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Alkylglycerol monooxygenase as a potential modulator for PAF synthesis in macrophages

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ABSTRACT

Alkylglycerol monooxygenase (AGMO, glyceryl ether monooxygenase) is an enzyme known to catalyze the cleavage of the *O*-alkyl bond of glyceryl ether lipids. Identification of the gene encoding AGMO was reported recently, however, the involvement of AGMO in modulating cellular lipids has not been reported until now. In this report, we investigate a possible role for AGMO in macrophage platelet-activating factor (PAF) production. AGMO mRNA expression levels decreased with lipopolysaccharide (LPS) treatments in mouse peritoneal macrophages and RAW264.7 cells. Tetrahydrobiopterin-dependent conversion of lyso-PAF to glycerophosphocholine in the microsomal fraction was also reduced in LPS-treated RAW264.7 cells. In the LPS-treated cells, both lyso-PAF and PAF levels increased. Moreover, exogenously expressed AGMO caused a reduction in cellular lyso-PAF and PAF levels in HEK293 cells. Collectively, our results suggest a possible mechanism for AGMO in modulating macrophage PAF production by regulating cellular lyso-PAF levels.

1. Introduction

Alkylglycerol monooxygenase (AGMO, glyceryl ether monooxygenase, EC 1.14.16.5) is the only enzyme, so far, known to catalyze the oxidative cleavage of *O*-alkyl bond of ether lipids. The enzymatic reaction was first described in 1964 as a tetrahydrobiopterin (BH_4, (6*R*)-5,6,7,8-tetrahydro-L-biopterin)-requiring enzyme from rat liver homogenates [1], and later, several detailed studies reported tissue distribution, substrate specificity, and apparent kinetics with the BH_4 cofactor [2,3]. In 2010, using an *in silico* database search and the recombinant gene expression of selected candidates, Watschinger et al. [4] reported that a gene with an unassigned function, transmembrane protein 195 (*TMEM195*), encodes AGMO. Alkylglycerols and their various metabolites, including ether phospholipids, are believed to have important biological roles as membrane components and mediators of cell responses [5–7]. However, the molecular basis of the alkylglycerolipid function, as well as the importance of AGMO in regulating metabolite levels, remain unclear.

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a phosphatidylcholine (PC) with *O*-alkyl (typically *O*-hexadecyl) and acetyl groups at *sn*-1 and *sn*-2 positions, respectively. PAF is a potent phospholipid mediator that activates a G protein-coupled receptor (PAFR) and causes pleiotropic biological effects, including inflammation, platelet activation, airway constriction, hypotension, and hypersensitive reactions in allergy [8]. PAF is synthesized in tissues and cells via two distinct pathways, the *de novo* and remodeling pathways [9]. The *de novo* pathway is thought to maintain physiological levels of PAF for normal cellular functions, whereas the remodeling pathway is activated by inflammatory stimuli and is thought to be the primary source of PAF under pathological conditions. Induction of PAF synthesis by various extracellular stimuli has been reported in peritoneal and alveolar macrophages, polymorphonuclear neutrophils (PMN), vascular endothelial cells, basophils, and bone marrow-derived mast cells [8].

Watschinger et al. [4] reported that AGMO mRNA expression level and enzymatic activity are high in the murine macrophage cell line RAW264.7. Stimulation of RAW264.7 cells or mouse peritoneal macrophages with lipopolysaccharide (LPS) induces enhanced production of PAF in the remodeling pathway through two enzymes, lysophosphatidylcholine acyltransferase 2 (LPCAT2, EC 2.3.1.23 and EC 2.3.1.67) and phospholipases A_2 (PLA_2, EC 3.1.1.4) [10,11]. Among PLA_2s, cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$, group IVA PLA_2) is a key enzyme for lyso-PAF (1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine) production in inflammatory cells, the major source of PAF production [11,12]. Lyso-PAF may either be converted to PAF or transformed back to PC by LPCAT2 [10]. PAF is then rapidly degraded to lyso-PAF by PAF acetylhydrolases (PAFAH) [13,14]. AGMO is the only identified enzyme that can irreversibly cleave the *O*-alkyl bond of lyso-PAF to produce glycerophosphocholine (GPC) [2–4].

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Thus, it is possible that this enzyme essentially eliminates lyso-PAF from the recycling system of lipid pool.

In this study, we investigated a possible role for AGMO in the modulation of macrophage PAF levels. The enzyme is primarily expressed in macrophages, and the expression as well as enzymatic activity is decreased by LPS stimulation, which coincides with increased cellular lyso-PAF and PAF levels. Exogenous expression of AGMO causes a reduction in cellular lyso-PAF and PAF levels. To our knowledge, this is the first report to demonstrate the biological importance of AGMO.

2. Materials and methods

2.1. Reagents

PAF, lyso-PAF, PAF-d4 (1-*O*-hexadecyl-(7,7,8,8-d4)-2-acetyl-*sn*-glycero-3-phosphorylcholine), and lyso-PAF-d4 (1-*O*-hexadecyl-(7,7,8,8-d4)-2-lyso-*sn*-glycero-3-phosphorylcholine) were purchased from Cayman Chemical (Ann Arbor, MI). Oligonucleotide primers were from Sigma–Aldrich (St. Louis, MO) and Greiner Bio-One (Frickenhausen, Germany). LPS, from *Salmonella minnesota*, was purchased from Sigma–Aldrich. ODN1826 and poly(I:C)-LMW were from InvivoGen (San Diego, CA). Other reagents were from Wako (Osaka, Japan).

2.2. Isolation of mouse peritoneal macrophages

C57BL/6J mice were obtained from CLEA Japan (Tokyo, Japan). Thioglycollate-induced peritoneal macrophages were isolated as previously described [15]. Cells were cultured for 2 h before stimulation and then incubated 16 h with or without 100 ng/ml LPS. The procedure was conducted in accordance with the guidelines for Animal Research at the University of Tokyo and was approved by the University of Tokyo Ethics Committee for Animal Experiments (M-P11-062).

2.3. Cell culture

Murine macrophage-like RAW264.7 cells and human embryonic kidney (HEK) 293 cells were maintained under 5% CO_2 in the air at 37 °C in Dulbecco's modified Eagle's medium (Nacali Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA). RAW264.7 cells were seeded onto a 60 mm dish (2.4×10^6 cells/dish) for preparation of the microsomal fraction, or onto 12-well plates (4×10^5 cells/well) for the preparation of RNA and lipids. HEK293 cells were seeded onto a 60 mm dish (1.75×10^6 cells per dish) for preparation of the microsomal fraction, or onto 12-well plates (3.5×10^5 cells/well) for the preparation of lipids.

2.4. Quantitative real-time PCR

Total RNAs were prepared using Absolutely RNA RT-PCR Miniprep Kit (Agilent Technologies, Santa Clara, CA) and RNeasy Mini Kit (Qiagen, Hilden, Germany) for peritoneal macrophages and RAW 264.7 cells, respectively. First strand cDNA was subsequently synthesized using Superscript III (Invitrogen). Real-time PCR was performed using FastStart DNA Master SYBR Green I and LightCycler 1.5 (Roche Applied Science, Mannheim, Germany). The following primers were used: *Agmo*, forward, 5′-GTTTTCTACTGTCCACTGGCCCT-3′ and reverse, 5′-GGGTTCTGATGATCTCTGTGTGG-3′; *Gapdh*, forward, 5′-TGACAATGAATACGGCTACAGCA-3′ and reverse, 5′-CTCCTGTTATTATGGGGGTCTGG-3′; *Rplp0*, forward, 5′-GTCCTGGCATTGTCTGTGGAG-3′ and reverse, 5′-GACTCTTCCTTTGCTTCAGCTTTG-3′.

2.5. Preparation of the microsomal fraction

Cells from 60 mm dishes were scraped into 400 μl of ice-cold buffer containing 20 mM Tris–HCl (pH 7.4), 300 mM sucrose, and Complete proteinase inhibitor mixture (Roche), and, then, sonicated on ice. After centrifugation for 5 min at 800*g*, the supernatant was collected and centrifuged at 100,000*g* for 1 h at 4 °C. The resulting pellets were resuspended in buffer containing 20 mM Tris–HCl (pH 7.4) and 300 mM sucrose. The protein concentration was measured using Bio-Rad protein assay kit (Bio-Rad, Hercules, CA).

2.6. Measurement of AGMO activity

AGMO activity was measured by the method reported by Werner et al. [16] with modifications. The reaction mixture contained 50 mM Tris–HCl (pH 8.2), 0.2 mM NADPH, 0.2 mM NAD+, 0.1 mg/ml catalase, 0.2 μg/ml dihydropteridine reductase, 0.2 mM BH_4, 0.1 mM lyso-PAF, and 3 μg of microsomal proteins, in a total volume of 50 μl. The enzyme reactions were performed on a heat block at 37 °C under darkness. After 10 min incubation, 500 μl of methanol containing 1% phosphoric acid was added to terminate the reaction. GPC was measured by liquid chromatography–mass spectrometry (LC–MS).

2.7. Cloning of AGMO

The entire coding region of mouse AGMO (Genbank accession number: NM_178767) was identified in the National Center for Biotechnology Information database. A 1344 bp cDNA clone encoding the full-length mAGMO was obtained by PCR amplification using a cDNA template from RAW264.7 cells. A FLAG epitope (DYKDDDDK) was attached to the C terminus by a second PCR run and cloned into a pCXN2.1 vector, which is a slightly modified version of pCXN2 [17]. Primers used for the first PCR run were: forward, 5′-CAAGGCTCGCTCTGTGCTAC-3′ and reverse 5′-TGGTGATGCAGGACGGAGAA-3′. Primers for the second PCR run were: forward, 5′-CTAGCTAGCCCACCATGAGGAGCCCAGGAGCCCAAG-3′ and reverse, 5′-CCGCTCGAGTTACTTGTCATCGTCATCCTTGTAATCAGGCTTTTTCCAAGAGC-3′.

2.8. Site-directed mutagenesis of AGMO

Mouse AGMO mutants (Q197A and D233A) were constructed by overlap extension PCR. The amplified PCR products were subcloned into the pCXN2.1 vector. Primers used were: Q197A, forward, 5′-CTCCTCTACGCATTTTGGATCCC-3′ and reverse, 5′-GGATCCAAAATGCGTAGAGGAG-3′; D233A, forward, 5′-CTGCATTGCTAAAAACTATGC-3′ and reverse, 5′-CAGCATAGTTTTTAGCAATGC-3′.

2.9. Overexpression of FLAG-tagged AGMO and catalytically inactive mutants

One day before transfection HEK293 cells were seeded onto a 60 mm dish or a 12-well plate. The cells were transfected with 12 μg (for 60 mm dish) or 2 μg (for 12-well plate) of expression vectors for FLAG-tagged wild-type AGMO, D223A or Q197A mutants, and empty vector as control using Lipofectamine 2000 (Invitrogen). The cells on the 12-well plate were also co-transfected with 0.5 μg of the LPCAT2 expression vector together with AGMO expression vectors. For PC composition analysis, cells on 12-well plates were extracted with 500 μl/well of methanol. For lyso-PAF and PAF analysis, 6 ng of lyso-PAF-d4 and 6 ng of PAF-d4 were added to the methanol extracts and cleaned up with Oasis HLB cartridges (Waters, Milford, MA) as described [18].

2.10. Western blot analysis

Total protein (2.5 μg) in the cell extracts was resolved by SDS–PAGE with 10% gels and transferred to a Hybond ECL nitrocellulose membrane (GE Healthcare, Little Chalfont, UK). The membrane was probed with either an anti-FLAG M2 antibody (Sigma–Aldrich) or an anti-calnexin antibody (BD, Franklin Lakes, NJ), and the HRP-conjugated anti-mouse IgG secondary antibody (GE). Immunoreactive signals were detected using ECL reagents (GE) and a LAS-4000 mini image analyzer (Fuji Film, Tokyo, Japan).

2.11. Liquid chromatography–mass spectrometry

Liquid chromatography MS (LC–MS) systems were used for quantitative analysis of GPC, lyso-PAF, and PAF, and profiling analysis of phosphatidylcholines. Acquity UPLC (Waters) or Nexera UHPLC (Shimadzu, Kyoto, Japan) were connected to TSQ Quantum Ultra (Thermo Fischer Scientific, Waltham, MA), TSQ Vantage (Thermo), LCMS-8040 (Shimadzu), or LCMS-8080 (Shimadzu) mass spectrometers. GPC was resolved by Acquity BEH HILIC column (1.0 × 50 mm, Waters) using 20 mM NH_4HCO_2 and acetonitrile as the mobile phases. GPC was monitored by selected reaction monitoring (SRM) transition of 258.2→104 in a positive electrospray ionization (ESI) mode and quantified by an external calibration method. Lyso-PAF, PAF, and their deuterated compounds were separated by either an Acquity BEH C8 column (1.0 × 100 mm, Waters) or a Kinetex C8 column (2.1 × 150 mm, Phenomenex, Torrance, CA) using either 20 mM NH_4HCO_3 and acetonitrile or 0.05% formic acid–water and acetonitrile, respectively. Lyso-PAF and lyso-PAF-d4 were monitored by a SRM transition of 482.3→184 and 486.3→184, respectively, in positive ESI. PAF and PAF-d4 were ionized as adduct ions in negative ESI, depending on the mobile phases used. SRM transitions of M→59 were used to detect PAF and PAF-d4, where M represents molecular related ions. Lyso-PAF and PAF were quantified by the internal standard method using deuterated compounds. A profiling analysis of PC was performed using Acquity BEH C8 column (2.1 × 100 mm) using 20 mM NH_4HCO_3 and acetonitrile as the mobile phases. SRM transitions of M→184 in positive ESI, where M is *m/z* of molecular related ions, were used. Peak areas of individual PC molecular species were normalized to the sum of the peak areas detected.

2.12. Statistics

All statistical calculations were performed using Prism 5 (GraphPad Software, San Diego, CA). $p < 0.05$ was considered statistically significant.

3. Results

3.1. Downregulation of AGMO following LPS stimulation in mouse peritoneal macrophages and RAW264.7 cells

We first examined the effects of LPS treatment on the expression levels of AGMO in thioglycollate-induced peritoneal macrophages and RAW264.7 cells. LPS, a TLR4 ligand, is one of the major activators of macrophages and induces enhanced production

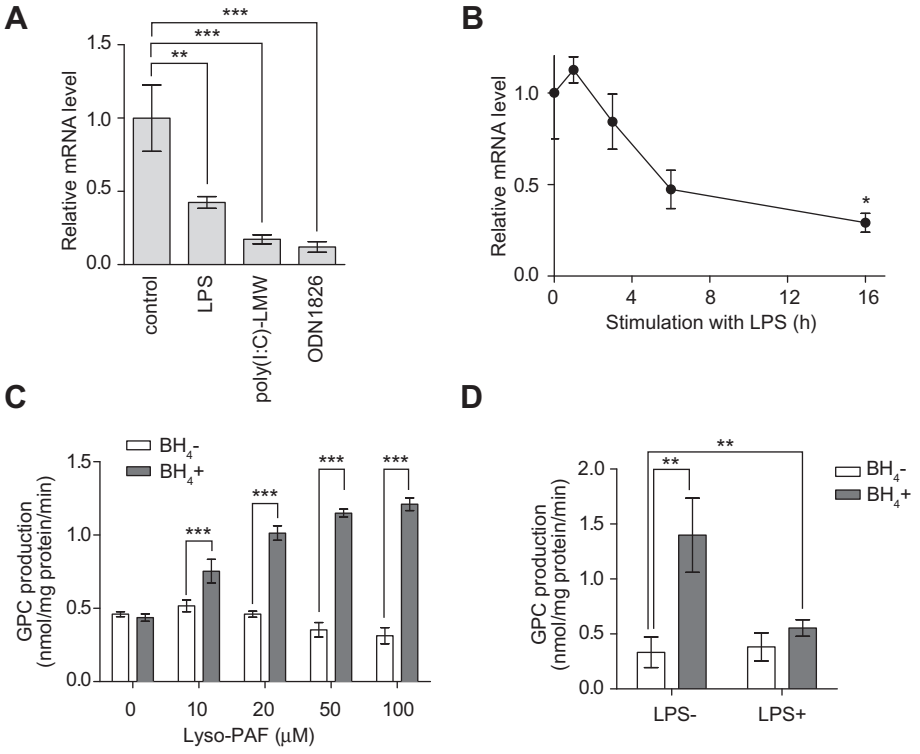

Fig. 1. LPS downregulates *Agmo* gene expression and increases lyso-PAF and PAF in macrophages. (A) Thioglycollate-induced mouse peritoneal macrophages were treated with TLR ligands (100 ng/ml LPS (TLR4 ligand), 1 μg/ml poly(I:C)-LMW (TLR3 ligand), or 0.8 μM ODN1826 (TLR9 ligand)) or PBS (control) for 16 h, and AGMO mRNA expression levels were determined. Data are expressed as normalized values using housekeeping *Rplp0* gene expression levels. Values represent mean ± s.d. ($n = 3$). $^{**}p < 0.01$, $^{***}p < 0.001$ by Bonferroni post hoc test after ANOVA. (B) AGMO mRNA expression levels in RAW 264.7 cells after LPS treatment (100 ng/ml). Results are shown as relative expression levels compared with time zero after normalization to *Gapdh* expression levels. Values represent means ± s.d. ($n = 3$). $^{*}p < 0.05$ vs. 0 h, by Dunnett's post hoc test after ANOVA. (C) GPC production activity of the microsomal fraction isolated from RAW 264.7 cells, in the presence or absence of BH_4. Concentrations of lyso-PAF added to the assay buffer are indicated. Data are expressed as mean ± s.d. ($n = 3$). $^{***}p < 0.001$, by Bonferroni post hoc test after two-way ANOVA. (D) Effect of LPS treatment on GPC production activity of RAW264.7 cells. Cells were treated with or without LPS for 16 h and microsomal membrane fractions were prepared. Data are expressed as mean ± s.d. ($n = 3$). $^{**}p < 0.01$, by Bonferroni post hoc test after ANOVA.

of PAF in the remodeling pathway. We found that AGMO mRNA expression was reduced following LPS-stimulation in both peritoneal macrophages and RAW264.7 cells (Fig. 1A and B). We also examined the effect of the TLR3 ligand (poly(I:C)) and the TLR9 ligand (ODN1826) on AGMO mRNA expression. Both ligands caused a reduction in AGMO mRNA expression levels, suggesting that a reduction in AGMO may be by common signaling in innate immune system (Fig. 1A). In RAW264.7 cells, AGMO mRNA levels decreased by 3 h after LPS stimulation and continued to decrease, resulting in a 70% reduction at 16 h (Fig. 1B).

Lyso-PAF is a precursor of PAF and a candidate substrate for AGMO [2,3]. We analyzed the AGMO enzymatic activity in

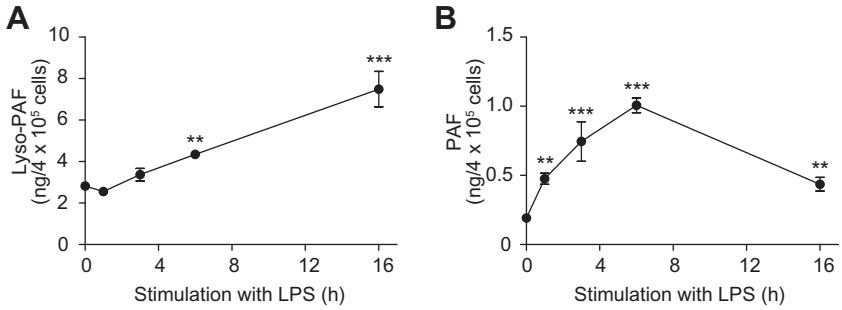

Fig. 2. Lyso-PAF and PAF levels in RAW264.7 cells after LPS treatment. Cells were plated onto a 12-well plate (4×10^5 cells/well) and cultured for 26 h in total. They were incubated with LPS (100 ng/ml) for the periods indicated before harvesting. Cellular lyso-PAF (A) and PAF (B) amounts were determined by LC–MS. Data are expressed as mean ± s.d. ($n = 3$). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. 0 h, by Dunnett's post hoc test after ANOVA.

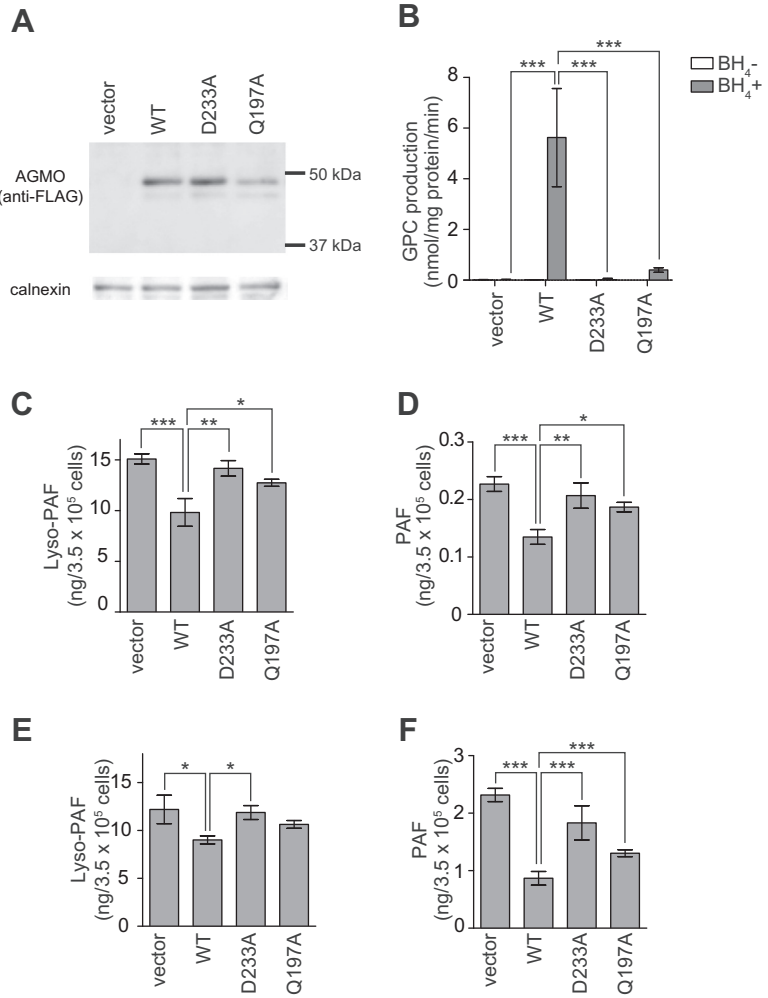

Fig. 3. Effects exogenous expression of AGMO on cellular lyso-PAF and PAF levels in HEK293 cells. (A) Western blot analysis of FLAG-tagged, wild-type, and mutant AGMO expressed in HEK293 cells. Cells were collected at 24 h post-transfection and microsomal fractions were analyzed. Vector, pCXN2.1 empty vector. Calnexin, loading control. (B) GPC production activity of AGMO and mutants expressed in HEK293 cells, using lyso-PAF as substrate. Data are expressed as mean ± s.d. ($n = 4$). (C–F) Lyso-PAF (C and E) and PAF (D and F) levels of HEK293 cells transfected with wild-type and mutant AGMO expression vectors. Cells were transfected in a 12-well plate, and lipids were extracted 16 h after transfection. In (E) and (F), cells were co-transfected with LPCAT2. Data are expressed as mean ± s.d. ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ by Bonferroni post hoc test after ANOVA.

microsomal fractions isolated from RAW264.7 cells. AGMO activity has been characterized as the activity that cleaves the *O*-alkyl bond of ether lipids and is dependent on a well-characterized cofactor (BH_4) [1,16]. In our assays of AGMO activity, lyso-PAF was used as a substrate, and the resulting GPC was quantified by LC–MS. Microsomal fractions isolated from RAW264.7 cells had activity that converts lyso-PAF to GPC in a BH_4-dependent manner (Fig. 1C). BH_4-independent GPC production did not increase with the addition of lyso-PAF, thus indicating that basal GPC production in the microsomal fraction is unrelated to AGMO activity (Fig. 1C). Next, we examined the AGMO activity of the microsomal fraction isolated from cells treated with LPS for 16 h. An 80% reduction of the BH_4-dependent GPC production activity was observed in microsomal fractions isolated from LPS-stimulated cells compared with untreated cells, while BH_4-independet GPC production was not altered (Fig. 1D). These observations are the first reports that AGMO expression in macrophages is regulated by inflammatory stimuli.

3.2. Increase in lyso-PAF and PAF levels by LPS, coinciding with a reduction in AGMO expression

We measured lyso-PAF and PAF levels in LPS-stimulated RAW264.7 cells. Lyso-PAF levels increased by 3 h after treatment with LPS and remained elevated for at least 16 h (Fig. 2A). These observations are similar to the time-course of the reduction in AGMO mRNA levels (Fig. 1A). PAF levels were also elevated in response to LPS, reaching its peak at 6 h (Fig. 2B). PAF levels then decreased at 16 h, though they were still at a higher level than that of untreated cells. The reduction in PAF levels at 16 h may be caused by the elevation of PAFAH that has been reported to occur several hours after LPS stimulation [19]. The up-regulation of lyso-PAF levels, coincided with the down-regulation of AGMO mRNA and enzymatic activity, raises the possibility that AGMO modulates cellular lyso-PAF levels and, subsequently, PAF production.

3.3. Effect of overexpression of AGMO on lyso-PAF and PAF levels in HEK 293 cells

To assess the relationship between cellular lyso-PAF and PAF levels, and AGMO enzymatic activity more directly, we performed transient transfection experiments in HEK293 cells. Microsomal fractions isolated from HEK293 cells transfected with AGMO expression plasmids showed BH_4-dependent AGMO activity, while cells transfected with an empty vector had no such activity (Fig. 3A and B). A previous report showed that several point mutations of human AGMO reduced enzymatic activity [20]. We made two mutant constructs for murine AGMO, Q197A, and D233A. The protein expression levels of the D233A mutant were similar to those of wild-type AGMO, and the Q197A mutant showed reduced protein expression (Fig. 3A). Consistent with the previous report, microsomal fractions isolated from cells expressing the D233A mutant had no activity in our assays, while the Q197A mutant retained minimal activity (Fig. 3B).

We next measured cellular lyso-PAF and PAF levels in the transfected cells. Consistent with the *in vitro* enzyme activities, cells transfected with the wild-type AGMO showed reduced lyso-PAF levels, while cells transfected with the mutants did not show a reduction in levels (D233A) or the levels were reduced to a lesser extent (Q197A) (Fig. 3C). Remarkably, a similar pattern was observed for PAF levels in the cells transfected with the wild-type or mutant AGMO (Fig. 3D). To examine the effect of AGMO expression on PAF levels under conditions of higher PAF production that occurs in LPS-stimulated macrophages, cells were co-transfected with LPCAT2 to promote PAF production from lyso-PAF. The effects of the wild-type and mutant AGMO on cellular lyso-PAF levels were very similar to those observed in cells not co-transfected with LPCAT2 (Fig. 3E and F). LPCAT2 elevated PAF levels by 10-fold, but co-expression of the wild-type AGMO markedly suppressed the levels. Compared to the wild-type AGMO, the two mutants had little or no effect on the reduction of cellular PAF levels (Fig. 3F).

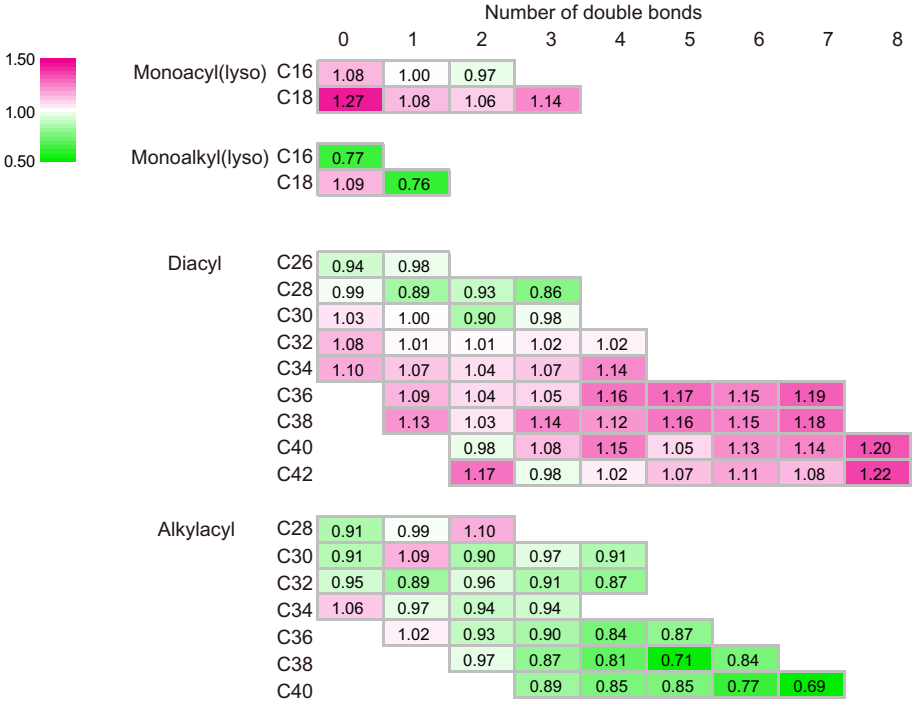

Fig. 4. Changes in PC composition by exogenous AGMO expression in HEK293 cells. Cells in 12-well plates were transfected with the wild-type AGMO or an empty vector for 24 h. Phospholipids were analyzed by LC–MS. PC molecular species are sorted by fatty carbon numbers and double bonds, and data are expressed as fold-changes to control vector-transfected cells (averages of quadruplicates).

These results indicate that AGMO enzymatic activity does, indeed, modulate PAF production levels.

3.4. Effect of AGMO expression on diradylPC composition

Lyso-PAF is not only a precursor for PAF, but can be also converted to PCs, which are major components of cellular membranes. To examine whether AGMO expression influences membrane lipid composition, we analyzed PC composition of HEK293 cells 24 h after transfection with AGMO. Expression of AGMO led to a reduction in the relative levels of several alkylacylPCs (Fig. 4). The reduction was most pronounced for PCs having long and polyunsaturated fatty chains. Several diacylPCs containing long and polyunsaturated fatty chains increased, which may be related to the reduction in alkylacylPCs through the relative availability of substrates for LPCATs or some other cellular compensatory mechanism (Fig. 4). Similar compensatory increase in dicayl phospholipids has been reported in ether-lipid-deficient mice [21]. Our results demonstrate that AGMO expression may modulate not only lyso-PAF (alkyl-lyso-PC, C16:0) but a wide-range of PCs.

4. Discussion

In the present report, we demonstrated a possible role for AGMO in macrophage PAF production. BH_4-dependent AGMO activity was first reported in 1964 using batyl alcohol (1-*O*-octadecylglycerol) as a substrate [1]. Later, substrate specificity of AGMO was investigated *in vitro*, and lyso-PAF was considered as a biological substrate candidate for AGMO [2,3]. Although AGMO could cleave the *O*-alkyl bond of PAF itself *in vitro*, previous studies showed that BH_4-dependent enzymatic activity was much lower for PAF when compared to lyso-PAF [2,3]. Moreover, the amount of the cellular PAF level was lower than lyso-PAF in RAW264.7 cells. Thus, we assume that the reduction in cellular PAF levels we observed was mainly caused by degradation of lyso-PAF by AGMO rather than that of PAF. The remodeling pathway for PAF synthesis is thought to be responsible for the formation of PAF from lyso-PAF in response to inflammatory and immune stimuli, while the *de novo* pathway for PAF synthesis is believed to be more constitutive. Despite structural similarities to lyso-PAF, previous studies showed that none of the 1-*O*-alkyllipids in the *de novo* pathway for PAF synthesis was a good substrate for BH_4-dependent enzyme activity [7,22]. Other ether lipids, such as plasmalogens (suggested to be involved in the myelination process [23]), monoalkylglycerol (which induces PKC inhibition in cell cultures of MDCK cells [24] and affects adipogenesis [25]), and alkyl-LPA (which may play a role in the nervous system [26], skeletal muscle [27] and platelets [28]), can be directly or indirectly affected by AGMO but await further investigation.

In conclusion, we propose a novel regulatory mechanism for PAF production mediated by AGMO, an enzyme whose expression is down-regulated by LPS by altering the cellular levels of lyso-PAF. The actual function of AGMO in the contribution to PAF levels will be revealed by experiments *in vivo* in the future.

Acknowledgments

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