The Effect of Magnesium Sulfate on Lipopolysaccharide Tolerance in Human Monocytes

Tzu-Yu Lin (drlin1971@gmail.com)
Far Eastern Memorial Hospital
Ya-Ying Chang
Far Eastern Memorial Hospital
Wei-Horng Jean
Far Eastern Memorial Hospital
Cheng-Wei Lu
Far Eastern Memorial Hospital
Jiann-Shing Shieh
Yuan Ze University

Research Article

Keywords: lipopolysaccharide tolerance, magnesium, migration, phagocytosis

Posted Date: January 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1246265/v1

Abstract

Binding of lipopolysaccharide (LPS) to toll-like receptor 4 induces release of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α) and leads to inflammatory pathologies. An immunosuppression status develops together with LPS-induced inflammation named LPS tolerance, which refers to downregulation of LPS signaling after pre-exposure of LPS, providing protection against hyperactive inflammation. During LPS tolerance, production of cytokines is mitigated, and phenotype of immune cells is altered. Magnesium is a crucial micronutrient, and used as an anti-inflammatory agent in clinical. Though the anti-inflammatory effect of magnesium through inhibiting LPS signaling has been demonstrated, the effect of magnesium on LPS tolerance remains unknown. In this study, we investigated modulation of magnesium sulfate ($MgSO_4$) on LPS tolerance. To induce LPS tolerance, THP-1 cells (a human leukemia monocyte cell line) were stimulated with LPS (200 ng/ml, 2 hours) after pre-exposure of LPS (200 ng/ml, 24 hours) with or without pretreatment of $MgSO_4$ (20 mM, 24 hours). Proliferation, morphological changes, adherence or TNF-α release, as well as the capacity of migration or phagocytosis were studied. From our results, $MgSO_4$ mitigated TNF-α release by LPS-tolerant cells. $MgSO_4$ also strengthened the inhibitory effect of LPS tolerance on proliferation or morphological changes. Besides, $MgSO_4$ enhanced LPS tolerance-triggered upregulation of migration, but not phagocytosis. In summary, $MgSO_4$ enhances LPS tolerance and alters activities of LPS-tolerant monocytes. Our findings addressed the role of $MgSO_4$ in immune system, and also provided evidence for a novel mechanism underlying the anti-inflammatory effect of $MgSO_4$.

Introduction

Lipopolysaccharide (LPS), a ligand of toll-like receptor 4 (TLR4), is a major pathogen associated molecular pattern [1, 2].Binding of LPS to TLR4 induces activation of nuclear factor-κB (NF-κB) pathway and production of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α) [1, 2], leading to a variety of inflammatory pathologies [1–3]. From previous report, LPS signaling develops together with a status of hypo-responsiveness to LPS, named LPS tolerance [3–5]. LPS tolerance, found in many inflammatory diseases including sepsis, is defined as downregulation of LPS-triggered inflammatory responses after pre-exposure of LPS. During LPS tolerance, reduced production of TNF-α or other pro-inflammatory cytokines provides protection against hyperactivation of inflammatory responses [3–6]. Furthermore, induction of LPS tolerance results in alternation of monocytes phenotypes [7, 8]. Phenotypes or activities of monocytes, such as cell adherence or proliferation, relates to innate immune function [9]. For example, adherence of monocytes to endothelial cells determines progress of atherosclerosis [10]. Proliferation of monocytes links to protection against infection [11]. In addition, alternation of monocyte phenotypes associates with outcomes of inflammatory diseases including sepsis [12]. Monocytes in septic patients exhibit hallmarks of LPS tolerance [7]. Reprogramming of monocytes or macrophages may indicate course of inflammatory diseases [8, 12, 13]. Therefore, activities of monocytes are critical to immunomodulation during inflammation.

Magnesium is one of essential element of life, and is indispensable for many physiological functions. The association between magnesium and inflammation has been extensively investigated. Magnesium protects against inflammatory reactions, while a low magnesium status often links to increased inflammation [14, 15]. Action of magnesium as an anti-inflammatory agent can be mediated by downregulation of LPS signaling. Magnesium inhibits LPS-triggered production of TNF-α or interleukin-1 [16, 17]. Magnesium also downregulates NF-κB pathway [16]. Though magnesium mitigates LPS-induced inflammation, the effect of magnesium on LPS tolerance or on LPS-tolerant cells remains unstudied. In this experiment, we employed THP-1 cells (a human leukemia monocytic cell line) to study the effect of magnesium sulfate ($MgSO_4$), a therapeutic drug for preeclampsia [18], on LPS tolerance. The modulation of $MgSO_4$ on proliferation, morphology, adherence, migration or phagocytosis of LPS-tolerant THP-1 cells was also investigated. Our results demonstrated the role of $MgSO_4$ in immune system. The modulation of $MgSO_4$ on monocytes during inflammation with LPS tolerance was also addressed in this study.

Materials And Methods

Cell culture and stimulation

Human monocytic THP-1 cells (American Type Culture Collection, Manassas, VA, USA) were maintained in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific, MA, USA) and 1% penicillin/streptomycin (Life Technologies, Carlsbad, CA, USA). Cells were incubated at 37°C in a mixture of 95% air and 5% CO_2. To induce LPS tolerance, cells were stimulated with LPS (1^{st} LPS, 200 ng/ml; Sigma-Aldrich, MO, USA) for 24 hours and then washed with phosphate buffered saline (PBS), followed by re-stimulation of LPS (2^{nd} LPS, 200 ng/ml) for 2 hours. In the groups with treatment of $MgSO_4$, $MgSO_4$ (20 mM; Sigma-Aldrich, MO, USA) was added 24 hours before 1^{st} LPS stimulation. The dose of $MgSO_4$ was based on our previous study [19]. THP-1 cells were randomly allocated into 6 groups (the PP, PL, LL, MPP, MPL and MLL group) illustrated in Figure 1. The experimental protocol of each group was illustrated in Figure 1. The PP and MPP group were control groups. LPS tolerance was induced in the LL and MLL group. The PL and MPL group received LPS stimulation without pre-exposure of LPS or development of tolerance.

Cell viability

The level of cell viability was measured using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, USA). In brief, cells were seeded in 96-well microplates, and incubated for 4 hours in a humidified atmosphere at 37 °C after MTT reagent was added to each well. Then, the absorbance was measured using a plate reader (Bio-Rad, Hercules, CA, USA) with a test wavelength at 570 nm and a reference wavelength at 630 nm. Cell viability was reported as the ratio of absorbance value in each group to that in the PP group.

Enzyme-linked immunosorbent assay (ELISA)

After treatment as previous description, the supernatant in each group was collected for assay of TNF-α. The concentration of TNF-α was quantified using the commercial ELISA kit of human TNF-α (R&D System, MN, USA) in accordance with the manufacturer's protocol.

Assay of cell proliferation and morphology

To determine proliferation rate, THP-1 cells were seeded at a density of 10^4 cells/well in 96-well microplates. At 0, 24 or 48 hours (e.g., Day 0, Day 1 or Day 2) after end of 2^{nd} LPS stimulation, viable cells in each group were determined using MTT assay after centrifugation. Then, ratio of proliferation was calculated as the ratio of absorbance value at each indicative time point relative to the absorbance value on Day 0. Finally, the growth curve in each group was plotted according to the average ratio of proliferation at each indicative time point. In addition, the ratio of proliferation in each group on Day 2 was compared relative to the PP group. To assay cell morphology, THP-1 cells were imaged using brightfield microscopy (Carl Zeiss, Inc., Oberkochen, Germany). For each group, we took at least 3 different fields under microscopy.

Measurement of cell adherence

Adherence of THP-1 cells is upregulated by LPS [20]. To determine cell adherence, the adherent cells were collected by scratching, and stained using trypan blue. We used a hemocytometer to calculate the number of adherent cells. The percentage of adherence in each group was calculated relative to total seeded cells that represent 100%.

Transwell migration assay

A 24-well Transwell system (Corning®, NY, USA) with microporous polycarbonate membranes (10 μm thickness, 5 μm pores) was employed to measure cell migration capacity. In brief, THP-1 cells were seeded at a density of 10^6 cells/ml in upper inserts with fresh medium. FBS, as a chemoattractant, was added in lower inserts, instead of upper inserts. After migration at 37°C for 6 hours, non-migrated cells (on the upper side of membranes) were removed using cotton swabs, and migrated cells (on the bottom side of membranes) were stained with diamidino-2-phenylindole (DAPI, Pierce). The stained cells were observed under fluorescence microscopy. We took at least 3 different fields for each group, and calculated the number of migrated cells per field.

Phagocytosis assay

After treatment as previous description, the phagocytic activity of cells was measured using the Phagocytosis Assay Kit (Abcam, Cambridge, UK) according to the manufacture's protocol. Briefly, zymosan was incubated with THP-1 cells at 37^0C, 5% CO_2 for 3 hours. Then cells were harvested by centrifugation. After washing and suspension in Phagocytosis Assay Buffer, cells were analyzed by the plate reader at Ex/Em of 490/520 nm. The level of phagocytosis in each group was reported as the ratio to the PP group. Furthermore, another set of cells were seeded on the glass slides. After incubation with zymosan, cells were fixation and imaged under fluorescence microscopy. We took at least 3 different fields for each group.

Statistical analysis

Statistical analysis was performed using a commercial software package (SigmaStat for Windows; SPSS Science, Chicago, IL, USA). All data were presented as mean ± standard deviations, and analyzed by one-way analysis of variance in conjunction with Tukey's post hoc test. A *P*-value < 0.05 was considered statistically significant.

Results

Cell viability

THP-1 cells were randomly allocated into the PP, PL, LL, MPP, MPL or MLL group. The experimental protocol of each group was illustrated in Figure 1. Cell viability in each group was measured and reported as the ratio to the PP group (Fig. 2, n = 4 in each group). Stimulation of LPS, with or without pre-exposure of LPS, did not alter cell viability. There was no significant difference between groups treated with and without $MgSO_4$.

$MgSO_4$ enhanced LPS tolerance

The concentration of TNF-α was assayed using ELISA and illustrated in Figure 3 (n = 3 in each group). The level of TNF-α in the PP group was low. LPS increased the concentration of TNF-α (P < 0.001, the PL group *versus* the PP group), while re-stimulation of LPS obviously decreased LPS-triggered upregulation of TNF-α (P < 0.001, the LL group *versus* the PL group). The reduced level of TNF-α after re-stimulation of LPS indicated successful induction of LPS tolerance in the LL group. In addition, the TNF-α expression in the MLL group was lower than that in the LL group (P = 0.033), suggesting $MgSO_4$ enhanced LPS tolerance through reducing TNF-α release by LPS-tolerant cells.

$MgSO_4$ strengthened the inhibitory effect of LPS tolerance on proliferation and morphological changes of THP-1 cells

We further studied effects of $MgSO_4$ on proliferation and morphology of THP-1 cells during LPS tolerance. Proliferation of THP-1 cells was assayed and illustrated in Figure 4a (n = 3 in each group). THP-1 cells kept growing from Day 0 to Day 2 in all groups except the MLL group. The comparison of proliferation ratio on Day 2 was shown in Figure 4b. On Day 2, the ratio of proliferation in the PL group was lower than in the PP group without statistical significance (Fig. 4b). Of note, LPS tolerance mitigated cell proliferation (P < 0.001, the LL group *versus* the PL group). Pre-treatment of $MgSO_4$ further inhibited proliferation of LPS-tolerant cells (P < 0.001, the MLL group *versus* the LL group).

Cell morphology was studied using bright microscopy, and the representative images were shown in Figure 4c. Since changes of cell morphology may be difficult to observed under brightfield microscopy within short duration of LPS stimulation (the PL group in Fig. 4c), we extended the duration of 2nd LPS stimulation to 24 hours (denominated as the PL24, LL24, MPL24 or MLL24 group). All THP-1 cells were of round-shape in the PP group. Compared to the LPS-triggered irregular or polygonal shape of cells in the PL24 group (indicated by the solid white arrow), cells in the LL24 group were more regular or round. It was suggested that re-stimulation of LPS mitigated LPS-induced morphological changes. Of note, the shape of cells in the MLL group was almost round-shaped, suggesting $MgSO_4$ inhibited cell morphological changes during LPS tolerance.

Adherence of THP-1 cells was not altered by $MgSO_4$

Levels of cell adherence were examined and illustrated in Figure 4d (n = 3 in each group). The level of cell adherence in the PP or MPP group was low. LPS stimulation increased adherence of THP-1 cells (P = 0.01, the PL group *versus* the PP group). The level of cell adherence in the LL group was lower than in the PL group (P < 0.01), indicating cellular adherence was reduced during LPS tolerance. Furthermore, $MgSO_4$ did not significantly alter THP-1 adherence, though the percentage of adherent cells in the MLL group seemed mild lower than in the LL group.

$MgSO_4$ upregulated ability of migration, but not phagocytosis, during LPS tolerance

We measured the migration capacity of THP-1 cells using the transwell migration assay and shown in Figure 5a (n = 4 in each group). In the PP group, the level of cell migration was low. Stimulation of LPS upregulated the number of migrated cells (P = 0.047, the PL group *versus* the PP group). Of note, re-stimulation of LPS (e.g. induction of LPS tolerance) further upregulated LPS-triggered cell migration (P = 0.008, the LL group *versus* the PL group). With pre-treatment of $MgSO_4$, the migration capacity significantly increased in LPS-tolerant THP-1 cells (P = 0.006, the MLL group *versus* the LL group).

The ability of phagocytosis was measured using the Phagocytosis Assay Kit and shown in Figure 5b (n = 4 in each group). In the PP group, the phagocytotic ability of THP-1 cells was weak. The level of

phagocytosis mildly increased by LPS stimulation. Of note, THP-1 phagocytosis in the LL group was significantly higher than in the PL group (P = 0.008, the LL group *versus* the PL group), suggesting LPS tolerance upregulated phagocytotic ability. With pre-treatment of $MgSO_4$, there was no change of phagocytosis level in LPS-tolerant cells.

Discussion

In this study, we sought to clarify the effect of $MgSO_4$ on LPS tolerance. Pre-treatment of $MgSO_4$ significantly reduced release of TNF-α and enhanced LPS tolerance. We further investigated modulation of $MgSO_4$ on LPS-tolerant monocytes. Effects of $MgSO_4$ on phenotypes and activities of LPS-tolerant THP-1 cells were summarized in Table 1. Based on our results, LPS tolerance mitigated LPS-triggered changes of morphology and adherence in THP-1 cells. Proliferation also reduced during LPS tolerance. The result that LPS tolerance slowed down proliferation was compatible with other previous report showing re-stimulation of LPS reduced cell proliferation [21]. From previous report, hallmarks of monocyte-to-macrophage differentiation of THP-1 cells include both reduced proliferative activity and increased adherence [22]. Notably, in this study, reduced proliferative activity accompanied reduced adherence was found in LPS-tolerant THP-1 cells. Development of LPS tolerance and monocyte-to-macrophage differentiation in THP-1 cells may not be mediated by the same pathway. More research is needed to fully explore the underlying mechanism. Our results addressed the role of $MgSO_4$ in the immune system. The possible role of magnesium in immune system or the modulation of magnesium on immune cells has been discussed in a few reports several decades ago [23]. For example, magnesium promotes M2 macrophage polarization [24]. Magnesium also involves T helper-B cell adherence [25]. To our knowledge, there is no published data about the association between magnesium and LPS tolerance. In this experiment, we confirmed the immunomodulatory effect of $MgSO_4$ on functions and activities of human monocytes during LPS tolerance.

Few published reports focused on regulation of LPS tolerance to cell migration. In this study, we examined migration of LPS-tolerant THP-1 cells since mobilization of monocytes to the inflammatory sites is critical to help host defense [26]. Based on our data, LPS tolerance increased monocyte migration. It is interesting that other immune cells such as neutrophils were also reported driven by LPS tolerance to infectious sites [27]. The induction of LPS tolerance may reprogram biological processes such as activating chemotaxis that upregulate the migration capacity of immune cells [7]. In addition, our results showed LPS tolerance increased cell phagocytosis, which may not be consistent with other study [28]. Perhaps the design of experiment or the type of cells may account for the discrepancy between studies. Furthermore, morphological change is an important factor contributes to ability of migration and phagocytosis. Close relationship between morphology and migration has been revealed in many reports [29–31]. In addition, change of cell shape and formation of pseudopodia is the key step to migration and phagocytosis of neutrophils [32]. We believe there is crosstalk between migration, phagocytosis and morphology of LPS-tolerant THP-1 cells during inflammation.

Multiple mechanisms mediate the anti-inflammatory effect of $MgSO_4$. Our previous report revealed $MgSO_4$ inhibits NLRP3 inflammasome pathway [19]. Other researchers found $MgSO_4$ reduces TLR4 expression and inhibits LPS/TLR4 pathway [16, 33]. In this study, we further found the enhancement effect of $MgSO_4$ on LPS tolerance. Since LPS tolerance helps mitigate overactivity of inflammatory reaction, $MgSO_4$ may also exert anti-inflammatory effects through enhancing LPS tolerance. Our data certainly had clinical impacts. In clinical, hypomagnesemia is not rare in septic patients [34]. Some clinical trials link the detrimental effect of hypomagnesemia to bad outcomes of sepsis [34, 35]. Of note, Inflammatory responses induced by $MgSO_4$ deficiency may links to alternation of cell migration or proliferation [36]. Therefore, the regulation by $MgSO_4$ to the activity of immune cells may determine prognosis of inflammatory pathologies with development of LPS tolerance such as sepsis.

Although our data was clear, there were still some limitations. First, we studied effects of $MgSO_4$ on intensity, instead of duration, of LPS tolerance. The effect of $MgSO_4$ on long-term LPS tolerance was not in scope of this experiment and sill remains unknown. Second, we only revealed the *in vitro* effect of $MgSO_4$ on LPS tolerance in this cellular experiment. Further research is needed to elucidate the *in vivo* effect of $MgSO_4$ on LPS tolerance. Third, the possible mechanism underlying the enhancing effect of $MgSO_4$ on LPS tolerance remains unmentioned in this manuscript. According to our literature review, induction of LPS tolerance relates to some microRNA, such as microRNA-221 [37] which was reported upregulated by $MgSO_4$ [38]. It is possible that miroRNA may account for the effect of $MgSO_4$ on LPS tolerance. More studies are required to demonstrate the involvement of microRNA in our findings.

In conclusion, $MgSO_4$ enhanced LPS tolerance. $MgSO_4$ also strengthened the effect of LPS tolerance on proliferation, morphological change or migration of human monocytes. Our findings revealed a novel mechanism underlying anti-inflammatory effects of $MgSO_4$, and also broaden the knowledge about the role of $MgSO_4$ in immune system.

Declarations

Compliance with ethical standards

Funding:

This work was supported by a grant from Far-Eastern Memorial Hospital (FEMH-2021-C-014) and Ministry of Science and Technology (MOST 109-2314-B-418-015-MY2), awarded to CYY.

Conflict of interests:

All the authors have no relevant financial or non-financial interests to disclose.

Author contributions:

All authors contributed to the study conception and design. YYC and TYL contributed to the conception and design, data collection, analysis and interpretation, writing and critical revision of the article. WHJ and CWL contributed to data analysis and interpretation as well as writing of the article. SJS contributed to critical revision of the article.

Ethical approval: There were no human tissues or animal experiments in this study.

References

1. Seeley JJ, Ghosh S (2017) Molecular mechanisms of innate memory and tolerance to LPS. J Leukoc Biol 101(1):107–119
2. Ciesielska A, Matyjek M, Kwiatkowska K (2021) TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci 78(4):1233–1261
3. Liu D et al (2019) Recent advances in endotoxin tolerance. J Cell Biochem 120(1):56–70
4. Lopez-Collazo E, del Fresno C (2013) Pathophysiology of endotoxin tolerance: mechanisms and clinical consequences. Crit Care 17(6):242
5. Collins PE, Carmody RJ (2015) The Regulation of Endotoxin Tolerance and its Impact on Macrophage Activation. Crit Rev Immunol 35(4):293–323
6. López-Collazo E, del Fresno C (2013) Pathophysiology of endotoxin tolerance: mechanisms and clinical consequences. Critical care 17(6):242
7. Pena OM et al (2011) Endotoxin tolerance represents a distinctive state of alternative polarization (M2) in human mononuclear cells. J Immunol 186(12):7243–7254
8. Widdrington JD et al (2018) Exposure of Monocytic Cells to Lipopolysaccharide Induces Coordinated Endotoxin Tolerance, Mitochondrial Biogenesis, Mitophagy, and Antioxidant Defenses. Front Immunol 9:2217
9. Otto NA et al (2021) Adherence Affects Monocyte Innate Immune Function and Metabolic Reprogramming after Lipopolysaccharide Stimulation In Vitro. J Immunol 206(4):827–838
10. Sato K et al (2018) Adropin Contributes to Anti-Atherosclerosis by Suppressing Monocyte-Endothelial Cell Adhesion and Smooth Muscle Cell Proliferation,Int J Mol Sci.19(5)
11. Ligon MM, Mysorekar IU (2018) Trans-mission control in the urinary tract: Local cytokine regulation of monocyte proliferation to combat infection. J Leukoc Biol 103(1):5–7
12. Pena OM et al (2014) An Endotoxin Tolerance Signature Predicts Sepsis and Organ Dysfunction at Initial Clinical Presentation. EBioMedicine 1(1):64–71
13. Shalova IN et al (2015) Human monocytes undergo functional re-programming during sepsis mediated by hypoxia-inducible factor-1alpha. Immunity 42(3):484–498

14. Weglicki WB (2012) Hypomagnesemia and inflammation: clinical and basic aspects. Annu Rev Nutr 32:55–71
15. Maier JA et al (2021) Magnesium and inflammation: Advances and perspectives. Semin Cell Dev Biol 115:37–44
16. Sugimoto J et al (2012) Magnesium decreases inflammatory cytokine production: a novel innate immunomodulatory mechanism. J Immunol 188(12):6338–6346
17. Amash A et al (2012) Placental secretion of interleukin-1 and interleukin-1 receptor antagonist in preeclampsia: effect of magnesium sulfate. J Interferon Cytokine Res 32(9):432–441
18. Duley L et al (2010) Magnesium sulphate and other anticonvulsants for women with pre-eclampsia,Cochrane Database Syst Rev(11):CD000025
19. Chang YY et al (2018) Effects of MgSO4 on inhibiting Nod-like receptor protein 3 inflammasome involve decreasing intracellular calcium. J Surg Res 221:257–265
20. Hmama Z et al (1999) Monocyte adherence induced by lipopolysaccharide involves CD14, LFA-1, and cytohesin-1. Regulation by Rho and phosphatidylinositol 3-kinase. J Biol Chem 274(2):1050–1057
21. Avendano-Ortiz J et al (2018) PD-L1 Overexpression During Endotoxin Tolerance Impairs the Adaptive Immune Response in Septic Patients via HIF1alpha. J Infect Dis 217(3):393–404
22. Park EK et al (2007) Optimized THP-1 differentiation is required for the detection of responses to weak stimuli. Inflamm Res 56(1):45–50
23. Tam M et al (2003) Possible roles of magnesium on the immune system. Eur J Clin Nutr 57(10):1193–1197
24. Li X et al (2021) Magnesium sulfate prophylaxis attenuates the postpartum effects of preeclampsia by promoting M2 macrophage polarization. Hypertens Res 44(1):13–22
25. Galland L (1988) Magnesium and immune function: an overview. Magnesium 7(5–6):290–299
26. Shi C, Pamer EG (2011) Monocyte recruitment during infection and inflammation. Nat Rev Immunol 11(11):762–774
27. Ariga SK et al (2014) Endotoxin tolerance drives neutrophil to infectious site. Shock 42(2):168–173
28. de Lima TM et al (2014) Phagocytic activity of LPS tolerant macrophages. Mol Immunol 60(1):8–13
29. Weber SM, Carroll SL (2021) The Role of R-Ras Proteins in Normal and Pathologic Migration and Morphologic Change. Am J Pathol 191(9):1499–1510
30. Jolivel V et al (2021) Microglial Cell Morphology and Phagocytic Activity Are Critically Regulated by the Neurosteroid Allopregnanolone: A Possible Role in Neuroprotection,Cells.10(3)
31. Bettegazzi B et al (2021) Galpha13 Contributes to LPS-Induced Morphological Alterations and Affects Migration of Microglia. Mol Neurobiol 58(12):6397–6414
32. Roberts RE, Hallett MB (2019) Neutrophil Cell Shape Change: Mechanism and Signalling during Cell Spreading and Phagocytosis,Int J Mol Sci.20(6)

33. Jiang J et al (2020) Magnesium sulfate ameliorates sepsis-induced diaphragm dysfunction in rats via inhibiting HMGB1/TLR4/NF-kappaB pathway. Neuroreport 31(12):902–908
34. Velissaris D et al (2015) Hypomagnesemia in Critically Ill Sepsis Patients. J Clin Med Res 7(12):911–918
35. Thongprayoon C, Cheungpasitporn W, Erickson SB (2015) Admission hypomagnesemia linked to septic shock in patients with systemic inflammatory response syndrome. Ren Fail 37(9):1518–1521
36. Bussiere L et al (1995) Triglyceride-rich lipoproteins from magnesium-deficient rats are more susceptible to oxidation by cells and promote proliferation of cultured vascular smooth muscle cells. Magnes Res 8(2):151–157
37. Quinn EM, Wang J, Redmond HP (2012) The emerging role of microRNA in regulation of endotoxin tolerance. J Leukoc Biol 91(5):721–727
38. Mi F, Liu F, Zhang C (2020) Magnesium protects mouse hippocampal HT22 cells against hypoxia-induced injury by upregulation of miR-221. J Cell Biochem 121(2):1452–1462

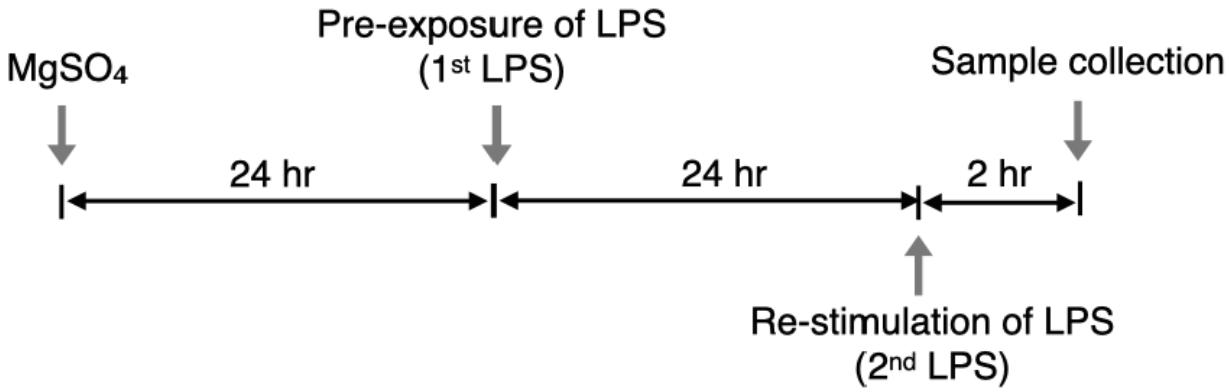
Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Fig. 1

(a)



(b)

Group	$MgSO_4$ →	1st LPS →	2nd LPS
PP	−	−	−
PL	−	−	+
LL	−	+	+
MPP	+	−	−
MPL	+	−	+
MLL	+	+	+

Figure 1

The experimental protocol. (a) To induce lipopolysaccharide (LPS) tolerance, THP-1 cells were re-stimulated with LPS (2^{nd} LPS, 200 ng/ml) for 2 hours after pre-exposure of LPS (1^{st} LPS, 200 ng/ml) for 24 hours. Then the samples were collected for analysis. In groups with treatment of magnesium sulfate ($MgSO_4$), $MgSO_4$ was administered 24 hours before stimulation of 1^{st} LPS. **(b)** Cells were randomly allocated into 6 groups (the PP, PL, LL, MPP, MPL and MLL groups). In the PP group, cells were stimulated with phosphate buffered saline (PBS), followed by PBS stimulation. In the PL group, cells were stimulated with PBS, followed by LPS stimulation. In the LL group (the LPS tolerance group), cells were stimulated with LPS, followed by LPS re-stimulation. In the MPP, MPL or MLL group, pre-treatment of $MgSO_4$ for 24 hours was employed in the PP, PL or LL group, respectively.

Figure 2

The cell viability. Cell viability was measured using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were randomly allocated into 6 groups (the PP, PL, LL, MPP, MPL and MLL groups). In the PP group, cells were stimulated with phosphate buffered saline (PBS), followed by PBS stimulation. In the PL group, cells were stimulated with PBS, followed by lipopolysaccharide (LPS) stimulation. In the LL group (the LPS tolerance group), cells were stimulated with LPS, followed by LPS re-stimulation. In the MPP, MPL or MLL group, pre-treatment of $MgSO_4$ for 24 hours was employed in the PP, PL or LL group, respectively. Data were derived from four independent experiments and expressed as means ± standard deviations.

Figure 3

The concentration of tumor necrosis factor-α (TNF-α). The concentration of TNF-α in the medium was measured by enzyme-linked immunosorbent assay (ELISA). Cells were randomly allocated into 6 groups (the PP, PL, LL, MPP, MPL and MLL groups). In the PP group, cells were stimulated with phosphate buffered saline (PBS), followed by PBS stimulation. In the PL group, cells were stimulated with PBS, followed by lipopolysaccharide (LPS) stimulation. In the LL group (the LPS tolerance group), cells were stimulated with LPS, followed by LPS re-stimulation. In the MPP, MPL or MLL group, pre-treatment of $MgSO_4$ for 24 hours was employed in the PP, PL or LL group, respectively. Data were derived from three independent experiments and expressed as means ± standard deviations. $^{*}P < 0.05$ *versus* the PP group. $^{\#}P < 0.05$ *versus* the PL group. $^{\&}P < 0.05$ *versus* the LL group.

Figure 4

Assay of cell proliferation, morphology and adherence. (a) Proliferation of THP-1 cells was assayed by measuring of viable cells using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Day 0, Day 1 and Day 2. The ratio of proliferation was calculated as the ratio of absorbance value at each indictive time point relative to the absorbance value on Day 0. **(b)** Ratio of proliferation on Day 2 in each group relative to the PP group. **(c)** Since morphological changes were not easily observable under brightfield microscopy within 2 hours of lipopolysaccharide (LPS) treatment (the PL group), we extended duration of 2^{nd} LPS stimulation to 24 hours in the PL, LL, MPL or MLL group (denoted as the PL24, LL24, MPL24 or MLL24 group, respectively). Cell morphology was imaged under bright field microscopy. The representative photography was shown. The white solid arrows indicated LPS-triggered irregular cellular shapes. **(d)** Adherence of THP-1 cells were measured by counting the number of adherent cells. We calculated the percentage of adherent cells in total seeded cells in each group. Cells were randomly allocated into 6 groups (the PP, PL, LL, MPP, MPL and MLL groups). In the PP group, cells were stimulated with phosphate buffered saline (PBS), followed by PBS stimulation. In the PL group, cells were stimulated with PBS, followed by LPS stimulation. In the LL group (the LPS tolerance group), cells

were stimulated with LPS, followed by LPS re-stimulation. In the MPP, MPL or MLL group, pre-treatment of $MgSO_4$ for 24 hours was employed in the PP, PL or LL group, respectively. Data were derived from three independent experiments and expressed as means ± standard deviations. *$P < 0.05$ *versus* the PP group. $^{\#}P < 0.05$ *versus* the PL group. $^{\&}P < 0.05$ *versus* the LL group.

Figure 5

The capacity of migration and phagocytosis. (a) Migration capacity of THP-1 cells was measured using the transwell migration assay. After 6 hours of migration, migrated THP-1 cells were stained with diamidino-2-phenylindole (DAPI) and imaged under fluorescence microscopy. We calculated the number of migrated cells in each group and reported as the ratio to the PP group. The representative photography was shown. **(b)** Phagocytosis capacity of THP-1 cells was measured using the Phagocytosis Assay Kit. After incubation with zymosan for 2 hours, the fluorescence intensity was measured and the photography under fluorescence microscopy was taken. Cells were randomly allocated into 6 groups (the PP, PL, LL, MPP, MPL and MLL groups). In the PP group, cells were stimulated with phosphate buffered saline (PBS), followed by PBS stimulation. In the PL group, cells were stimulated with PBS, followed by lipopolysaccharide (LPS) stimulation. In the LL group (the LPS tolerance group), cells were stimulated with LPS, followed by LPS re-stimulation. In the MPP, MPL or MLL group, pre-treatment of $MgSO_4$ for 24 hours was employed in the PP, PL or LL group, respectively. Data were derived from four independent experiments and expressed as means ± standard deviations. A.U.: anonymous unit. *$P < 0.05$ *versus* the PP group. $^{\#}P < 0.05$ *versus* the PL group. $^{\&}P < 0.05$ *versus* the LL group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.tiff