Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-021-15773-7

RESEARCH ARTICLE

Plasmalogens ensure the stability of non-neuronal (microglial) cells during long-term cytotoxicity

Fatma Ali[1] · Md. Shamim Hossain[2] · Ahmed Abdeen[3,4] · Md. Sahab Uddin[5,6] · Ahmed Gaber[7,8] · Walaa F. Alsanie[8,9] · Rania Waheed[3] · Obeid Shanab[10] · Hassan Ahmed[11]

Received: 18 June 2021 / Accepted: 29 July 2021

Abstract

Microglia (MG) are resident phagocytes in the brain responsible for neuronal maintenance. The regulation of MG necroptosis is required for protecting neurons during neurodegenerative diseases. Therefore, this study proposed to elucidate the molecular mechanisms underlying microglia necroptosis during long-time apoptotic stimuli (lipopolysaccharide, LPS). The protective role of plasmalogens (PLS) was also investigated against LPS insult in MG cells (including BV2 and MG6 cell lines). LPS produced time-dependent decreases in the survival of BV2 and MG6 cells mediated by the caspase signaling pathway. Interestingly, MG death was mediated by caspase-8 and 9 signaling pathways suggesting that MG necroptosis was actively attributed to long-time LPS treatment through intrinsic and extrinsic pathways. Notably, caspase signaling was markedly inhibited in the PLS-pretreated cells; thereby, PLS were capable of maintaining the MG cell population and inhibit the MG necroptosis against the longtime of LPS administration via its antioxidant and anti-inflammatory properties.

Keywords Lipopolysaccharide · Plasmalogens · Microglia cells · Necroptosis · Caspase signaling

Introduction

Microglia (MG) cells, primary immune cells, represent 5-12% of the total neuronal cells in rodents and 0.5%–16% in humans (Gomez-Nicola and Perry 2015; Shen et al. 2018). Since MG cells play a pivotal role in neuronal maintenance through constant monitoring of the neuronal physiology, scavenging the damaged neurons, infectious microorganisms, junk proteins, and toxic agents. These functional roles highlight the necessity of MG in protecting the neuronal cells during neurodegenerative disorders (Hristovska and Pascual 2016; Pierre et al. 2017; Fujino et al. 2020). They are documented as a potentially sensitive indicator for pathological alterations in the CNS (Colonna and Butovsky 2017).

Responsible Editor: Lotfi Aleya

✉ Obeid Shanab
Shanab.bio@vet.svu.edu.eg

✉ Hassan Ahmed
hassan-younes@vet.svu.edu.eg

[1] Physiology Department, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt

[2] Department of Neuroinflammation and Brain Fatigue Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

[3] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt

[4] Center of Excellence in Screening of Environmental Contaminants (CESEC), Benha University, Toukh 13736, Egypt

[5] Department of Pharmacy, Southeast University, Dhaka, Bangladesh

[6] Pharmakon Neuroscience Research Network, Dhaka, Bangladesh

[7] Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

[8] Center of Biomedical Sciences Research (CBSR), Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

[9] Department of Clinical Laboratories Sciences, The Faculty of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

[10] Biochemistry Department, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt

[11] Physiology Department, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt

Published online: 07 August 2021

MG cells are abnormally activated in neurodegenerative disorders such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease (Fujino et al. 2017; Heneka 2017; Valori et al. 2019). Several cytokines and oxygen reactive radicals are abundantly released after such activation, followed by neuronal death and initiation of neurodegenerative diseases (Colonna and Butovsky 2017). It is hypothesized that over-activated MG cells undergo auto-regulation via necroptotic mechanisms to protect the neuronal cells from the harmful MG over-activation impacts (Ali et al. 2019). Accumulating evidence has demonstrated MG necroptosis in ischemic stroke (Yang et al. 2018), spinal cord damage (Fan et al. 2016), and various disorders of the nervous system (Caccamo et al. 2017; Royce et al. 2019). The recognition receptors Toll-like receptors 4 (TLR4) expressed in MG are strongly involved in such necroptotic regulation (Shen et al. 2018).

Lipopolysaccharide (LPS) is the main constituent of the gram-negative bacteria cell membrane and is defined as TLR4-ligand (Luo et al. 2017). Interestingly, the TLR4-ligands are strongly associated with the mechanisms involved in MG necroptosis and inflammatory response (Maeda and Fadeel 2014; Huang et al. 2018). Therefore, inhibiting MG necroptosis is essential to control the progressive neuronal damage (Oliveira et al. 2018). Furthermore, several lines of evidence point to caspases' involvement in the inflammatory process linked to neurodegenerative diseases (Julien and Wells 2017; Zhang et al. 2018). Complex different signaling pathways such as death receptor signaling, endoplasmic reticulum, and mitochondria-mediated pathways orchestrate a diversity of cell death processes including necroptosis, apoptosis, and autophagy (Fulda 2009; Vanden Berghe et al. 2015; Peña-Blanco and García-Sáez 2018).

Plasmalogens (PLS) are a class of phospholipids content of scallop and contain an ether bond in position S_N1 to an alkenyl group. PLS are distributed in various tissues including mainly cell membranes of nervous, immune, and cardiovascular tissues. They are strongly involved in nervous system development, inflammatory pathways, and cellular antioxidant defense mechanisms (Fujino et al. 2020). PLS has been shown to have a modulatory role in reducing LPS-induced inflammation in MG cells through inhibiting TLR-4 endocytosis (Ali et al. 2019). Accumulative evidence underpin the significant protective activity of PLS against AD and some neurodegenerative diseases (Fujino et al. 2017, 2020; Su et al. 2019). However, the mechanistic insights underlie the apoptotic pathways and the protective role of PLS in LPS-long-time treated MG cells remain unclear.

Therefore, the goal of this research was to look at the molecular processes underlying the effects of serum starvation and long-term LPS exposure in BV2 and MG6 cells, as well as the responsive modulatory role of PLS in injured cells. Opposite to neuronal cells, the results revealed that the non-neuronal MG cells were insensitive to serum starvation-induced death. However, prolonged LPS exposure stimulates necroptosis of the MG cells via caspase signaling pathways. Western blotting was performed to understand the molecular mechanism of the necroptosis cascade produced by LPS and/or PLS treatment. The findings support a role for PLS in protecting the MG cells against long-term LPS-induced injury.

Materials and methods

Cultured cells and reagents

MG6 cell lines were purchased (RCB catalog #RCB2403, RRID: CVCL_8732) from Health Science Research Resources Bank, Japan; BV2 immortalized cells were given as a gift from Dr. Hidetoshi Saitoh, Kyushu University, Japan. The BV2 and MG6 cells were held in a DMEM medium with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 50 μg/ml penicillin, and 50 μg/ml streptomycin (Invitrogen). At 37 °C, cells were cultured in a humidified chamber with 5% CO_2. Highly purity LPS was purchased from Sigma-Aldrich, St. Louis, MO, USA. PLS were extracted and purified from scallop as previously described (Sejimo et al. 2018). The primary antibodies for cleaved PARP-1, cleaved caspase-9, 3, and 8 were obtained from Cell Signaling Technology, Massachusetts, USA. While, β-actin antibody was provided by Santa-Cruz Biotechnology Inc., Texas, USA.

TUNEL assays

The suggested protocol of the In Situ Cell Death Detection Kit, TMR red, was used to quantify cell apoptosis using TUNEL assays (Roche). MG6 and BV2 cells were seeded in 12 chamber plates at a density of 1×10^5 cells/ chamber. The cultivated cell was rinsed in PBS and fixed for 15 min at room temperature in 2% paraformaldehyde. After a PBS wash, cells were permeabilized with a solution containing 0.1 % Triton X-100 in 0.1 % sodium citrate for 5 min. The cells were then rinsed again in PBS before incubating for 60 min at 37 °C with the TUNEL combination. Finally, the cells were thoroughly rinsed in cold PBS to eliminate any remaining TUNEL mixture and treated for 20 min with 1 μg/ml DAPI. After mounting, the cells were visualized by fluorescence lifetime imaging microscopy (Keyence, BZ-9000 series and BZ-X700 series, Japan).

Western blot analysis

MG6 and BV2 cells were cultured in 6 cm dishes at a density of 1×10^6/ml. Immunoblotting was performed as noted earlier (Hossain et al. 2013). SDS-PAGE (8 to 15 %) was used to

isolate the whole-cell lysate protein. The separated proteins were then transferred from the gel to nitrocellulose membrane (BIO-RAD) and incubated in 0.1% Tween-tris-buffered saline containing 5 % skimmed milk for blocking. Next, all proteins were probed against their specific antibodies (cleaved PARP-1, cleaved caspase-9, 3, and 8) at dilution of 1:1000. β-actin antibody was set as an endogenous control for this study. After that, the membranes were rinsed in TBS and incubated with the secondary antibodies for 120 min at 25 °C. Finally, protein bands were visualized by exposure to Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, Massachusetts) using LAS4000 Biomolecular Imager. Quantification of the band intensity was analyzed by densitometric analysis using the Image-J program.

Cell proliferation assays

MG6 and BV2 cells were seeded in 96 well plates at a density of 1×10^4 cells/well. As previously defined, the Cell Counting Kit-8 (cell proliferation assay kit; Dojindo, Kumamoto, Japan) was used to test the vitality of the cells (Hossain et al. 2013). At a wavelength of 450 nm, the absorbance value was measured using an ELISA plate reader.

Data statistics

All values are presented as means ± SE. One-way ANOVA and Student's *t*-test were used to investigate the effects of LPS in more than two groups and only two groups, respectively. Significant was described as a p value of less than 0.05. The Graph Pad Prism 5.0 program was used to perform all of the statistics.

Results

Insensitivity of BV2 cells to serum starvation-induced cytotoxicity

MTT assay was used to measure cell death in comparison to control cells. In contrast to the neuronal cells, treatment of BV2 cells with serum starvation (FBS 2%) for 72 h did not affect the cell viability (Fig. 1A). No changes were observed in the cultured BV2 were observed (Fig. 1B). Furthermore, there were no increases in the percentage of TUNEL-positive cells (Fig. 1C, D).

Serum starvation failed to induce cytotoxicity in MG6 cells

MG6 cells treated with low serum medium (FBS 2%) for 72 h, MTT assay was examined to the cell death compared with control cells (FBS 10%), treatment with FBS 2% did not affect the cell viability (Fig. 2A). Microscopic cell imaging that following serum starvation of microglial MG6 cells showed no decrease in cell number (Fig. 2B). Besides, there were no recorded increases in the TUNEL-positive cells (Fig. 2C, D).

Sensitivity of BV2 microglial cells to LPS cytotoxicity

LPS treatment resulted in a substantial reduction in the BV2 cell viability after 36 h following treatment with 1 μL LPS and switch from inflammatory activation to cell death, at different time points (Fig. 3A, B). However, in BV2 treated with 1 μL LPS for 6, 12, and 24 h, cell viability showed no alterations (Fig. 3A). In comparison to serum starvation, LPS treatment resulted in a substantial increase in the percentage of TUNEL-positive cells in BV2 cells (Fig. 3C. D).

Sensitivity of MG6 cells to LPS cytotoxicity

Indeed, the detection of MG6 cell viability by MTT assay showed that LPS treatment caused a time-dependent dramatic inhibition of cell viability. Exposure to 1 μL LPS for 6, 12, and 24 h did not cause changes in the cell viability (Fig. 4A). While, treatment for a long-time 36 h remarkably lowered the viability of cells (Fig. 4A, B). In addition, the TUNEL assay also revealed that LPS positively increases the number of TUNEL-positive cells (Fig. 4C, D).

Effect of LPS treatment on the apoptotic pathways in BV2 cells

Treatment of the BV2 cells with 1 μL LPS for 36 h triggered the up-regulation of caspase-8 expression (Fig. 5A, B) along with a drastic increase in the cleaved caspase-3 (Fig. 5A, C). Enhancement of the cleaved PARP1 expression was also detected in LPS-treated BV2 cells (Fig. 5A, D). However, there was no alteration in the expression level of cleaved caspase-9 after 36 h of LPS treatment (Fig. 5A, E).

Effect of LPS treatment on the apoptotic pathways in MG6 cells

Immunoblot analysis of the caspase signaling pathway in LPS-treated MG cells was performed in an attempt to observe the molecular events that activate MG death. The results revealed a marked increase in the expression of the cleaved caspase-9 (Fig. 6A, B), caspase-3 (Fig. 6A, C), and caspase-8 (Fig. 6A, E). In addition, a remarkable increase in the expression of the apoptotic marker, cleaved PARP-1 (Fig. 6A, D) was observed in LPS-treated MG6 cells.

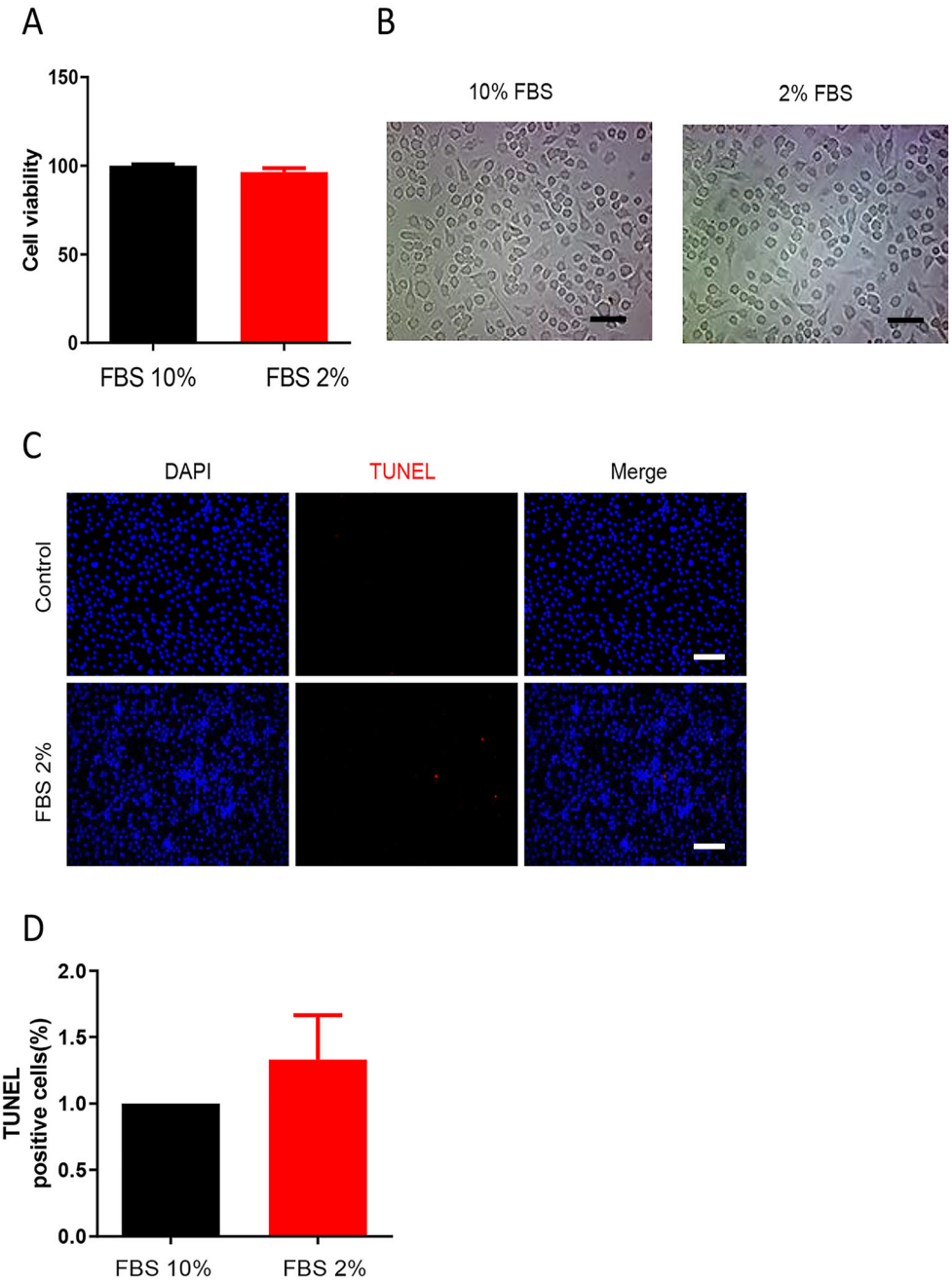

Fig. 1 Effect of serum starvation on BV2 cells. **A** Quantitative analysis of cell viability after 72 h culturing with 10% FBS (control cells) and 2% FBS (serum-starved cells). **B** Representative image for BV2 cells (scale bar = 50 μm). **C** Serum-starved cells, TUNEL assay revealed no apoptotic cells, scale bar = 50 μm. **D** Quantitative analyses of apoptotic cells

PLS modulated LPS-induced necroptosis in BV2 cells

To examine the modulatory effects of PLS on LPS-induced cytotoxicity in BV2 cells, cell viability and TUNEL-assay were assessed. As depicted in Fig. 7A and B, the treatment of BV2 cells with 1 μL LPS for 36 h caused a necroptosis and, consequently, marked reduction of cell viability was observed. However, pre-treatment with PLS could significantly mitigate the LPS-enhanced cell death (Fig. 7A, B). In addition, TUNEL assay analysis has identified an observable increase in the positive necroptosis cells after 36 h LPS treatment along with a significant reduction in the apoptotic cells in the PLS-pretreated group compared with LPS sole treatment (Fig. 7C, D).

PLS modulated LPS-induced necroptosis in MG6 cells

To investigate whether PLS could attenuate LPS-induced necroptosis in MG cells, MG6 cells were used. Short-course PLS incubation time was analyzed to ascertain if LPS-induced necroptosis were promoted. MG6 cells were treated with and/or without PLS and cell viability was determined. The data support a diminished

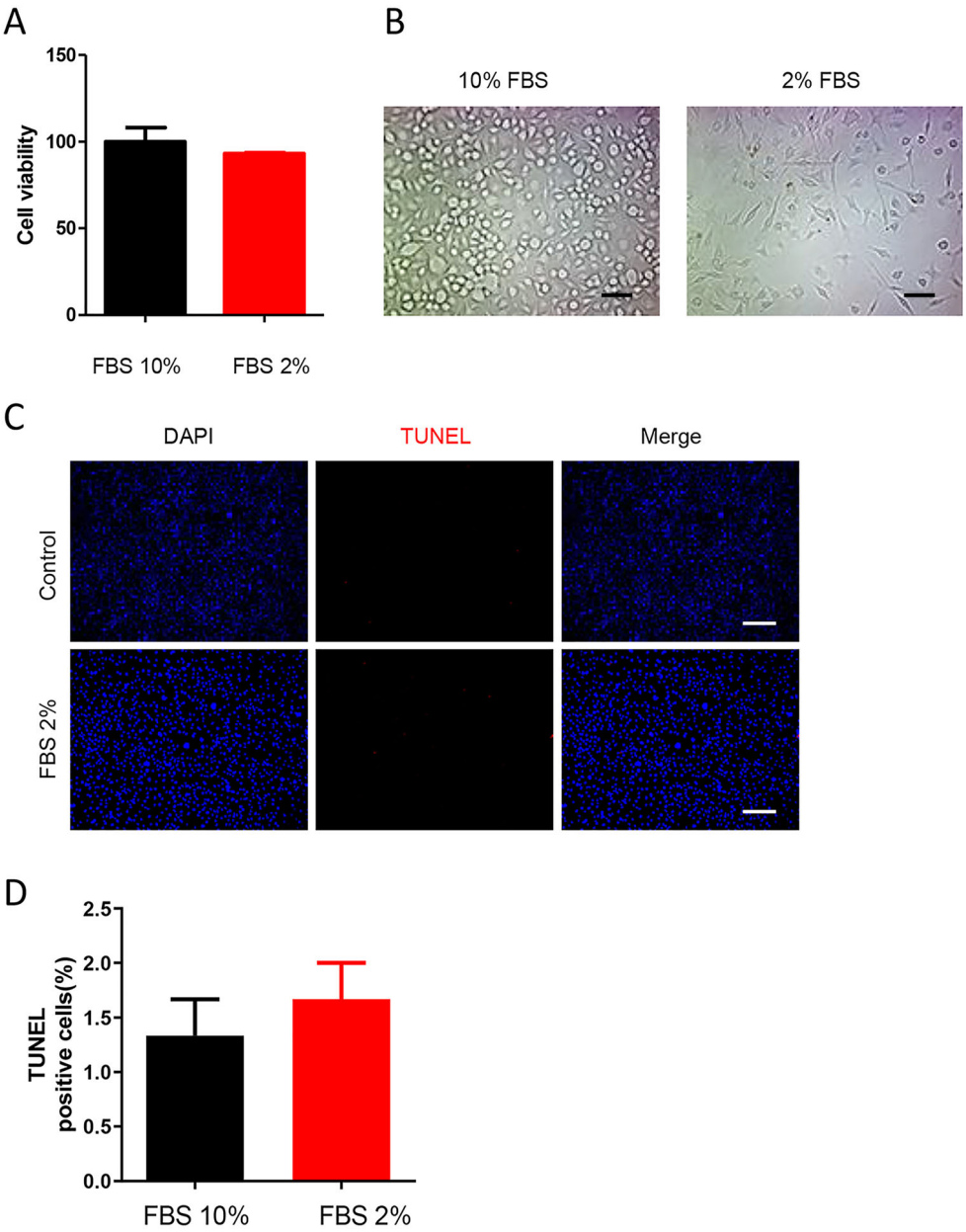

Fig. 2 Effect of serum starvation on MG6 cells. **A** Quantitative analysis of cell viability after 72 h culturing with 10% FBS (control cells) and 2% FBS (serum-starved cells). **B** Representative image for MG6 cells (scale bar = 50 μm). **C** Serum-starvedcells, TUNEL assay revealed no apoptotic cells, scale bar = 50 μm. **D** Quantitative analyses of apoptotic cells

LPS insult capacity to induce cell necroptosis as presented in Fig. 8 A and B. In comparison to LPS individual treatment, PLS-pretreated cells demonstrated a substantial decrease in necroptosis (Fig. 8C, D). Moreover, the western blot analyses exhibited that the pretreated cells with PLS for 12 h showed a significant reduction in the up-regulated expressions of the cleaved caspase 8 and 3 as well as PARP-1 compared to the LPS sole treatment (Fig. 9). The data obtained from Figs. 7, 8, and 9 suggested the existence of inhibitory activity of PLS against LPS-induced caspase signaling in MG cells.

Discussion

The present study explored the roles of caspase signaling in LPS-induced necroptosis as well as the mitigating effect of PLS in MG cells via long-time exposing BV2 and MG6 to LPS treatment. The obtained data revealed that MG cell viability was affected by the LPS treatment, but not by the serum starvation. LPS mediated the extrinsic and intrinsic pathways of necroptosis in BV2 and MG6 cells. However, PLS significantly inhibited the LPS-induced necroptosis in both cells.

In the current study, BV2 and MG6 cultured in serum-starved media for 72 h revealed no changes in the cell viability

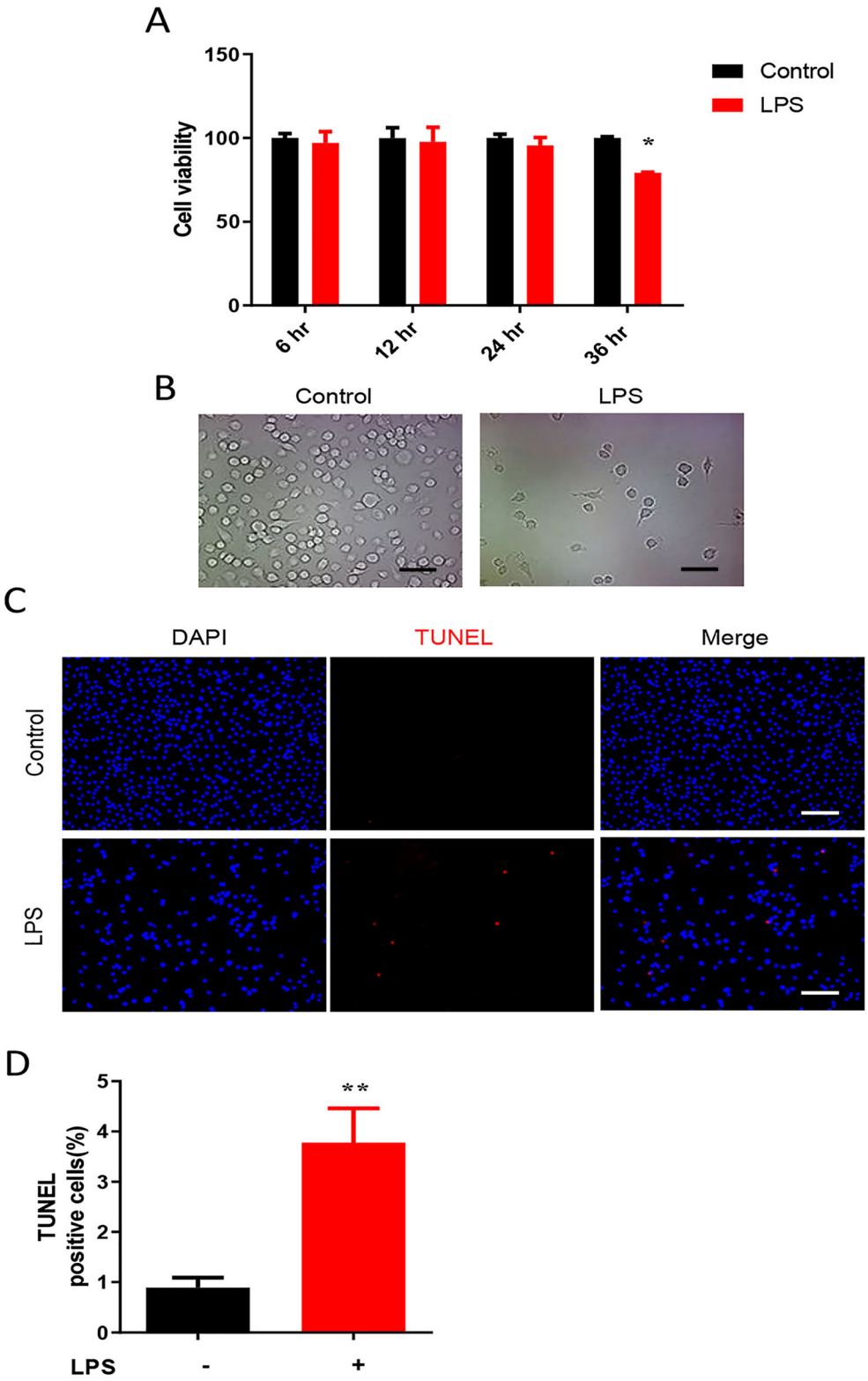

Fig. 3 LPS treatment induces BV2 cells apoptosis. **A** Quantitative analysis of BV2 cell viability after LPS exposure at different time-points (*$p < 0.05$ *vs* control group). **B** Representative image for BV2 cells (scale bar = 50 μm). **C** Apoptotic cells were examined by TUNEL assay with corresponding DAPI picture after being exposed to 1 μg/ml LPS for 36 h; scale bar = 50 μm. **D** The percentage of positive apoptotic cells is shown in the bar graph (**$p < 0.01$ *vs* control group)

and number of TUNEL-positive cells as well. These data suggest that serum starvation for 72 h could not induce the caspase signaling in the non-neuronal cells, BV2, and MG6 cells. Our findings are consistent with that obtained by Hossain et al. (2013), who investigated the effect of serum starvation on the mitochondrial apoptotic pathways in the non-neuronal cells. On the other hand, Burguillos and his group have reported a MG activation under LPS short time treatment (6 h)

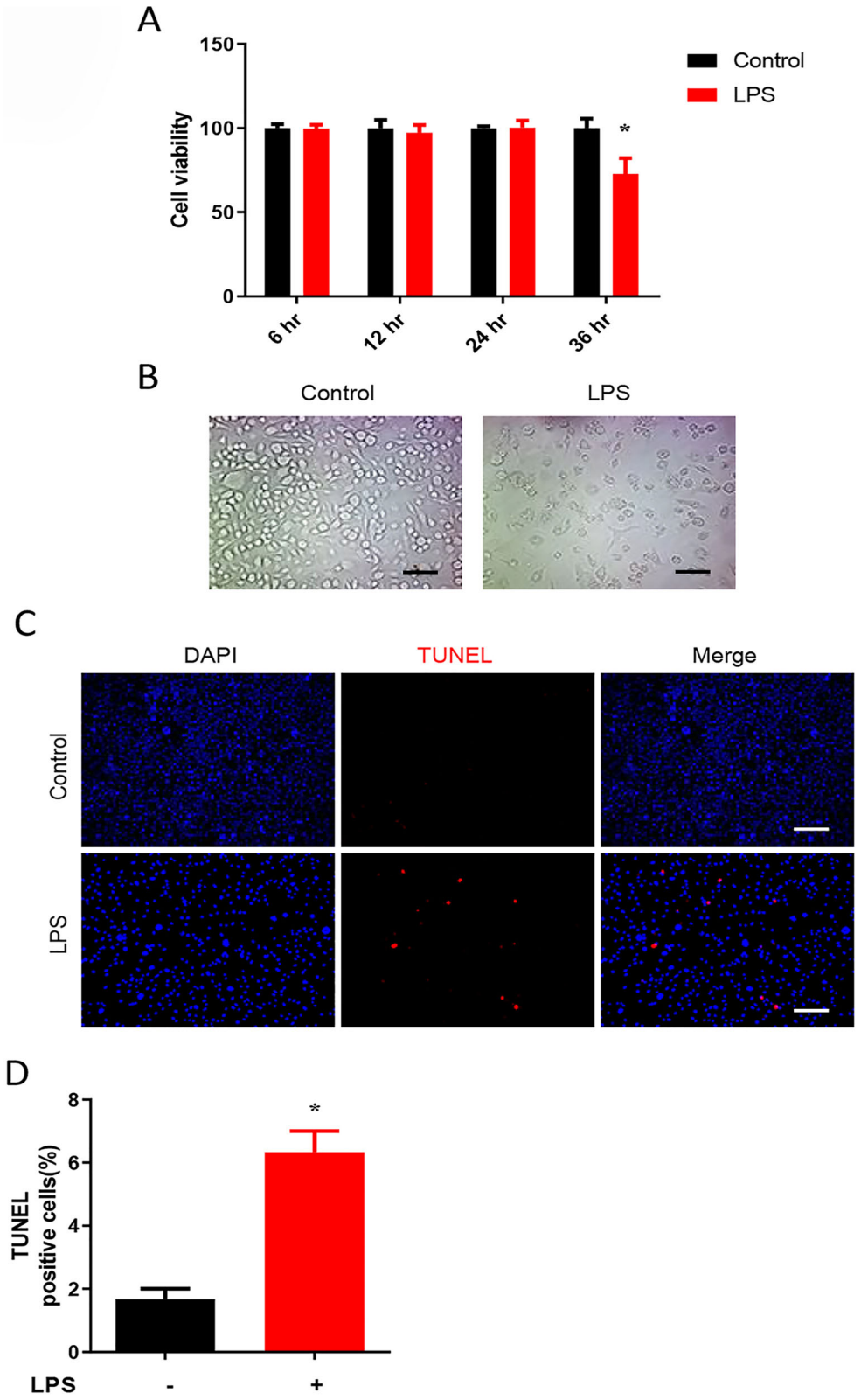

Fig. 4 LPS treatment induces MG6 cells apoptosis. **A** Quantitative analysis of MG6 cell viability after LPS exposure at different time-points (*$p < 0.05$ *vs* control group). **B** Representative image for MG6 cells (scale bar = 50 μm). **C** Apoptotic cells were examined by TUNEL assay with corresponding DAPI picture after being exposed to 1 μg/ml LPS for 36 h; scale bar = 50 μm. **D** The percentage of positive apoptotic cells is shown in the bar graph (*$p < 0.05$ *vs* control group)

(Burguillos et al. 2011). It is well documented that activation of MG caspase-3 is required to initiate the inflammatory response and production of cytokine in order to protect the neuronal cells. However, these events may be accompanied by the over-activation of MG cells making the matter worse and enhancing the neuronal damage (Colonna and Butovsky

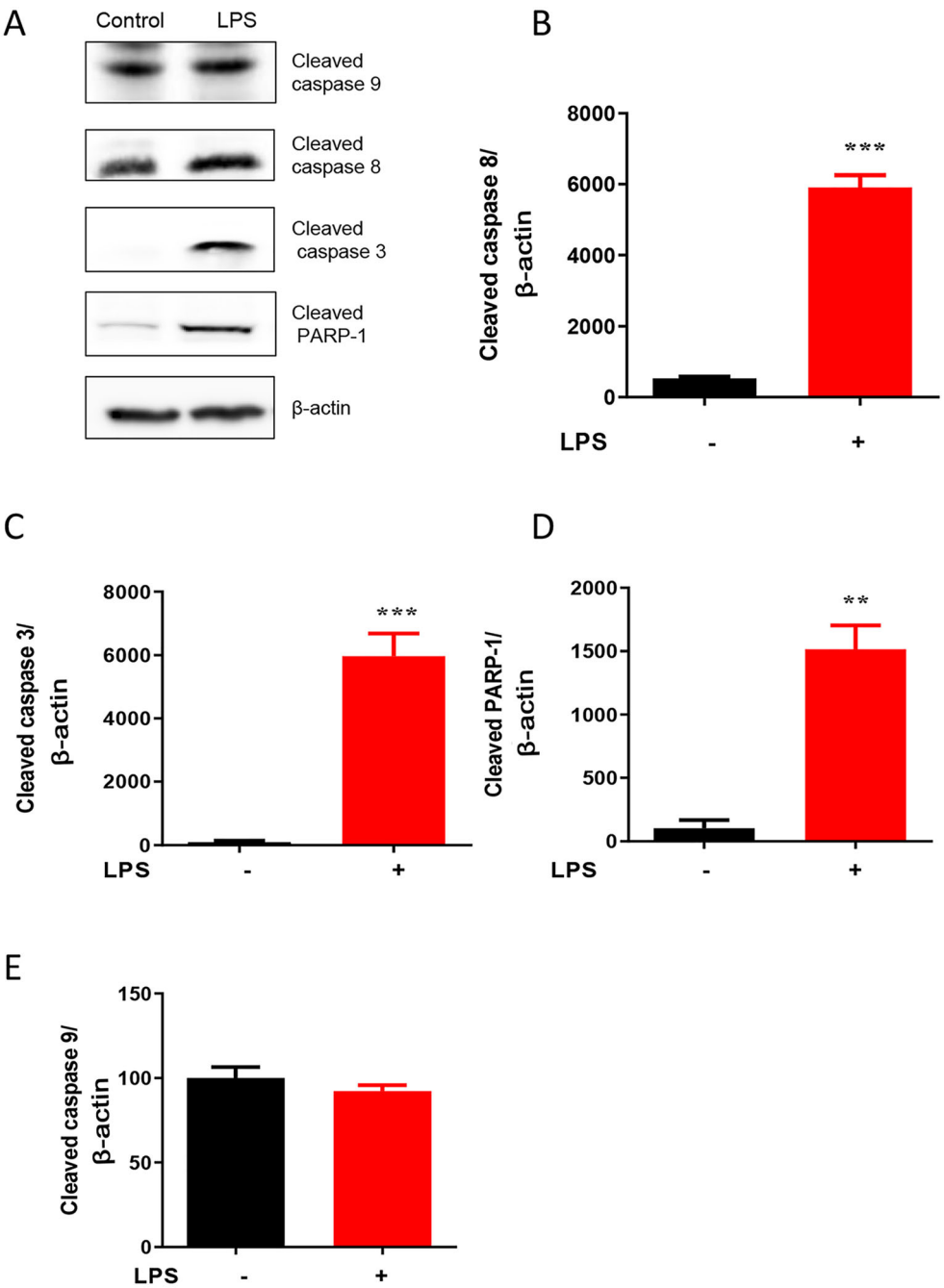

Fig. 5 LPS induces extrinsic apoptosis in BV2 cells. **A** BV2 cells were subjected to 1 μg/mL LPS for 36 h and western blot was performed. **B** Cleaved caspase-8 is elevated in response to LPS (***$p < 0.001$, n = 3). **C** Caspase-8 activation leads to increase the cleaved caspase-3 expression (***$p < 0.001$, n = 3). **D** Increased expression of the cleaved PARP-1 (**$p < 0.01$, n = 3). **E** Caspase-9 expression was not changed in response to LPS treatment. For densitometry, β-actin was used as a loading control. All data are displayed as means ± SEM

2017), which dedicates the importance of regulatory mechanisms required for MG survival and activation.

Along with the present study, several studies support the implication of extrinsic caspase signaling in MG apoptosis. Extrinsic apoptosis is known to begin by activation of caspase-8 which derives activated caspase-3; thereby, the cleaved PARP-1 is up-regulated triggering the MG apoptosis (Vanden Berghe et al. 2015). In a study performed by Xie et al. (2010a), BV2 cells and mouse primary culture were exposed to morphine. That experiment concluded that caspase-8 plays a central role in the MG apoptosis induced by morphine exposure. In another study conducted by the same research group, extrinsic apoptosis was stimulated in the BV2 MG cell after valproic acid treatment (Xie et al. 2010b). Moreover, short time LPS treatment has been reported to induce caspase-8 and caspase-3 activation which regulates the activation of the MG cells via a PKCδ-dependent pathway and eventually triggers a pro-inflammatory response required for mitigating the neuronal injury (Burguillos et al. 2011; Shen et al. 2018; Ali et al. 2019). In the same line, our results indicated that a long-time (36 h) treatment of LPS in BV2 cell showed enhanced expression of caspase 8 and 3

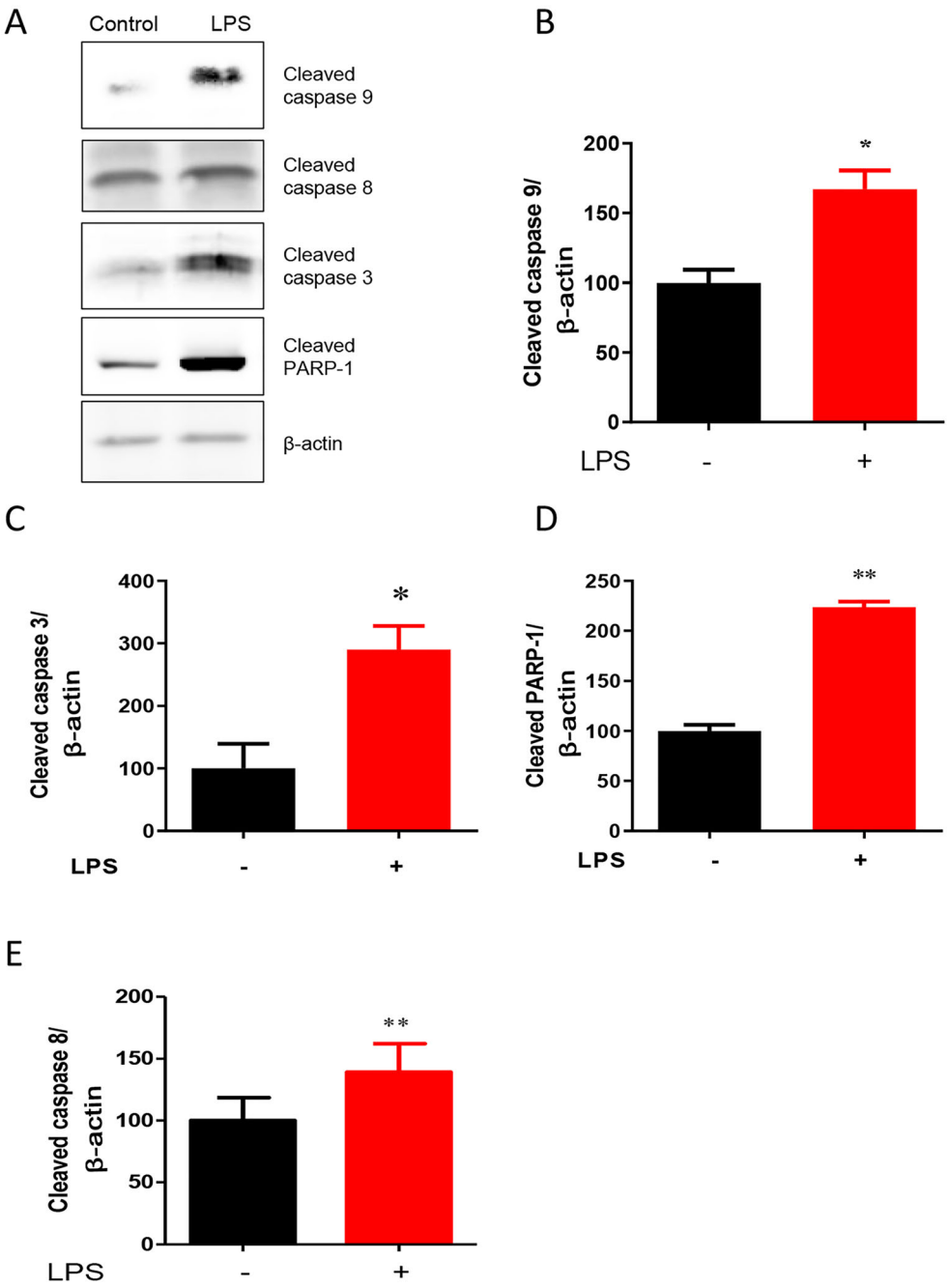

Fig. 6 Mitochondrial apoptosis is specific for MG6 toward LPS treatment. **A** Western blot analysis was performed for MG6 cultured with and without LPS 1 μg/mL for 36 h. **B** Western blot analysis and corresponding densitometry identified an increase in cleaved caspase-9 levels in LPS whole cell lysates compared with controls (*$p < 0.05$, n = 3). **C** Caspase-9 activation results in increase the cleaved caspase-3 expression (*$p < 0.05$, n = 3). **D** Increased expression of the cleaved PARP-1 (**$p < 0.01$, n = 3). **E** Caspase-8 expression was significantly elevated in response to LPS treatment (**$p < 0.01$, n = 3). For densitometry, β-actin was used as a loading control. All data are displayed as means ± SEM

without changes in caspase 9. Later on, these events progressed to activation of the downstream target, PARP-1 (apoptosis marker) suggesting that the observed BV2 cell death has occurred via the extrinsic apoptotic pathway in TLR4-dependent mechanisms (Lemmers et al. 2007).

Interestingly, in the present study, long-time-LPS treatment triggers intrinsic and extrinsic apoptosis in MG6 cells. There is evidence that showed a crosstalk between the intrinsic and extrinsic pathways of apoptosis after prolonged LPS exposure which occurred via the mitochondrial disintegration associated with caspase-3 activation in a process mediated by the cleaved caspase-9 along with up-regulation of caspase 8. In this study, it is assumed that LPS treatment promotes the TLR4 endocytosis and further caspase-8 activation with nuclear factor kappa B ($NF\text{-}_kB$) up-regulation and downstream production of pro-inflammatory cytokines (Ali et al. 2019). By another way, LPS treatment increased the production of ROS leading to DNA damage and further translocation of Bax; thereby, the mitochondria lose their membrane potential in a process that progressed to the release of cytochrome c and caspase-9 activation for the induction of apoptotic cascade (Wu et al. 2014). A significant cell death indicated by a

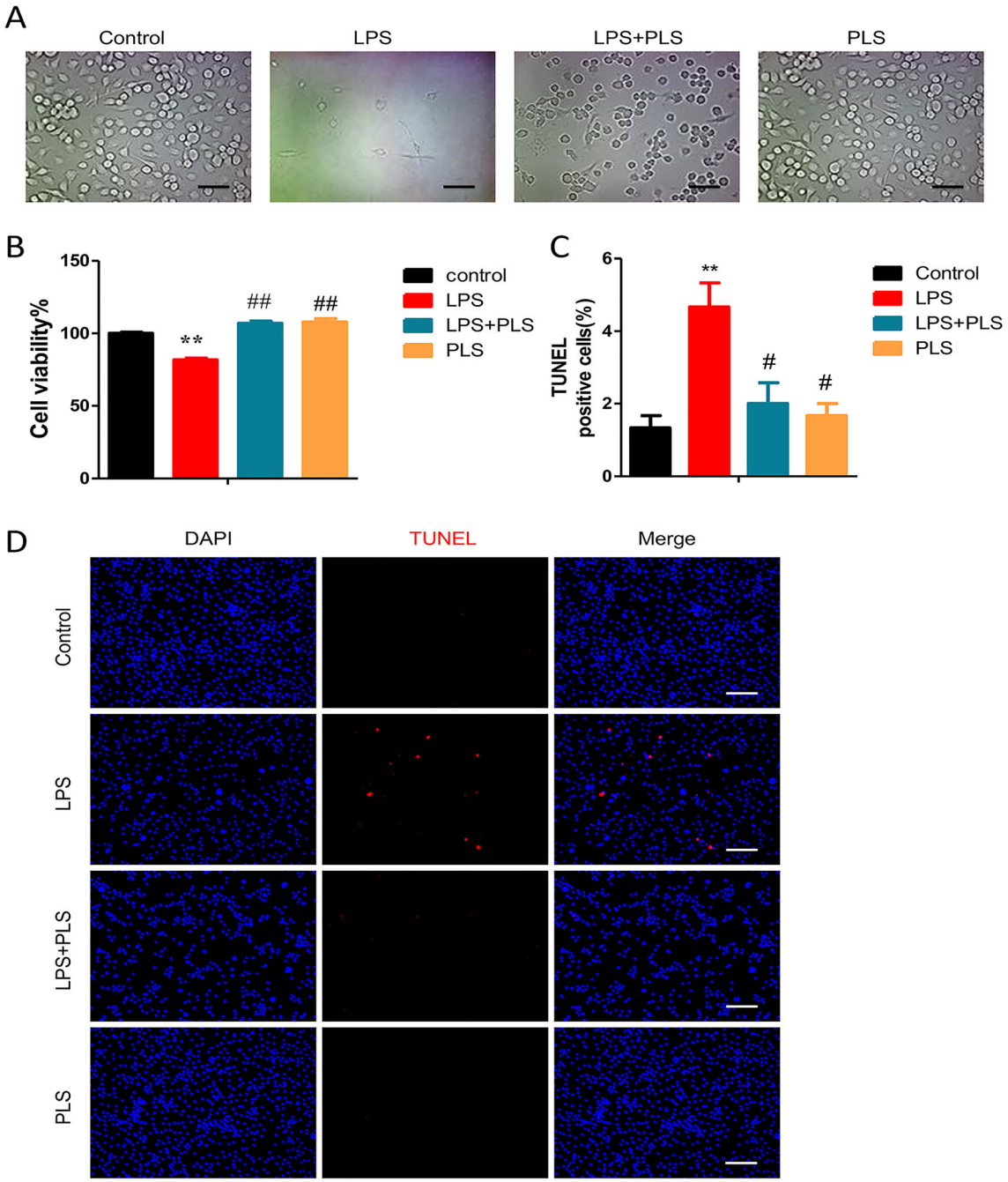

Fig. 7 PLS pretreatment inhibits LPS-induced apoptosis in BV2 cells. **A** Representative image for BV2 cells after treatment with LPS and/or PLS (scale bar = 50 μm). **B** Quantitative analysis of cell viability. **C** The bar graph depicts the percentage of BV2 cells that were positive for apoptosis after being exposed to LPS for 36 h and PLS (5 μg/ml) for 12 h; apoptotic cells were examined using the TUNEL assay with a corresponding DAPI image; scale bar = 50 μm (**D**). All data are displayed as means ± SEM (n = 3; * *vs* control group; # *vs* LPS group; ** $p < 0.01$; # $p < 0.05$; ## $p < 0.01$)

remarkable reduction of cell viability and increased positive apoptotic cells were denoted in MG6 cells after long-time treatment by LPS. These events may be attributed to the LPS-enhanced expression of the cleaved caspase-9 and cleaved caspase 8. The implication of caspases 3, 8, and 9 in the LPS-induced apoptosis confirms the data obtained by previous reports supporting the crosstalk between the extrinsic and intrinsic apoptosis in various cell types (Hu et al. 2015; Elkin et al. 2018; Wang et al. 2018; Xu et al. 2019; Abdeen et al. 2020).

PLS are a class of phospholipids integrated in the neuronal membranes. PLS has shown anti-inflammatory and antioxidant activities during neurodegenerative diseases (Su et al. 2019; Fujino et al. 2020). Fujino and his group have documented the efficacy of oral administration of PLS in improving cognitive function in patients with mild AD (Fujino et al.

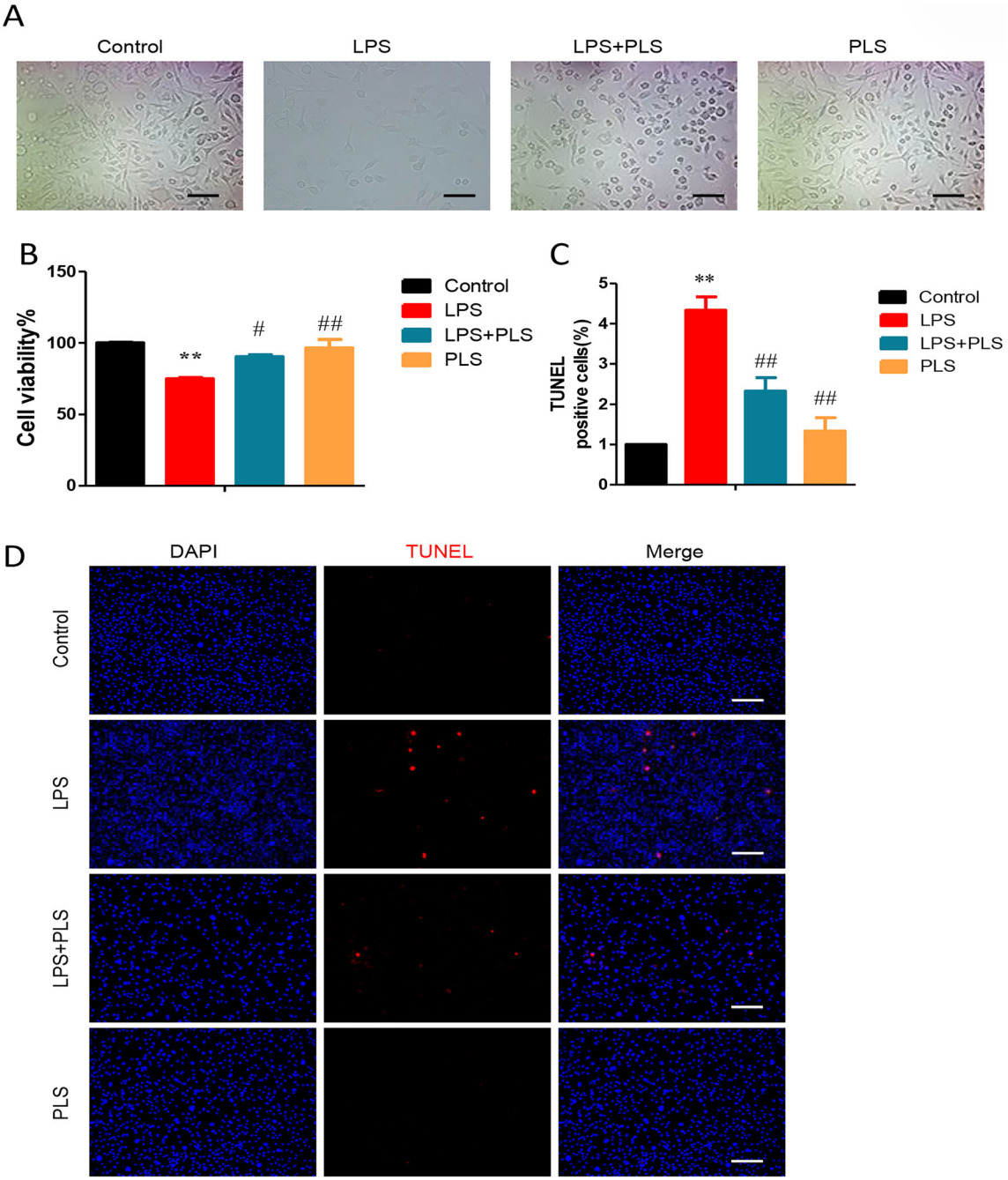

Fig. 8 PLS pretreatment inhibits LPS-induced apoptosis in MG6 cells. **A** Representative image for MG6 cells after treatment with LPS and/or PLS (scale bar = 50 μm). **B** Quantitative analysis of cell viability. **C** The bar graph depicts the percentage of MG6 cells that were positive for apoptosis after being exposed to LPS for 36 h and PLS (5 μg/ml) for 12 h; apoptotic cells were examined using the TUNEL assay with a corresponding DAPI image; scale bar = 50μm (**D**). All data are displayed as means ± SEM (n = 3; * *vs* control group; # *vs* LPS group; ** $p < 0.01$; # $p < 0.05$; ## $p < 0.01$)

2017). Recent work has implied that MG cells necroptosis contributes to caspase signaling and neuroinflammatory reactions via TLR4 activation (Huang et al. 2018). In the current study, PLS could potentially inhibit the accelerated LPS-induced up-regulation of caspase-8 and 3 and PARP-1 to levels near to controls. These findings explain the role of PLS in inhibiting the caspase-dependent apoptotic pathways after long LPS treatment. As a sequent, marked improvement in the cell viability along with significantly diminished TUNEL-positive cells after long-time-LPS treatment were observed. Such improvements might be attributed to the ability of PLS to reduce LPS-induced TLR4 endocytosis and consequently, inhibition of caspase signaling and production of inflammatory mediators (Ali et al. 2019). Moreover, the antioxidant properties of PLS might have a role in decreasing ROS-induced apoptosis.

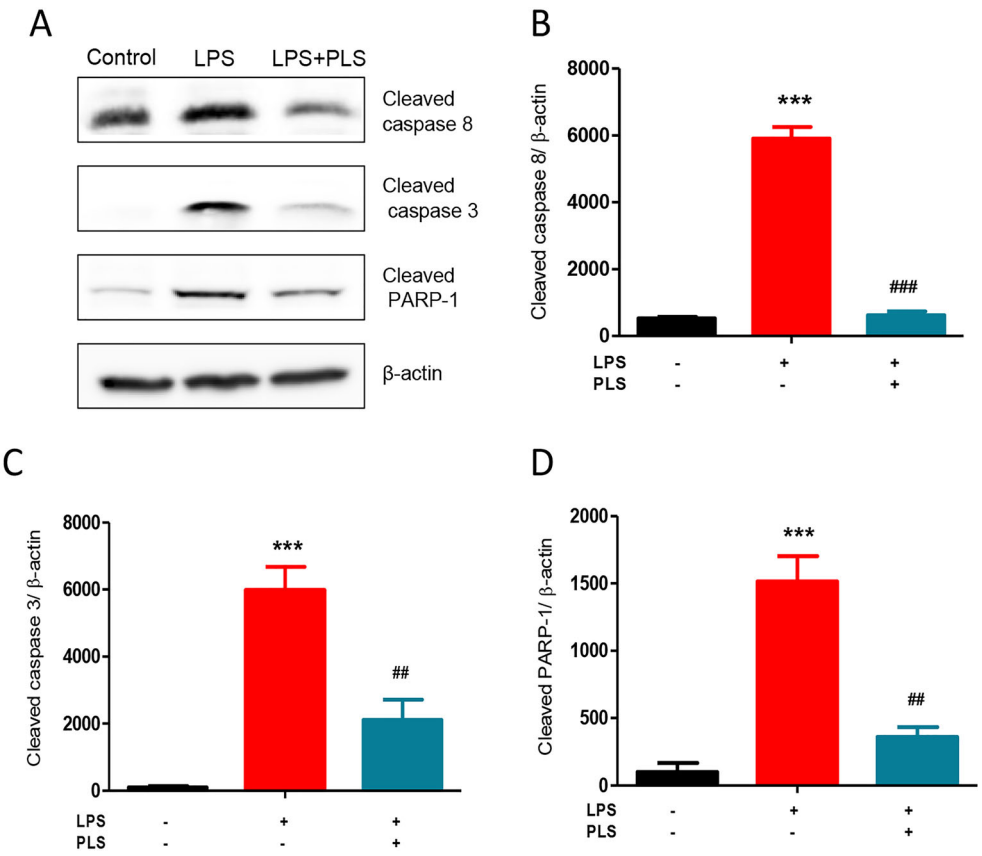

Fig. 9 PLS pretreatment inhibits LPS-triggered caspase signaling in cells. **A** Immunoblots of cleaved caspase-8 and 3, PARP-1, and β-actin after treatments with LPS and/or PLS. **B** Semiquantitative analysis of cleaved caspase-8 protein expression. **C** Semiquantitative analysis of cleaved caspase-3 protein expression. **D** Semiquantitative analysis of cleaved PARP-1 protein expression. For densitometry, β-actin was used as a loading control. All data are displayed as means ± SEM. (n = 3; * *vs* control group; # *vs* LPS group; ***$p < 0.001$; ##$p < 0.01$; ###$p < 0.001$)

These data confirm the potential role of PLS in attenuating the LPS-inflicted necroptosis in MG cells, after LPS long-time treatment, via its anti-inflammatory and antioxidant properties. Fig. 10 summarizes the possible protective mechanisms of PLS pretreatment in LPS long-time treated MG cell.

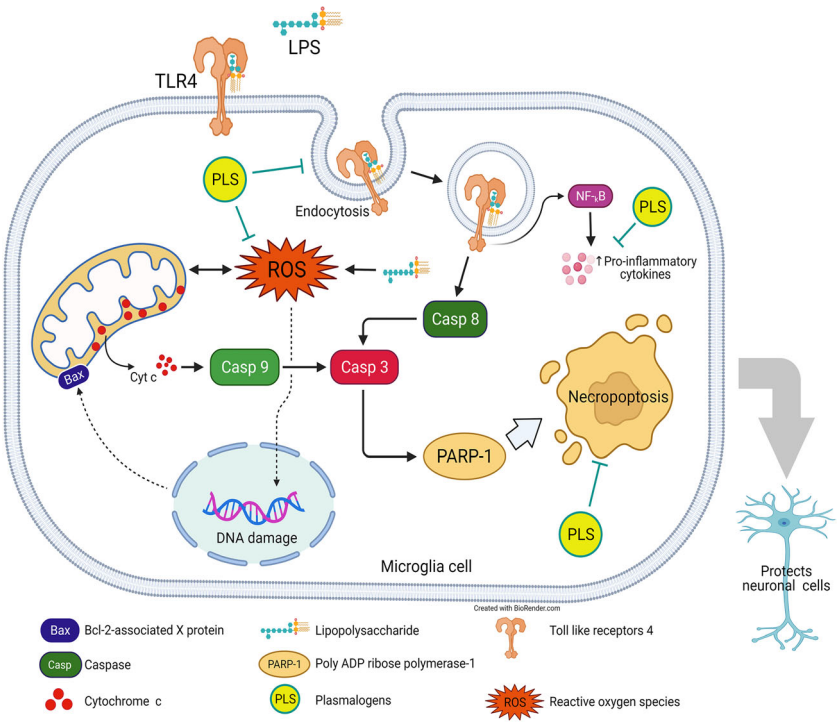

Fig. 10 Schematic diagram illustrates the possible protective mechanisms of PLS pretreatment in LPS-long-time-treated microglia cell

Conclusions

Altogether the findings suggest that long-time LPS treatment could induce necroptosis in MG cells. The caspase signaling pathway is implicated in the LPS-induced MG death. A crosstalk between the extrinsic and intrinsic apoptosis has been reported. The neuronal cell serum starvation could not affect the non-neuronal cell/MG survival. PLS could mitigate the LPS-induced necroptosis in MG cells via its anti-inflammatory and antioxidant properties. We anticipate that our finding may elucidate some mechanistic insights related to the effect of long-time LPS treatment on MG survival. Besides, the potential use of PLS in the regulation of MG cell activation in order to control neuronal death during neurodegenerative diseases.

Acknowledgments The authors are grateful for the resources offered by the Center of Excellence in Screening of Environmental Contaminants (STDF grant no. 31290). Taif University Researchers Supporting Project number (TURSP-2020/53), Taif University, Taif, Saudi Arabia, is also acknowledged by the authors.

Authors' contributions Conceptualization and methodology, F.A., and S.H.; software, A.A., S.U., W.A., and H.A.; validation, F.A. and S.H.; formal analysis, S.H., R.W., and H.A.; resources, F.A., S.H., and W.A; data curation, F.A., S. H, S.U., and A.G; writing—original draft preparation, all authors; writing-review and editing, F.A., A.A, R.W, and H.A.

Data Availability The data used to support the findings of this study are available from the corresponding authors upon request.

Declarations

Ethical Approval Not applicable.

Consent for publication Not applicable.

Consent to Participate Not applicable.

Conflict of Interest The authors have indicated that the article's content does not interfere with their personal interests.

References

Abdeen A, Abdelkader A, Elgazzar D, Aboubakr M, Abdulah OA, Shoghy K, Abdel-Daim M, el-Serehy HA, Najda A, el-Mleeh A (2020) Coenzyme Q10 supplementation mitigates piroxicam-induced oxidative injury and apoptotic pathways in the stomach, liver, and kidney. Biomed Pharmacother 130:110627. https://doi.org/10.1016/j.biopha.2020.110627

Ali F, Hossain MS, Sejimo S, Akashi K (2019) Plasmalogens inhibit endocytosis of toll-like receptor 4 to attenuate the inflammatory signal in microglial cells. Mol Neurobiol 56:3404–3419. https://doi.org/10.1007/s12035-018-1307-2

Burguillos MA, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, Cano J, Brundin P, Englund E, Venero JL, Joseph B (2011) Caspase signalling controls microglia activation and neurotoxicity. Nature 472:319–324. https://doi.org/10.1038/nature09788

Caccamo A, Branca C, Piras IS, Ferreira E, Huentelman MJ, Liang WS, Readhead B, Dudley JT, Spangenberg EE, Green KN, Belfiore R, Winslow W, Oddo S (2017) Necroptosis activation in Alzheimer's disease. Nat Neurosci 20:1236–1246. https://doi.org/10.1038/nn.4608

Colonna M, Butovsky O (2017) Microglia function in the central nervous system during health and neurodegeneration. Annu Rev Immunol 10:441–468. https://doi.org/10.1146/annurev-immunol-051116-052358

Elkin ER, Harris SM, Loch-Caruso R (2018) Trichloroethylene metabolite S-(1,2-dichlorovinyl)-L-cysteine induces lipid peroxidation-associated apoptosis via the intrinsic and extrinsic apoptosis pathways in a first-trimester placental cell line. Toxicol Appl Pharmacol 338:30–42. https://doi.org/10.1016/j.taap.2017.11.006

Fan H, Zhang K, Shan L, Kuang F, Chen K, Zhu K, Ma H, Ju G, Wang YZ (2016) Reactive astrocytes undergo M1 microglia/macrohpages-induced necroptosis in spinal cord injury. Mol Neurodegener 11:1–16. https://doi.org/10.1186/s13024-016-0081-8

Fujino T, Yamada T, Asada T et al (2017) Efficacy and blood plasmalogen changes by oral administration of plasmalogen in patients with mild Alzheimer's disease and mild cognitive impairment: a multicenter, randomized, double-blind, placebo-controlled. EBioMedicine 17:199–205. https://doi.org/10.1016/j.ebiom.2017.02.012

Fujino T, Hossain MS, Mawatari S (2020) Peroxisome biology: experimental models, peroxisomal disorders and neurological diseases. Springer Nature, Switzerland

Fulda S (2009) Apoptosis pathways and neuroblastoma therapy. Curr Pharm Des 15:430–435. https://doi.org/10.2174/138161209787315846

Gomez-Nicola D, Perry VH (2015) Microglial dynamics and role in the healthy and diseased brain: a paradigm of functional plasticity. Neuroscientist 21:169–184. https://doi.org/10.1177/1073858414530512

Heneka MT (2017) Inflammasome activation and innate immunity in Alzheimer's disease. Brain Pathol 27:220–222. https://doi.org/10.1111/bpa.12483

Hossain MS, Ifuku M, Take S et al (2013) Plasmalogens rescue neuronal cell death through an activation of AKT and ERK survival signaling. PLoS One 8:1–14. https://doi.org/10.1371/journal.pone.0083508

Hristovska I, Pascual O (2016) Deciphering resting microglial morphology and process motility from a synaptic prospect. Front Integr Neurosci 9:1–7. https://doi.org/10.3389/fnint.2015.00073

Hu L, Su C, Song X, Shi Q, Fu J, Xia X, Xu D, Song E, Song Y (2015) Tetrachlorobenzoquinone triggers the cleavage of Bid and promotes the cross-talk of extrinsic and intrinsic apoptotic signalings in pheochromocytoma (PC) 12 cells. Neurotoxicology 49:149–157. https://doi.org/10.1016/j.neuro.2015.06.005

Huang Z, Zhou T, Sun X, Zheng Y, Cheng B, Li M, Liu X, He C (2018) Necroptosis in microglia contributes to neuroinflammation and retinal degeneration through TLR4 activation. Cell Death Differ 25:180–189. https://doi.org/10.1038/cdd.2017.141

Julien O, Wells JA (2017) Caspases and their substrates. Cell Death Differ 24:1380–1389. https://doi.org/10.1038/cdd.2017.44

Lemmers B, Salmena L, Bidère N, Su H, Matysiak-Zablocki E, Murakami K, Ohashi PS, Jurisicova A, Lenardo M, Hakem R, Hakem A (2007) Essential role for caspase-8 in toll-like receptors and NFκB signaling. J Biol Chem 282:7416–7423. https://doi.org/10.1074/jbc.M606721200

Luo Q, Yan X, Bobrovskaya L, Ji M, Yuan H, Lou H, Fan P (2017) Anti-neuroinflammatory effects of grossamide from hemp seed via suppression of TLR-4-mediated NF-κB signaling pathways in lipopolysaccharide-stimulated BV2 microglia cells. Mol Cell Biochem 428:129–137. https://doi.org/10.1007/s11010-016-2923-7

Maeda A, Fadeel B (2014) Mitochondria released by cells undergoing TNF-α-induced necroptosis act as danger signals. Cell Death Dis 5: e1312–e1319. https://doi.org/10.1038/cddis.2014.277

Oliveira SR, Amaral JD, Rodrigues CMP (2018) Mechanism and disease implications of necroptosis and neuronal inflammation. Cell Death Dis 9:10–12. https://doi.org/10.1038/s41419-018-0872-7

Peña-Blanco A, García-Sáez AJ (2018) Bax, Bak and beyond — mitochondrial performance in apoptosis. FEBS J 285:416–431. https://doi.org/10.1111/febs.14186

Pierre WC, Smith PLP, Londono I, Chemtob S, Mallard C, Lodygensky GA (2017) Neonatal microglia: the cornerstone of brain fate. Brain Behav Immun 59:333–345. https://doi.org/10.1016/j.bbi.2016.08.018

Royce GH, Brown-Borg HM, Deepa SS (2019) The potential role of necroptosis in inflammaging and aging. GeroScience 41:795–811. https://doi.org/10.1007/s11357-019-00131-w

Sejimo S, Hossain MS, Akashi K (2018) Scallop-derived plasmalogens attenuate the activation of PKCδ associated with the brain inflammation. Biochem Biophys Res Commun 503:837–842. https://doi.org/10.1016/j.bbrc.2018.06.084

Shen X, Venero JL, Joseph B, Burguillos MA (2018) Caspases orchestrate microglia instrumental functions. Prog Neurobiol 171:50–71. https://doi.org/10.1016/j.pneurobio.2018.09.007

Su XQ, Wang J, Sinclair AJ (2019) Plasmalogens and Alzheimer's disease: a review. Lipids Health Dis 18:1–10

Valori CF, Guidotti G, Brambilla L, Rossi D (2019) Astrocytes in Motor Neuron Diseases. In: Advances in Experimental Medicine and Biology. Springer Nature, Singapore, pp 227–272

Vanden Berghe T, Kaiser WJ, Bertrand MJM, Vandenabeele P (2015) Molecular crosstalk between apoptosis, necroptosis, and survival signaling. Mol Cell Oncol 2:1–13. https://doi.org/10.4161/23723556.2014.975093

Wang S, Xu Y, Li C, Tao H, Wang A, Sun C, Zhong Z, Wu X, Li P, Wang Y (2018) Gambogic acid sensitizes breast cancer cells to TRAIL-induced apoptosis by promoting the crosstalk of extrinsic and intrinsic apoptotic signalings. Food Chem Toxicol 119:334–341. https://doi.org/10.1016/j.fct.2018.02.037

Wu H, Che X, Zheng Q, Wu A, Pan K, Shao A, Wu Q, Zhang J, Hong Y (2014) Caspases: a molecular switch node in the crosstalk between autophagy and apoptosis. Int J Biol Sci 10:1072–1083. https://doi.org/10.7150/ijbs.9719

Xie N, Li H, Wei D, LeSage G, Chen L, Wang S, Zhang Y, Chi L, Ferslew K, He L, Chi Z, Yin D (2010a) Glycogen synthase kinase-3 and p38 MAPK are required for opioid-induced microglia apoptosis. Neuropharmacology 59:444–451. https://doi.org/10.1016/j.neuropharm.2010.06.006

Xie N, Wang C, Lin Y, Li H, Chen L, Zhang T, Sun Y, Zhang Y, Yin D, Chi Z (2010b) Neuroscience Letters The role of p38 MAPK in valproic acid induced microglia apoptosis. Neurosci Lett 482:51–56. https://doi.org/10.1016/j.neulet.2010.07.004

Xu B, Sui YL, Fan TJ (2019) Gatifloxacin inducing apoptosis of stromal fibroblasts through cross-talk between caspase-dependent extrinsic and intrinsic pathways. Int J Ophthalmol 12:1524–1530. https://doi.org/10.18240/ijo.2019.10.02

Yang J, Zhao Y, Zhang L, Fan H, Qi C, Zhang K, Liu X, Fei L, Chen S, Wang M, Kuang F, Wang Y, Wu S (2018) RIPK3/MLKL-mediated neuronal necroptosis modulates the M1/M2 polarization of microglia/macrophages in the ischemic cortex. Cereb Cortex 28: 2622–2635. https://doi.org/10.1093/cercor/bhy089

Zhang C-J, Jiang M, Zhou H, Liu W, Wang C, Kang Z, Han B, Zhang Q, Chen X, Xiao J, Fisher A, Kaiser WJ, Murayama MA, Iwakura Y, Gao J, Carman J, Dongre A, Dubyak G, Abbott DW et al (2018) TLR-stimulated IRAKM activates caspase-8 inflammasome in microglia and promotes neuroinflammation. J Clin Invest 128:5399–5412. https://doi.org/10.1172/JCI121901