BBA - Molecular and Cell Biology of Lipids 1863 (2018) 1458–1468

Contents lists available at ScienceDirect

BBA - Molecular and Cell Biology of Lipids

journal homepage: www.elsevier.com/locate/bbalip

Lysophosphatidylinositols in inflammation and macrophage activation: Altered levels and anti-inflammatory effects

Julien Masquelier[1], Mireille Alhouayek[1], Romano Terrasi, Pauline Bottemanne, Adrien Paquot, Giulio G. Muccioli*

Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Belgium

ARTICLE INFO

Keywords:
Lysophospholipids
Lipid mediator
Phosphatidylinositol
IBD

ABSTRACT

Lysophosphatidylinositols (LPI) are bioactive lipids that are implicated in several pathophysiological processes such as cell proliferation, migration and tumorigenesis and were shown to play a role in obesity and metabolic disorders. Often, these effects of LPI were due to activation of the G protein-coupled receptor GPR55. However, the role of LPI and GPR55 in inflammation and macrophage activation remains unclear. Therefore, we thought to study the effect of macrophage activation and inflammation on LPI levels and metabolism. To do so, we used J774 and BV2 cells in culture activated with lipopolysaccharides (LPS, 100 ng/mL) as well as primary mouse alveolar and peritoneal macrophages. We also quantified LPI levels in the cerebellum, lung, liver, spleen and colon of mice with a systemic inflammation induced by LPS (300 μg/kg) and in the colon of mice with acute colitis induced by dextran sulfate sodium (DSS) or trinitrobenzene sulfonic acid (TNBS) and chronic DSS-induced colitis.

Our data show that LPS-induced macrophage activation leads to altered LPI levels in both the cells and culture medium. We also show that cytosolic phospholipase A2α (cPLA2α) and α/β-hydrolase domain 6 (ABHD6) are among the enzymes implicated in LPI metabolism in J774 macrophages. Indeed, ABHD6 and cPLA2α inhibition increased 20:4-LPI levels in LPS-activated macrophages. Furthermore, incubation of LPS-activated cells with LPI decreased J774 activation in a GPR55-dependent manner. In vivo, LPI levels were altered by inflammation in the liver, spleen and colon. These alterations are tissue dependent and could highlight a potential role for LPI in inflammatory processes.

1. Introduction

Lysophospholipids are glycerophospholipids in which the glycerol moiety is substituted by a single acyl chain. They are constituted of a constant “polar” moiety linked to the glycerol backbone (defining the family) and, within each family, of a variable acyl chain, on the sn2 or sn1 position of the glycerol. These lipids, once considered as simple intermediates in the metabolism of phospholipids, are now recognized as bioactive lipids. The most studied lysophospholipid is certainly lysophosphatidic acid (LPA) which has been implicated in cell proliferation and survival, cancer, inflammation, pain, and metabolic syndrome [1,2]. Lysophosphatidylcholines (LPC), the most abundant lysophospholipids, seem to play a role both in the initiation and resolution of inflammation, possibly through a G protein-coupled receptor (GPCR), GPR132 [2,3]. Lysophosphatidylserine (LysoPS) production by activated and apoptotic neutrophils is a signal leading to their phagocytosis by macrophages, thus triggering resolution of inflammation [4]. However, much less is known on the properties of other lysophospholipids such as the lysophosphatidylinositols (LPI) which remain to date the less studied lysophospholipids. Early studies reported their effects on insulin secretion and on the cell cycle [5–8]. More recently, the GPCR GPR55 was put forth as an important molecular target mediating LPI's effects [9–11]. The molecular LPI species were shown to have different agonist potencies towards GPR55 [9]. Indeed, 2-arachidonoyl LPI is the most potent agonist of GPR55, while 1-palmitoyl LPI is a weak partial agonist. Linoleoyl, oleoyl and stearoyl

Abbreviations: LPS, lipopolysaccharides; LPI, lysophosphatidylinositols; LPE, lysophosphatidylethanolamines; LPG, lysophosphatidylglycerols; LysoPS, lysophosphatidylserines; LPC, lysophosphatidylcholines

* Corresponding author at: Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Av. E.Mounier, 72 (B1.72.01), 1200 Bruxelles, Belgium.

E-mail address: giulio.muccioli@uclouvain.be (G.G. Muccioli).

[1] Both authors share first authorship.

https://doi.org/10.1016/j.bbalip.2018.09.003
Received 26 April 2018; Received in revised form 26 August 2018; Accepted 9 September 2018
Available online 11 September 2018
1388-1981/

LPI were less potent agonists than arachidonoyl LPI [9]. Since then, LPI and GPR55 have been associated with several pathophysiological processes [12]. For instance LPI and GPR55 have been implicated in cell proliferation, migration and tumorigenesis [13]. Moreover, the LPI-GPR55 axis clearly plays a role in obesity and metabolic disorders [14]. However, the role of LPI and GPR55 in inflammation and immune cell activation remains unclear.

Complicating the study of the effects of LPI is the intricacy of their metabolic pathway. Phosphatidylinositols (PI) are the key precursors of LPI. Upon hydrolysis by a phospholipase A1 (PLA1) or a phospholipase A2 (PLA2), PI will generate 2-acyl LPI and 1-acyl LPI, respectively (Suppl. Fig. S1). Among the PLA1 enzymes, DDHD1 (also known as PA-PLA1) was described for its ability to produce 2-arachidonoyl LPI in vitro and in vivo [15]. PLA2 enzymes are expected to generate LPI, however PLA2 isoforms have been mostly studied in regards to arachidonic acid and eicosanoid synthesis. Among these, cPLA2α, which has a preference for releasing arachidonic acid from the sn-2 position of phospholipids, is implicated in the biosynthesis of LPI [16–18]. However, while activation of cPLA2α and the release of arachidonic acid from membrane phospholipids constitute key features of inflammation, little is known on the effect of inflammation on the cell and tissue levels of LPI.

Following their synthesis, LPI can be reacylated to PI by acyltransferases [19,20] or deacylated by lysophospholipases. LPI-specific PLA and PLC activities have been reported although the molecular characterization of these specific lysoPLA and lysoPLC enzymes is still lacking [19]. Of note, cPLA2α has been reported as capable of deacylating LPI into glycerophosphoinositol [21], therefore it could be implicated both in the synthesis and catabolism of LPI. More recently, α/β hydrolase domain 6 (ABHD6), a monoacylglycerol hydrolase, was also implicated in lysophospholipid hydrolysis, although this was not directly shown for LPI [22]. Finally, autotaxin, a lysoPLD responsible for LPA biosynthesis from LPC, is also able to convert LPI to LPA although less efficiently than LPC [23].

Considering (i) the complexity of the metabolic pathways of LPI, (ii) the fact that several enzymatic activities of the pathway have not been characterized and (iii) that inflammation could affect several enzymes of these pathways, we decided to study the interplay between LPI and inflammation. Thus, we used an in vitro approach to study the consequences of macrophage activation on LPI and other lysophospholipid levels. We then show, in vivo, that inflammation alters the levels of LPI in numerous tissues. As LPI levels are altered upon inflammation and LPS-induced macrophage activation, we also wondered whether LPI could modulate J774 cell activation. Thus, this report points to LPI as interesting bioactive lipids in the context of inflammation.

2. Materials and methods

2.1. Materials

Bovine liver LPI (major specie 18:0 LPI), bovine brain LysoPS (major specie 18:0 LysoPS), egg LPE (major specie 18:0 LPE), egg LPC (major species 16:0 and 18:0 LPC), 18:0 LPG and 18:0 LPA as well as internal standards were purchased from Avanti polar lipids. WWL70 was purchased from Cayman Chemicals. HEPES, sucrose, EGTA, glycerol, $CaCl_2$, PI from bovine liver, dipalmitoylglycerol (DAG), arachidonoyltrifluoromethylketone (ATFMK), bromoenol lactone (E-6-(Bromoethylene) tetrahydro-3-(1-naphthyl)-2H-pyran-2-one, BEL) and CID16020046 (4-[4,6-Dihydro-4-(3-hydroxyphenyl)-3-(4-methylphenyl)-6-oxopyrrolo [3,4-*c*]pyrazol-5(1H)-yl]-benzoic acid) were all bought from Sigma Aldrich. Lipopolysaccharides (LPS) from *E. coli* (O55:B5) and fetal bovine serum were also purchased from Sigma Aldrich. Dextran sulfate sodium (DSS) was purchased from TdB Consultancy. pDream2.1 vector containing the human GPR55 coding sequence was obtained from GenScript corporation. Lipofectamine LTX plus was purchased from Life Technologies. Cell culture media, trypsin and penicillin-streptomycin were purchased from Gibco.

2.2. Animal models

C57BL/6 J mice (8–10 weeks of age) were purchased from Charles River Laboratories (Brussels, Belgium) and were housed under standard conditions (12 hours light/dark cycle, and controlled 22 °C temperature) and supplied with water and food ad libitum. All animal care and experimental procedures were conducted in accordance with the guidelines of the local ethics committee and in accordance with European directive 2010/63/EU, which was transformed into the Belgian Law of May 29, 2013 regarding the protection of laboratory animals. All experimental protocols were approved by the Université catholique de Louvain animal ethics committee (study agreement 2014/UCL/MD/001, laboratory agreement LA1230314).

For LPS-induced inflammation in mice, LPS (300 μg/kg, in saline containing 0.1% Tween 80) or vehicle were administered i.p and mice (7 mice/group) were sacrificed by cervical dislocation 4 or 8 h later [24,25].

Three models of colitis were used.

TNBS-induced colitis: Mice (10 mice/group) were food-deprived for 24 h before administration of trinitrobenzene sulfonic acid (TNBS). Mice were anesthetized by intraperitoneal (i.p.) injection of a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). TNBS (100 mg/kg, in 50 μL 0.9% NaCl-ethanol, 50:50, v/v) was administered intrarectally into the colon (4 cm from the anus) using a cannula. Control mice received 50 μL of a 0.9% NaCl-ethanol (50:50, v/v) solution. To ensure a homogeneous distribution and retention of TNBS (or vehicle) within the colon, mice were held by the tail, in a vertical position, for 60 s after administration [26].

DSS-induced colitis:

Acute colitis: Colitis was induced by adding dextran sulfate sodium (DSS, 5%) to the drinking water of mice for 5 days. Mice (8 per group) were sacrificed once the colitis was well established i.e. at day 7 [26,27].

Chronic colitis: Chronic colitis was induced by following 3 cycles consisting in 7 days of DSS (2.5%) in the drinking water followed by 3 weeks of normal drinking water [28]. Mice (8 per group) were sacrificed during the recovery phase after the third cycle of DSS.

For all models, body weight and food and water intake were monitored daily for the duration of the study. Following sacrifice of the mice, colons were quickly recovered and snap-frozen in liquid nitrogen before storage at −80 °C until their analysis.

2.3. Cell culture

The murine macrophage cell line J774 (a generous gift from M.-P. Mingeot-Leclercq, LDRI, UCL, Belgium and from J. Leclercq, LDRI, UCL, Belgium) was cultured under standard conditions (37 °C in a humidified 5% CO2 incubator) in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and antibiotics. BV2 microglial-like cells (a generous gift from E. Hermans, IONS, UCL, Belgium) were grown in high-glucose DMEM medium with 10% FBS and antibiotics.

Cells were seeded overnight into 24-well plates (2.5×10^5 cells per well) for qRT-PCR experiments, and into 35 mm dishes (10×10^6 cells per dish) for lipid quantification, and the experiments performed the next morning. Cells were then incubated with fresh culture medium containing 100 ng/mL of LPS for 8 h (based on previous experiments [24,28]). For all experiments, a control condition was performed, where cells were seeded concomitantly but were only incubated with vehicle (DMSO, 0.05%) in the absence of LPS. When the cells were treated, the compounds of interest were added 1 h prior to LPS.

2.4. Primary macrophage isolation

2.4.1. Murine peritoneal macrophages were obtained by eliciting an acute peripheral inflammatory reaction with an i.p. injection of thioglycolate as previously described [24]. Briefly, adult NMRI female mice were given an i.p. injection of an aged 4% thioglycolate solution. Three days later, peritoneal cells were recovered in 5 mL PBS, pelleted and resuspended in culture medium (RPMI supplemented with 10% FBS and antibiotics).

2.4.2. Murine alveolar macrophages were recovered from bronchoalveolar lavage as described by Zhang et al. with slight modifications [29,30]. Briefly, 7 to 8 weeks old mice were anesthetized and then sacrificed by cervical dislocation. After a surgical incision, the trachea was exposed and a cannula fixed in it. Five consecutive lung lavages were performed through this cannula with 1 ml phosphate buffered saline (PBS) solution containing 0.5 mM of EDTA. Alveolar cells recovered in PBS were pelleted by centrifugation at 250 ×*g* for 5 min at 4 °C and resuspended in culture medium (RPMI supplemented with 10% FBS and antibiotics).

For both primary peritoneal and alveolar macrophages, 3 h after the initial plating, cells were washed with PBS to remove non-adherent cells, growth medium was replenished and cells (0.5×10^6 cells/well) were incubated overnight. The next day, cells were incubated with fresh culture medium containing 100 ng/mL of LPS for 8 h before lipid extraction and quantification.

2.5. Real-time quantitative PCR

For real-time quantitative PCR (RT-qPCR) experiments on cells, media was removed and TriPure reagent (Roche) was added to each well of the tissue culture plates, the plates were then stored at −80 °C for later assessment. For in vivo experiments, tissues were snap frozen in liquid nitrogen at the time of sacrifice and stored at −80 °C for later assessment. TriPure reagent was added on the day RNA extraction was performed.

In both cases, the same procedure was performed. Total RNA was extracted using the TriPure reagent according to the manufacturer's instructions. cDNA was synthesized using a reverse transcription kit (Promega corporation) from 1 μg of total RNA. qPCR was performed with a STEP one PLUS instrument and software (Applied Biosystems) as previously described [31]. Products were analyzed by performing a melting curve at the end of the PCR reaction. Data are normalized to the 60S ribosomal protein L19 (RPL19) mRNA expression. Primer sequences are given in Table 1.

2.6. Transfection of J774 cells with hGPR55

J774 cells were seeded overnight in 24-well plates (1.5×10^5 cells per well) and stable transfection performed the following day using lipofectamine LTX plus and 0.5 μg of pDream2.1 plasmid containing the coding sequence for human GPR55. Transfected cells were selected by addition of G418 to the culture medium, then a clonal selection was performed by serial dilution of the initial cell population.

2.7. Lysophospholipid quantification

Cell were recovered by scraping using 7 mL of PBS, and transferred to a vial containing the internal standards (17:1 LPI; 17:1 LysoPS; 17:0 LPC; 17:0 LPE; 17:1 LPG; 17:0 FA), chloroform (14 ml) and methanol (7 mL). The mixture was acidified using HCl 2 N (300 μL). Lipids in the media (7 mL) were extracted using a similar procedure but with 14 mL of chloroform and 7 mL of methanol. Following vigorous mixing and sonication, the samples were centrifuged and the organic layer was recovered and then dried under a stream of N_2. The lipid fraction was pre-purified by solid-phase extraction (SPE) over silica using methanol as the eluting solvent [32]. The resulting lipid fractions were analyzed by HPLC-MS using a LTQ Orbitrap mass spectrometer (ThermoFisher Scientific) coupled to an Accela HPLC system (ThermoFisher Scientific) equipped with an electrospray ionization (ESI) source and operated using the Xcalibur software (Thermo Fisher Scientific). Chromatographic separations were performed using a C18 column (Kinetex, 150 mm × 4,6 mm, 5 μm, from Phenomenex). The gradient elution (400 μL/min) consisted of solvent A (methanol-water-ammonium hydroxide (50:50:0,1 v/v/v)) and solvent B (methanol-ammonium hydroxide (100:0,1 v/v)), starting at 100% A and reaching linearly 100% B in 30 min, this was followed by 15 min at 100% B before reequilibrating at 100% A [32]. The ESI spray voltage was set at 5000 V and the capillary temperature at 270 °C. The sheath gas flow was at 40 arbitrary units. In the orbitrap, lysophospholipids (except LPC) and PI were detected as $[M\text{-}H]^-$ ions whereas LPC were detected as acetate adducts. The area under the curve (AUC) for each lipid of interest was normalized using the AUC obtained for the relevant internal standard. Absolute quantification of LPI species was obtained using a validated HPLC-MS method previously reported [33].

2.8. cPLA2 activity

The procedure was based on a protocol described by Leslie and Gelb [34]. Briefly, cells were homogenized in an HEPES buffer (10 mM, pH 7.4) containing sucrose (0.34 M), glycerol (10%, v/v), EGTA (1 mM) and a phosphatase inhibitors cocktail (524629, Sigma) before ultracentrifugation (100,000 ×*g*, 60 min, 4 °C). Dithiothreitol (1%, v/v) and a protease inhibitors cocktail (P8340, Sigma) were then added to the resulting cytosolic fraction. The substrate preparation was obtained by vigorous mixing, followed by ultrasound application, of a 100 μL suspension containing 75 nmol of PI and 22.5 nmol of DAG in HEPES buffer (50 mM, pH 7.4). cPLA2 activity was assessed by incubating

Table 1
Primer sequences.

Product	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
RPL19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT
IL-1β	TCGCTCAGGGTCACAAGAAA	CATCAGAGGCAAGGAGGAAAAC
COX-2	TGACCCCCAAGGCTCAAATAT	TGAACCCAGGTCCTCGCTTA
TNFα	AGCCCCCAGTCTGTATCCTT	GGTCACTGTCCCAGCATCTT
IL-6	ACAAGTCGGAGGCTTAATTACACAT	TTGCCATTGCACAACTCTTTTC
MCP-1	GCAGTTAACGCCCCACTCA	CCCAGCCTACTCATTGGGATCA
ABHD6	CTGTCCATAGTGGGGCAAGT	TCAGATGGGTAGTAAGCGGC
Autotaxin	AGCACCTGACCAACTGTGT	TGCATCATATTTCGCTTCTGGG
PA-PLA1	TAACATTGGCAAAGCAAGCA	TGATGCTGAAGAACGTCCAA
cPLA2α	GTGAGGGGCTTTATTCCACA	ACAGTGCCTTCATCACACCA
mGPR55[a]	CATCATGGGCTTCTGTTCCT	TCCAGATGCAGGCTCTCTTT
hGPR55[b]	TCTACATGATCAACCTGGCAGTCT	CTGGGACAGGACCATCTTGAA

[a] Murine GPR55.
[b] Human GPR55.

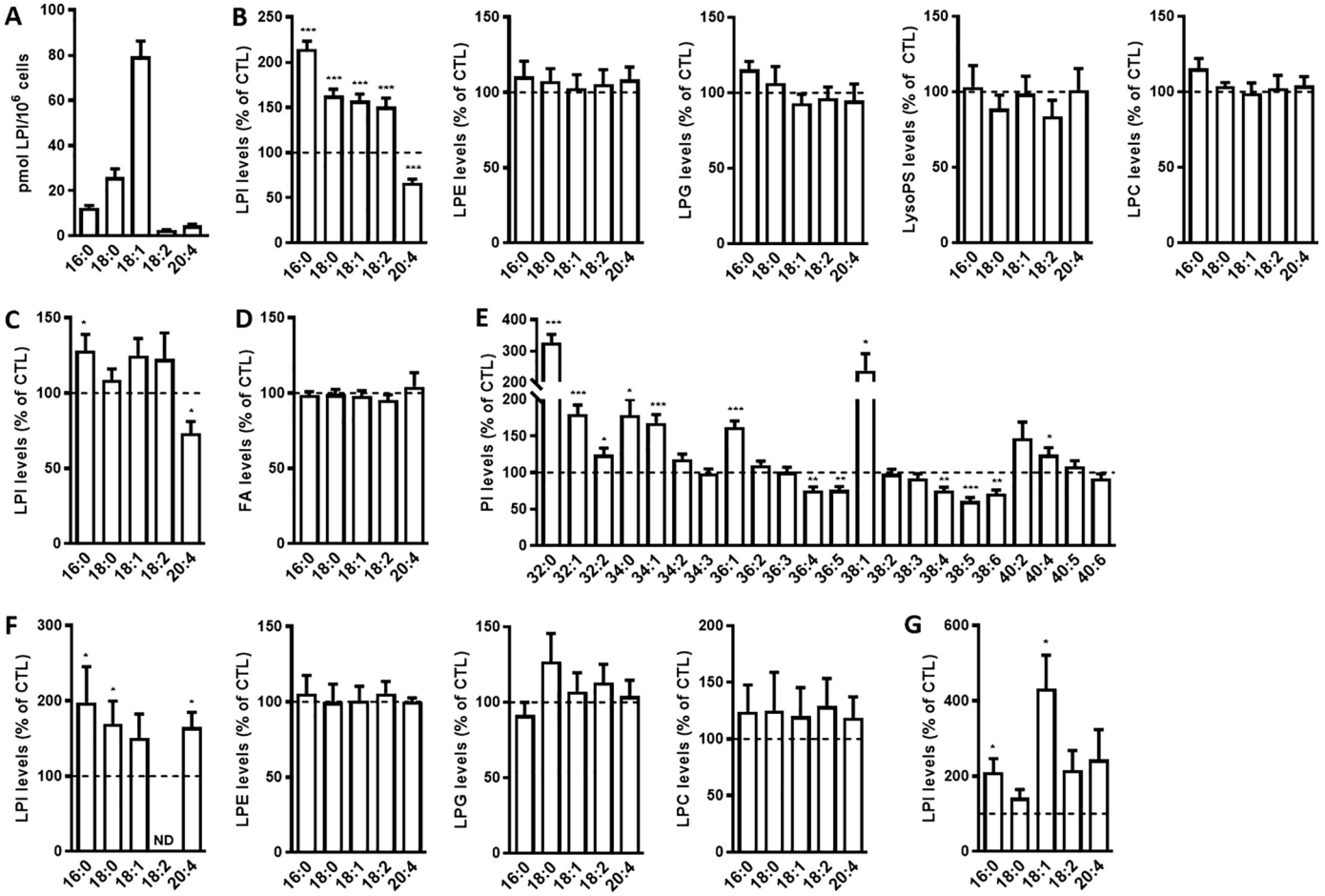

Fig. 1. Effect of 8 h activation of J774 macrophages on lysophospholipid, phosphatidylinositol and fatty acid levels.
(A) Lysophosphatidylinositol (LPI) levels measured by HPLC-MS in J774 control cells in pmol/10^6 cells. (B, C) Lysophospholipid levels in (B) the cells and (C) the culture medium were measured by HPLC-MS following activation of J774 cells with 100 ng/mL of lipopolysaccharides (LPS) for 8 h. (D) Fatty acid (FA) and (E) phosphatidylinositol (PI) levels in the cells were measured by HPLC-MS following activation of J774 cells with 100 ng/mL of LPS for 8 h. (F) Lysophospholipid levels were measured by HPLC-MS following activation of primary alveolar macrophages with 100 ng/mL of LPS. LysoPS were below detection limit in alveolar macrophages. (G) LPI levels were measured by HPLC-MS in primary peritoneal macrophages activated with 100 ng/mL of LPS for 8 h. The acyl chain and the degree of unsaturation of the individual species are shown on the x axis. Data are expressed for each individual species in percentage of the control condition (cells not incubated with LPS, dotted line). Values are mean ± SEM from three separate experiments (measurements in quadruplicates). $^*P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ for LPS activation vs. control cells.
LPE: lysophosphatidylethanolamine, LPG: lysophosphatidylglycerol, LysoPS: lysophosphatidylserine, LPC: lysophosphatidylcholine.

(20 min, 37 °C, final volume 50 µL) the cytosolic fraction (40 µg of proteins) with the substrate preparation (7.5 nmol of PI and 2.25 nmol of DAG) in HEPES buffer containing BSA (50 µg), NaCl (7.5 µmol) and $CaCl_2$ (0.25 µmol). At the end of the incubation, tubes were placed on ice and chloroform (2 mL), methanol (1 mL) and 0.5 mL of water (pH 3) were subsequently added. Following mixing and centrifugation, the organic layer containing the products of the reaction was recovered and dried under a nitrogen stream. The resulting residue was dissolved in methanol and analyzed by HPLC-MS as described above. Calcium content, protein amount and incubation time were optimized before assessing the effect of LPS incubation on cPLA2 activity (data not shown).

2.9. Statistical analysis

Results are expressed as mean ± SEM. Significance between two distinct groups was determined by unpaired Student *t*-test and comparisons between various groups were assessed by one-way anova followed by a Dunnett post-test for comparisons against a "control" condition or by a Bonferroni post-test for comparisons between different conditions. Data were analyzed using GraphPad Prism 7.0 for Windows (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Macrophage activation for 8 h alters LPI and PI levels

To study the effect of macrophage activation on LPI levels, we used the J774 murine macrophage cell line. We started by quantifying the major LPI species in these cells and found 18:1 LPI to be the most abundant LPI in these cells (Fig. 1A). Upon incubation with lipopolysaccharides (LPS, 100 ng/mL) for 8 h, J774 cells are activated and express pro-inflammatory markers such as interleukin (IL)-6, IL-1β and tumor necrosis factor α (Suppl. Fig. S2A) [24,30]. We used this model to investigate the effect of LPS-induced J774 macrophage activation on lysophospholipid levels. Among the lysophospholipids investigated here, we found that the levels of LPI were significantly altered, while LPE, LPG, LysoPS and LPC levels were not affected (Fig. 1B). Interestingly, J774 cell activation has a different effect depending on the LPI species, as 20:4 LPI levels were decreased whereas 16:0, 18:0, 18:1 and

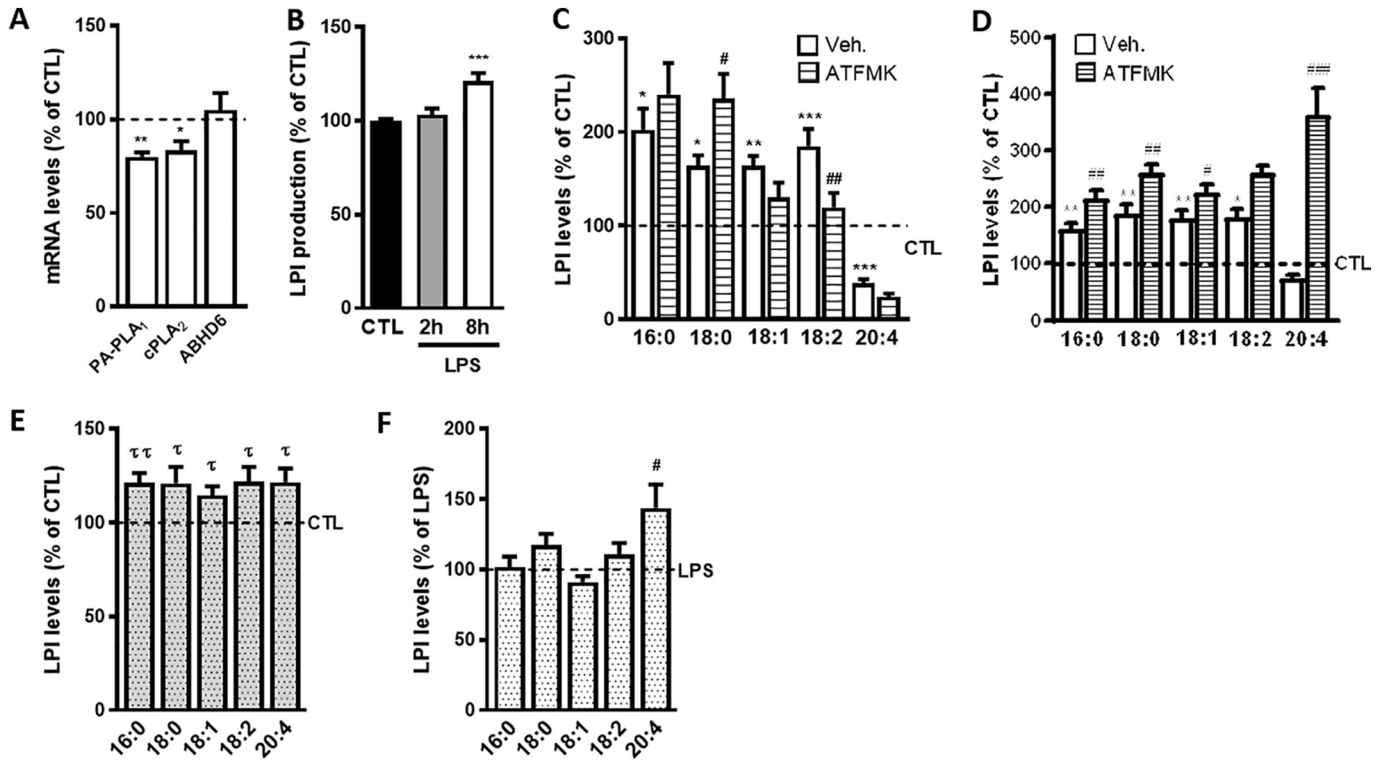

Fig. 2. Effect of lipopolysaccharide-induced activation of J774 macrophages on the metabolic pathways of lysophosphatidylinositols.
(A) mRNA expression of PA-PLA1, cPLA2α and ABHD6 was measured by RT-qPCR in J774 cells activated by incubation with 100 ng/mL of lipopolysaccharides (LPS) for 8 h. RPL19 was used as reference gene. (B) cPLA2 activity was measured in J774 cells incubated with 100 ng/mL of LPS for 2 h or 8 h. (C, D) Effect of cPLA2 inhibition with ATFMK (10 μM) on LPI levels in the (C) cells and (D) media of J774 cells activated with LPS (100 ng/mL). (E, F) Effect of ABHD6 inhibition with WWL70 (10 μM) on LPI levels in (E) control J774 cells and (F) J774 cells activated with LPS (100 ng/mL). (A–E) Data are expressed in percentage of the control condition. (F) Data are expressed in percentage of the LPS condition. Values are mean ± SEM from three separate experiments (measurements in quadruplicates). $^{*}P < 0.05$, $^{**}P < 0.01$ for LPS activation vs. control cells. $\tau P < 0.05$, $\tau\tau P < 0.01$ for ABHD6 inhibition vs. control cells. $\#P < 0.05$ for ABHD6 inhibition vs LPS-activated cells.

18:2 LPI levels were increased. 20:4 LPI levels could be decreased due to secretion of LPI into the culture medium, therefore we also measured LPI levels in the medium of J774 cells and found decreased levels of 20:4 LPI, while 16:0 LPI was increased, similarly to what we observed in the cells (Fig. 1C). Because LPI levels were altered upon TLR4 activation, we also assessed the effect of J774 cells activation on fatty acid and PI levels. While fatty acid levels were not affected by LPS-induced activation (Fig. 1D), PI levels were altered. Interestingly, several PI species that could lead to the production of 20:4 LPI, including 38:4 PI which is the most abundant PI, were decreased by macrophage activation (e.g. 36:4 PI, 36:5 PI, 38:5 PI, 38:6 PI) while other PI species were increased (Fig. 1E).

To see whether the observed alteration of LPI levels was cell-line-dependent, we used, in addition to J774 cells, the microglial BV2 cell line. Similarly to J774, LPS incubation induces a strong increase in IL-1β and IL-6 mRNA expression in BV2 cells (Suppl. Fig. S2B) as well as changes in LPI levels similar to those observed in J774 cells, with a decrease in 20:4 LPI and increased levels of 16:0, 18:0 and 18:1 LPI (Suppl. Fig. S2D).

Both BV2 cells and J774 cells are immortalized cell lines in culture. To determine whether alteration of LPI levels could be considered as a common trait of macrophage activation, we also used primary murine peritoneal and alveolar macrophages. LPS-induced activation of these primary macrophages led to a general increase in LPI levels (Fig. 1F–G). In the alveolar macrophages, 16:0, 18:0 and 20:4 LPI levels were increased while the levels of the other lysophospholipids were not altered (Fig. 1F). In primary peritoneal macrophages, the levels of 16:0 and 18:1 LPI were significantly increased (Fig. 1G).

3.2. Macrophage activation alters LPI metabolism

In order to generate hypotheses on the mechanisms responsible for the changes in LPI levels, we determined the effect of LPS activation on mRNA expression of the enzymes involved in LPI metabolism. These enzymes include cPLA2α, PA-PLA1, ABHD6 and Autotaxin [15,16,22]. Activation of J774 cells with LPS results in decreased cPLA2α and PA-PLA1 mRNA expression, with no variation in the expression of ABHD6 while Autotaxin was not expressed by J774 cells (Fig. 2A). Conversely, in BV2 cells, LPS incubation increased the expression of the two phospholipases as well as the expression of Autotaxin, which was not expressed in the control condition (Suppl. Fig. S2E).

In macrophages, changes in cPLA2α activity are generally the consequence of its Ca^{2+}-dependent recruitment to membranes and post-translational modifications such as phosphorylation [18]. Therefore, we developed a method to assess cPLA2α activity in cells by measuring the production of LPI from PI. Following incubation of cytosolic fractions of J774 cells with the PI substrate for 20 min, we extracted the lipid fractions and quantified LPI levels by HPLC-MS. By doing so, we measured an increase in the production of 16:0 LPI, 18:0 LPI and 18:1 LPI, but not 20:4 LPI (Suppl. Fig. 3) which suggests a cPLA2α activity. The measured PLA2 activity was sensitive to inhibition by arachidonoyltrifluoromethylketone (ATFMK, 10 μM), which inhibits both cPLA2α and iPLA2, but not to inhibition by the iPLA2 inhibitor bromoenolactone (BEL, 600 nM) (Suppl. Fig. 3). Having characterized this enzymatic activity, we performed similar experiments on homogenates from control J774 cells and J774 cells activated with 100 ng/mL of LPS for 2 h or 8 h and found LPI production to be

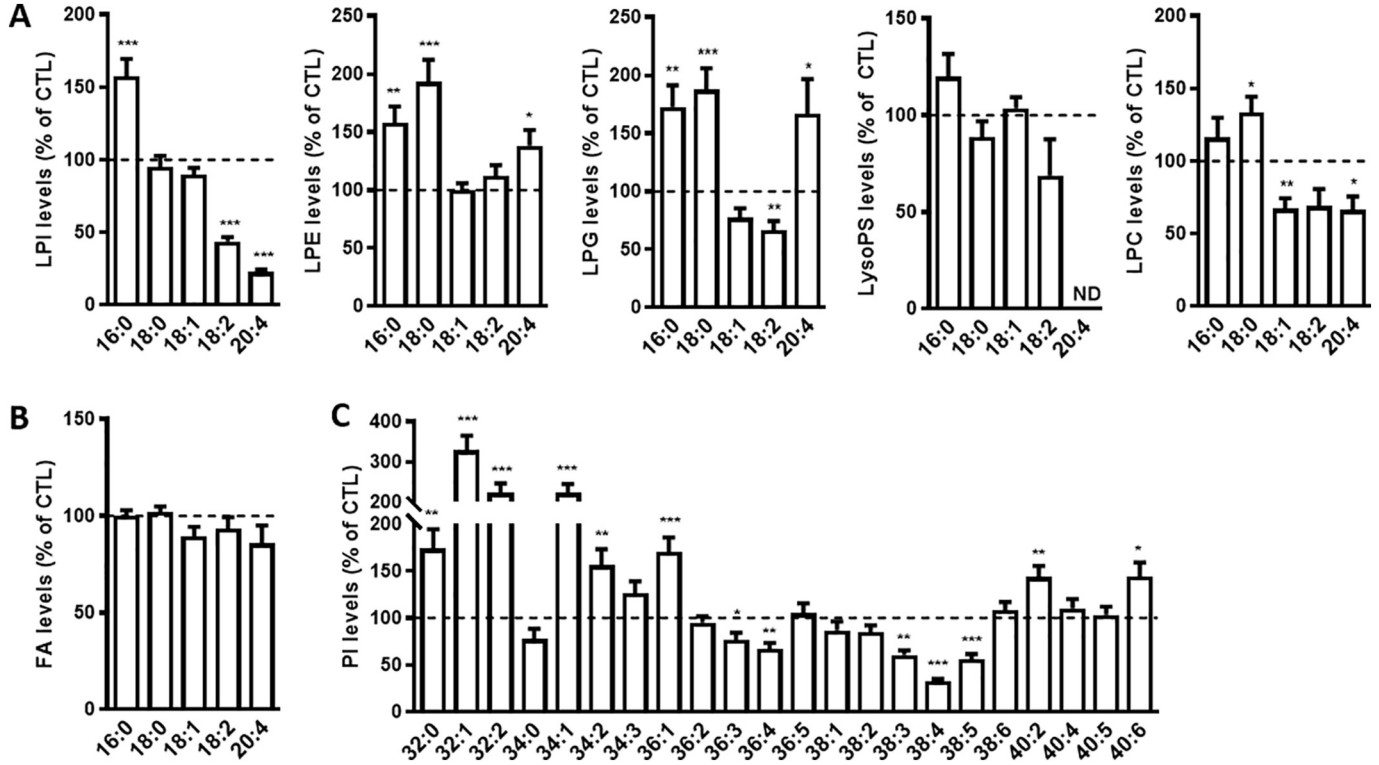

Fig. 3. Effect of 24 h activation of J774 macrophages on lysophospholipid, phosphatidylinositol and fatty acid levels.
(A) Lysophospholipid, (B) fatty acid (FA) and (C) phosphatidylinositol (PI) levels in the cells were measured by HPLC-MS following activation of J774 cells with 100 ng/mL of lipopolysaccharides (LPS) for 24 h. The acyl chain and the degree of unsaturation of the individual species are shown on the x axis. Data are expressed for each individual species in percentage of the control condition (cells not incubated with LPS, dotted line). Values are mean ± SEM from three separate experiments (measurements in quadruplicates). $*P < 0.05$ $**P < 0.01$ $***P < 0.001$ for LPS activation vs. control cells.
LPE: lysophosphatidylethanolamine, LPG: lysophosphatidylglycerol, LysoPS: lysophosphatidylserine, LPC: lysophosphatidylcholine.

increased only following 8 h of LPS (Fig. 2B). The same was observed in BV2 cells activated with 100 ng/mL of LPS for 8 h (Suppl. Fig. S2F). In order to ascertain further the involvement of cPLA2α in the alteration of LPI levels, we incubated LPS-activated J774 cells with ATFMK for 8 h and quantified LPI levels in the cells and media. In both the cells and the media, we found again that LPS increased LPI levels, except for 20:4 LPI that is decreased (Fig. 2C). Interestingly we found different effects of ATFMK on LPI levels in the cells and the media. Indeed, in the cells, ATFMK did not affect the LPS-induced alteration in LPI levels, except in the case of 18:2 LPI that was decreased and 18:0 LPI that was further increased with ATFMK incubation (Fig. 2C). Conversely, in the media, ATFMK further increased the levels of 16:0, 18:0 and 18:1 LPI (Fig. 2D). The biggest change however was in LPI 20:4 levels which were decreased by LPS and increased > 5 fold by ATFMK (Fig. 2D).

As mentioned, ABHD6 has been put forth as a lysophospholipase [22], therefore we wanted to study whether it was implicated in LPI metabolism in J774 cells both in control and activated conditions. Interestingly, the effect of ABHD6 inhibition on LPI levels was different between control cells and cells stimulated with LPS. Indeed, in control cells, incubation with the ABHD6 inhibitor WWL-70 (10 μM) for 6 h [35] led to a small, albeit significant, increase of the levels of all the LPI measured (Fig. 2E). However, in cells stimulated with LPS, WWL-70 only increased the levels of 20:4 LPI (Fig. 2F). This could implicate ABHD6 in the decreased levels of 20:4 LPI in activated J774 macrophages.

3.3. Macrophage activation for 24 h alters lysophospholipid and PI levels

LPI levels were altered following activation of macrophages for 8 h with LPS. However, the levels of the other lysophospholipids measured were not altered. We wondered whether longer activation times would have different effects on lysophospholipid levels and whether the alteration of LPI levels would be sustained. Therefore, to see the impact of a longer macrophage activation time, we incubated J774 macrophages with 100 ng/mL of LPS for 24 h and measured lysophospholipid, fatty acid and PI levels. At this time point, pro-inflammatory cytokine expression is decreased compared to the earlier time points (Suppl. Fig. S2A). Here again, we observed variations in LPI levels with 16:0 LPI still increased at 24 h and 20:4 LPI further decreased by macrophage activation (Fig. 3A). The other LPI that were increased at 8 h were either unchanged or even decreased at 24 h of activation (Fig. 3A). LPE and LPG levels that were not affected by macrophage activation at 8 h were altered at 24 h. Indeed LPE and LPG with 16:0, 18:0 and 20:4 acyl chains were increased (Fig. 3A). Concerning LPC, we observed an increase in the levels of 18:0 LPC and a decrease in 18:1 and 20:4 LPC (Fig. 3A). On the other hand, LysoPS (Fig. 3A) and fatty acid (Fig. 3B) levels were still not significantly altered at 24 h. Finally, we measured PI levels and found that most PI that were altered at 8 h were still altered at 24 h (Fig. 3C).

3.4. LPI decrease LPS-induced macrophage activation

Bioactive lipids could serve as autocrine signaling molecules regulating macrophage activation [24,36,37]. It is thus possible for the different lysophospholipids studied here to affect LPS-induced activation of J774 cells. Therefore, we incubated the cells with 10 μM of the various lysophospholipids and activated them with 100 ng/mL of LPS for 8 h. LPI consistently decreased several parameters of macrophage activation, such as LPS-induced mRNA expression of IL-1β, IL-6 and COX-2 (Fig. 4A). LPC decreased IL-1β and IL-6 expression, while LysoPS

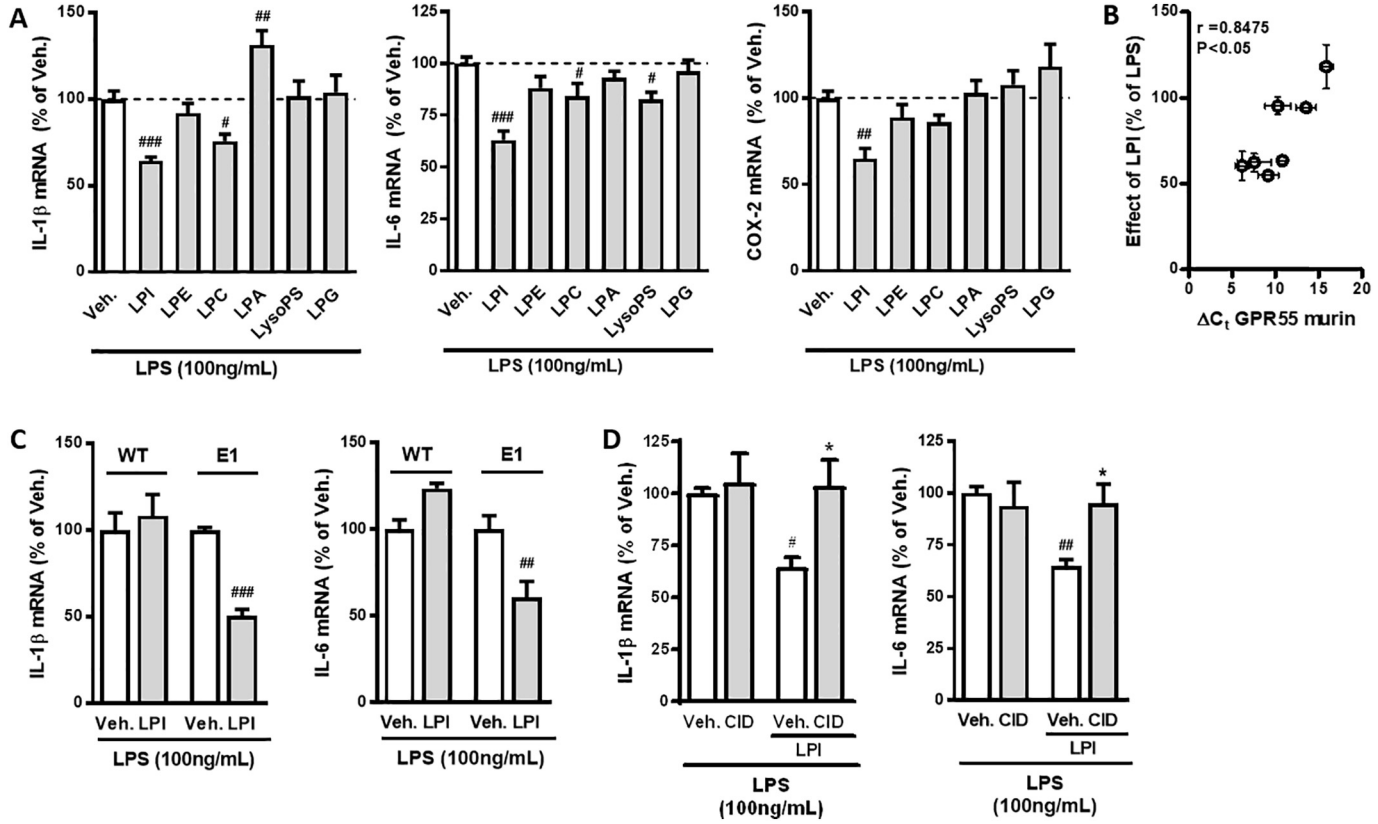

Fig. 4. LPI decrease lipopolysaccharides-induced activation of J774 cells.
J774 cells were activated with 100 ng/mL of lipopolysaccharides (LPS) for 8 h. Lysophospholipids were added at 10 μM 1 h prior to incubation of cells with LPS. mRNA expression of pro-inflammatory markers (IL-1β, IL-6, COX-2) was measured by RT-qPCR with RPL19 as reference gene. Data are expressed in percentage of the LPS-induced mRNA expression of each marker (Veh.). (A) Effect of lysophospholipids on LPS-induced mRNA expression of pro-inflammatory markers. (B) Correlation between the endogenous expression of GPR55 (ΔCt) in different clones of J774 cells and the effect of LPI on LPS-induced IL-1β expression. (C) Effect of LPI on LPS-induced IL-1β and IL-6 mRNA expression in wild-type J774 cells with low GPR55 expression (WT) and the same clone transfected with human GPR55 (E1). (D) Effect of LPI and the GPR55 antagonist CID16020046 (CID, 10 μM) on LPS-induced IL-1β and IL-6 mRNA expression in the E1 clone transfected with GPR55. Values are mean ± SEM from three separate experiments (measurements in triplicates) except for B (seven different experiments in triplicates). For A and C: $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ for lysophospholipid treatment vs. Veh. LPI: lysophosphatidylinositol, LPE: lysophosphatidylethanolamine, LPG: lysophosphatidylglycerol, LysoPS: lysophosphatidylserine, LPC: lysophosphatidylcholine.

only decreased IL-6 expression (Fig. 4A). LPE and LPG had no effect on LPS-induced activation of J774 cells (Fig. 4A). Of note, we have previously shown in these cells that mRNA expression of IL-6 and COX-2 are correlated with the production of IL-6 and prostaglandins, respectively [30].

3.5. LPI actions on LPS-induced macrophage activation are mediated by GPR55

LPI bind and activate GPR55 receptors. However, although some reports show that GPR55 is involved in the effects of LPI on cancer cell proliferation or osteoclast formation, little is known on the involvement of GPR55 in the effects of LPI on macrophage activation [12]. Thus we asked whether the effect of LPI on LPS-induced J774 activation was mediated by this receptor. Interestingly, we serendipitously noted that the magnitude of effect of LPI on J774 activation was positively correlated with the expression of GPR55 mRNA (Fig. 4B). Indeed, we had J774 cells from different origins and we noticed that in the cells where the ΔCt of GPR55 (threshold cycle of GPR55 compared to that of the reference gene RPL19) was higher, i.e. where GPR55 expression was lower, LPI had less effect on LPS-induced expression of IL-1β (Fig. 4B). To confirm this observation and clearly establish the role of GPR55, we selected J774 cells showing low GPR55 expression and little effect of LPI in reducing LPS-induced macrophage activation (Fig. 4C, WT columns). Using these cells we then generated, by stable transfection, clones of J774 cells expressing human GPR55 (hGPR55) and tested the effect of LPI on one of those clones (clone E1). hGPR55 mRNA was well expressed in transfected J774 cells and at a more constant level compared to murine GPR55 (Suppl Fig. S4). As illustrated in Fig. 4C, LPI reduce LPS-induced IL-1β and IL-6 mRNA expression in J774-hGPR55 clone E1 but not in J774 WT cells. To ascertain further the involvement of GPR55 in the effects of LPI, we co-incubated the hGPR55 clone E1 with LPI and the GPR55 antagonist CID16020046 (CID). CID blocked the effects of LPI on IL-6 and IL-1β mRNA expression (Fig. 4D).

3.6. LPI tissue levels are altered by inflammation

In order to study further the metabolism and levels of LPI in inflammatory settings, we thought to quantify LPI levels in vivo during inflammation. Thus, we used a model of LPS-induced inflammation in mice obtained by i.p. injection of a low dose of LPS (300 μg/kg) for 4 or 8 h [24,25]. This dose of LPS induces a time-dependent increase in the expression of inflammatory markers in many tissues. Indeed, IL-1β and monocyte chemoattractant protein-1 (MCP-1) mRNA expression were increased in the colon, liver, spleen, lung and cerebellum at 4 h and 8 h post-LPS injection (Figs. 5–7). Of note, their expression was lower in the 8 h group compared to the 4 h group, although still higher than the control group, in line with a progressive spontaneous resolution of inflammation.

In two tissues, the lung and the cerebellum, LPI levels were

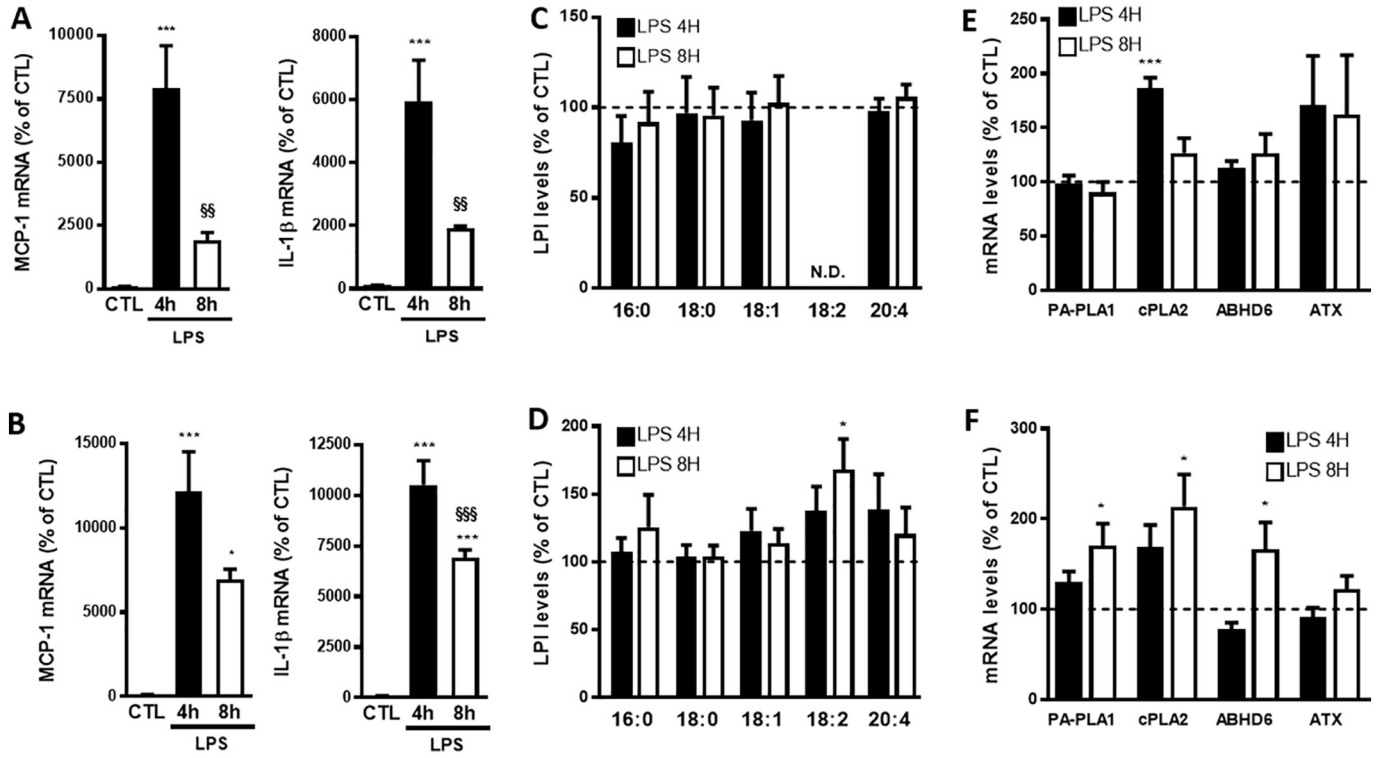

Fig. 5. Impact of lipopolysaccharide-induced inflammation on lysophosphatidylinositol levels and their metabolic pathway in the cerebellum and lung.
Systemic inflammation in mice was induced by intraperitoneal administration of 300 μg/kg of lipopolysaccharides (LPS) for 4 and 8 h. (A, B) mRNA expression of pro-inflammatory markers IL-1β and MCP-1 measured by RT-qPCR in (A) the cerebellum and (B) the lung with RPL19 as reference gene. (C, D) Lysophosphatidylinositol (LPI) levels measured by HPLC-MS in (C) the cerebellum and (D) the lung. (E, F) mRNA expression of cPLA2α, PA-PLA1, ABHD6 and Autotaxin (ATX) were measured by RT-qPCR in (E) the cerebellum and (F) the lung with RPL19 as reference gene. Data are expressed in percentage of the control condition (dotted line in C–F). Values are mean ± SEM with 7 mice per group. $^{*}P < 0.05$, $^{***}P < 0.001$ for mice receiving LPS vs. control mice. $\$\$P < 0.01$, $\$\$\$P < 0.001$ for 8 h of LPS vs. 4 h of LPS.

generally not affected by inflammation either after 4 h or 8 h of LPS administration (Fig. 5C–D), with the exception of 18:2 LPI which is increased in the lung. Note that we did not detect 18:2 LPI in the brain similarly to a previous report by Oka et al. [9]. Conversely, all the measured LPI were strongly increased by inflammation in the liver and spleen (Fig. 6C–D). However, this increase was only sustained at 8 h in the liver (Fig. 6C) and proved transient in the spleen, with a return to baseline at 8 h (Fig. 6D). The picture was different in the colon where we found a significant decrease in 16:0, 18:0, 18:2 and 20:4 LPI levels already 4 h post LPS injection, and this decrease was sustained at 8 h (Fig. 7B).

3.7. LPS-induced inflammation alters the expression of enzymes involved in the metabolism of LPI

Because LPI levels were affected by inflammation in some tissues, we assessed mRNA expression of several enzymes implicated in the metabolism of lysophospholipids in general and LPI in particular. Similarly to what was done for the cells, we studied cPLA2α, PA-PLA1, ABHD6, and autotaxin. Changes in mRNA expression of these enzymes in inflammatory settings were quite different depending on the tissue considered. In the cerebellum where LPI levels were not affected, a transient increase of cPLA2α at 4 h was observed (Fig. 5E). In the lung, there were no significant differences in enzyme expression at 4 h, but all the enzymes, except for autotaxin, were increased at 8 h (Fig. 5F). A similar increase was observed for cPLA2α, PA-PLA1 and ABHD6 in the colon at 8 h, while the expression of autotaxin was decreased at both time points (Fig. 7C). In the liver, expression of PA-PLA1 and cPLA2α exhibited a trend to increase, which only reached significance for cPLA2α at 8 h. Conversely, mRNA expression of ABHD6 was decreased in this tissue (Fig. 6E). In the spleen, PA-PLA1 mRNA expression was increased at both time points (Fig. 6F).

3.8. Intestinal LPI levels are altered in mouse models of colitis

Because LPI levels were affected by LPS-induced inflammation and were only decreased in the colon (Fig. 7B), we asked whether these levels would be affected in a similar way in murine models more closely mimicking inflammatory bowel diseases (IBD). Indeed, several bioactive lipids, such as endocannabinoids and sphingolipids have been implicated in the pathogenesis of IBD. Therefore we used three well characterized models of IBD, the DSS-induced and TNBS-induced acute models of colitis and the DSS-induced chronic model of colitis (Fig. 7D–F), in order to determine the impact of colitis on LPI levels [26,28,31]. Similarly to what we observed in the LPS model, we found that LPI levels, with the exception of 18:0 LPI, were decreased in the colon of mice with acute DSS-induced colitis (Fig. 7D). In the TNBS-induced colitis model, only 16:0 and 18:1 LPI were decreased (Fig. 7E). In the chronic DSS-induced colitis model, all LPI, except 20:4-LPI were decreased (Fig. 7F).

4. Discussion

Among all lysophospholipids, LPI are certainly the less studied of the lot. However, there has been a renewed interest in these bioactive lipids mainly since GPR55 was identified as the receptor for LPI [9,38]. Since then, LPI and GPR55 have been implicated in several pathophysiological processes [12]. However, the role of LPI in inflammation remains unclear. A study recently reported differential changes of LPI levels in M1 and M2 RAW264 cells but did not investigate the enzymes

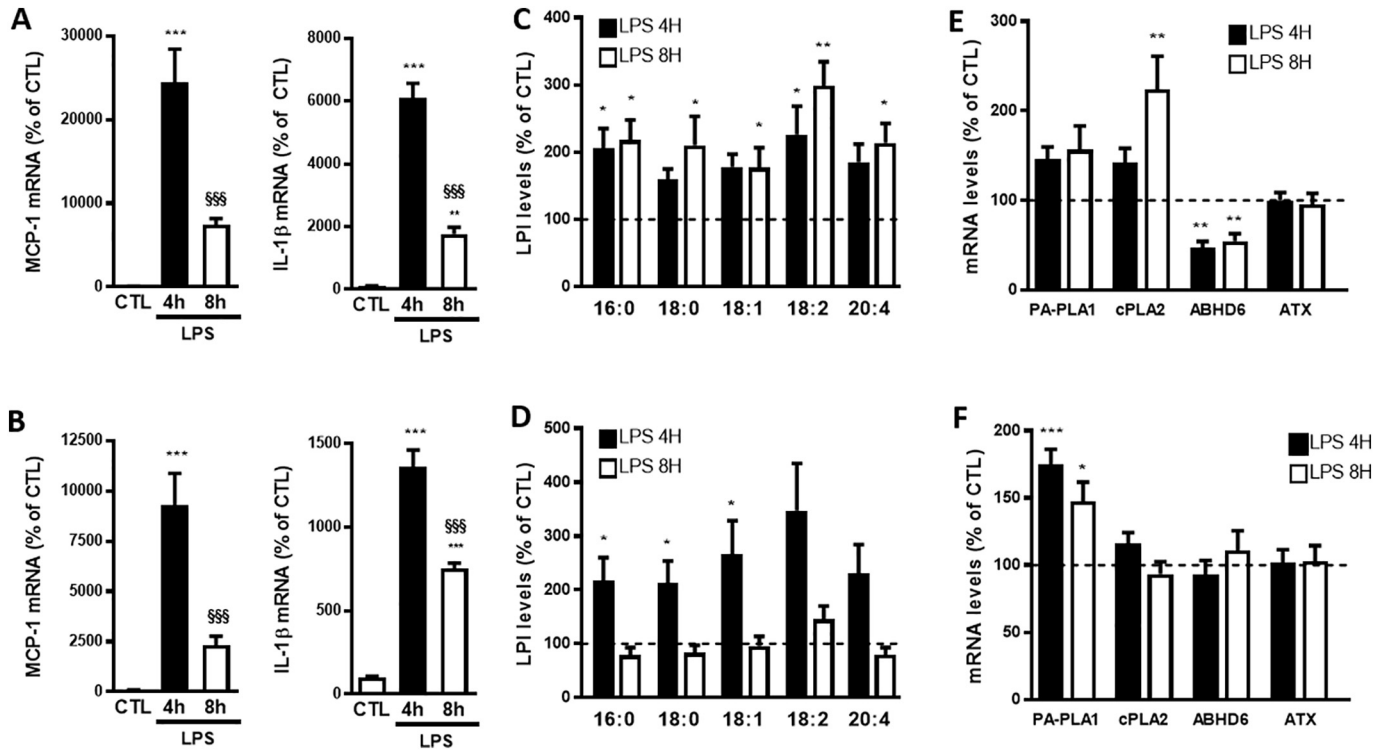

Fig. 6. Impact of lipopolysaccharide-induced inflammation on lysophosphatidylinositol levels and their metabolic pathway in the liver and spleen.
Systemic inflammation in mice was induced by intraperitoneal administration of 300 μg/kg of lipopolysaccharides (LPS) for 4 and 8 h. (A, B) mRNA expression of pro-inflammatory markers IL-1β and MCP-1 measured by RT-qPCR in (A) the liver and (B) the spleen with RPL19 as reference gene. (C, D) Lysophosphatidylinositol (LPI) levels measured by HPLC-MS in (C) the liver and (D) the spleen. (E, F) mRNA expression of cPLA2α, PA-PLA1, ABHD6 and Autotaxin (ATX) were measured by RT-qPCR in (E) the liver and (F) the spleen with RPL19 as reference gene. Data are expressed in percentage of the control condition (dotted line in C–F). Values are mean ± SEM with 7 mice per group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ for mice receiving LPS vs. control mice. $\$\$\$P < 0.001$ for 8 h of LPS vs. 4 h of LPS.

involved [39]. Another study showed a pro-inflammatory role of GPR55 in colitis and a decrease of macrophage activation with a GPR55 antagonist but did not investigate the effect of inflammation on the levels of GPR55's endogenous ligand [40]. On the other hand, LPI showed neuroprotective effects in rat hippocampal slices [41]. Therefore, we decided here to study the metabolism of LPI in inflammation. These studies were conducted in vitro, on activated macrophages, and in vivo.

We started by evaluating the effect of macrophage activation on LPI levels. To do so, we used LPS, a potent macrophage activator. Interestingly, activation of the J774 murine macrophage cell line for 8 h only led to variations in the levels of LPI, but not the other lysophospholipids. However, activation of these cells for 24 h also led to variations in the levels of other lysophospholipids. Actually, while the levels of most of the detected LPI were increased following 8 h of J774 and BV2 activation, the levels of 20:4 LPI were decreased. Moreover, 20:4 LPI was further decreased following 24 h of J774 activation. This could be expected as during inflammation or macrophage activation, there is a release of arachidonic acid which could lead to a decrease in 20:4-containing lysophospholipids species. We could not evidence such an increase in arachidonic acid in the cells. This is however not so surprising as we have previously shown increased prostaglandin synthesis in J774 cells activated with LPS [24]. Because an inflammatory response is often thoroughly regulated, mixing both signals to initiate and terminate the process, we thought that LPI could play a role in macrophage activation. Therefore, we incubated the LPS-activated J774 cells with various lysophospholipids and we show that incubation with LPI decreases LPS-induced macrophage activation.

As macrophage activation affected LPI levels, we took a closer look at its effects on the metabolic enzymes of LPI. Phosphatidic acid-preferring phospholipase A1, PA-PLA1, also known as DDHD1, is involved in the hydrolysis of fatty acids at the sn-1 position of phospholipids, to produce fatty acids and 2-acyl lysophospholipids [42]. Yamashita et al. found that PA-PLA1 was implicated in the formation of 20:4 LPI in particular. cPLA2α is implicated in the hydrolysis of sn-2 fatty acids from phospholipids. Mariggiò et al. reported that cPLA2α may be one of the synthesizing enzymes of 1-stearoyl LPI [21]. These roles for PA-PLA1 and cPLA2α are based on the fact that, among PI, 1-stearoyl-2-arachidonoyl PI is the most abundant. However, cPLA2α is not only responsible for LPI biosynthesis but can also hydrolyze LPI esterified in the sn2 position, such as 20:4 LPI formed through PA-PLA1 activity. We thought of assessing the variations in mRNA enzyme expression and possibly link them with changes in LPI levels. Interestingly, we found different trends for enzyme expression in the J774 and BV2 cell lines, despite similar trends for LPI levels upon activation of these two cell lines, suggesting different metabolic pathways in these cells. In J774 cells, a decrease in mRNA levels of PA-PLA1 and the increase in cPLA2α activity could explain the decrease of 20:4 LPI levels. Supporting this hypothesis is the fact that inhibiting cPLA2α counteracted the LPS-induced decrease in 20:4 LPI levels and led to a drastic increase of 20:4 LPI.

ABHD6 is another enzyme that has been implicated in lysophospholipid catabolism with LPI levels increasing in the liver of ABHD6 knock-out mice [22]. This enzyme could be involved in the hydrolysis of 20:4 LPI as ABHD6 inhibition in LPS-activated J774 cells led to an increase in 20:4 LPI levels. However, the metabolic pathways of LPI are quite complex (Suppl. Fig. S1), with possible reacylations and subsequent deacylations. Therefore, it is difficult to link the levels of a particular LPI with the expression or activity of a single enzyme. This reinforces the notion that measuring bioactive lipid levels will offer a global and terminal view of all the changes that might occur in a given metabolic pathway.

This is also true in vivo, where we assessed the impact of a systemic inflammation on LPI levels in the colon, liver, spleen, lung and cerebellum of mice receiving LPS (300 μg/kg) for 4 or 8 h. This dose of LPS

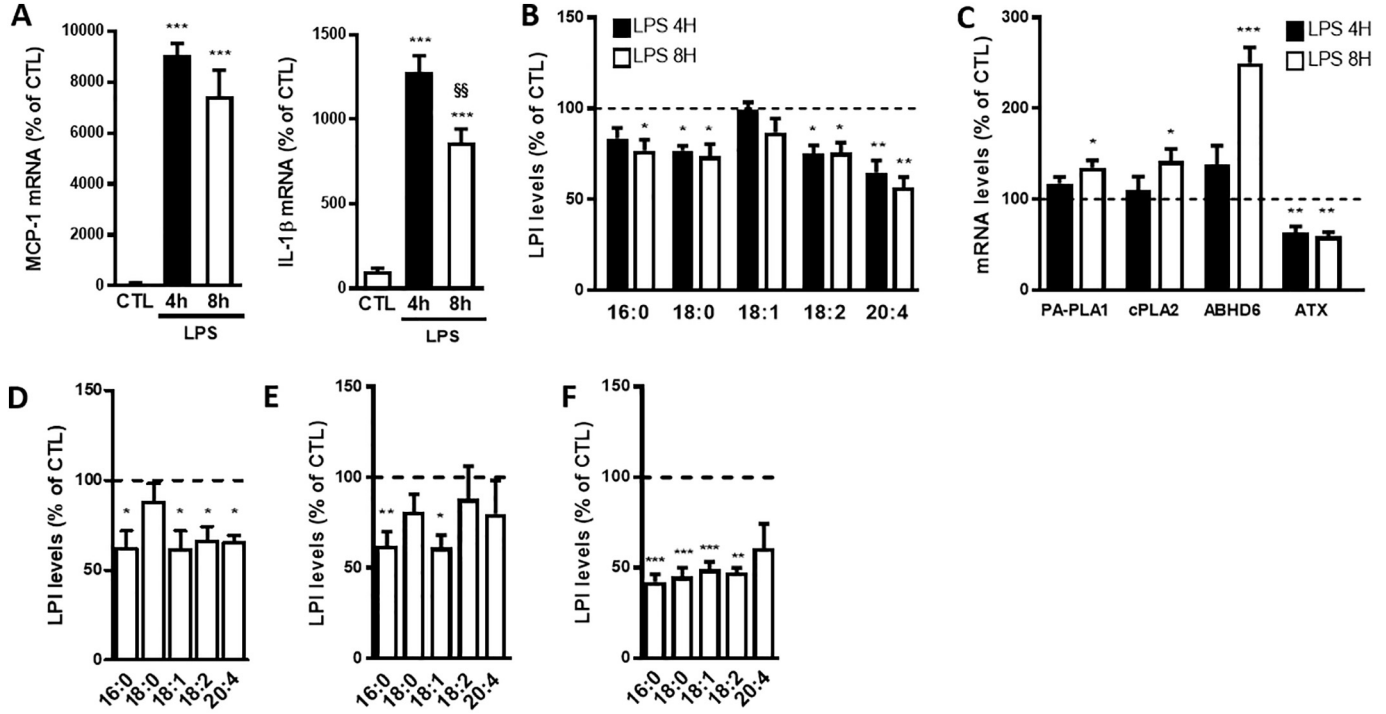

Fig. 7. Impact of colon inflammation on lysophosphatidylinositol levels and their metabolic pathways.
(A–C) Systemic inflammation in mice was induced by intraperitoneal administration of 300 μg/kg of lipopolysaccharides (LPS) for 4 and 8 h. (A) mRNA expression of pro-inflammatory markers IL-1β and MCP-1 measured by RT-qPCR in the colon with RPL19 as reference gene. (B) Lysophosphatidylinositol (LPI) levels measured by HPLC-MS in the colon. (C) mRNA expression of cPLA2α, PA-PLA1, ABHD6 and Autotaxin (ATX) were measured by RT-qPCR in the colon with RPL19 as reference gene.
(D–F) LPI levels measured by HPLC-MS in the colon of mice with (D) acute DSS-induced colitis, (E) acute TNBS-induced colitis and (F) chronic DSS-induced colitis. Data are expressed in percentage of the control condition (dotted line in B–F). Values are mean ± SEM with 7 mice per group (A–C), 8 mice per group (D and F) and 10 mice per group (E). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ for inflamed mice (LPS, DSS or TNBS) vs. control mice. $\$\$P < 0.01$ for 8 h of LPS vs. 4 h of LPS.

was chosen on the basis of previous studies performed in our laboratory to mimic the LPS-induced inflammation present during inflammatory pathologies such as colitis or the metabolic syndrome [24,25,31]. Although we found clear changes in LPI levels induced by inflammation, the changes were different depending on the tissue. Here also, as with the macrophages, it is difficult to link LPI levels with mRNA expression of the metabolic enzymes. Therefore, further studies will be needed to pinpoint the changes in metabolic pathways leading to these changes.

Interestingly, in the colon of mice receiving LPS, LPI levels are decreased and we could observe a similar decrease in LPI levels in three models of colon inflammation. These alterations in LPI levels could be a mere consequence of inflammation or could be implicated in the inflammatory response as GPR55 was suggested to be involved in colitis [40]. Further studies will help in understanding the role of LPI in colon inflammation.

Finally, as we showed in this study that LPI could reduce macrophage activation, we wanted to study the implication of GPR55 in these effects. Although GPR55 has been put forth as the receptor for LPI [19,43], not much is known on its effects on inflammation or macrophage activation. A serendipitous observation led us to suspect that this receptor could be involved in the effects of LPI on macrophage activation. Indeed in cells were GPR55 was well expressed, LPI decreased macrophage activation, whereas in cells where GPR55 was less expressed, the effect of LPI was lost. To confirm our hypothesis, we transfected J774 cells showing low expression of GPR55 and little effect of LPI on macrophage activation with human GPR55 and were able to rescue the effect of LPI on macrophage activation. Our results suggest that LPI could have anti-inflammatory properties when GPR55 is highly expressed. However, these results were obtained using a mix of LPI from bovine liver, which contains several species of LPI including small amounts of 20:4 LPI that is decreased following LPS-induced J774 macrophage activation. It is noteworthy that LPI were shown to have different potencies on GPR55 depending on the acyl chain with 2-arachidonoyl LPI showing the highest potency [9]. As acyl chain length and insaturation can influence the biological activity of lysophospholipids [44], and our data show an effect of LPI on macrophage activation, it will be interesting to study the effect of individual LPI species on macrophage activation and inflammation.

In conclusion, as LPI levels were altered by macrophage activation, we hypothesized that they could be implicated in the control of macrophage activation. We were able to show that this was indeed the case, through a GPR55-dependent mechanism. Moreover, inflammation also affects LPI levels in vivo. These alterations are tissue dependent and could highlight a potential role for LPI in inflammatory processes.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

We wish to thank Thérèse Timmerman and, Elise Bonte for their assistance. MA is a postdoctoral researcher from the Fonds de la Recherche Scientifique (FRS-FNRS), Belgium. PB is a research fellow of the “Fonds pour la Recherche dans l'Industrie et l'Agriculture” (FRIA, Belgium). GGM is the recipient of grants from the F.R.S. - FNRS, Belgium (grants CC 1.5.034.10, PDR T.0148.14) and from the Fonds Spéciaux de Recherches (FSR, Université catholique de Louvain). The MASSMET platform, from the Université catholique de Louvain, is acknowledged for access to the HLPC-MS.

Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbalip.2018.09.003.

References

[1] H. Y Kihara, J. Mizuno, Chun, Lysophospholipid receptors in drug discovery, Exp. Cell Res. 333 (2015) 171–177.
[2] I. Sevastou, E. Kaffe, M.A. Mouratis, V. Aidinis, Lysoglycerophospholipids in chronic inflammatory disorders: the PLA(2)/LPC and ATX/LPA axes, Biochim. Biophys. Acta 1831 (2013) 42–60.
[3] A. Grzelczyk, E. Gendaszewska-Darmach, Novel bioactive glycerol-based lysophospholipids: new data – new insight into their function, Biochimie 95 (2013) 667–679.
[4] S.C. Frasch, R.F. Fernandez-Boyanapalli, K.A. Berry, R.C. Murphy, C.C. Leslie, J.A. Nick, P.M. Henson, D.L. Bratton, Neutrophils regulate tissue Neutrophilia in inflammation via the oxidant-modified lipid lysophosphatidylserine, J. Biol. Chem. 288 (2013) 4583–4593.
[5] S.A. Metz, Lysophosphatidylinositol, but not lysophosphatidic acid, stimulates insulin release. A possible role for phospholipase A2 but not de novo synthesis of lysophospholipid in pancreatic islet function, Biochem. Biophys. Res. Commun. 138 (1986) 720–727.
[6] S.A. Metz, Putative roles for lysophospholipids as mediators and lipoxygenase-mediated metabolites of arachidonic acid as potentiators of stimulus-secretion coupling: dual mechanisms of p-hydroxymercuribenzoic acid-induced insulin release, J. Pharmacol. Exp. Ther. 238 (1986) 819–832.
[7] M. Falasca, D. Corda, Elevated levels and mitogenic activity of lysophosphatidylinositol in k-ras-transformed epithelial cells, Eur. J. Biochem. 221 (1994) 383–389.
[8] M.G. M Falasca, A. Silletta, A.L. Carvelli, A. Di Francesco, V. Fusco, D. Ramakrishna, Corda, Signalling pathways involved in the mitogenic action of lysophosphatidylinositol, Oncogene 10 (1995) 2113–2124.
[9] S. Oka, T. Toshida, K. Maruyama, K. Nakajima, A. Yamashita, T. Sugiura, 2-Arachidonoyl-sn-glycero-3-phosphoinositol: a possible natural ligand for GPR55, J. Biochem. 145 (2009) 13–20.
[10] C.M. Henstridge, N.A. Balenga, R. Schroder, J.K. Kargl, W. Platzer, L. Martini, S. Arthur, J. Penman, J.L. Whistler, E. Kostenis, M. Waldhoer, A.J. Irving, GPR55 ligands promote receptor coupling to multiple signalling pathways, Br. J. Pharmacol. 160 (2010) 604–614.
[11] S. Oka, K. Nakajima, A. Yamashita, S. Kishimoto, T. Sugiura, Identification of GPR55 as a lysophosphatidylinositol receptor, Biochem. Biophys. Res. Commun. 362 (2007) 928–934.
[12] M. Alhouayek, J. Masquelier, G.G. Muccioli, Lysophosphatidylinositols, from cell membrane constituents to GPR55 ligands, Trends Pharmacol. Sci. 39 (6) (2018) 586–604.
[13] M. Falasca, R. Ferro, Role of the lysophosphatidylinositol/GPR55 axis in cancer, Adv. Biol. Regul. 60 (2016) 88–93.
[14] C.M. Henstridge, A.J. Brown, M. Waldhoer, GPR55: metabolic help or hindrance? Trends Endocrinol. Metab. 27 (2016) 606–608.
[15] A. Yamashita, T. Kumazawa, H. Koga, N. Suzuki, S. Oka, T. Sugiura, Generation of lysophosphatidylinositol by DDHD domain containing 1 (DDHD1): possible involvement of phospholipase D/phosphatidic acid in the activation of DDHD1, Biochim. Biophys. Acta 1801 (2010) 711–720.
[16] Y. Kita, T. Ohto, N. Uozumi, T. Shimizu, Biochemical properties and pathophysiological roles of cytosolic phospholipase A2s, Biochim. Biophys. Acta 1761 (2006) 1317–1322.
[17] R. Pineiro, T. Maffucci, M. Falasca, The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation, Oncogene 30 (2011) 142–152.
[18] J.E. Burke, E.A. Dennis, Phospholipase A2 structure/function, mechanism, and signaling, J. Lipid Res. 50 (Suppl) (2009) S237–S242.
[19] A. Yamashita, S. Oka, T. Tanikawa, Y. Hayashi, Y. Nemoto-Sasaki, T. Sugiura, The actions and metabolism of lysophosphatidylinositol, an endogenous agonist for GPR55, Prostaglandins Other Lipid Mediat. 107 (2013) 103–116.
[20] A. Yamashita, Y. Hayashi, Y. Nemoto-Sasaki, M. Ito, S. Oka, T. Tanikawa, K. Waku, T. Sugiura, Acyltransferases and transacylases that determine the fatty acid composition of glycerolipids and the metabolism of bioactive lipid mediators in mammalian cells and model organisms, Prog. Lipid Res. 53 (2014) 18–81.
[21] S. Mariggio, J. Sebastia, B.M. Filippi, C. Iurisci, C. Volonte, S. Amadio, V. De Falco, M. Santoro, D. Corda, A novel pathway of cell growth regulation mediated by a PLA2alpha-derived phosphoinositide metabolite, FASEB J. 20 (2006) 2567–2569.
[22] G. Thomas, J.L. Betters, C.C. Lord, A.L. Brown, S. Marshall, D. Ferguson, J. Sawyer, M.A. Davis, J.T. Melchior, L.C. Blume, A.C. Howlett, P.T. Ivanova, S.B. Milne, D.S. Myers, I. Mrak, V. Leber, C. Heier, U. Taschler, J.L. Blankman, B.F. Cravatt, R.G. Lee, R.M. Crooke, M.J. Graham, R. Zimmermann, H.A. Brown, J.M. Brown, The serine hydrolase ABHD6 is a critical regulator of the metabolic syndrome, Cell Rep. 5 (2013) 508–520.
[23] H. Yukiura, K. Hama, K. Nakanaga, M. Tanaka, Y. Asaoka, S. Okudaira, N. Arima, A. Inoue, T. Hashimoto, H. Arai, A. Kawahara, H. Nishina, J. Aoki, Autotaxin regulates vascular development via multiple Lysophosphatidic acid (LPA) receptors in Zebrafish, J. Biol. Chem. 286 (2011) 43972–43983.
[24] M. Alhouayek, J. Masquelier, P.D. Cani, D.M. Lambert, G.G. Muccioli, Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 17558–17563.
[25] B. V Mutemberezi, J. Buisseret, O. Masquelier, M. Guillemot-Legris, G.G. Alhouayek, Muccioli, Oxysterol levels and metabolism in the course of neuroinflammation: insights from in vitro and in vivo models, J. Neuroinflammation 15 (2018) 74.
[26] M. Alhouayek, P. Bottemanne, K.V. Subramanian, D.M. Lambert, A. Makriyannis, P.D. Cani, G.G. Muccioli, N-acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis, FASEB J. 29 (2015) 650–661.
[27] M. Alhouayek, B. Buisseret, A. Paquot, O. Guillemot-Legris, G.G. Muccioli, The endogenous bioactive lipid prostaglandin D2-glycerol ester reduces murine colitis via DP1 and PPARgamma receptors, FASEB J. 32 (9) (2018) 5000–5011 (p.fj201701205R).
[28] S. Wirtz, C.F. Neufert, B.F. Weigmann, M.F. Neurath, Chemically induced mouse models of intestinal inflammation, Nat. Protoc. 2 (3) (2007) 541–546.
[29] X. Zhang, R. Goncalves, D.M. Mosser, The isolation and characterization of murine macrophages, Curr. Protoc. Immunol. Chapter 14 (2008) (p.Unit).
[30] M. Alhouayek, P. Bottemanne, A. Makriyannis, G.G. Muccioli, N-acylethanolamine-hydrolyzing acid amidase and fatty acid amide hydrolase inhibition differentially affect N-acylethanolamine levels and macrophage activation, Biochim. Biophys. Acta 1862 (2017) 474–484.
[31] M. Alhouayek, D.M. Lambert, N.M. Delzenne, P.D. Cani, G.G. Muccioli, Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation, FASEB J. 25 (2011) 2711–2721.
[32] O. Guillemot-Legris, J. Masquelier, A. Everard, P.D. Cani, M. Alhouayek, G.G. Muccioli, High-fat diet feeding differentially affects the development of inflammation in the central nervous system, J. Neuroinflammation 13 (1) (2016) 206.
[33] J. Masquelier, G.G. Muccioli, Development and validation of a specific and sensitive HPLC-ESI-MS method for quantification of lysophosphatidylinositols and evaluation of their levels in mice tissues, J. Pharm. Biomed. Anal. 126 (2016) 132–140.
[34] C.C. Leslie, M.H. Gelb, Assaying phospholipase A2 activity, Methods Mol. Biol. 284 (2004) 229–242.
[35] W.R. Marrs, J.L. Blankman, E.A. Horne, A. Thomazeau, Y.H. Lin, J. Coy, A.L. Bodor, G.G. Muccioli, S.S. Hu, G. Woodruff, S. Fung, M. Lafourcade, J.P. Alexander, J.Z. Long, W. Li, C. Xu, T. Moller, K. Mackie, O.J. Manzoni, B.F. Cravatt, N. Stella, The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors, Nat. Neurosci. 13 (2010) 951–957.
[36] A.L. Groeger, C. Cipollina, M.P. Cole, S.R. Woodcock, G. Bonacci, T.K. Rudolph, V. Rudolph, B.A. Freeman, F.J. Schopfer, Cyclooxygenase-2 generates anti-inflammatory mediators from omega-3 fatty acids, Nat. Chem. Biol. 6 (2010) 433–441.
[37] H.B. Cohen, D.M. Mosser, Extrinsic and intrinsic control of macrophage inflammatory responses, J. Leukoc. Biol. 94 (2013) 913–919.
[38] C.M. Henstridge, N.A. Balenga, L.A. Ford, R.A. Ross, M. Waldhoer, A.J. Irving, The GPR55 ligand L-alpha-lysophosphatidylinositol promotes RhoA-dependent Ca^{2+} signaling and NFAT activation, FASEB J. 23 (1) (2009) 183–193.
[39] C. Zhang, Y. Wang, F. Wang, Z. Wang, Y. Lu, Y. Xu, K. Wang, H. Shen, P. Yang, S. Li, X. Qin, H. Yu, Quantitative profiling of glycerophospholipids during mouse and human macrophage differentiation using targeted mass spectrometry, Sci. Rep. 7 (2017) 412.
[40] A. Stancic, K. Jandl, C. Hasenohrl, F. Reichmann, G. Marsche, R. Schuligoi, A. Heinemann, M. Storr, R. Schicho, The GPR55 antagonist CID16020046 protects against intestinal inflammation, Neurogastroenterol. Motil. 27 (2015) 1432–1445.
[41] S. Kallendrusch, S. Kremzow, M. Nowicki, U. Grabiec, R. Winkelmann, A. Benz, R. Kraft, I. Bechmann, F. Dehghani, M. Koch, The G protein-coupled receptor 55 ligand l-alpha-lysophosphatidylinositol exerts microglia-dependent neuroprotection after excitotoxic lesion, Glia 61 (2013) 1822–1831.
[42] J. Aoki, A. Inoue, K. Makide, N. Saiki, H. Arai, Structure and function of extracellular phospholipase A1 belonging to the pancreatic lipase gene family, Biochimie 89 (2007) 197–204.
[43] K. Makide, A. Uwamizu, Y. Shinjo, J. Ishiguro, M. Okutani, A. Inoue, J. Aoki, Novel lysophosphoplipid receptors; their structure and function, J. Lipid Res. 55 (10) (2014) 1986–1995.
[44] P.J. Ojala, T.E. Hirvonen, M. Hermansson, P. Somerharju, J. Parkkinen, Acyl chain-dependent effect of lysophosphatidylcholine on human neutrophils, J. Leukoc. Biol. 82 (2007) 1501–1509.