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Monoacylglycerol lipase promotes Fcγ receptor-mediated phagocytosis in microglia but does not regulate LPS-induced upregulation of inflammatory cytokines

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ABSTRACT

Monoacylglycerol lipase (MAGL) is important for neuroinflammation. However, the regulatory mechanisms underlying its expression and function remain unknown. Lipopolysaccharide (LPS) treatment post-translationally upregulated MAGL expression, whereas it downregulated MAGL transcription through a Stat6-mediated mechanism in microglia. Neither MAGL knockdown nor JZL-184, a selective MAGL inhibitor, suppressed LPS-induced upregulation of inflammatory cytokines in microglia. Moreover, exogenous expression of MAGL in BV-2 microglial cell line, which lacks endogenous MAGL, did not promote the induction of inflammatory cytokines by LPS treatment. Interestingly, MAGL knockdown reduced Fcγ receptor-mediated phagocytosis in primary microglia, and introduction of MAGL into the BV-2 cells increased Fcγ receptor-mediated phagocytosis. Collectively, these results suggest that MAGL regulates phagocytosis, but not LPS-mediated cytokine induction in microglia.

1. Introduction

Monoacylglycerol lipase (MAGL) hydrolyzes 2-arachidonoylglycerol (2-AG) and terminates endocannabinoid (eCB) signaling, which regulates production of arachidonic acid (AA) and prostaglandins (PG) that mediate neuroinflammation in the brain [1]. Recent studies have shown that MAGL mediates the pathogenesis of several neurological disorders, such as Alzheimer's disease, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -induced dopaminergic neurodegeneration, multiple sclerosis, and ischemia [2–6]. MAGL knockout mice or JZL-184-pretreated mice that were administered LPS neither exhibit microglial activation nor produce inflammatory cytokines such as interleukin 1β (IL1β) or tumor necrosis factor α (TNFα). Moreover, A significant reduction in production of AA and PG is observed, indicating that MAGL has a predominant role in neuroinflammation through 2-AG hydrolysis in brain [7]. However, the identity of the cells that play a predominant role in MAGL signaling during pathological progression in the brain is yet to be determined [1–7].

In microglia, several Toll-like receptors (TLRs) are activated in response to pathogen- or danger-associated molecular patterns, thus initiating innate and adaptive immune responses [8,9]. Studies using knockout mice have shown that activation of TLR signaling in microglia has important roles in regulation of pathological outcome, and NF-κB functions as a nuclear effector molecule inducing several inflammatory cytokines through signaling cascades emanating from TLRs [10–13]. These studies strongly suggest that MAGL in microglia plays a crucial role in TLR-mediated neuropathology and mediates neuroinflammation. Moreover, several transcription factors involved in anti-inflammatory signaling such as Stat6 and c-myc have been shown to mediate the induction of pro-inflammatory cytokines by preventing NF-κB activation in inflammatory reactions [14–19]. Under normal conditions, Stat6 interacts with CBP/p300 independent of treatment with anti-inflammatory cytokines, including IL-4 [17,20]. In inflammatory conditions, CBP/p300 is recruited to DNA-bound p65-p50 heterodimer, resulting in the production of inflammatory cytokines. Integration of these factors is required for maximal NF-κB signaling in inflammatory conditions [21], suggesting that Stat6 may compete with NF-κB for the limited amounts of these

Abbreviations: MAGL, monoacylglycerol lipase; AA, arachidonic acid; 2-AG, 2-arachidonoylglycerol; FcγR, Fcγ receptor; IL1β, interleukin1β; KD, knockdown; LPS, lipopolysaccharide; PLA, phospholipase A; TLR, toll-like receptor; TNFα, tumor necrosis factor α.

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transcriptional co-activators and regulates the neuroinflammatory condition in microglia [16].

Here, the regulatory mechanism of MAGL expression upon LPS treatment was analyzed by focusing these transcription factors. Interestingly, Stat6 is required for microglial MAGL transcription, and its expression is regulated by transcriptional downregulation as well as by potentiation of its stability in the presence of LPS. Furthermore, MAGL function in microglia during inflammatory conditions was elucidated by using shRNA-mediated knockdown (KD) or treatment with JZL-184, a MAGL-specific inhibitor. Unexpectedly, loss of MAGL in microglia or introduction of FLAG-MAGL into BV-2 cells did not affect the induction of inflammatory cytokines upon LPS treatment but regulated Fcγ receptor (FcγR)-mediated phagocytosis.

2. Materials and methods

2.1. Reagents and cell culture

2-AG and BAY11-7082 were purchased from Cayman Chemicals and SantaCruz Biotechnology, respectively. LPS was obtained from Sigma. AS1517499 and 10058-F4 were obtained from Axon Medchem and Focus Biomolecules, respectively. pSIH-copGFP H1 short hairpin (sh)RNA expression lentivector was obtained from System Biosciences. The primary antibodies used were anti-MAGL (Cayman Chemicals), anti-GAPDH (Millipore, clone 6C5), and anti-FLAG (Wako Chemicals). The sequences of the two shRNA targeting MAGL and the scrambled negative control are 5′-gaaggtc cttgctgccaaact-3′ (KD1), 5′-ggaacactgtggccgttatga-3′ (KD2), and 5′-caacaagatgaagagcaccaa-3′ (scrambled), respectively. These expression cassettes were subcloned into the pSIH-H1 vector by using BamHI and EcoRI. The cDNA encoding MAGL was subcloned from mouse brain and integrated into 3xFLAG-pcDNA7.1 (Sigma) and pLVSIN (Invitrogen) vectors. The mouse microglial cell line BV-2 was a kind gift from Dr. Tsuda (Kyushu University). Primary culture of microglia was prepared from mouse pups at postnatal day 2 (P2). Mixed glia were cultured in a poly-D-lysine-coated flask in DMEM containing 10% FBS for 10–14 days in a CO_2 incubator. The mixed glial culture was shaken to dislodge microglia that were loosely attached on astrocytes, and purified microglia were seeded on poly-D-Lys-coated tissue culture dishes.

2.2. Transfection and lentiviral KD/expression

In total, 1.5×10^5 BV-2 microglial cells were plated on 24-well tissue culture dishes in DMEM containing 10% FBS for 24 h, and cells were transfected with MAGL expression vector by using Lipofectamine LTX (Invitrogen). Cells were treated with LPS (1 μg/ml) 48 h after transfection and were incubated for 24 h. Cells were fixed with 4% paraformaldehyde in PBS and immunostained with anti-FLAG antibody. For enzymatic assay for MAGL activity, 2-AG was used as a substrate for the lysates of cells expressing FLAG-MAGL, and the assay was performed using the glycerol colorimetric assay kit, as described previously [22].

pSIH-copGFP shRNA containing scrambled oligonucleotides or target sequences against MAGL (MAGL KD1 and KD2) was

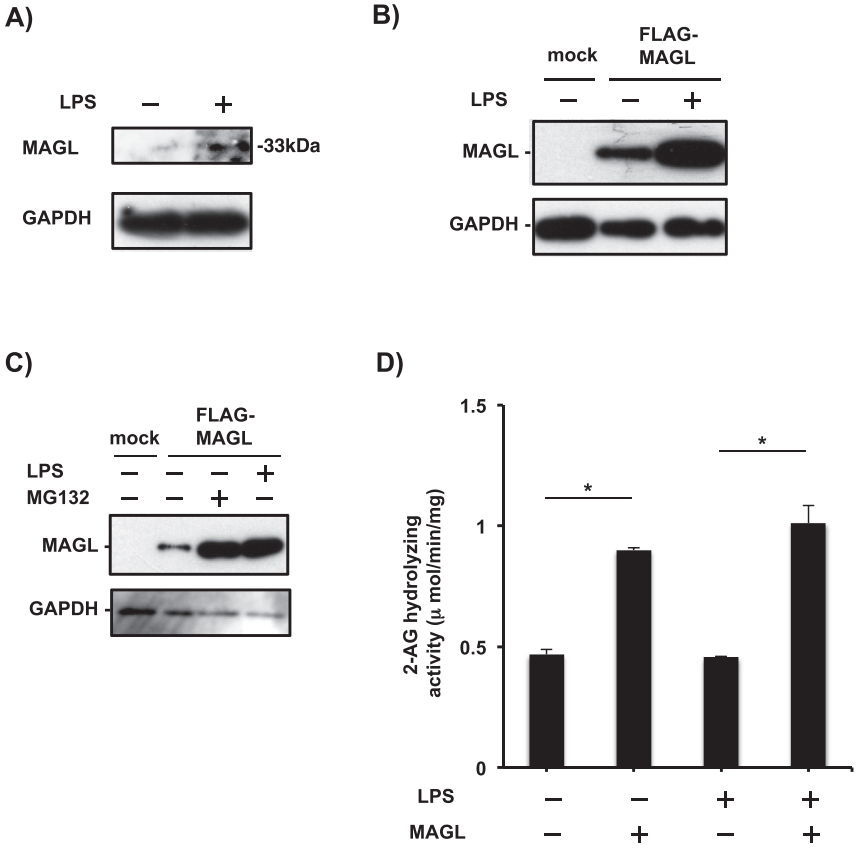

Fig. 1. LPS regulates MAGL expression and activity in primary microglia and the BV-2 cell line. A) Cell lysates from mouse microglia, treated with or without LPS, were resolved by SDS-PAGE and immunoblotted for MAGL and GAPDH. B) Lysates from BV-2 microglial cell lines, with or without transient expression of FLAG-MAGL, were subjected to immunoblotting with anti-FLAG or GAPDH antibodies. C) BV-2 cells with stable FLAG-MAGL expression were treated with LPS or MG132, and the amounts of FLAG-MAGL were determined by immunoblots probed with anti-FLAG or GAPDH antibodies. D) 2-AG hydrolyzing activity in BV-2 lysates transiently expressing FLAG-MAGL was measured using the glycerol colorimetric assay method and was indicated as specific activity [22]. Data are represented as mean ± SEM of three experiments. Tukey's test: $^*p < 0.05$.

transfected into HEK293T cells (Packaging cells) with LP1, LP2, and VSVG-LP vectors (Invitrogen) by using Lipofectamine LTX. Lentiviral particles were collected after transfection. Viral particles at multiplicity of infection (MOI) of ~10 were incubated with the microglial culture for 72 h, and cells were treated with 1 μg/ml LPS for 24 h. For the stable expression of MAGL, pLVSIN vector encoding FLAG-MAGL was transfected into HEK293T cells, as described above, and transduced cells were selected in DMEM containing 10% FBS in the presence of 1 μg/ml puromycin. For the transient transfection of MAGL, control vector or 3xFLAG pcDNA7.1 encoding MAGL was introduced into BV-2 cells, and transfected cells were used for immunocytochemistry.

2.3. Expression analysis of MAGL and inflammatory cytokines

RNA from primary microglia or BV-2 cells was extracted by using ReliaPrep™ RNA Cell Miniprep System (Promega), and quantitative real-time RT-PCR (qRT-PCR) was performed using THUNDERBIRD™ SYBR qPCR system. The following primers were used for qRT-PCR analysis: 5′-ggaacactgtggccgttatg-3′ and 5′-ctgacaaaaacttggaagtccga-3′ for *MAGL*; 5′-agtccatgccatcactgccac-3′ and 5′-tgtcatcatacttggcaggtttc-3′ for *GAPDH*; 5′-accacttcacaagtcggaggc-3′ and 5′-ggtagcatccatcatttctttg-3′ for *IL-6*; 5′-ccatgagctttgtacaaggaga-3′ and 5′-tgcagactcaaactccactttg-3′ for *IL-1β*; 5′-acccagtgccctgctttgtg-3′ and 5′-ttcttcctgatagaggtggtcc-3′ for *iNOS*; 5′-gctcttctgtctactgaacttc-3′ and 5′-ggttgtctttgagatccatgcc-3′ for *TNFα*. For immunoblot analysis, cells were lysed with lysis buffer (Tris–HCl (pH 7.4), 1% SDS, 0.15 M NaCl, and protease inhibitor cocktail), and lysates were subjected to SDS-PAGE, transferred to PVDF membrane (Millipore), and probed with the antibodies mentioned above. Blots were incubated with secondary antibodies for 30 min (Dako Cytomation) and the signals were detected using an electrochemiluminescence (ECL) system (Wako Chemicals).

2.4. Fcγ receptor-mediated phagocytosis of IgG beads

Texas-Red-labeled anti-streptavidin IgG antibody was incubated with avidin-coated polystyrene particles (1 or 3 μm in diameter, Spherotech) in PBS containing 1% BSA at 37 °C for 1 h, then washed 3 times, and suspended in PBS with 1% BSA. Mouse microglia or BV-2 cells were cultured on poly-D-Lys-coated coverslips in 24-well tissue culture dishes, and incubated with prepared Texas-Red-conjugated IgG beads (1 μm in diameter) for 1 h, or with 3 μm IgG beads for 30 min, respectively. After washing with PBS three times, cells were fixed in PBS containing 4% paraformaldehyde for 15 min. Fixed cells were stained with DAPI, and phagocytic assay was performed by counting GFP-positive microglia or transfected

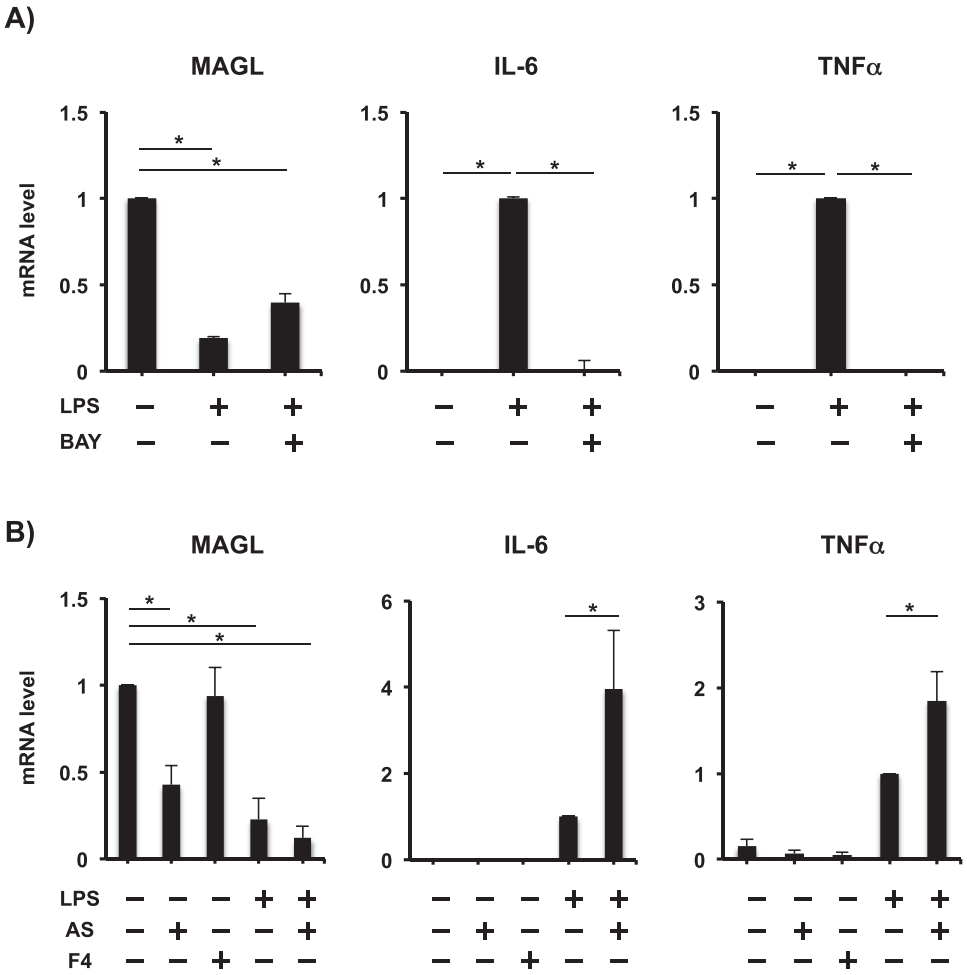

Fig. 2. MAGL is transcriptionally regulated by Stat6, but not by c-myc, in response to LPS. A) Mouse microglia were pretreated for 1 h with 3 μM BAY11-7082, a IκB inhibitor, prior to LPS treatment. RNA was extracted 8 h after LPS treatment, and qRT-PCR analysis was performed for MAGL, IL-6, and TNFα. B) Effects of AS1517499 and 10058F-4 on MAGL expression were analyzed by qRT-PCR. Treatment with AS1517499 exhibited significant reduction in MAGL expression, suggesting that Stat6 regulates MAGL transcription in microglia. Data are represented as mean ± SEM of three experiments. Dunnet's test: $^{*}p < 0.05$.

BV-2 cells by using a confocal laser-scanning microscope (OLYMPUS FV1000).

2.5. Statistical analysis

Each experiment was performed with at least three independent preparations and mean ± SEM values are shown in bar diagrams. Data from expression analysis and phagocytic assay were analyzed by *post-hoc* test of pairwise difference followed by Tukey's or Dunnett's method. A p value of <0.05 was considered to be significant.

3. Results and discussion

3.1. MAGL is transcriptionally and post-translationally regulated by LPS in microglia

MAGL expression is variable among cell types, and the mechanism by which MAGL expression in microglia is regulated during inflammation remains unknown [23–26]. Since MAGL is important for inflammatory response mediated by prostaglandins in the brain [7], primary microglia isolated from neonatal mice were incubated in presence or absence of LPS for 24 h. Upregulation of MAGL

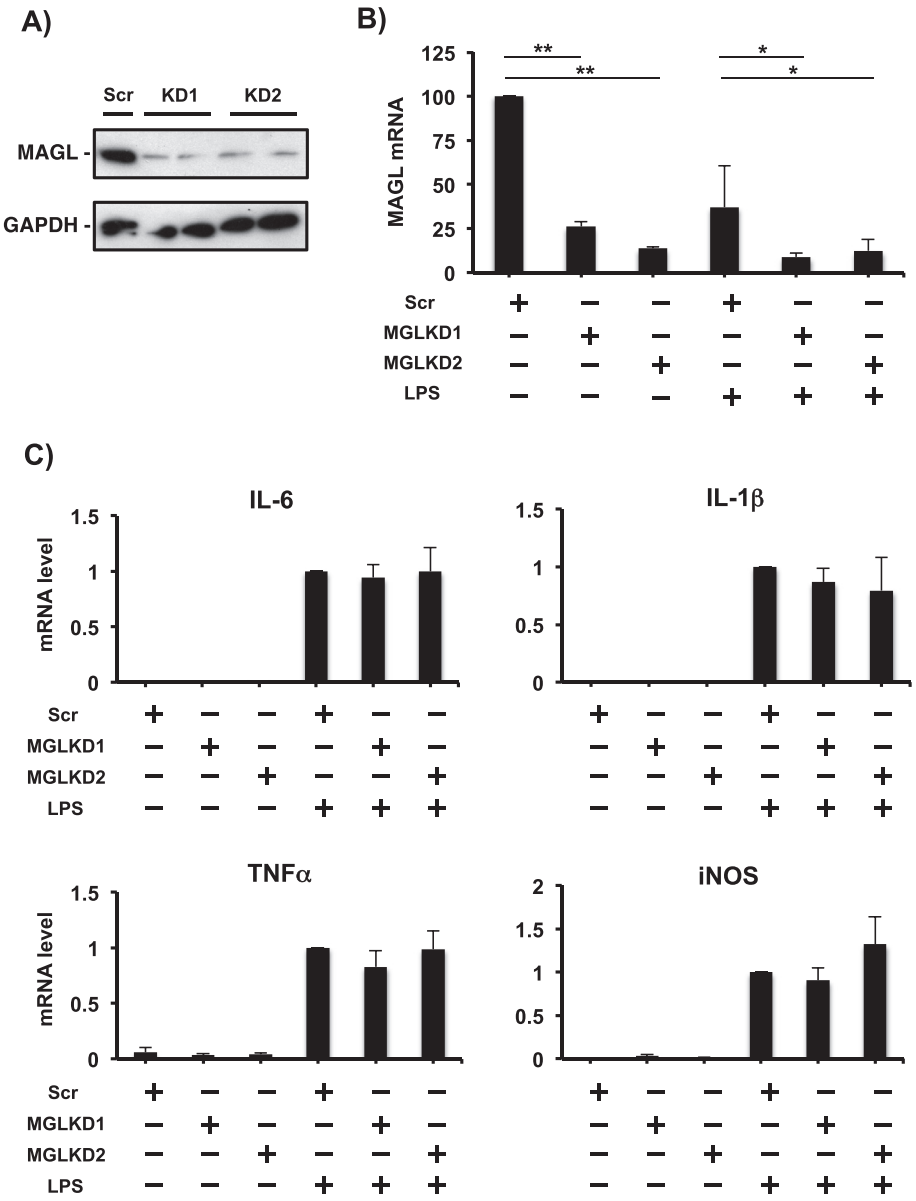

Fig. 3. Neither MAGL KD nor JZL-184 treatment affects induction of inflammatory cytokines in mouse microglia. A) HEK293 cells transiently expressing FLAG-MAGL were simultaneously transfected with lentiviral vectors carrying scrambled or specific target sequences against MAGL, and expression of FLAG-MAGL was analyzed by immunoblotting with anti-FLAG or GAPDH antibodies. B) MAGL mRNA levels in primary microglia transduced with scramble or MGL shRNAs were analyzed by qRT-PCR. Data are presented as mean ± SEM of three experiments. Dunnet's test: $^{*}p < 0.05$, $^{**}p < 0.01$. C) mRNA levels of inflammatory cytokines from control or MAGL KD microglia treated with or without LPS were determined by qRT-PCR. GAPDH was used as an internal control, and quantification of mRNAs levels was performed in duplicate and represented as mean ± SEM from three experiments. There was no significant difference in the cytokine expression between control and MAGL KD cells. D) mRNA levels of inflammatory cytokines from primary microglia treated with or without LPS and JZL-184 were quantified by qRT-PCR. Data are presented as mean ± SEM of three experiments. There was no significant difference in the cytokine expression between control and JZL-184 treated cells. E) mRNA levels of inflammatory cytokines from control or FLAG-MAGL transfected BV-2 cells treated with or without LPS were determined by qRT-PCR analysis. Data were represented as mean ± SEM of three experiments and there was no significant difference in *IL-6, IL-1β, and TNFα* expression between control and FLAG-MAGL transfected BV-2 cells.

expression was detected in the presence of LPS by western blot analysis (Fig. 1A). FLAG-MAGL was also introduced into the microglial BV-2 cell line, which lacks endogenous MAGL expression [25]. LPS enhanced the stability of the exogenous FLAG-MAGL expressed in BV-2 cells (Fig. 1B). It has been shown that its primary sequence possesses a putative destruction box motif (D-box) and MAGL turnover is spatiotemporally regulated by proteasomal degradation system in axon and dendrites [26]. Therefore, BV-2 cell lines stably expressing FLAG-MAGL were also established by lentiviral transduction and its proteolytic regulation was analyzed. When MAGL-transfected cells were treated with the proteasome inhibitor MG132, their expression levels were restored to levels similar to that in LPS-treated cells, suggesting that MAGL is rapidly degraded by proteasomes in the absence of LPS (Fig. 1C). Although the control BV-2 cell lysate possessed 2-AG hydrolyzing activity in the absence of MAGL, as described previously [25], introduction of FLAG-MAGL induced higher 2-AG hydrolyzing activity, and LPS treatment promoted MAGL activity, suggesting that functional MAGL is increased during inflammatory conditions (Fig. 1D).

Expression of several lipid-metabolizing enzymes is transcriptionally regulated by LPS treatment [27]. Therefore, *MAGL* mRNA levels were examined by qRT-PCR analysis in primary microglia. Transcription of *MAGL* mRNA was downregulated, although that of *IL-6* and *TNFα* mRNA were induced in the presence of LPS (Fig. 2A, middle and right panel). NF-κB is known to regulate the production of several cytokines in microglia under the inflammatory condition [12,15]. Unexpectedly, treatment with BAY11-7082, an irreversible IκBα inhibitor, partially restored *MAGL* mRNA expression, whereas it completely inhibited *IL-6* and *TNFα* expression, suggesting that NF-κB may not play a major role in MAGL suppression (Fig. 2A, left panel). Several anti-inflammatory transcription factors such as Stat6 and c-myc are known to be involved in the regulation of the progress of acute inflammatory responses [14,16]. Stat6 and c-myc have been shown to be associated with CBP in resting condition and

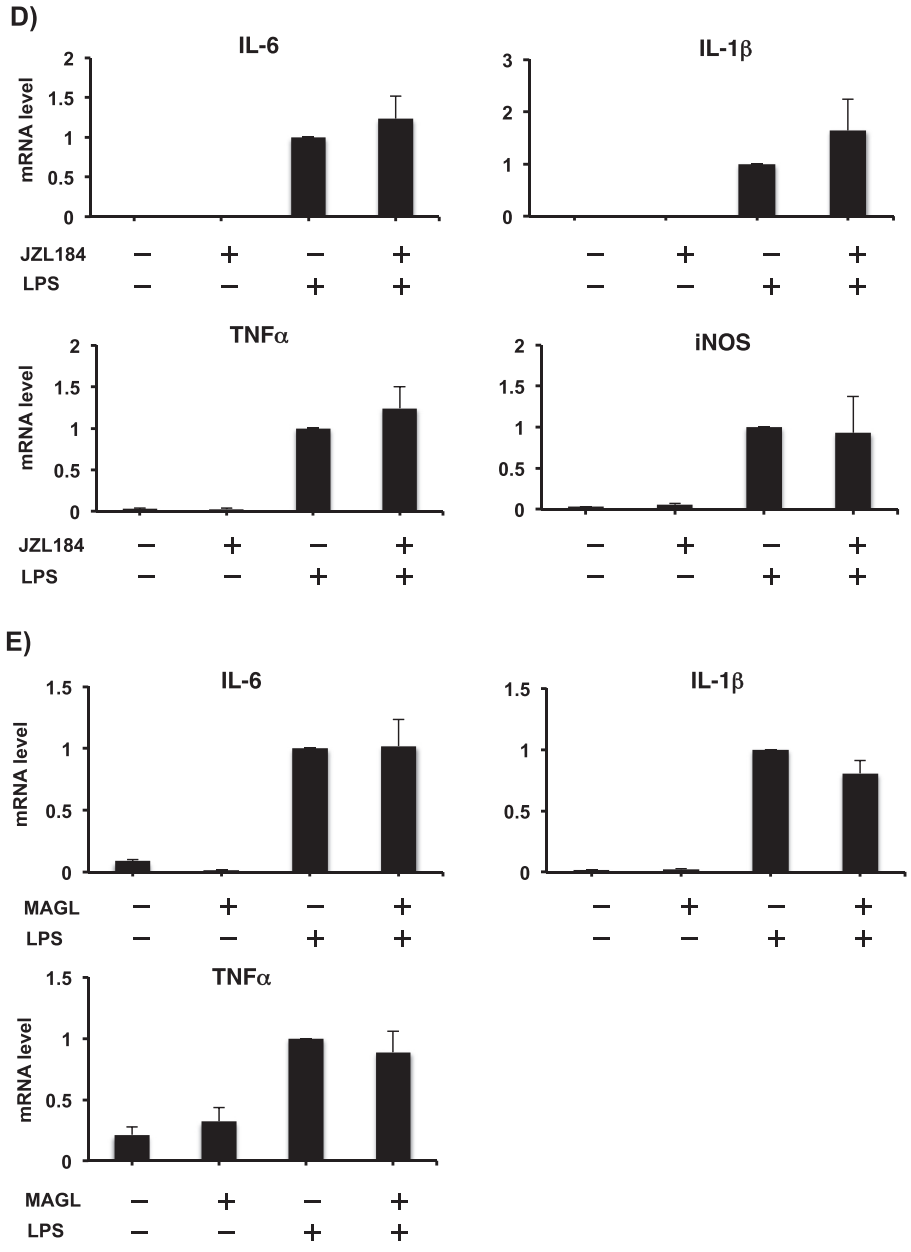

Fig. 3. (*continued*).

in the presence of anti-inflammatory cytokines; however, they counteract NF-κB signaling in inflammatory conditions [16–20]. CBP/p300 is required for full activation of one of the NF-κB complexes and is sequestered from these transcription factors in the absence of LPS [21]. Therefore, there is a possibility that *MAGL* expression may be regulated by one of these anti-inflammatory transcription factors. To investigate whether they could be involved in MAGL expression microglia were treated for 24 h with 1 μM AS1517499, a specific Stat6 inhibitor, or with 60 μM 10058F-4, an inhibitor of c-myc transcriptional activity, prior to LPS treatment [18,19]. Treatment with AS1517499 caused significant reduction in *MAGL* mRNA level, whereas 10058-F4 did not affect *MAGL* expression, suggesting that Stat6, but not c-myc, is a major transcription factor regulating *MAGL* expression in microglia (Fig. 2B, left panel). *MAGL* expression was also decreased when cells were treated with Stat6 inhibitor in the presence of LPS; however, as expected, AS1517499 promoted *IL-6* and *TNF* induction by NF-κB upon LPS treatment, indicating that Stat6 negatively regulate NF-κB-dependent transactivation (Fig. 2B, middle and right panel). These results suggest that Stat6-mediated *MAGL* expression could be repressed by LPS-induced NF-κB-mediated sequestration of transcription factors such as CBP/p300 away from the Stat6-regulated MAGL promoter.

3.2. MAGL inhibition does not affect induction of inflammatory cytokines in microglia

Although MAGL knockout mice did not exhibit microglial activation or production of inflammatory cytokines when LPS was administered, it remains unknown which cell-derived MAGL plays a critical role in inflammation [7]. Therefore, the role of microglial MAGL in the induction of inflammatory cytokines was addressed by using lentivirus-mediated KD or treatment with JZL-184. When MAGL shRNA was introduced into HEK293T cells or primary microglia, efficient (approximately 80%–90%) MAGL KD was confirmed by expression analysis and GFP expression in transduced cells (Fig. 3A, B, Fig. 4A). Unexpectedly, MAGL KD in primary microglia did not affect production of inflammatory cytokines,

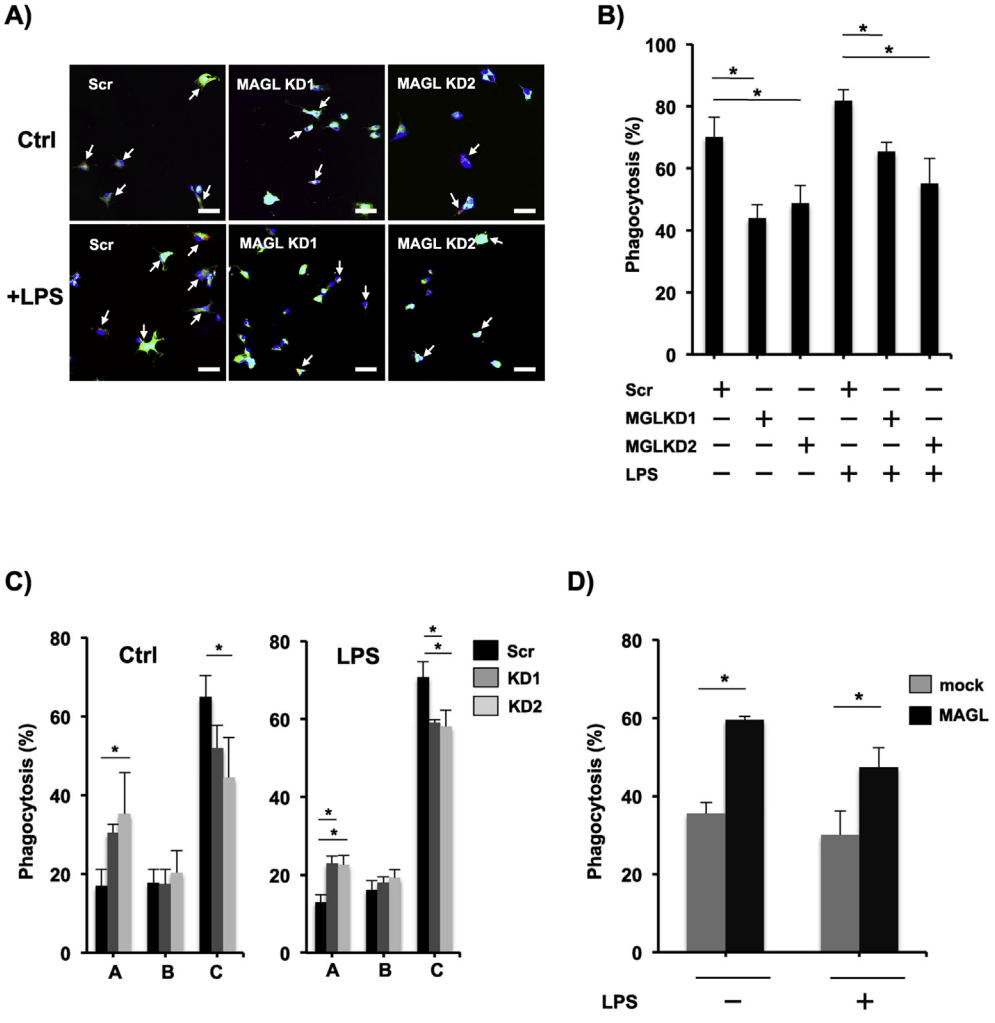

Fig. 4. MAGL promotes FcγR-mediated phagocytosis. A) GFP-positive control or MAGL KD microglia (green) were incubated with Texas-Red labeled IgG beads (red) for 1 h and DAPI (blue) was used to label cell nuclei. Arrows indicate GFP-positive phagocytotic microglia. Scale bars: 20 μm. B, C) FcγR-mediated phagocytosis was represented as the percentage of GFP-positive control or MAGL KD cells with IgG beads against total GFP-positive cells. The columns represent the mean ± SEM of three experiments and Dunnett's test showed the significant differences between control and KD cells (*$p < 0.05$). The population of control or MAGL KD phagocytes was categorized as the number of IgG beads per cell (A = 1, 2; B = 3, 4; C≧5), and the ratio of the corresponding GFP-positive phagocytes (B) and total phagocytes (C) to GFP-positive cells were represented by a bar graph. D) BV-2 cells transiently transfected with control or FLAG-MAGL were incubated with Texas-Red IgG beads for 30 min and subjected to immunofluorescence microscopy analysis, as described previously. Control cells or FLAG-MAGL expressing cells engulfing Texas-Red IgG were counted with respect to the total control or MAGL expressing cells. The data in the bar graph were presented as the mean ± SEM of three experiments. Dunnett's test showed the significant differences between control and MAGL-expressing cells (*$p < 0.05$).

including *IL-6*, *IL-1β*, *TNFα*, and *iNOS*, upon LPS treatment (Fig. 3C). To confirm the effects of MAGL KD JZL-184 (1 μM) was used as a specific MAGL inhibitor that shows stronger specific inhibitory preference against mouse MAGL in 2-AG hydrolysis (IC_{50} ~ 2 nM) than against rat MAGL [28]. Mouse microglia were pretreated with JZL-184 for 1 h, following which LPS was added to the control or MAGL KD cells for 24 h. Inflammatory cytokine induction was not affected by JZL-184 treatment, indicating that microglial MAGL is not required for cell-autonomous production of inflammatory cytokines (Fig. 3D). The effects of MAGL expression on the induction of inflammatory cytokines was also examined in BV-2 cells by qRT-PCR analysis. Although 2-AG hydrolyzing activity was promoted by MAGL expression in the presence of LPS (Fig. 1D), induction of *IL-6, IL-1β, and TNFα* was not upregulated by MAGL expression in BV-2 cells (Fig. 3E). These results indicate that lack of MAGL effect on LPS-dependent production of inflammatory cytokines in microglia is due to the absence of cell-intrinsic transactivating mechanism of the cytokines by MAGL in microglia. It has recently been reported that JZL-184 pretreatment with simultaneous exposure to LPS and INFγ inhibits NO and IL-1β production in astrocytes and microglia, respectively [29]. However, IFNγ response such as NO production is mainly regulated by Stat1, which is induced subsequently to NFκB signaling, and IFNγ has an inhibitory effect on LPS-induced IL1β production in macrophage and microglia [30–32]. Considering the pathological phenotype of MAGL knockout mice [7], it is likely that MAGL activity in neurons and other glia regulate the production of inflammatory cytokines when LPS administration was performed *in vivo* or MAGL function in cytokine signaling in neuron-glia network could be modulated by persistent inflammatory condition.

3.3. MAGL regulates Fcγ receptor-mediated phagocytosis in microglia

Several neurodegeneration models have elucidated the pathophysiological features of MAGL in microglia [2,7], and several AA metabolizing enzymes including soluble phospholipase A2 (sPLA2) are known to be activated by proinflammatory cytokines with morphological changes in astrocytes [33]. These findings raised the possibility that MAGL might regulate microglial homeostasis and phagocytosis in LPS treatment. When primary microglia were treated with LPS, the morphology of control microglia changed from amoeboid to polarized (Fig. 4A). However, MAGL KD microglia exhibiting GFP fluorescence showed shrunken morphology irrespective of LPS treatment (Fig. 4A). Systemic inflammation induced by LPS challenge is known to modulate FcγR expression, and an increase in FcγRIII expression was detected in mouse microglia upon LPS treatment (data not shown) [34]. When Texas Red-labeled IgG beads were added to cultured microglia, FcγR-mediated phagocytosis was promoted in the presence of LPS, and MAGL KD GFP-positive cells showed reduced phagocytic activities irrespective of LPS treatment (Fig. 4B, C). Furthermore, whether exogenous MAGL expression could promote FcγR-mediated phagocytosis was examined by using BV-2 cells, which lack MAGL expression, since BV-2 cells were also used for the FcγR-mediated phagocytic assay [35]. When BV-2 cells were transiently transfected with FLAG-MAGL, it stimulated their phagocytic activity. LPS treatment and MAGL expression did not affect FcγR expression (data not shown), indicating that MAGL positively regulates phagocytosis of microglia (Fig. 4D). Although cytosolic PLA catalyze AA release and translocate to phagosomes in phagocytes, MAGL was localized mainly in the cytosol as previously shown [2]. Furthermore, MAGL KD affected cell morphology, suggesting that MAGL regulates Fcγ receptor-mediated phagocytosis in microglia, independent of the PLA-dependent mechanism [36,37].

Conflict of interest

There is no conflict of interest in this manuscript.

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References

[1] G. Labar, J. Wouters, D.M. Lambert, A review on the monoacylglycerol lipase: at the interface between fat and endocannabinoid signaling, Curr. Med. Chem. 17 (2010) 2588–2607.
[2] J. Mulder, M. Zilberter, S.J. Pasquaré, et al., Molecular reorganization of endocannabinoid signaling in Alzheimer's disease, Brain 134 (2011) 1041–1060.
[3] J.R. Piro, D.I. Benjamin, J.M. Duerr, et al., A dysregulated-endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease, Cell. Reports 1 (2012) 617–623.
[4] R. Chen, J. Zhang, Y. Wu, et al., Monoacylglycerol lipase is a therapeutic target for Alzheimer's disease, Cell. Reports 2 (2012) 1–11.
[5] A. Bernal-Chico, M. Canedo, A. Manterola, et al., Blockade of monoacylglycerol lipase inhibits oligodendrocyte excitotoxicity and prevents demyelination *in vivo*, Glia 63 (2015) 163–176.
[6] S. Carloni, D. Alonso-Alconada, S. Girelli, et al., Pretreatment with the monoacylglycerol lipase inhibitor URB602 protects from the long-term consequences of neonatal hypoxic-ischemic brain injury in rats, Transl. Invest 72 (2012) 400–406.
[7] D.K. Nomura, B.E. Morrison, J.L. Blankman, et al., Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation, Science 334 (2011) 809–813.
[8] T. Kawai, S. Akira, Toll-like receptors and their crosstalk with other innate receptors in infection and immunity, Immunity 34 (2011) 637–650.
[9] K. Saijo, C.K. Glass, Microglial cell origin and phenotypes in health and disease, Nat. Rev. Immunol. 11 (2011) 775–787.
[10] T. Kawai, O. Adachi, T. Ogawa, et al., Unresponsiveness of MyD88-deficient mice to endotoxin, Immunity 11 (1999) 115–122.
[11] X. Wang, L. Stridh, W. Li, et al., Lipopolysaccharide sensitizes neonatal hypoxic-ischemic brain injury in a MyD88-dependent manner, J. Immunol. 183 (2009) 7471–7477.
[12] T.D. Gilmore, Introduction to NF-κB: players, pathways, perspectives, Oncogene 25 (2006) 6680–6684.
[13] A.E. Frakes, L. Ferraiuolo, A.M. Haidet-Phillips, et al., Microglia induce motor neuron death via the classical NF-κB pathway in amyotrophic lateral sclerosis, Neuron 81 (2014) 1009–1023.
[14] A.B. Lentsch, A. Kato, B. Davis, et al., Stat4 and Stat6 regulate systemic inflammation and protect against lethal endotoxemia, J. Clin. Investig. 108 (2001) 1475–1482.
[15] W.V. Berghe, K. De Bosscher, E. Boone, et al., The nuclear factor-κB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter, J. Biol. Chem. 274 (1999) 32091–32098.
[16] Y. Ohmori, T.A. Hamilton, Interleukin-4/STAT6 represses STAT1 and NFκB-dependent transcription through distinct mechanisms, J. Biol. Chem. 275 (2000) 38095–38103.
[17] C.H. Shen, J. Stavnezer, Interaction of Stat6 and NF-κB: direct association and synergistic activation of interleukin-4-induced transcription, Mol. Cell. Biol. 18 (1998) 3395–3404.
[18] O.M. Pello, M. De Pizzol, M. Mirolo, et al., Role of c-myc in alternative activation of human macrophages and tumor-associated macrophage biology, Blood 119 (2012) 411–421.
[19] Y. Chiba, M. Todoroki, Y. Nishida, et al., A novel Stat6 inhibitor AS1517499 ameliorates antigen-induced bronchial hypercontractility in mice, Am J Respir. Cell. Mol. Biol. 41 (2009) 516–524.
[20] S. Gingras, J. Simard, B. Groner, et al., p300/CBP is required for transcriptional induction by interleukin-4 and interacts with Stat6, Nucleic Acids Res. 27 (1999) 2722–2729.
[21] W.V. Berghe, K. De Bosscher, E. Boone, et al., The nuclear factor-κB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter, J. Biol. Chem. 274 (1999), 32091–23098.
[22] D. Navia-Paldanius, J.R. Savinainen, J.T. Laitinen, Biochemical and pharmacological characterization of human α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12), J. Lipid Res. 53 (2012) 2413–2424.
[23] F. Chouinard, J.S. Lefebvre, P. Navarro, et al., The endocannabinoid 2-arachidonoyl-glycerol activates human neutrophils: critical role of its hydrolysis and *de novo* leukotriene B_4 biosynthesis, J. Immunol. 186 (2011) 3188–3196.

[24] M. Karlsson, J.A. Contreras, U. Hellman, et al., cDNA cloning, tissue distribution, and identification of the catalytic triad of monoacylglycerol lipase, J. Biol. Chem. 272 (1997) 27218–27223.
[25] G.G. Muccioli, C. Xu, E. Odah, et al., Identification of a novel endocannabinoid-hydrolyzing enzyme expressed by microglial cells, J. Neurosci. 27 (2007) 2883–2889.
[26] E. Keimpema, K. Barabas, Y.M. Morozov, et al., Differential subcellular recruitment of monoacylglycerol lipase generates spatial specificity of 2-arachidonyl glycerol signaling during axonal pathfinding, J. Neurosci. 30 (2010) 13992–14007.
[27] J. Zhang, S. Fujii, Z. Wu, et al., Involvement of COX-1 and up-regulated prostaglandin E synthases in phosphatidylserine liposome-induced prostaglandin E2 production by microglia, J. Neuroimmunol. 172 (2006) 112–120.
[28] J.Z. Long, D.K. Nomura, B.F. Cravatt, et al., Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism, Chem. Biol. 16 (2009) 744–753.
[29] R. Pihlaja, J. Takkinen, O. Eskola, et al., Monoacylglycerol lipase inhibitor JZL184 reduces neuroinflammatory response in APdE9 mice and in adult mouse glial cells, J. Neuroinflammation 12 (2015) 1–6.
[30] M.A. Meraz, J.M. White, K.C.F. Sheehan, et al., Targeted disruption of the *Stat1* gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway, Cell 84 (1996) 431–442.
[31] J. Gao, D.C. Morrison, T.J. Parmely, et al., An interferon-γ-activated site (GAS) is necessary for full expression of the mouse *iNOS* gene in response to interferon-γ and lipopolysaccharide, J. Biol. Chem. 272 (1997) 1226–1230.
[32] A.J. Loughlin, M.N. Woodroofe, Inhibitory effect of interferon-gamma on LPS-induced interleukin 1 beta production by isolated adult rat brain microglia, Neurochem. Int. 29 (1996) 77–82.
[33] W. Sheng, Y. Zong, A. Mohammad, et al., Pro-inflammatory cytokines and lipopolysaccharide induce changes in cell morphology, and upregulation of ERK1/2, iNOS and sPLA2-IIA expression in astrocytes and microglia, J. Neuroinflammation 8 (2011) 1–15.
[34] K. Lunnon, J.L. Teeling, A.L. Tutt, et al., Systemic inflammation modulates Fc receptor expression on microglia during chronic neurodegeneration, J. Immunol. 186 (2011) 7215–7224.
[35] J. Koenigsknecht-Taboo, G.E. Landreth, Microglial phagocytosis induced by fiblillar β-amyloid and IgGs are differentially regulated by proinflammatory cytokines, J. Neurosci. 25 (2005) 8240–8249.
[36] M. Girotti, J.H. Evans, D. Burke, et al., Cytosolic phospholipase A_2 translocates to forming phagosomes during phagocytosis of zymosan in macrophages, J. Biol. Chem. 279 (2004) 19113–19121.
[37] M.R. Lennartz, A.F.C. Yuen, S.M. Masi, et al., Phospholipase A_2 inhibition results in sequestration of plasma membrane into electronlucent vesicles during IgG-mediated phagocytosis, J. Cell. Sci. 110 (1997) 2041–2052.