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Group X Secretory Phospholipase A2 Enhances TLR4 Signaling in Macrophages

Preetha Shridas,*‡ William M. Bailey,*‡ Kayla R. Talbott,*‡ Rob C. Oslund,§ Michael H. Gelb,§,* Nancy R. Webb,*‡

Secretory phospholipase A2s (sPLA2) hydrolyze glycerophospholipids to liberate lysophospholipids and free fatty acids. Although group X (GX) sPLA2 is recognized as the most potent mammalian sPLA2 in vitro, its precise physiological function(s) remains unclear. We recently reported that GX sPLA2 suppresses activation of the liver X receptor in macrophages, resulting in reduced expression of liver X receptor-responsive genes including ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1), and a consequent decrease in cellular cholesterol efflux and increase in cellular cholesterol content (Shridas et al. 2010. Arterioscler. Thromb. Vasc. Biol. 30: 2014–2021). In this study, we provide evidence that GX sPLA2 modulates macrophage inflammatory responses by altering cellular cholesterol homeostasis. Transgenic expression or exogenous addition of GX sPLA2 resulted in a significantly higher induction of TNF-α, IL-6, and cyclooxygenase-2 in J774 macrophage-like cells in response to LPS. This effect required GX sPLA2 catalytic activity, and was abolished in macrophages that lack either TLR4 or MyD88. The hypersensitivity to LPS in cells overexpressing GX sPLA2 was reversed when cellular free cholesterol was normalized using cyclodextrin. Consistent with results from gain-of-function studies, peritoneal macrophages from GX sPLA2-deficient mice exhibited a significantly dampened response to LPS. Plasma concentrations of inflammatory cytokines were significantly lower in GX sPLA2-deficient mice compared with wild-type mice after LPS administration. Thus, GX sPLA2 amplifies signaling through TLR4 by a mechanism that is dependent on its catalytic activity. Our data indicate that this effect is mediated through alterations in plasma membrane free cholesterol and lipid raft content. The Journal of Immunology, 2011, 187: 000–000.

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inflammatory angiogenesis (12). Thus, available data concerning such effects have been contradictory.

Recent studies in our laboratory provide an alternative mechanism whereby GX sPLA₂ may regulate macrophage inflammatory responses. We have shown that GX sPLA₂ suppresses macrophage expression of ABCA1 and ABCG1, leading to reduced cellular cholesterol efflux and increased cellular cholesterol content (13). In this study, we conducted comprehensive gain-of-function and loss-of-function studies to more clearly define the role of GX sPLA₂ in macrophage inflammatory responses. Because TLR4 signaling pathways have been shown to be modulated by plasma membrane free cholesterol and are implicated in atherosclerosis (14), we investigated whether GX sPLA₂ modulates macrophage responses to LPS.

Materials and Methods

Animals and experimental treatments

Targeted deletion of the GX sPLA₂ gene was performed by InGenious Targeting Laboratory (Stony Brook, NY) using embryonic stem cells derived from C57BL/6 mice (15). Heterozygous GX sPLA₂+/- mice were bred to produce GX sPLA₂+/- (wild-type [WT]) and GX sPLA₂-/- mice (GX knockout [KO]) mice that were used for the experiments. Mice deficient in TLR4 and MyD88 (16) were generously provided by Dr. L. Curtiss (Scripps Research Institute) and K. Moore (Harvard Medical School), respectively. Male and female mice (3–5 mo old) were maintained on a 10-h light/14-h dark cycle and received standard mouse chow and water ad libitum. For LPS treatments, mice were injected with 3 mg/kg mGX sPLA₂ in lipoprotein-deficient medium or PBS and then incubated for 15 min at 4°C, washed with PBS, and then incubated for 15 min at 4°C with anti–CT-B Ab to cross-link CT-B and lipid rafts. After washing with PBS, cells were fixed in 4% paraformaldehyde for 20 min at 4°C. Fixed cells were mounted with a Prolong Antifade Kit (Molecular Probes). Confocal microscopy was performed at the University of Kentucky Imaging Facility using a Leica laser-scanning confocal microscope with argon (488 nm) and krypton (568 nm) lasers. For FACS analysis, J774 cells were incubated with 100 ng/ml LPS in the presence or absence of 0.1 μg/ml mGX sPLA₂ in lipoprotein-deficient medium (DMEM supplemented with 10% lipoprotein-deficient serum, 100 U/ml penicillin, 100 μg/ml streptomycin) for 8 h before analysis. J774 cells were treated with 10 μM lysophosphatidylcholine (Sigma, St. Louis, MO), 10 μM arachidonic acid (Sigma, St. Louis, MO), or vehicle (ethanol) in DMEM containing 1% fatty acid-free BSA for 16 h before treatment with 100 ng/ml LPS for 8 h.

Confocal microscopy and flow cytometry (FACS) to visualize lipid rafts

J774 cells were seeded on glass coverslips and grown until confluent in complete medium. All subsequent procedures were performed using pre-chilled solutions according to the manufacturer’s directions (Vybrant Lipid Raft Labeling Kit; Invitrogen). Cells were washed once with complete medium before the addition of Alexa Fluor 488-labeled cholera toxin B (CT-B) conjugate (1 μg/ml). Cells were incubated for 15 min at 4°C, washed with PBS, and then incubated for 15 min at 4°C with anti–CT-B Ab to cross-link CT-B and lipid rafts. After washing with PBS, cells were fixed in 4% paraformaldehyde for 20 min at 4°C. Fixed cells were mounted with a Prolong Antifade Kit (Molecular Probes). Confocal microscopy was performed at the University of Kentucky Imaging Facility using a Leica laser-scanning confocal microscope with argon (488 nm) and krypton (568 nm) lasers. For FACS analysis, J774 cells were incubated with 100 ng/ml LPS in the presence or absence of 0.1 μg/ml mGX sPLA₂ in lipoprotein-deficient medium (DMEM supplemented with 10% lipoprotein-deficient serum, 100 U/ml penicillin, 100 μg/ml streptomycin) for 8 h before analysis. J774 cells were treated with 10 μM lysophosphatidylcholine (Sigma, St. Louis, MO), 10 μM arachidonic acid (Sigma, St. Louis, MO), or vehicle (ethanol) in DMEM containing 1% fatty acid-free BSA for 16 h before treatment with 100 ng/ml LPS for 8 h.

RNA extraction and quantitative PCR

Total RNA was isolated from J774 cells or MPMs using RNeasy Mini kit (Promega). RNA (0.2–1 μg) was reverse transcribed into cDNA using a reverse transcription system (Promega). After 4-fold dilution, 5 μl was used as a template for real-time PCR. Amplification was done for 40 cycles using Power SYBR Green PCR master Mix Kit (Applied Biosystem) and IQ 5 real-time PCR machine (I Cycler; Bio-Rad). Quantification was performed in duplicate using the standard curve method and normalized to 18S. Sequences of primers are as follows: 18s, 5'-GTT AGA AGG ACA CAG ACT GG-3' (forward) and 5'-GGT AGA CCG CCC GCT CCT-3' (reverse); Tnf-α, 5'-GGG ACC TCT ACT TGT GCA TTG-3' (forward) and 5'-GTG ACT ATG GAT CTC CAG AAG-3' (reverse); Il-6, 5'-CAA CGA TGA TGC ACT TGC AG-3' (forward) and 5'-GTA GCT ATG GTA CTC CAG AAG-3' (reverse); cyclooxygenase-2 (COX-2), 5'-CCA GCA CTT CAC CCA TCA GTT-3' and 5'-ACC CAG CTC CTC GAT TA-3' (reverse).

Cholesterol depletion and repletion

Cholesterol depletion and repletion of J774 cells was conducted as described by Zhu et al. (4). In brief, cells were incubated with or without prewarmed 10 mM methyl-β-cyclodextrin (MβCD; Sigma) at 37°C for 30 min to deplete cholesterol. Macrophages were then washed with PBS and incubated in the presence or absence of cholesterol coupled with MβCD (80 μg/ml; Sigma) at 37°C for 1 h to replete cholesterol. The cells were then incubated for 8 h with 100 ng/ml LPS before collection of culture media and cells.

Biochemical reagents and assays

FLAG-tagged fusion protein (GX sPLA₂) was detected by Western blot analysis using anti-FLAG M2 Ab (Stratagene, La Jolla, CA). Total JNK Ab was from R&D Systems (Minneapolis, MN); pErk, total Erk, and pJNK Abs were from Cell Signaling (Danvers, MA). Tnf-α and IL-6 protein concentrations in culture media were determined by ELISA (R&D Systems). The cholesterol content of J774 cells was quantified as described by Boyanova et al. (17).

Statistical analyses

Data are expressed as mean ± SEM. Results were analyzed by Student t test or one-way ANOVA followed by Bonferroni’s posttest. The p values <0.05 were considered statistically significant. Data were tested for normality and equal variance before analysis.
Results

**GX sPLA2 promotes macrophage inflammatory responses**

To determine whether GX sPLA2 modulates macrophage inflammatory responses, MPMs isolated from WT and GX sPLA2-deficient (GX KO) mice were treated with 100 ng/ml LPS for 6 h. Notably, GX KO MPMs exhibited a significantly blunted upregulation (~60% reduction) of TNF-α, IL-6, and COX-2 mRNAs in response to LPS compared with WT cells (Fig. 1A). There was no significant difference in the expression of these genes when WT and GX KO cells were incubated in the absence of LPS (not shown).

As another approach to define the role of GX sPLA2 in macrophage inflammatory responses, we performed gain-of-function studies in J774 macrophage-like cells. J774 cells were stably transfected with C-terminally FLAG-tagged GX sPLA2 (J774-GX cells). J774-GX cells secreted significantly more phospholipase activity compared with J774-C cells (4- to 5-fold increase; data not shown). Overexpression of GX sPLA2 did not alter the basal expression of the inflammatory mediators TNF-α, IL-6, or COX-2 in J774 cells (data not shown). However, incubations with 100 ng/ml LPS resulted in a significantly increased induction of TNF-α (1.7-fold), IL-6 (2.2-fold), and COX-2 (4-fold) in J774-GX cells compared with J774-C cells (Fig. 1B). Similarly, J774 cells treated with LPS in the presence of recombinant mGX sPLA2 (0.1 μg/ml; ~7 nM) exhibited a significant increase in TNF-α (2.7-fold), IL-6 (1.6-fold), and COX-2 (3.6-fold) mRNA expression compared with cells treated with LPS in the absence of GX sPLA2 (Fig. 1C). Thus, our studies in MPMs and J774 cells consistently show that GX sPLA2 augments macrophage inflammatory responses.

**GX sPLA2 promotes macrophage inflammatory responses through a mechanism that is dependent on its catalytic activity**

GX sPLA2 is known to elicit biological effects that are independent of its catalytic function (7, 12). To determine whether GX sPLA2 catalytic activity is required for its effect on macrophage responses to LPS, we developed J774 cells stably overexpressing a catalytically inactive GX sPLA2 mutant (J774-H46Q cells). As we previously reported, the amount of FLAG-tagged recombinant GX sPLA2 secreted into the media was similar for J774-H46Q and J774-GX cells, whereas the phospholipase activity in conditioned media from J774-H46Q cells was not increased compared with J774-C cells (13). Consistent with data from the analysis of mRNA (Fig. 1C), J774-GX cells treated with 100 ng/ml LPS secreted significantly more TNF-α (1.7-fold) and IL-6 (1.7-fold) protein compared with J774-C cells (Fig. 2A, 2B). In contrast, there was no significant difference in cytokine secretion between LPS-treated J774-H46Q and J774-C cells. To verify that the hyperresponsiveness of J774-GX cells was a specific effect of the transgene, we treated cells with LPS in the presence of indoxam, a specific inhibitor of sPLA2 activity (18). Indoxam significantly reduced LPS-induced TNF-α secretion by J774-GX cells to a level that was similar to LPS-induced secretion by J774-C cells (Fig. 2C). These results indicate that GX sPLA2 catalytic activity is required for its ability to produce hypersensitivity to inflammatory stimuli. Although there was a trend for a blunted inflammatory response when J774-C cells were treated with indoxam, this did not reach statistical significance. Previously published studies indicate that lipoprotein particles are substrates for GX sPLA2 hydrolysis (19). Therefore, to rule out the possibility that GX sPLA2 modulates inflammation by modifying lipoproteins in the media, we conducted additional experiments with media supplemented with lipoprotein-deficient serum. Under these conditions, TNF-α and IL-6 expression by J774 cells treated with LPS was blunted compared with the same cells incubated with media supplemented with FBS. Nevertheless, the addition of GX sPLA2 significantly increased the induction of cytokine expression, suggesting that lipoprotein hydrolysis was not required for this effect (Supplemental Fig. 1). Although our data are consistent with the conclusion that GX sPLA2 amplifies the effect of LPS by generating a lipid mediator, we were unable to recapitulate the enzyme’s effect by exogenous addition of 10 μM arachidonic acid or lysophosphatidylcholine, the two major products of GX sPLA2 hydrolysis (Supplemental Fig. 2).

**GX sPLA2 augments TLR4-dependent signaling in macrophages**

TLR4/CD14/MD2 act as coreceptors that mediate LPS-induced signaling in macrophages, which is initiated by the recruitment of cytosolic adapter proteins such as MyD88 to the coreceptor complex (20, 21). Thus, it was of interest to define the role of TLR4 and MyD88 and downstream signaling pathways in GX sPLA2-mediated...
hyperresponsiveness. MPMs from TLR4+/+ and TLR4−/− and MyD88+/+ and MyD88−/− mice were treated with LPS in the presence or absence of 0.1 mg/ml mGX sPLA2. Consistent with the data in Fig. 1C, the addition of GX sPLA2 resulted in significantly increased cytokine expression in WT MPMs treated with LPS (Fig. 3A,3B). As expected, the expression of the inflammatory mediators was markedly blunted in TLR4−/− and MyD88−/− MPMs treated with LPS in the absence of GX sPLA2. Notably, LPS also failed to induce inflammatory mediators in TLR4−/− and MyD88−/− MPMs in the presence of GX sPLA2, indicating that GX sPLA2 enhances inflammatory responses through a TLR4- and MyD88-dependent mechanism.

NF-κB and MAPK signaling are major signaling pathways involved in TLR4-mediated inflammatory cytokine induction (22). Using an NF-κB promoter luciferase reporter assay, we previously reported that LPS-induced NF-κB activation is enhanced in cells overexpressing GX sPLA2 (13). Interestingly, deficiency of GX sPLA2 in MPMs was associated with a considerable reduction in LPS-induced phosphorylation of Erk1/2 and JNK that was restored by the addition of recombinant GX sPLA2 (Fig. 3C), indicating that GX sPLA2 enhances the activation of the MAPK pathway downstream of TLR4. Taken together, our data indicate that GX sPLA2 increases macrophage inflammatory responses by augmenting TLR4-dependent signaling.

GX sPLA2 modulates cellular free cholesterol and lipid raft content

We previously demonstrated that hydrolytic products generated by GX sPLA2 negatively regulate liver X receptor (LXR) activation in macrophages, and thus significantly reduce the expression of LXR target genes ABCA1 and ABCG1. As a consequence, cholesterol efflux to extracellular acceptors is blunted in macrophages by GX sPLA2 (13). Membrane free cholesterol and lipid raft content are known to modulate TLR signaling in macrophages (23–26), and the absence of ABCA1 and ABCG1 leads to the accumulation of cell membrane free cholesterol and enhanced macrophage inflammatory responses. These observations prompted us to consider whether GX sPLA2 regulates TLR4 signaling by modulating macrophage free cholesterol and lipid raft content.
Overexpression of GX sPLA₂ in J774 cells was associated with a significant (30%) increase in cellular free cholesterol (Fig. 4A), which compares with the 12–14% increase reported for ABCA1-deficient macrophages (4). Other published studies reported that similar changes in cellular free cholesterol content cause physiologically relevant differences in inflammatory responses (5, 24). Similarly, treatment with recombinant GX sPLA₂ significantly increased the free cholesterol content of nontransfected J774 cells (Fig. 4B). Alterations in GX sPLA₂ did not affect cellular cholesterol ester content in either model system (data not shown). These findings are consistent with our previous report that MPMs deficient in GX sPLA₂ have significantly reduced free cholesterol compared with WT MPMs (13). Cell surface lipid rafts were assessed by staining cells with Alexa Fluor-488 labeled CT-B, which binds to a ganglioside GM1 in lipid rafts (27). When viewed by confocal laser microscopy, J774-GX cells stained with CT-B showed increased fluorescence and more punctuate staining compared with control cells (Fig. 4C). Analysis by flow cytometry confirmed significantly increased CT-B labeling of J774-GX cells, consistent with increased lipid rafts and plasma membrane free cholesterol (Fig. 4D) (28). Thus, our data point to a previously unrecognized role for GX sPLA₂ in regulating plasma membrane lipid raft content.

The hyperresponsiveness to LPS mediated by GX sPLA₂ is related to altered cellular free cholesterol and lipid raft content

To determine whether increased inflammatory responses by J774-GX cells was related to increased membrane free cholesterol and lipid rafts, we manipulated plasma membrane free cholesterol content by treating cells with MβCD before LPS exposure.

Treatment with 10 mM MβCD for 30 min reduced free cholesterol content in J774-C and J774-GX cells by 54 and 73%, respectively (Fig. 5A). Subsequent incubation of cholesterol-depleted cells for 60 min with cholesterol-loaded MβCD increased free cholesterol content in both cell types (Fig. 5A). Notably, cholesterol depletion and repletion resulted in free cholesterol levels that were no longer significantly different in the two cell types (Fig. 5A). After each of these cholesterol manipulations, cells were treated for 8 h with LPS and the amount of TNF-α in the media was quantified. As previously shown, in the absence of cholesterol depletion/repletion, LPS induced significantly more TNF-α secretion in J774-GX cells compared with J774-C cells (Fig. 5B). After cholesterol depletion, the response to LPS in both cell types was significantly reduced and the hyperresponsiveness of J774-GX cells was no longer evident. In the case of cholesterol-repleted cells, the responsiveness to LPS was restored to a level that was similar for the two cell types (Fig. 5B). These data demonstrate a direct relationship between plasma membrane free cholesterol and the response to LPS, and also indicate that the hyperresponsiveness of J774-GX cells is secondary to increased plasma membrane free cholesterol content.

**FIGURE 4.** GX sPLA₂ enhances macrophage free cholesterol and lipid raft content. A. Free cholesterol content of J774-C and J774-GX cells normalized to cell protein. B. Free cholesterol content of untreated J774 cells and cells treated with 0.1 μg/ml GX sPLA₂ for 20 h normalized to cell protein. C. J774-C and J774-GX cells were labeled with Alexa Fluor 488-CT-B to visualize lipid rafts. Original magnification ×100. D. J774-C and J774-GX cells were labeled with Alexa Fluor 488-CT-B and analyzed by flow cytometry to determine mean fluorescence intensity of cells. A, B, and D, Data (means ± SEM; n = 4) are representative of three independent experiments. *p < 0.05; ***p < 0.001.

**FIGURE 5.** GX sPLA₂-mediated hyperresponsiveness to LPS is reversed by plasma membrane cholesterol depletion and repletion. A. Free cholesterol content of J774-C and J774-GX cells normalized to cell protein. Cells were incubated in the presence or absence of 10 mM MβCD at 37°C for 30 min to deplete cholesterol. Subsets of cholesterol-depleted cells were then incubated for 60 min with 1 mM MβCD-loaded cholesterol to replete cholesterol before LPS treatments. B. TNF-α in conditioned media from cells depicted in A after treatment with 100 ng/ml LPS for 8 h. A and B, Data are means ± SEM (n ≥ 3). **p < 0.01; ***p < 0.001. The analysis of IL-6 secretion produced similar results.

GX KO mice exhibit reduced response to LPS

To determine whether GX sPLA₂ impacts inflammatory responses in vivo, we injected WT and GX KO mice with LPS (3 μg/kg body weight) or saline, and plasma cytokine levels were determined 3 h after treatment. Plasma levels of IL-6, IL-1β, and TNF-α were below the level of detection in both strains of mice after saline
GX sPLA₂ REGULATES MACROPHAGE INFLAMMATORY RESPONSES

Discussion

Macrophages play a key role in atherosclerosis by influencing the extent of lipid deposition and inflammation in the vessel wall (29). GX sPLA₂ is expressed by macrophages and is present in atherosclerotic lesions, where it has been implicated in proatherogenic and proinflammatory processes (19, 30, 31). We recently reported that GX sPLA₂ negatively regulates ABCA1 and ABCG1 expression, and hence reduces cellular cholesterol efflux in macrophages. These effects were dependent on GX sPLA₂ hydrolytic activity and the expression of LXRα/β (13). In this study, we extend these findings to show that GX sPLA₂ modulation of cholesterol homeostasis in macrophages is associated with an altered response to inflammatory stimuli. Using gain-of-function and loss-of-function approaches, we demonstrated that GX sPLA₂ enhances macrophage responses to LPS, a known ligand for TLR4. This hyperresponsiveness is associated with reduced ABCA1 and ABCG1 expression, reduced cellular cholesterol efflux, and increased plasma membrane free cholesterol and lipid rafts. The finding that GX KO mice exhibit a blunted response to LPS provides compelling evidence that GX sPLA₂ plays an important role in modulating inflammation in vivo.

Our conclusion that the enhancement of TLR4 signaling by GX sPLA₂ is secondary to alterations in plasma membrane cholesterol and lipid raft content is consistent with previous reports that cholesterol accumulation in macrophages is associated with a proinflammatory phenotype (4, 5, 24, 32). Li et al. (3) concluded that free cholesterol accumulation in macrophages leads to the induction and secretion of TNF-α and IL-6 by inducing an ER stress response, which activates several inflammatory pathways including IkB kinase/NF-κB and MAPK. More recent studies (4, 5) suggest that changes in plasma membrane free cholesterol/lipid raft content modulate macrophage inflammatory responses through an MyD88-dependent signaling pathway that is independent of an ER stress response. Lipid rafts also play an important role in trafficking and secretion of TNF-α (23). In this study, we show that increased expression of GX sPLA₂ leads to significantly increased cellular free cholesterol and lipid raft content, consistent with our previous finding that GX sPLA₂ deficiency is associated with reduced cholesterol content in MPMs (13). We further demonstrated that the hyperresponsiveness to LPS mediated by GX sPLA₂ is completely abrogated when cellular free cholesterol is normalized using cyclodextrin, a water-soluble compound capable of transferring cholesterol directly to and from the plasma membrane (28, 33). Thus, our findings are analogous to previous studies in ABCG1- and/or ABCA1-deficient macrophages, where augmented TLR4 signaling was related to increases in plasma membrane lipid rafts (4, 5). Although we did not directly test whether GX sPLA₂ leads to enhanced signaling through other TLRs, increased inflammatory gene responses to TLR2, TLR3, and TLR4 ligands have been demonstrated for bone marrow-derived macrophages deficient in both ABCA1 and ABCG1 (5).

According to our model (Fig. 7), GX sPLA₂ modulates macrophage free cholesterol content by suppressing the activation of LXRα/β and hence the expression of LXR target genes ABCA1 and ABCG1.
and ABCG1. Interestingly, in addition to being cholesterol sensors, LXRs are known to act as negative regulators of inflammatory signaling in macrophages through a mechanism that is independent of its ability to trans-activate target genes (34). On activation by some ligands, LXRs are capable of blunting macrophage responses to inflammatory stimuli through trans-repression of NF-κB (35, 36). Importantly, in our previous study using an NF-κB promoter luciferase construct, we ruled out the possibility that GX sPLA2 alters the trans-repressive effect of the LXR ligand T0901317 (13). Likewise, the ability of T0901317 to suppress LPS-mediated induction of IL-6 and TNF-α is similar in J774-C and J774-GX cells, indicating that GX sPLA2 does not modulate the trans-repressive effect of LXR activation on inflammatory cytokine expression (data not shown).

GX sPLA2 is speculated to exhibit properties independent of its catalytic function by binding to sPLA2 receptors including the M-type receptor (12, 37), although direct evidence is lacking. Granata et al. (7) reported that catalytically inactive GX sPLA2 was equally effective as WT GX sPLA2 in enhancing cytokine production in human lung macrophages. Our data that GX sPLA2, but not the catalytically inactive mutant H46Q, enhances LPS-induced cytokine production and that indoxam blocks the effect provides strong evidence that phospholipid hydrolysis is required. However, exogenous treatment of J774 cells with hydrolytic products of GX sPLA2, arachidonic acid or lysophosphatidylcholine, did not significantly increase cytokine production in response to LPS treatment. This does not rule out the possibility that arachidonic acid or lysophosphatidylcholine mediates GX sPLA2’s effect, however, because it is possible that supplementing arachidonic acid or lysophosphatidylcholine in the media complexed to BSA does not recapitulate what occurs when GX sPLA2 is continuously hydrolyzing cellular membranes. Real-time PCR analysis of J774 cells and MPMs indicated absence of M-type receptor expression (data not shown), further excluding a role for this sPLA2 receptor.

Studies in gene-targeted mice support the conclusion that GX sPLA2 promotes inflammatory processes in the setting of acute and chronic asthma (10) and in inflammatory lung disease (8). Most recently, Sato et al. (38) reported that pharmacological inhibition of sPLA2 significantly attenuates the acute lung inflammation and injury induced by LPS in C57BL/6J mice. The authors concluded that the protective effect was most likely due to inhibition of group V sPLA2 and GX sPLA2 activities. In a recently published study, we determined that GX sPLA2 deficiency significantly reduces abdominal aortic aneurysm formation in apolipoprotein E-deficient mice infused with angiotensin II (39). This protective effect was associated with a significantly blunted induction of inflammatory mediators in the aortas of GX sPLA2-deficient apolipoprotein E-deficient mice after angiotensin II infusion. Although GX sPLA2 is generally considered to induce proinflammatory responses in macrophages in vitro (7), one study concluded that overexpression of GX sPLA2 in RAW264.7 cells has an anti-inflammatory effect (8). Although GX sPLA2 enhanced LPS-stimulated IL-6 production in RAW264.7 cells in line with our study, TNF production was significantly suppressed (8). The basis for the discrepant effect of GX sPLA2 on TNF induction in the previously published study and this study is not clear, but it may be related to the different macrophage-like cell lines used. Our data indicate that GX sPLA2 is not transcriptionally regulated by LPS in macrophages (data not shown). Current evidence suggests that GX sPLA2 is regulated posttranslationally through proteolytic cleavage of an inactive proenzyme. Although the proteases(s) responsible for its proteolytic activation have yet to be identified, it is known that GX sPLA2 is expressed in an inactive form that requires removal of 11 aa residues at the N terminus for catalytic activity (40). Studies using transgenic mice indicate that GX sPLA2 enzymatic activity is under tight regulation and suggest that during inflammation the inactivezymogen is proteolytically activated (41). These data and our current study provide the interesting possibility that GX sPLA2 acts in a feed-forward loop to augment macrophage responses to inflammatory stimuli.

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Disclosures

The authors have no financial conflicts of interest.

References

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