantly reduced cholesterol efflux to apoA1 (64% reduction) and high-density lipoprotein (20% reduction) compared with J774-C and J774-H46Q cells (Figure 3B and C), consistent with reduced expression of ABCA1 and ABCG1, respectively. MPMs from GX knockout mice showed a modest, but significant, increase in cholesterol efflux to apoA1 compared with WT cells (Figure 3D), demonstrating that endogenous GX $sPLA_2$ modulates efflux. The enhanced effluxing capacity of GX knockout MPMs was associated with a significant decrease in cellular free cholesterol (FC) content (Figure 3E).

AA Suppresses ABCA1 and ABCG1 Expression Through a Cyclooxygenase 1/2–Independent Mechanism

Our data indicating that increased GX $sPLA_2$ catalytic activity leads to suppressed ABCA1 and ABCG1 expression prompted us to investigate which of the enzyme's lipolytic products recapitulate its effects. Although treatment of J774 cells with 10-μmol/L lysophosphatidylcholine did not significantly alter ABCA1 or ABCG1 mRNA levels (Figure 4A), the expression of both genes was significantly blunted in cells treated with 10-μmol/L AA (Figure 4B). J774-GX cells demonstrated significantly increased AA release compared with J774-C cells (Figure 4C), consistent with GX $sPLA_2$'s known ability to hydrolyze intact cell membranes and release AA.[12] Similarly, the exogenous addition of recombinant GX $sPLA_2$ led to significantly enhanced AA release from non-transfected J774 cells (Figure 4D). On the other hand, the deficiency of GX $sPLA_2$ in MPMs was associated with a modest, but detectable, 16% decrease in AA (data not shown). As expected, the increase in AA release in GX $sPLA_2$-overexpressing cells led to a significant increase in LPS-stimulated PGE_2 generation, providing the possibility that GX $sPLA_2$ may alter ABCA1 and ABCG1 expression through the generation of a downstream AA metabolite. To investigate this possibility, J774-C and J774-GX cells were treated with the nonselective cyclooxygenase 1/2 inhibitor indomethacin, which effectively blocked PGE_2 production in

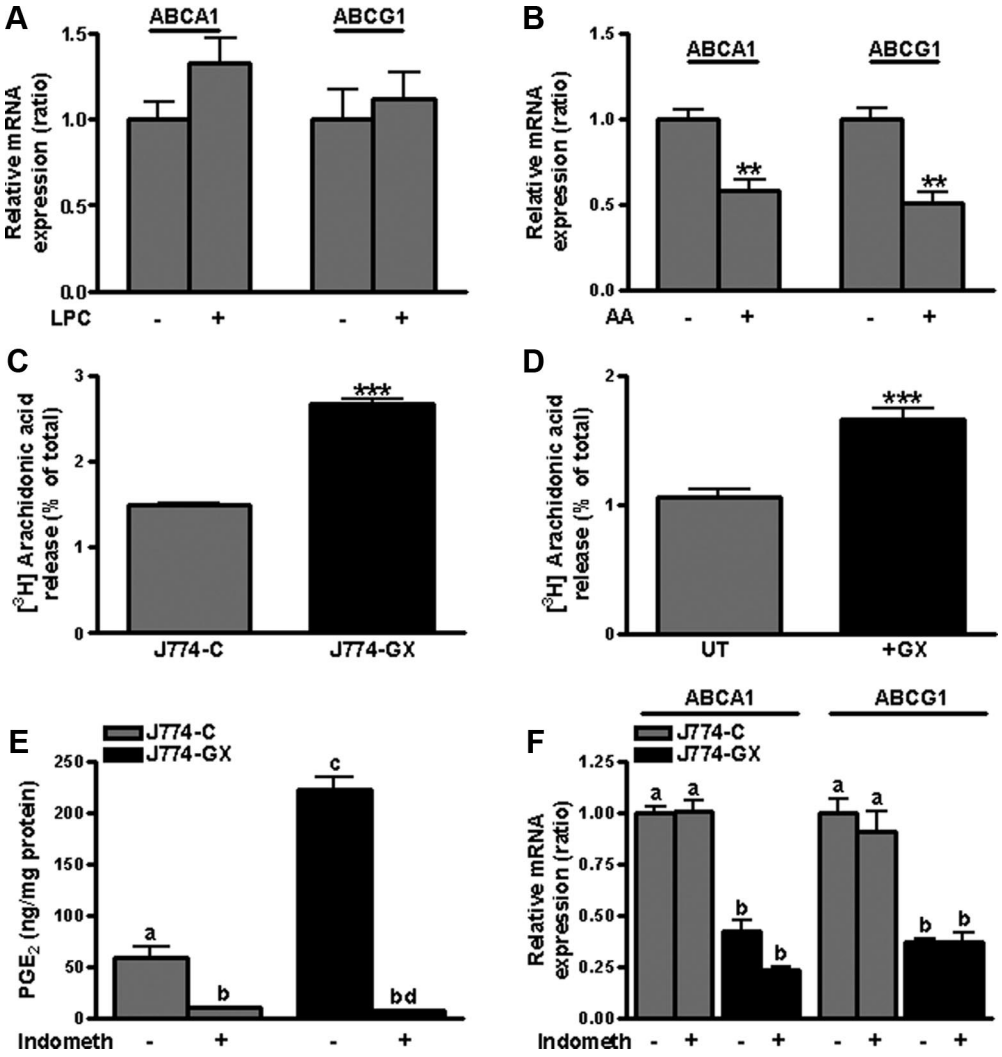

Figure 4. A, Relative expression of ABCA1 and ABCG1 mRNA in J774 cells treated for 20 hours with either 10-μmol/L lysophosphatidylcholine (LPC) or vehicle (n=6). B, Relative expression of ABCA1 and ABCG1 mRNA in J774 cells treated with either 10-μmol/L arachidonic acid (AA) or vehicle for 8 hours (n=4). $^{**}P<0.01$ vs untreated cells. C, [^{3}H]-AA release during 6 hours was quantified and expressed as the percentage of total cellular [^{3}H]-AA. D, [^{3}H]-AA release during 6 hours by untreated J774 cells (UT) and J774 cells treated with 0.1-μg/mL GX $sPLA_2$ (+GX) expressed as the percentage of total cellular [^{3}H]-AA. $^{***}P<0.001$. E, PGE_2 in conditioned media of J774-C and J774-GX cells treated for 6 hours with 100-ng/mL LPS in the presence or absence of 1-μmol/L indomethacin (indometh). F, Relative expression of ABCA1 and ABCG1 mRNA in J774-C and J774-GX cells incubated with either 1-μmol/L indomethacin or vehicle for 6 hours. Data are representative of at least 2 independent experiments. Data that are not significantly different ($P>0.05$) are indicated with the same letter.

both cell types on treatment with LPS (Figure 4E). However, indomethacin did not normalize ABCA1 or ABCG1 expression in J774-GX cells (Figure 4F), indicating that GX $sPLA_2$ does not regulate gene expression by amplifying prostanoid synthesis.

GX $sPLA_2$ Regulates LXR Target Gene Expression

The coregulation of ABCA1 and ABCG1 suggested the interesting possibility that GX $sPLA_2$ modulates the activity of LXRα/β, known transcriptional activators of these transporters.[13,14] Additional RT-PCR analyses demonstrated that expression of several other known LXR targets (sterol regulatory element-binding protein 1c, lipoprotein lipase, matrix metalloproteinse 9, phospholipid transfer proteins (PLTP) was suppressed in J774-GX cells compared with J774-C cells (supplemental Figure IIA). However, the expression of apolipoprotein E was not altered in J774-GX cells, perhaps in line with studies showing that modulation of LXR action may be different among diverse target promoters.[15] GX $sPLA_2$ overexpression was not associated with alterations in transcripts regulated by peroxisome proliferator–activated receptor (PPAR) γ (CD36 or aP2) or sterol regulatory element-binding protein 2 (low-density lipoprotein receptor or 3-hydroxy-3-methylglutaryl coenzyme A reductase) or other genes involved in cellular cholesterol metabolism (scavenger receptor BI [SR-BI], acetyl-coenzyme A acetyl transferase [ACAT], scavenger receptor [SRA]) (supplemental Figure IIB).

To investigate whether GX $sPLA_2$ modulates LXR activation, J774-C, J774-H46Q, and J774-GX cells were incubated in the presence or absence of the LXR agonist T0901317. As

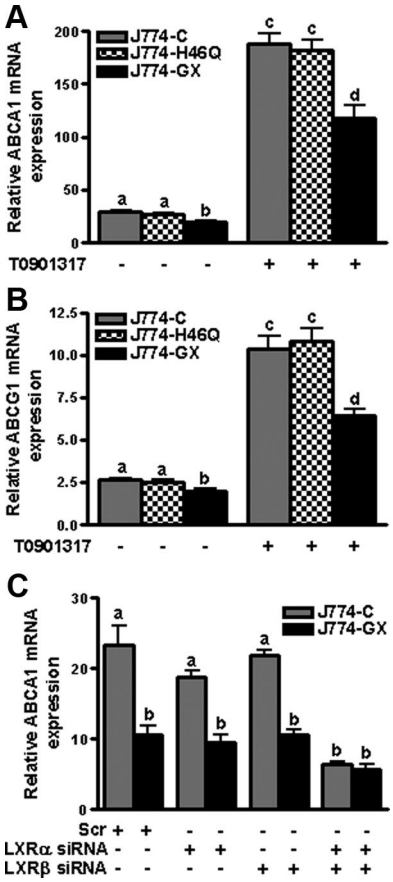

Figure 5. A and B, J774-C, J774–H46Q, and J774-GX cells were incubated in the presence or absence of 1-μmol/L T0901317 for 20 hours, as indicated, before RT-PCR. Data are given as the mean±SEM (n=4) and are representative of 3 experiments; values that are not significantly different ($P>0.05$) are indicated with the same letter. C, J774-C and J774-GX cells were transfected with control siRNA (scr), siRNA targeting LXRα, siRNA targeting LXRβ, or siRNA targeting both LXRα and LXRβ for 24 hours, and then treated with 1-μmol/L T0901317 for an additional 20 hours before RT-PCR. Data are given as the mean±SEM (n=4) and are representative of 2 experiments. Data that are not significantly different ($P>0.05$) are indicated with the same letter.

expected, ABCA1 and ABCG1 expression was significantly induced in J774-C cells after treatment with T0901317 (Figure 5A and B). Interestingly, the extent of induction was significantly blunted in T0901317-treated J774-GX cells, but not in J774-H46Q cells, a finding that was consistent with the protein data presented in Figure 3A. This reduced induction was not because of altered LXRα or LXRβ mRNA (supplemental Figure IIB) or LXRα protein (supplemental Figure IIC). We also excluded the possibility that GX $sPLA_2$ modulates LXR activation by regulating the expression of cholesterol sulfotransferase, which catabolizes oxysterols that may serve as LXR agonists,[16] or activating signal cointegrator-2, an LXR coactivator[17] (supplemental Figure IID). The expression of oxidosqualene cyclase, which generates 24,25-epoxycholesterol, was significantly reduced in J774-GX cells, possibly as a result of increased cellular FC in J774-GX cells.[18] However, the suppressive effect of GX $sPLA_2$ might be reversible by an LXR agonist if the effect was mediated through decreased oxidosqualene cyclase expression.

Next, we determined whether GX $sPLA_2$ decreased LXR target gene expression when LXRα/β was suppressed. Consistent with previous data, ABCA1 mRNA expression was 55% lower in J774-GX cells compared with J774-C cells transfected with control siRNA (Figure 5C). This difference in expression of ABCA1 between J774-C and J774-GX did not significantly change when the cells were transfected with siRNA targeting either LXRα or LXRβ, but the difference was completely abolished when cells were cotransfected with siRNAs targeting both LXRα and LXRβ. The residual ABCA1 expression in both J774-C and J774-GX cells likely reflects LXR-independent ABCA1 expression[19] and the fact that LXRα/β expression was only partially (60%–70% LXRα and 80%–90% LXRβ) suppressed (supplemental Figure III). These data provide direct evidence that the negative effect of GX $sPLA_2$ on LXR target gene expression is LXRα/β dependent.

Increased GX $sPLA_2$ Activity Is Linked to Suppressed Activation of LXR

Taken together, our data suggest that catalytically active GX $sPLA_2$ inhibits the ability of LXR to transactivate target genes. To investigate whether GX $sPLA_2$ inhibits LXR transactivation through liver X receptor response element (LXRE), we performed luciferase reporter assays in human embryonic kidney 293 (HEK293) cells. Compared with control cells, LXRE promoter reporter expression was reduced in cells cotransfected with GX $sPLA_2$ (Figure 6A). Although the addition of increasing concentrations of T0901317 dose dependently increased reporter expression in control cells, the ability of an LXR agonist to activate reporter expression was significantly blunted in cells overexpressing GX $sPLA_2$. The expression of H46Q had no effect on T0901317-induced reporter expression (Figure 6B). Reporter assays performed in RAW 264.7 macrophages confirmed that GX $sPLA_2$ blunted agonist-induced reporter expression with either T0901317 or 25-hydroxy cholesterol (supplemental Figure IVA and B).

Next, we performed reporter assays using a chimeric construct composed of the galactosidase-4 (GAL4)-DNA-binding domain fused to the ligand-binding domain (LBD) of mouse LXRα and a GAL4 response element-driven luciferase reporter construct (Figure 6C). Reporter activity was significantly reduced when GX $sPLA_2$ was coexpressed, suggesting that the ability of GX $sPLA_2$ to blunt target gene expression is mediated through the LXR LBD. More important, GX $sPLA_2$ had no effect on reporter expression driven by a glucocorticoid receptor/GAL4 chimeric construct, demonstrating the specificity of GX $sPLA_2$'s inhibitory effect (Figure 6D).

Accumulating evidence suggests that some LXR agonists can negatively regulate macrophage responses to inflammatory stimuli through transrepression of nuclear factor (NF) κB target genes.[20] Thus, it was of interest to determine whether GX $sPLA_2$ modulates the transrepressive effect of LXR on NF-κB. Murine RAW 264.7 macrophages were transfected with GX $sPLA_2$, along with mouse LXRα and an NF-κB

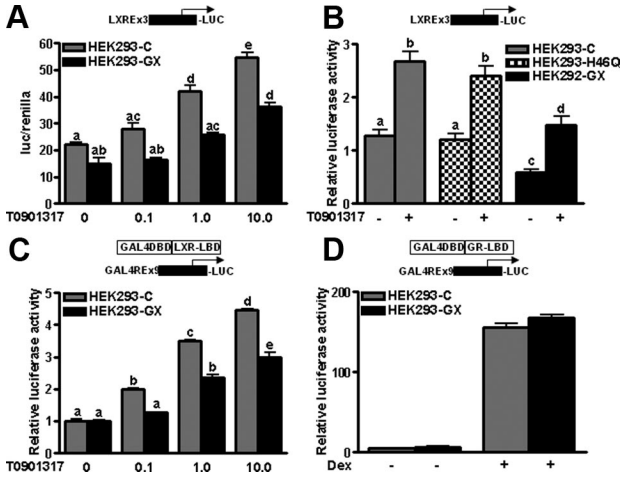

Figure 6. A, Reporter activity in HEK293 cells transiently transfected with pTK-3xLXRE-Luc reporter construct along with plasmids encoding mLXRα, renilla luciferase, and either mGX $sPLA_2$ expression vector (HEK293-GX) or the corresponding pcDNA3.0 empty expression vector (HEK293-C). Twenty hours after transfection, cells were incubated for 24 hours with fresh media containing the indicated concentrations of T0901317 (T09). B, Reporter activity in HEK293 cells transiently transfected with pTK-3xLXRE-Luc reporter construct, along with plasmids encoding mLXRα, renilla luciferase, and mGX $sPLA_2$ expression vector, H46Q (HEK293–H46Q), or the corresponding pcDNA3.0 empty expression vector. Twenty hours after transfection, cells were incubated for 24 hours with fresh media containing 1-μmol/L T0901317. C, HEK293 cells were cotransfected with Gal4-driven luciferase reporter construct and an expression vector containing Gal4–DNA-binding domain fused to LXR-LBD, along with GX $sPLA_2$ expression plasmid or control plasmid. After 24 hours, the cells were treated with fresh media containing indicated concentrations of T0901317 for 24 hours. Data are given as the mean±SEM (n=4) and are representative of at least 3 independent experiments. D, The Gal4-driven luciferase reporter construct was cotransfected into HEK293 cells with an expression vector containing Gal4–DNA-binding domain fused to LBD of glucocorticoid receptor (GR-LBD) and expression plasmid for GX $sPLA_2$ or vector control. After 24 hours of transfection, the cells were treated with fresh media containing 10-μmol/L dexamethasone (DEX) for a further 24 hours before analyzing the cell lysates for luciferase activity. Data are representative of at least 2 independent experiments. Data that are not significantly different ($P>0.05$) are indicated with the same letter.

promoter luciferase construct, and then treated with LPS. As reported previously,[20] NF-κB promoter activation was dampened in control cells treated with LPS in the presence of T0901317 (supplemental Figure IVC). Promoter activity was reduced to similar levels in cells transfected with GX $sPLA_2$, indicating that GX $sPLA_2$ does not alter the transrepressive effects of LXR agonists on NF-κB.

Discussion

ABCA1 and, to a lesser extent, ABCG1 play key roles in macrophage cholesterol homeostasis by exporting excess cellular FC to extracellular acceptors. LXRs are nuclear hormone receptors that act as intracellular cholesterol sensors by inducing the expression of ABCA1 and ABCG1 in response to cellular FC loading.[13,14] In this study, we identified a novel mechanism for regulating the macrophage expression of ABCA1 and ABCG1. To our knowledge, we show for the first time that MPMs deficient in GX $sPLA_2$ have significantly increased ABCA1/ABCG1, increased cholesterol efflux, and, consequently, decreased cellular FC. This regulatory effect requires GX $sPLA_2$ catalytic activity, is mimicked by the addition of AA to cells, is abrogated when LXRα/β is suppressed, and is partially reversed by LXR agonists T0901317 and 25-hydroxy cholesterol. The results from luciferase reporter assays support the conclusion that GX $sPLA_2$ suppresses ABCA1 and ABCG1 expression by inhibiting the ability of LXR to transactivate target genes.

GX $sPLA_2$ is expressed in a number of different tissues, including brain, spleen, lung, thymus, intestine, uterus, and testis,[2,21] where it has the potential to modulate LXR signaling. It was recently determined that GX $sPLA_2$ is expressed in the adrenal gland, where it regulates steroidogenesis by modulating the expression of steroidogenic acute regulatory protein,[22] a known LXR target.[23] It was also recently reported that hydrolytic products generated by GX $sPLA_2$ negatively regulate adipogenesis, possibly by suppressing LXR activation.[24] Given its potent ability to hydrolyze cell membranes and its potential role in multiple metabolic processes, it is important to consider how GX $sPLA_2$ may be regulated in vivo. Studies in vitro implicate the M-type $sPLA_2$ receptor in mediating uptake of extracellular GX $sPLA_2$; however, RT–quantitative PCR analysis of J774 cells and MPMs suggests that this receptor is not expressed in macrophages (data not shown). Unlike most members of the $sPLA_2$ family, GX $sPLA_2$ is expressed in an inactive form, requiring removal of an N-terminal propeptide for catalytic activity.[2] Recent data from the analysis of transgenic mice support the conclusion that GX $sPLA_2$ activity is under tight regulation and suggest the interesting possibility that proteolytic activation may occur during inflammation.[25] Thus, GX $sPLA_2$ may play a key role in coordinating metabolic responses in multiple cell types during inflammation.

Evidence suggests that GX $sPLA_2$ induces some biological effects through a mechanism that is independent of its phospholipase activity.[6] Our data that GX $sPLA_2$, but not the inactive mutant H46Q, modulates LXR target gene expression provide strong evidence that phospholipid hydrolysis is required for its suppressive effect. This conclusion is further supported by our finding that indoxam (a specific $sPLA_2$ inhibitor) blocked GX $sPLA_2$'s effect.

Our data are consistent with the possibility that GX $sPLA_2$ regulates ABCA1 and ABCG1 through the generation of AA. Forced overexpression or exogenous addition of GX $sPLA_2$ resulted in significantly enhanced AA release in J774 cells, whereas GX $sPLA_2$ deficiency in MPMs was associated with a 16% reduction in AA release. As expected, accompanying the enhanced AA release in J774-GX cells was a significant increase in LPS-stimulated PGE_2 generation. However, we excluded a role for prostanoids because the nonselective cyclooxygenase 1/2 inhibitor indomethacin failed to normalize ABC transporter expression in J774 macrophages overexpressing GX $sPLA_2$. According to several reports, polyunsaturated fatty acids, including AA, act as endogenous LXR antagonists, although the mechanism is unclear. AA suppresses transactivation by interacting with the LXR LBD to inhibit LXR/retinoid X receptor binding to LXREs.[26,27] Gel shift mobility and LBD activation assays indicate that poly-

unsaturated fatty acids (PUFAs) compete with LXR agonists to block LXR activation.[26] Also, PUFAs inhibit LXR function through the activation of PPARα and PPARγ, which, in turn, reduces the formation of LXR/retinoid X receptor heterodimers.[28] Our data demonstrate that GX $sPLA_2$ suppresses LXRE promoter activity through a mechanism involving the C-terminal portion of LXR that spans the LBD. However, the ability of GX $sPLA_2$ to suppress LXR activation, as assessed in luciferase reporter assays, was not completely reversed by T0901317, suggesting that hydrolytic products generated by GX $sPLA_2$ do not act in a manner that involves direct competition for agonist binding. Nevertheless, the effect of GX $sPLA_2$ was specific to LXR LBD because there was no suppression of transactivation when the LXR LBD was replaced by the glucocorticoid receptor LBD. In addition to ligand binding, the LXR LBD mediates the interaction with corepressors and coactivators,[29] providing the possibility that GX $sPLA_2$ modulates LXR activation through a mechanism that does not involve competition with an agonist.

In addition to playing a central role in macrophage cholesterol homeostasis, considerable evidence suggests that, on activation by some ligands, LXRs may also regulate inflammatory responses by suppressing NF-κB–mediated gene induction.[30] However, our finding that the LXR agonist T0901317 suppresses the activation of an NF-κB promoter construct in response to LPS to a similar extent in J774-C and J774-GX cells suggests that GX $sPLA_2$ overexpression does not interfere with LXR transrepression of NF-κB. This finding is in keeping with other studies demonstrating that not all LXR ligands known to upregulate ABCA1 transcription are capable of repressing inflammatory gene expression.[31] In the absence of LXR ligand, NF-κB activation induced by LPS was significantly enhanced in macrophages overexpressing GX $sPLA_2$. Although the molecular basis for this effect requires further investigation, GX $sPLA_2$ has been previously implicated in both positive and negative regulation of inflammatory responses.[3,6]

The possibility that $sPLA_2$ can also provide lipid mediators (free fatty acids and PGs) that serve as ligands for PPARs cannot be excluded; however, evidence that this occurs is controversial.[32–34] We found no evidence that CD36 and adipocyte protein-2 (aP2), known PPAR-γ targets, were regulated by GX $sPLA_2$. What remains to be investigated is whether other members of the $sPLA_2$ family can also regulate LXR target gene expression or if this is a unique property of GX $sPLA_2$ because of its potent hydrolytic activity. Interestingly, a suppressive effect of cytosolic phospholipase A_2-mediated AA generation on LXR target gene expression was recently reported in primary mouse aortic smooth muscle cells.[35] This finding and the findings from the present study highlight a new mechanism for regulating gene expression involving hydrolytic products generated by cellular phospholipase A_2.

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Disclosures

None.

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