Unbound IRF2 to IRF2BP2 mediates KLF4 signaling leading to anti-inflammatory phenotype of microglia

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Research Article

Keywords: Neuroinflammation, Agmatine, Microglia, IRF2BP2, IRF2, KLF4

Posted Date: November 9th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2232738/v1

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Abstract

Background
Following central nervous system (CNS) injury, the investigation for neuroinflammation is vital because of its pleiotropic role in both acute injury and long-term recovery. Agmatine (Agm) is well known for its neuroprotective effects and anti-neuroinflammatory properties. However, Agm's mechanism for neuroprotection is still unclear. We screened target proteins that bind to Agm using a protein microarray; the results showed that Agm strongly binds to interferon regulatory factor 2 binding protein (IRF2BP2), which partakes in the inflammatory response.

Methods
To confirm the relationship between Agm and IRF2BP2 in neuroinflammation, we used microglia cell-line (BV2) and treated with lipopolysaccharide (LPS; 20 ng/ml) and interleukin (IL)-4 (20 ng/ml). Although Agm bound to IRF2BP2, it failed to enhance IRF2BP2 expression in BV2.

Therefore, we shifted our focus onto interferon regulatory factor 2 (IRF2), which is a transcription factor and interacts with IRF2BP2.

Results
IRF2 was highly expressed in BV2 after LPS treatment but not after IL-4 treatment. When Agm bound to IRF2BP2 following Agm treatment, the free IRF2 translocated to the nucleus of BV2. The translocated IRF2 activated the transcription of Kruppel-like factor 4 (KLF4), causing KLF4 to be induced in BV2. The expression of KLF4 increased the CD206-positive cells in BV2.

Conclusion
Taken together, unbound IRF2, resulting from the competitive binding of Agm to IRF2BP2, may provide protection against neuroinflammation via an anti-inflammatory mechanism of microglia involving the expression of KLF4.

Introduction
Agmatine (Agm), an arginine-derived primary amino acid found in the nerve cell body and synaptic terminals, has been effective in ameliorating neuropathological damages in vitro and in in vivo models depicting central nervous system (CNS) injury [1, 2, 3, 4]. Agm also prevented neuroinflammation by regulating proinflammatory factors, such as nuclear factor kappa B (NF-κB) and matrix metalloproteinases (MMPs) during brain injury in rodents [5, 6]. Although the neuroprotective effect of Agm is clear, a neuroprotective mechanism has still not been elucidated. To this end, we used Agm-FITC and a protein microarray chip with 30,000 types of proteins. Interferon regulatory factor 2 binding protein
2 (IRF2BP2) exhibited a strong binding affinity, amongst the proteins that bound to Agm-FITC (Fig. 1a and b).

IRF2BP2 is a key player in macrophage-associated inflammation and lipid homeostasis. Recently, it has been shown to modulate macrophage plasticity by increasing anti-inflammatory (M2) and decreasing proinflammatory (M1) marker genes [7]. In IRF2BP2 deficient mice, macrophages induced inflammation and impaired cholesterol efflux [8]. These mice also demonstrated that the infarct increased and neurological function decreased in the ischemic brain injury model [9].

Neuroinflammation after an ischemic stroke leads to a variety of acute neurological insults. In this event, microglia, mentioned brain macrophages, mediate the immune responses in the brain. In neuroinflammation following CNS injury, microglia activation induced acute lesions [10] [11, 12]. The cytotoxic effects, as a result of activated microglia, are initiated shortly after the insult and can continue to exacerbate the injury for a few days afterward [13]. It has been hypothesized that the later effects of microglia could contribute to tissue repair and wound healing. This is because microglia, like other macrophages, are also classified into M1 and M2 phenotypes, depending on their functions [14] [15]. Therefore, it is very important to induce the M2 phenotype of microglia in the early neuroinflammatory stages following CNS injury.

Agm has been demonstrated to play a vital role in neuroprotection, however, currently, there are no specific reports regarding this mechanism. After neuroinflammation, IRF2BP2 in the microglia has not been studied extensively. Therefore, in this study, we aimed to explore the mechanism of microglia activation as a result of IRF2BP2 binding to Agm, and to confirm the polarization of microglia by this mechanism.

**Materials And Methods**

**Agm-fic and Protein Microarray**

Agm-FITC has been described in previous studies [16]. Briefly, Agm (0.16 mg, Sigma-Aldrich) was added to a DMF (1 ml, Fisher) solution containing FITC (50 mg, Thermo) and TEA (18 ul, Sigma-Aldrich) at room temperature (RT). The organic solution was washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified using column chromatography (CH₂Cl₂: MeOH = 10:1) to obtain a product with a 60% yield. To screen the target proteins that bind to Agm, we used the HuProt human proteome microarray v4.0 (CDI Laboratories). This chip was blocked with 20 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.1% Tween-20, and 5% BSA on an orbital shaker for 2 h, at RT. For screening Agm binding proteins, the blocked chip was washed 3 times with a microarray buffer for 10 min, followed by incubation with Agm-FITC (2 ug/ml) for 8 h at 4°C. After washing each chip three times with 1x PBS-T and Milli-Q water, the microarray was centrifuged for 5 min in a 50 mL centrifuge tube and scanned with an Axon GenePix 4000B Microarray Scanner (Molecular Devices). The probe signals of the microarray were acquired using the GenePix Pro6.0 software.
(Molecular Devices). After scanning, the probes were considered detectable when the z-scores for both duplicates were over three. The mean of the signal intensities from all proteins on the chip was calculated.

**Cell Culture And Drug Treatment**

The murine microglia cell-line (BV2) was obtained from the Ajou University College of Medicine, Chronic Inflammatory Disease Research Center. BV2 was cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Thermo) with 10% fetal bovine serum (FBS; Thermo) and 1% penicillin/streptomycin (Thermo) and maintained in a 37°C environment containing 5% CO₂. To induce the M1 phenotype activated by lipopolysaccharides (LPS) or the M2 phenotype activated by IL-4, BV2 was treated with LPS (20 ng/ml, Sigma-Aldrich) and IL-4 (20 ng/ml, cell signaling), respectively. The cells were also simultaneously treated with Agm (100uM, Sigma-Aldrich). Cultured media containing the BV2 cells were transferred to serum-free RPMI-1640 media after 24 h of stimulation, where they were cultured for a further 48 h.

**Co-immunoprecipitation (Co-ip)**

BV2 lysates were precleared using Protein A/G Agarose (50 ml, Santa Cruz). The rabbit anti-IRF2BP2 (2.5 mg, Thermo), rabbit anti-Interferon regulator factor 2 (IRF2; 2.5 mg, Abcam), and mouse anti-IgG isotype control (2.5 mg, Santa Cruz) were added to the precleared lysates (200 ml) and incubated overnight at 4°C. The Protein A/G Agarose was collected and the supernatant was aspirated by micro-centrifugation (3,000 g for 2 min at 4°C). This procedure was repeated twice. After washing all the reactions three times, the products were boiled for 5 min and thereafter micro-centrifuged briefly to pellet Protein A/G Agarose.

**Nucleus And Cytoplasm Fractionation**

To isolate the nucleus and cytoplasm of BV2, a NE-PER Nuclear Cytoplasmic Extraction Reagent kit (Thermo) was used according to the manufacturer’s instructions. Briefly, The BV2 pellet was centrifuged at 500 g for 3 min and thereafter suspended in 200 µl of cytoplasmic extraction reagent (CER) I by vertexing. Following this, 11 µl of CRE II was added to the suspension and centrifuged at 16000 g for 5 min. The cytoplasmic extract was transferred to a pre-chilled tube, to which 100 µl of the nuclear extract was added, and centrifuged at 16000 g for 10 min. The resulting supernatant, constituting of both nuclear and cytoplasm extracts, was used for further experiments.

**Immunoblotting (Ibt)**

Whole proteins were isolated using ice-cold RIPA lysis buffer (Tech & Innovation) containing 1 mM PMSF (Thermo). The whole protein (50 ug), Co-ip sample (50 ug), and nuclear and cytoplasm extract (30 ug) were separated on 10% sodium dodecyl sulfate-polyacrylamide electrophoresis gels (Bio-Rad), and
thereafter electro-transferred onto a polyvinylidene difluoride membrane (Milipore). The intensity of the proteins was probed with rabbit anti-IRF2BP2 (1:1000, Thermo), rabbit anti-IRF2 (1:1000, Abcam), mouse anti-INF- (1:1000, Abcam), mouse anti-KLF4 (1:1000, R&D system), mouse anti-CD86 (1:1000, Abcam), rabbit anti-CD206 (1:1000, Abcam), goat anti-Lamin B (Santa Cruz), and rabbit anti-β-actin (1:1000, Abcam) overnight at 4°C. The membrane was reacted with goat anti-Mouse IgG(H + L)-Peroxidase conjugated HRP, goat anti-rabbit IgG(H + L)-Peroxidase conjugated HRP, and rabbit anti-goat IgG(H + L)-Peroxidase conjugated HRP for 1 h at RT. Immunoreactive bands were detected using the ECL system (Thermo) and visualized using the LAS-4000 (Fuji).

**Immunocytochemistry (Icc)**

BV2 were fixed using 4% PFA for 15 min and permeabilized using 0.1% Triton X-100 for 7 min. All the cell groups were washed three times for 3 min at each step with PBS. Before treating them with the antibodies, the cells were blocked using 3% BSA for 1 h. Rabbit anti-IRF2BP2 (1:1000, Thermo), rabbit anti-IRF2 (1:1000, Abcam), mouse anti-KLF4 (1:1000, R&D system), mouse anti-CD86 (1:1000, Abcam), and rabbit anti-CD206 (1:1000, Abcam) were incubated overnight at 4°C. After washing the cells three times with PBS, we treated them with donkey anti-Rabbit IgG Alexa flour 488 (1:1000, Invitrogen,) and donkey goat IgG Alexa flour 568 (1:1000, Abcam). Finally, the cells were stained with the mounting solution DAPI (Thermo) and visualized using the LSM 700 (Carl Zeiss).

**Electrophoretic Mobility Shift (Emsa) Assay**

To confirm the interaction between IRF2 and Kruppel-like factor 4 (KLF4) promoter, an EMSA kit (Thermo) was used according to the manufacturer's instructions. DNA probes of KLF4 (5′-GGT AGT GGG GAA TGG GAA AAG GAG T-3′, IDT) were also prepared. Briefly, the nuclear extract was obtained according to the nuclear fractionation method mentioned above. Biotin-labeled DNA-binding oligonucleotides were incubated with 10 mg of nuclear extract at 15°C for 30 min, to allow the formation of the KLF complex. The DNA binding complexes were separated from the free probes using a 6% polyacrylamide gel. Following electrophoresis, the gel was transferred to a nylon membrane at 380 mA for 45 min. DNA crosslinking was performed. Once the blocking process was complete, the membrane was washed with washing buffer three times at RT. Signals were observed using enhanced chemiluminescence reagents. Images were captured using the LAS-4000 (Fuji).

**Flow Cytometry**

To analyze BV2 polarization by Agm, the cells (5 x 10⁶/ml) were washed with FACS buffer (PBS with 0.5% BSA) and blocked using 3% BSA. The cells from each sample were treated with Mouse anti-CD86 labeled with APC (BD, 1:1000) and Mouse anti-CD206 labeled with PE-Cy7 (BD, 1:1000), for 1 h. The negative controls were treated with compensation beads negative control (BD, 1 drop) and compensation beads
anti-mouse Ig,k bead (BD, 1 drop). The positive control samples were only treated with compensation beads anti-mouse Ig,k bead (BD, 1 drop); thereafter the APC or PE-Cy7 control antibody (BD,1:1000) was added to each sample. Finally, all the samples were analyzed by following BD FACS LSR II SORP (BD).

Results

Interaction Of Agm With Irf2bp2 In Protein Microarray And Bv2

To screen the proteins that interact with Agm, we conducted a protein microarray chip with Agm-FITC (Fig. 1a). Agm-FITC strongly bound to four proteins, namely dopa decarboxylase (DDC), annexin A11 (ANXA11), interferon regulatory factor 2 binding protein 2 (IRF2BP2), and Obg-like ATPase 1 (OLA1) (Fig. 1b). Amongst the four proteins, we mainly focused on IRF2BP2, because it is involved in the macrophage-related inflammation response [7]. To confirm that Agm and IRF2BP2 interact in BV2 (Fig. 1c), we conducted Co-ip. First, we induced inflammatory conditions in BV2 via LPS and IL-4 treatment. The amount of IRF2BP2 bound to Agm significantly increased by ~ 8-fold in the Agm groups compared with the non-Agm treatment groups (Fig. 1d). However, in the control (Con) and inflammation condition groups (LPS and IL-4), the level of IRF2PB2 bound to Agm did not change.

Irf2bp2 Expression Is Not Regulated By Agm In Bv2

To confirm the regulation of IRF2BP2 expression by Agm, we evaluated the IRF2BP2 expression in BV2 using ICC. The expression of IRF2BP2 showed no significant difference, regardless of treatment or non-treatment with Agm (Fig. 2a). We also performed IBT to accurately quantify the expression of IRF2BP2. The IBT results also demonstrated that there were no changes in IRF2BP2 expression owing to Agm treatment (Fig. 2b: Fig. 2c).

Irf2 Expression By Lps In Bv2

After confirming that the expression of IRF2BP2 was not regulated by Agm, we focused on IRF2 because its transcription is regulated by IRF2BP2 and it induced macrophage polarization. [17] [18]. We confirmed the expression of IRF2 by ICC and found that IRF2 was highly expressed in the LPS group compared with the Con and IL-4 groups (Fig. 3A). IBT showed that the level of IRF2 expression also increased in the LPS group compared with the Con and IL-4 groups. However, there was no statistical difference between the Agm-treated and non-treated groups (Fig. 3b). Here, we could find that IRF2 was not expressed in the IL-4 group. Looking for this reason, it was found that IRF2 is induced by IFN- amongst the several inflammatory factors [19], [20] [21]. When we examined the expression of IFN- by LPS, IFN- was significantly expressed in the LPS group compared with the Con group, with a difference of ~ 8-fold (Fig. 3d: Fig. 3e). Additionally, while confirming the level of IRF2 expression after LPS and IFN- treatment, it was found that IRF2 was expressed in both groups, however, it was significantly highly expressed in the LPS group compared with the IFN- group, by ~ quarter (Fig. 3f: Fig. 3g). Therefore, we excluded the IL-4
group, since it did not affect IRF2 expression. We also investigated how Agm induces the transcription of IRF2 into the BV2 nucleus, and how IRF2 transcription regulates the BV2 phenotype.

**Irf2 Translocation By Agm Into The Bv2 Nucleus**

To unambiguously confirm the translocation of IRF2 into the BV2 nucleus, we analyzed the intensity of IRF2 expression in the BV2 nuclear area. IRF2 was highly expressed in the BV2 nucleus of the LPS group treated with Agm but not in the cytoplasm (Fig. 4a). We used mean intensity analysis for the measurement of IRF2 fluorescence intensity in BV2 nucleus (Supplement 1). The IRF2 intensity in the BV2 nucleus of the LPS group treated with Agm was ~ 3-fold higher compared with the Con and LPS groups (Fig. 4b). We also attempted nuclear and cytoplasm fractionation to confirm IRF2 translocation into the BV2 nucleus. The IBT of nuclear and cytoplasmic fractions showed that the quantity of IRF2 increased in the BV2 nucleus of the LPS group treated with Agm (Fig. 4c). The quantity of IRF2 increased in the BV2 nucleus of the LPS group treated with Agm by ~ 6-fold, compared with the Con and LPS groups (Fig. 4d). We confirmed that the translocation of IRF2 was due to the interaction of IRF2 and IRF2BP2 using Co-ip (Fig. 4e). The association of IRF2 and IRF2BP2 was decreased in the LPS group treated with Agm by ~ 2.5-fold compared with the LPS group (Fig. 4f). This suggests that IRF2 was translocated into the BV2 nucleus because the interaction between IRF2 and IRF2BP2 was blocked as a result of Agm's affinity to IRF2BP2.

**Klf4 Expression Induced By Irf2 Translocation Into The Nucleus**

Recently, a study reported that the IRF2 translocated to the macrophage nucleus interacted with the KLF4 promoter, and enhanced KLF4 expression [22]. We tested the interaction between IRF2 and the KLF4 promoter in BV2; the EMSA assay showed that IRF2 was bound to the KLF4 promoter (Fig. 5a). In the LPS group treated with Agm, IRF2 strongly bound to the KLF4 promoter, with a ~ 2-fold difference compared with the Con and LPS groups (Fig. 5b). We also performed ICC and IBT to verify the expression of KLF4 in BV2. ICC showed that Agm induced the expression of KLF4 (Fig. 5c). IBT also showed that KLF4 was significantly expressed in the LPS group treated with Agm with a ~ 5-fold compared with the Con and LPS group (Fig. 5d: Fige).

**Induction Of The M2 Phenotype Of Bv2 By Agm**

KLF4 expression induced the M2 phenotype of macrophages [23] [24]. To confirm the phenotype of BV2, we used CD86 (M1 phenotype) and CD206 (M2 phenotype) antibodies. ICC indicated that CD206 expression significantly increased in the LPS group treated with Agm (Fig. 6a). IBT demonstrated that CD86 expression increased in the LPS group, however CD206 expression in the LPS group treated with Agm was higher by ~ 10-fold (Fig. 6b: Fig. 6c). Flow cytometry also confirmed that although the number of CD86 positive BV2 was increased in the LPS group, CD206 positive BV2 in the LPS group treated with Agm was higher by ~ 3-fold (Fig. 6d: Fig. 6e).
Discussion

In neuroinflammatory conditions, the binding of IRF2BP2 to Agm induced the translocation of IRF2 into the microglia nucleus. Further, IRF2 induced the expression of KLF4 and the M1 phenotype of the microglia was converted to the M2 phenotype. This is consistent with our hypothesis that the binding of IRF2BP2 to Agm prevents microglia-induced neuroinflammation (Fig. 7). Therefore, for the first time, we suggest a mechanism that depicts that the regulation of the microglia phenotype, by Agm, improving neuroinflammatory conditions.

In the neuroinflammatory state following CNS injury, microglia, which are the resident macrophages, release a variety of inflammatory and cytotoxic mediators. These mediators contribute to neuronal cell damage and cell death, which in turn exacerbates CNS injury [15]. Therefore, it is very important to control microglia activation following CNS injury. The mechanism by which Agm modulates microglia activation in neuroinflammation has been of much interest.

Agm, an arginine-derived primary amino acid, is capable of regulating several nonreceptor-mediated functions in mammalian cells, including the intracellular polyamine content and nitric oxide generation [25]. It has also shown effectiveness in decreasing hypoxic brain tissue damage; many studies showed that, in this condition, Agm mainly interrupted neurotoxicity and apoptosis related to cell death [26], [27] [28]. In neuroinflammatory diseases, exogenous Agm treatment also attenuated the neuroinflammatory response by suppressing pro-inflammatory factors [6] [29]. Despite the proven neuroprotective effects of Agm, the precise mechanism and factors remained unclear. To elucidate these unknown factors, we screened potential target factors that could interact with Agm using a protein microarray chip that had 30,000 proteins. We managed to identify four proteins, namely DDC, ANXA11, IRF2BP2, and OLA1 (Fig. 1), that bound to Agm. From amongst these proteins, we chose to focus on IRF2BP2, which is involved in innate and adaptive immune responses, because we were interested in neuroinflammation.

It has been indicated that IRF2BP2 may be a key player in macrophage regulation and lymphocyte activation in immune responses. Mice with IRF2BP2-deficient macrophages displayed an M1 phenotype, with pro-inflammatory factors [7]. When these mice experienced an ischemic stroke, it led to an increase in the inflammatory cytokines of the microglia [9]. IRF2BP2 is also associated with T lymphocytes. In patients with multiple sclerosis, IRF2BP2 was down-regulated in T lymphocytes; this suggests that IRF2BP2 could be involved in the regulation of T lymphocyte-mediated disease inflammation [30]. Our Co-IP data showed that Agm interacted with IRF2BP2 in BV2 after LPS treatment (Fig. 1). Contrary to what we expected, there was no change in the expression of IRF2BP2 upon Agm treatment, as per the immunoblotting result. Therefore, we focused on downstream proteins that interact with IRF2BP2. IRF2BP2 can interact with IRF2, acting as an IRF2-dependent transcriptional repressor. Therefore, we investigated the role of IRF2 in neuroinflammatory conditions.

IRF2, which belongs to the IRF family, is expressed via IFN- stimulation [31]. The transcription of IRF1, which activates many inflammatory cytokines and induces the M1 phenotype of macrophages, is blocked due to IRF2 binding to the same DNA sequences [32] [33]. IRF2 overexpression showed a
protective effect by limiting IRF1-induced inflammation and hepatic damage in an ischemia-reperfusion model of the liver [34]. IRF2-deficient mice are easily exposed to listeriosis and exhibit delayed oxidative effects on IFN- treatment [35]. Instead of IRF1, IRF2 interacted with the promoter of IL-4 and suppressed its expression, thereby inducing the M2 phenotype of the macrophages [31]. These findings illustrate the anti-inflammatory and immunosuppressive activities of IRF2. Therefore, IRF2 seems to exhibit anti-inflammatory properties in sterile inflammations and may be a key regulator in macrophage polarization. Recently, Cui et al. suggested that IRF2 selectively affects the binding of HIF-1α and KLF4 to the promoters of their respective target genes; IRF2 interrupted the binding of HIF-1α, but improved that of KLF4, in activated macrophages [22].

The KLF family is a subfamily of the zinc finger class of DNA-binding transcriptional regulators. Members of this family have been shown to be involved in both the regulation of proliferation and differentiation of cells in several tissues [36] [37]. Amongst these family, KLF4 is particularly involved in monocyte differentiation [38]. Liao et al. demonstrated that KLF4 worked together with Stat6 to induce the M2 phenotype of macrophage targets, such as Arg-1, after IL-4 treatment [23]. KLF4 also suppressed the TGF-β signaling pathway by inhibiting the binding of nuclear factor-kappa B (NF-κB) and activation of its cofactor (p300/CBP-PCAF). This caused the induction of the M1 phenotype macrophage [39] [40]. In myeloid KLF4 deficient mice (ApoE-/ background), the expression of M1 markers is increased and that of M2 markers is decreased [41].

Collectively, the results obtained from this study reveal a previously unknown mechanism of protection by Agm, in neuroinflammation, and identify IRF2BP2 as a potential therapeutic target. Pharmacologic Agm can also induce the M2 phenotype of microglia, which is related to anti-inflammation. These characteristics can be utilized not only in neuroinflammation but also in other inflammatory responses. Therefore, we suggest that Agm can be considered as a therapeutic option for neuroinflammatory conditions related to CNS injury.

**Declarations**

**Ethical Approval and Consent to participate**

The animal study was reviewed and approved by The Institutional Animal Care and Use Committee of Yonsei University Health System and according to the National Institutes of Health guidelines.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.
Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1A2C2008034 to JEL), and NRF funded by the Ministry of Education (NRF-2020R1I1A1A01064803 to JYK).

Author contributions

J.Y.K and J.E.L contributed to study conception, design, discussion and written. Experimental material preparation, data collection, and analysis were performed by J.K, A.Y.S and S.B All authors contributed to the article and approved the submitted version.

Acknowledgement

We would like to thank Editage (www.editage.co.kr) for English language editing.

References


Figures

Figure 1

The interaction of Agm and IRF2BP2. (a and b) The protein microarray chip contains over 21,000 full-length human recombinant proteins that cover more than 80% of the annotated human genome. It shows that Agm-FITC significantly interacts with DDC, ANXA11, IRF2BP2, and OLA1. (c) Co-ip shows the binding of Agm and IRF2BP2 (61~72kDa) upon Agm treatment of the Con, LPS, and IL-4 groups of BV2. (d) The
binding quantification graph indicates that Agm treatment groups significantly increased the binding of Agm and IRF2BP2 compared with the non-treatment groups. However, there was no statistical difference among Agm treatment groups. (n=3/group, *P<0.01)

**Figure 2**

Agm treatment did not affect IRF2BP2 expression in BV2. a) ICC shows IRF2BP2 expression in the BV2 cytosol. b) IBT showing IRF2BP2 expression. c) The quantification of IRF2BP2 expression showed no statistical difference. (n=3/group, Scale bar=10 µm)
IRF2 expression by IFN- and LPS in BV2. a) IRF2, binding to IRF2BP2, expressed in the LPS group compared to the Con and IL-4 groups. IRF2 expression was induced in the BV2 nucleus of the Agm treatment group. b) IBT showed IRF2(43~55kDa) expression in Non and Agm of LPS groups. c) The quantification measured result showed that IRF2 expression significantly increased in the Non and Agm treatment of the LPS groups compared to the Con and IL-4 groups. d and e) IFN- (40~45kDa) expression highly increased in LPS group. f) IRF2 expressed in LPS and IFN- groups. g) In LPS and IFN- groups, IRF2 expression had no significant, but significant compared to the Con group. (n=3/group, Scale bar=10 µm, *P<0.01)
Figure 4

IRF2 translocation to the nucleus of BV2. a) In the Agm group of LPS, IRF2 translocated to the nucleus of BV2. b) Significant Overlapping of IRF2-FITC and nucleus-DAPI in the Agm group of LPS. c) In IBT of the cytosolic and nuclear subfractions of BV2 treated with LPS and Agm, IRF2 was found in the nuclear subfraction. It appeared in the cytosolic subfraction of BV2 treated with LPS alone. d) Quantification showing that IRF2 expression was significantly increased in the nuclear subfraction of the Agm group of LPS and cytosolic subfraction of the Non group of LPS. e and f) Co-ip and quantification showing that
the interaction between IRF2BP2 and IRF2 highly increased in the Non group of LPS, but decreased in the Agm group of LPS. (n=3~5/group, Scale bar=10 µm, *P<0.01)

Figure 5

KLF4 expression in BV2. a) EMSA showing the DNA-binding capacity of IRF2 to the KLF4 promoter. b) The IRF2-binding activity was significantly increased in the Agm group of LPS compared with the other
groups. c) ICC showing that KLF4 expression increased in the Agm group of LPS. d) IBT indicating that KLF4 (60kDa) expression was highly induced in the Agm group of LPS. e) The relative intensity of KLF4 expression was quantified. KLF4 expression was significantly increased in the Agm group of LPS. (n=3~5/group, Scale bar=10 µm, *P<0.01, **P<0.05)

Figure 6

M2 phenotype induction of BV2 by Agm. a) and b) ICC and IBT showing CD206 (180kDa) expression in the Agm group of LPS. c) The quantification indicates that CD206 expression increased in the Agm group of LPS compared with the Non group of LPS. d) Flow cytometry confirming that CD206 was induced in the Agm group of LPS. e) The percentage of CD206-positive cells increased in the Agm group of LPS compared with the Non group of LPS. (n=3~5/group, Scale bar=10 µm, *P<0.01)
Figure 7

Working model of the induction of the M2 phenotype of microglia as a result of the interaction of IRF2BP2 and Agm under neuroinflammatory conditions.

Supplementary Files

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- Fig4CNeud1.jpg
- Fig6bCD206.jpg
- Fig6bCD86.jpg