



# Lipopolysaccharide-induced inflammation in human peritoneal mesothelial cells is controlled by ERK1/2-CDK5-PPAR $\gamma$ axis

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**Background:** Peritonitis is a common complication in which the peritoneum becomes inflamed. Peroxisome proliferator-activated receptor (PPAR) $\gamma$  agonists and extracellular signal-regulated kinases 1/2 (ERK1/2) inactivation have been found to restore damage caused by lipopolysaccharide-induced (LPS) inflammation. This study aimed to investigate the association between PPAR $\gamma$  and ERK1/2 in LPS-induced inflammation in peritonitis.

**Methods:** Human peritoneal mesothelial cells were maintained in Dulbecco's Modified Eagle Medium and treated with LPS under a series of different concentrations and treatment times. Cellular interleukins-1 $\beta$  (IL-1 $\beta$ ), cellular interleukins-6 (IL-6), cellular interleukins-12 (IL-12) were measured by enzyme-linked immunosorbent assay (ELISA) assay. Expression or activation of cyclin-dependent kinase (CDK)5, ERK1/2, and PPAR $\gamma$  was detected using quantitative real-time PCR and/or western blot.

**Results:** LPS induced dose- and time-dependent increments in the cellular IL-1 $\beta$ , IL-6, and IL-12 contents, cyclin-dependent kinase 5 (CDK5) expression, and PPAR $\gamma$ <sup>Ser273</sup> phosphorylation. Treatment with 1  $\mu$ g/mL LPS for 12 hours was the optimal experimental design for inflammation stimulation. The concentration of LPS over 1  $\mu$ g/mL or treatment more than 12 hours reduced the inflammatory status. LPS stimulation also activated ERK1/2 and increased its interaction with CDK5. Further, ERK1/2 inhibition by AZD0364 prevented IL-1 $\beta$ , IL-6, IL-12, and CDK5 expression, as well as activation of ERK1/2 and phosphorylation of PPAR $\gamma$ , induced by LPS. Knockdown of CDK5 using its siRNA caused similar changes as AZD0364, minus ERK1/2 inactivation.

**Conclusions:** Our results suggested that LPS-induced inflammation in human peritoneal mesothelial cells can be partly suppressed by inhibiting the ERK1/2/CDK5/PPAR $\gamma$  axis.

**Keywords:** Extracellular signal-regulated kinases 1/2 (ERK1/2); lipopolysaccharide (LPS); peritonitis; cyclin-dependent kinase 5 (CDK5); peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ )

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## Introduction

Peritonitis is a common complication of peritoneal dialysis (PD), accounting for approximately 15% of mortality in patients with PD (1-3). It refers to inflammation of the peritoneum. Increased episodes of peritonitis in patients with PD are correlated with or are predictive of death (1).

Typical causes of peritonitis include spontaneous bacterial or fungal infection, visceral perforation or damage, inflammation, appendicitis, inflammatory bowel diseases, and feline infectious peritonitis virus (4-7). Widely used animal models of peritonitis include intraperitoneal inoculation of the gut bacterium *Bacteroides fragilis* (*B. fragilis*), which secretes lipopolysaccharide (LPS) and LPS promotes inflammation by stimulating the production of interleukins (ILs) such as IL-1 $\beta$ , IL-6 and IL-8, tumor necrosis factor (TNF)- $\alpha$  via Toll-like receptor 2 (TLR2) activation (5). Research using LPS-induced peritonitis models has suggested that peroxisome proliferator-activated receptor (PPAR) $\gamma$  plays crucial anti-inflammatory roles (8,9), with the expression of PPAR $\gamma$  exerting a protective effect against LPS-induced inflammation injury.

The anti-inflammatory effect of PPAR $\gamma$  is related to the inhibition of various inflammatory pathways, including the nuclear factor-kappa B (NF- $\kappa$ B) pathway (8,10). Activation of extracellular signal-regulated kinases 1/2 (ERK1/2) is associated with LPS-induced mastitis (11,12), acute lung injury (13), and proinflammatory cytokine release (14). Some studies have suggested the possibility that LPS modulates both the ERK1/2 and PPAR $\gamma$  signaling pathways, although there is little information on the association or interaction between these pathways in LPS-induced inflammation and peritonitis.

The interaction between cyclin-dependent kinase (CDK)5 and ERK/PPAR $\gamma$ , however, is known to control and enrich the roles of PPAR $\gamma$  in cellular modulation (15,16). In Alzheimer's disease, LPS has been reported to induce tau-phosphorylation in a CDK5-dependent manner (17). The present study was performed to investigate the association of the ERK/CDK5 axis and PPAR $\gamma$  in LPS-induced peritonitis *in vitro*, with the aim of providing novel insights into the pathogenesis of LPS-induced peritonitis.

We present the following article in accordance with the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/atm-21-1623>).

## Methods

### Cell culture and LPS induction

The simian virus 40 (SV40)-immortalized human peritoneal mesothelial cell line HMrSV5 (ATCC, USA, ATCC16880) was maintained in Dulbecco's Modified Eagle Medium (DMEM, Hyclone, Logan, UT, USA) supplemented with 10% fetal-bovine serum (FBS) and different concentrations of LPS (0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, and 100  $\mu$ g/mL; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at 37  $^{\circ}$ C with 5% CO $_2$  for 12 hours. Further, cells were cultured with the optimal LPS concentration of 1  $\mu$ g/mL for 3, 6, 12, 24, 36, 48, and 72 hours.

### Cell transfection and treatment

CDK5 small interfering RNA (siRNA) and scrambled sequence were purchased from Genechem Co. Ltd. (Shanghai, China) and transfected into HPMCs using Lipofectamine 2000 reagent (Santa Cruz, CA, USA) according to the manufacturer's instructions. The target sequence of CDK5-siRNAs was #266: 5'-AAUCUUUCCAGUUUCUCGUA-3', #406: 5'-ACAGAAACAGGGUUUUCUCAU-3', #809: 5'-AAUAUCGUGCUGUAGUAUCAU-3'. Cells were incubated in DMEM with 5% CO $_2$  at 37  $^{\circ}$ C for 24 hours.

To inhibit ERK signaling, HPMCs were treated with 1  $\mu$ M AZD0364 (Sigma-Aldrich; Merck KGaA) (18) for 4 hours, followed by LPS treatment. Each experiment was performed with 3 independent replications.

### Enzyme-linked immunosorbent assay

The contents of IL-1 $\beta$ , IL-6, and IL-12 in the cellular medium were determined using commercial ELISA kits purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., (Shanghai, China). A Thermo microplate reader (Thermo Fisher Scientific Inc., Rockford, IL, USA) was used to determine the optical density at 450 nm. Each experiment was performed with 3 independent replications.

### Quantitative real-time PCR analysis

Total RNA extraction was performed with TRIzol reagent

(TaKaRa, Tokyo, Japan), and was followed by the synthesis of first-strand complementary DNA (cDNA) using a Bestarq PCR RT Kit (DBI Bioscience, Ludwigshafen, Germany). For PCR amplification, 1 µg DNA template was used with primers purchased from Sangon (Shanghai, China) and DBIBestar® SYBRGreen qPCR master Mix (DBI Bioscience). PCR amplification was conducted on an Agilent Stratagene Mx3000P Real-time PCR machine (DBI Bioscience) with the following reaction cycles: 95 °C for 2 minutes; 94 °C for 20 seconds, 58 °C for 20 seconds, and 72 °C for 20 seconds, for 40 cycles. Relative expression levels were calculated using the  $2^{-\Delta\Delta C_t}$  method, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) serving as an internal control. The sequence of primers used was CDK5 forward: 5'-GGAAGGCACCTACGGAACTG-3', CDK5 reverse: 5'-GGCACACCCTCATCATCGT-3'.

#### *Co-immunoprecipitation assay*

Cells were treated with LPS for 12 hours. Interaction between CDK5 and ERK1/2 was detected by co-immunoprecipitation (co-IP) assay using a Pierce® Co-IP Kit (Thermo Scientific Inc.; #26149). Total protein (1 mg) from the lysate was mixed with 2 µg of ERK1/2 antibody in a column at 4 °C overnight. The immune complexes were analyzed by western blot with the indicated antibodies. Immunoglobulin G (IgG) was employed as the normal control.

#### *Western blot analysis*

Protein lysates were isolated, quantified, and electrophoretically fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 10% gel (Sangon), and then transferred onto Whatman polyvinylidene difluoride (PVDF) membranes (Millipore, Whatman, Germany) according to standard methods. The membranes were blocked with 5% skim milk and tris-buffered saline (Sigma-Aldrich; Merck KGaA) at 4 °C overnight. Then, the membranes were subjected to primary antibody incubation with antibodies against CDK5 (1:1,000; Cell Signaling Technology, CST, Danvers, MA, USA), ERK1/2 (1:1,000; CST), p-ERK1/2 (1:1,000; CST), PPAR $\gamma$  (1:1,000; CST), p-PPAR $\gamma^{\text{Ser273}}$  (1:1,000; CST), and GAPDH (1:10,000; Boster Biotechnology, Wuhan, China) at 4 °C overnight. Incubation with secondary antibody (horseradish peroxidase goat anti-rabbit/mouse IgG; 1:20,000; Boster Biotechnology) was conducted at room temperature for 40 minutes. An enhanced chemiluminescence (ECL) system

(Millipore) was used for signal detection, and the Image-Pro Plus 6.0 software analysis system (Media Cybernetics, Inc., Bethesda, MD, USA) was used to analyze protein signals. Data were presented as fold change, meaning the expression of the detected protein relative to the control protein. Each detection was replicated for 3 times.

#### *Statistical analysis*

All data were expressed as mean  $\pm$  standard deviation (SD) of 3 replicates. Statistical analyses were performed with SPSS 20.0 software (IBM, USA). The Student's *t*-test was employed to analyze differences between groups. Statistical significance was recognized as  $P < 0.05$ .

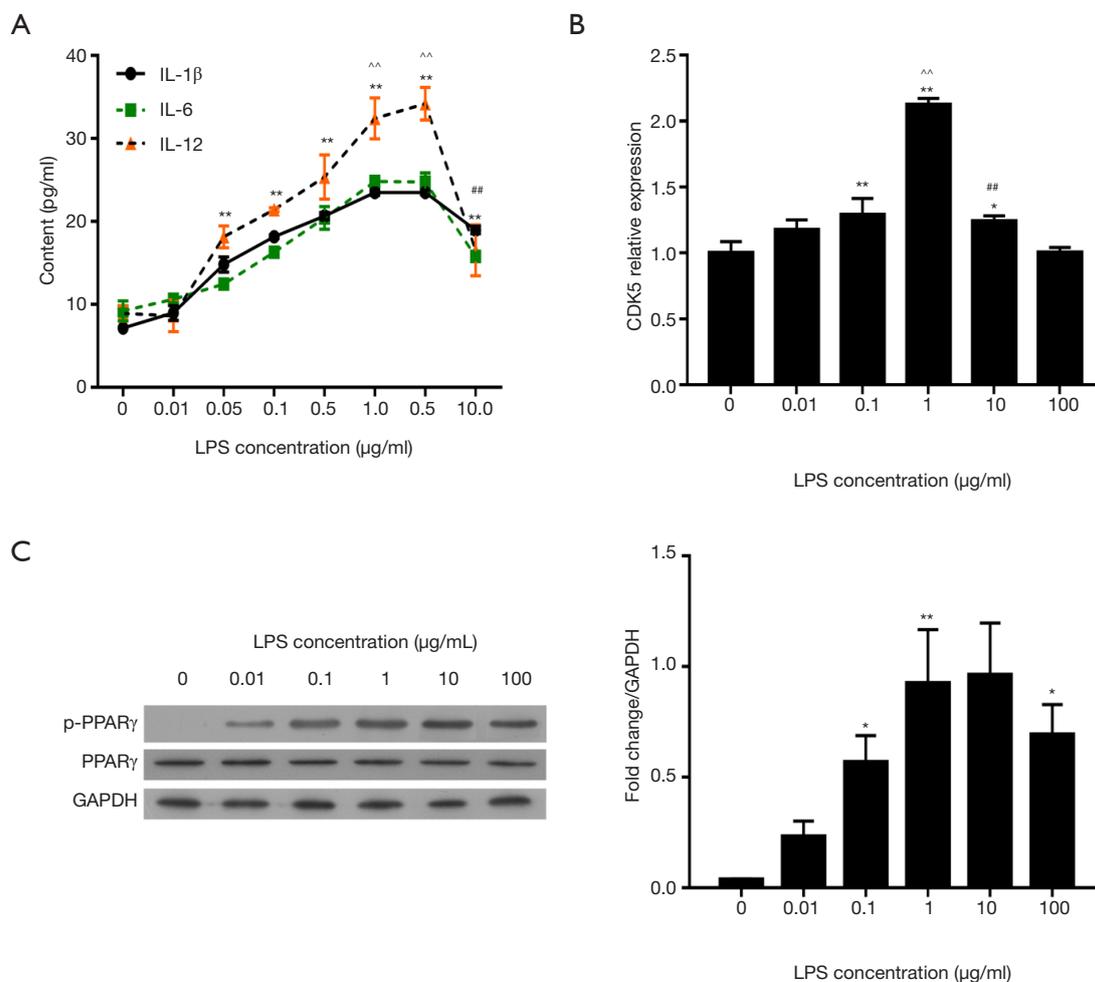
## **Results**

#### *LPS induced dose-dependent inflammation and PPAR $\gamma$ phosphorylation*

First, we determined whether LPS could dose-dependently induce the upregulation of inflammatory cytokines (IL-1 $\beta$ , IL-6, and IL-12) in HPMCs. ELISA assay demonstrated that treatment with more than 0.05 µg/mL LPS for 12 hours significantly elevated the contents of IL-1 $\beta$ , IL-6, and IL-12 compared with the control (0 µg/mL LPS;  $P < 0.01$ , *Figure 1A*). No continuous or obvious increase in IL-1 $\beta$  and IL-6 was induced by 5 µg/mL LPS, while 10 µg/mL LPS reversed the increasing trend in the contents of the cytokines. Similar trends were observed for CDK5 expression detected by qRT-PCR (*Figure 1B*) and for PPAR $\gamma^{\text{Ser273}}$  phosphorylation detected by western blot (*Figure 1C*). Therefore, 1 µg/mL LPS induced the highest levels of inflammation, along with the highest levels of CDK5 expression and PPAR $\gamma$  phosphorylation.

#### *LPS induced time-dependent inflammation and PPAR $\gamma$ phosphorylation*

Next, we tested whether LPS could induce the upregulation of inflammatory cytokines (IL-1 $\beta$ , IL-6, and IL-12) in HPMCs in a time-dependent manner. For this end, 1 µg/mL LPS was employed as the optimal concentration. Under treatment times up to 24 hours, LPS induced time-dependent increases in the expression levels of IL-1 $\beta$ , IL-6, and IL-12 in HPMC cells, as detected by ELISA (*Figure 2A*); however, treatment times longer than 24 hours resulted in comparative reductions in the contents of IL-



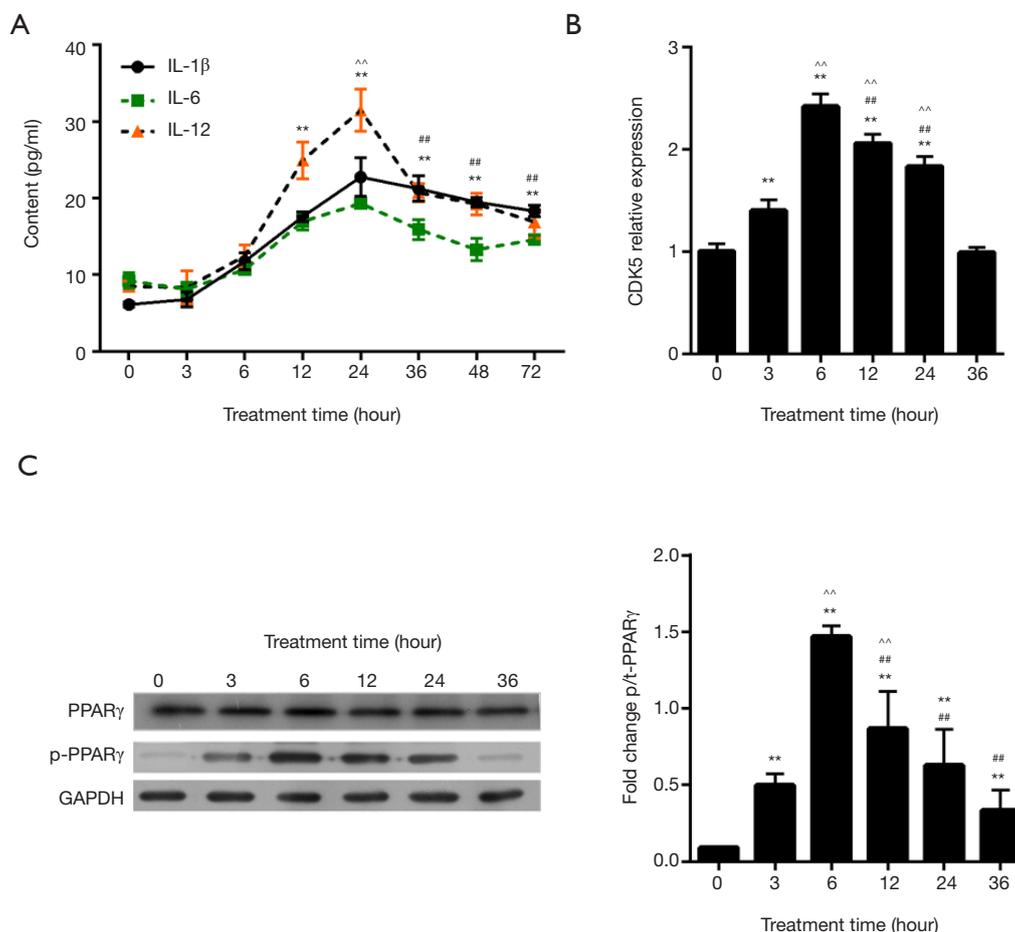
**Figure 1** LPS induces inflammation in a dose-dependent manner. (A) ELISA assay to determine IL-1 $\beta$ , IL-6, and IL-12 content in HPMCs under the administration of LPS at different concentrations. (B) qRT-PCR of CDK5 mRNA expression under the indicated treatments. (C) Western blot analysis of the levels of PPAR $\gamma$  and its phosphorylation under the indicated treatments, along with quantitative analysis of p-PPAR $\gamma$ /total PPAR $\gamma$ . \* $P < 0.05$  vs. control (0  $\mu\text{g/ml}$ ). \*\* $P < 0.01$  vs. control. ^ $P < 0.01$  vs. the neighbor (0.5 or 0.1  $\mu\text{g/ml}$ ). ## $P < 0.01$  vs. 1  $\mu\text{g/ml}$ . ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; CDK5, cyclin-dependent kinase 5; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ .

1 $\beta$ , IL-6, and IL-12 to compare with 12 hours ( $P < 0.01$ ). LPS induced the highest levels of CDK5 mRNA expression (Figure 2B) and PPAR $\gamma$ <sup>Ser273</sup> phosphorylation (Figure 2C) at 6 hours post stimulation. Interestingly, 3 hours of LPS treatment caused obvious increases in CDK5 expression and PPAR $\gamma$  phosphorylation, although a similar trend was not observed in the expression levels of IL-1 $\beta$ , IL-6, and IL-12 (Figure 2A,B). These data showed that CDK5 expression and PPAR $\gamma$  phosphorylation increased in the early stage of LPS stimulation, which was followed later by the elevated production of inflammatory factors. An long treatment time

more than 12 hours significantly decreased the expression levels of CDK5 mRNA and PPAR $\gamma$  phosphorylation ( $P < 0.01$  for 12 vs. 6 hours; Figure 2B,C). However, we chose 12 hours as the treatment time for further experiments based on the inflammation profiles.

#### ***LPS activated ERK1/2 and increased interaction between ERK1/2 and CDK5***

ERK1/2 activation has been reported to be related to LPS-induced inflammation (19). To identify the association

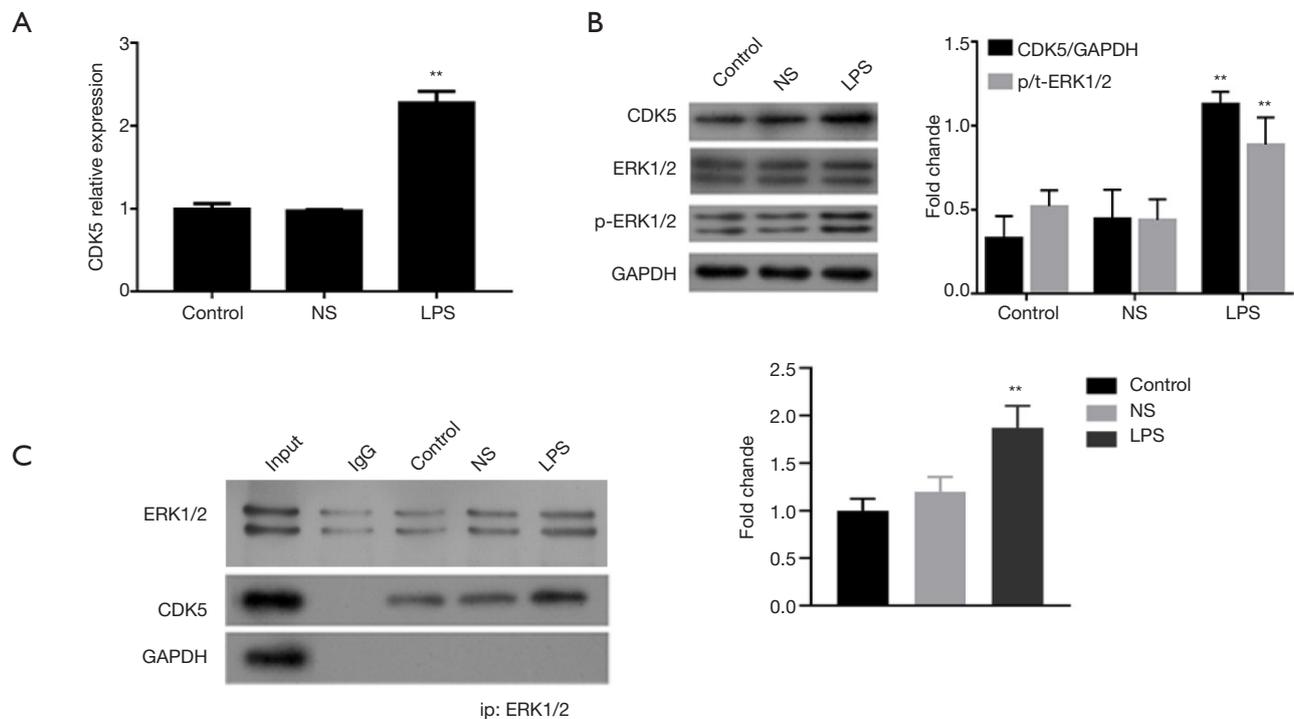


**Figure 2** LPS induces inflammation in a time-dependent manner. (A) ELISA assay to determine IL-1 $\beta$ , IL-6, and IL-12 content in HPMCs under the administration of LPS under different treatment times. (B) qRT-PCR assay of CDK5 mRNA expression under the indicated treatment times. (C) Western blot analysis of the levels of PPAR $\gamma$  and its phosphorylation under the indicated treatment times, along with quantitative analysis of p-PPAR $\gamma$ /total PPAR $\gamma$ . \*\* $P < 0.01$  vs. control (0 hours). ^^ $P < 0.01$  vs. the neighbor (3, 6, or 12 hours). ## $P < 0.01$  vs. the highest value (24, 36 hours). ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; CDK5, cyclin-dependent kinase 5; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ .

between ERK1/2 and CDK5 in LPS-treated HPMC cells, we examined the effect of LPS on the activation of ERK1/2. The results showed that LPS (1  $\mu$ g/mL, 12 hours) increased the mRNA and protein expression of CDK5, as well as ERK1/2 phosphorylation (Figure 3A,B). Furthermore, using a co-IP assay, we observed the interaction between CDK5 and ERK1/2 proteins, which was strengthened by the administration of LPS (Figure 3C). Together, these results suggested that LPS activated ERK1/2 signaling and interaction between ERK1/2 and CDK5 proteins.

### ***ERK1/2 inhibition prevented LPS-induced inflammation and PPAR $\gamma$ phosphorylation***

Subsequently, we investigated whether ERK1/2 was involved in the LPS-induced changes in CDK5 expression and PPAR $\gamma$  phosphorylation in HPMC cells. For this purpose, ERK1/2 inhibitor AZD0364 (1  $\mu$ M) (19) was used in combination with LPS treatment. Our results showed that AZD0364 significantly decreased the LPS-induced IL-1 $\beta$ , IL-6, and IL-12 elevation ( $P < 0.01$ , Figure 4A), CDK5



**Figure 3** LPS induces the activation of ERK1/2 and enhances ERK1/2-CDK5 interaction. (A) The relative expression level of CDK5 mRNA detected by qRT-PCR under the indicated conditions. (B) Western blot analysis of the expression levels of CDK5, ERK1/2, and phosphorylated ERK1/2, along with quantitative analysis of CDK5/GADPH and p-ERK1/2/total ERK1/2 under the indicated conditions. \*\* $P < 0.01$  vs. control. (C) Co-IP assay was used to detect the interaction between ERK1/2 and CDK5 under control or LPS treatment. LPS, lipopolysaccharide; ERK1/2, extracellular signal-regulated kinases 1/2; CDK5, cyclin-dependent kinase 5.

expression and ERK1/2 phosphorylation ( $P < 0.01$ , Figure 4B), and PPAR $\gamma$  phosphorylation ( $P < 0.01$ , Figure 4C), suggesting that ERK1/2 functioned upstream of CDK5 and PPAR $\gamma$  during LPS-induced inflammation.

#### **CDK5 siRNA prevented LPS-induced inflammation and PPAR $\gamma$ phosphorylation**

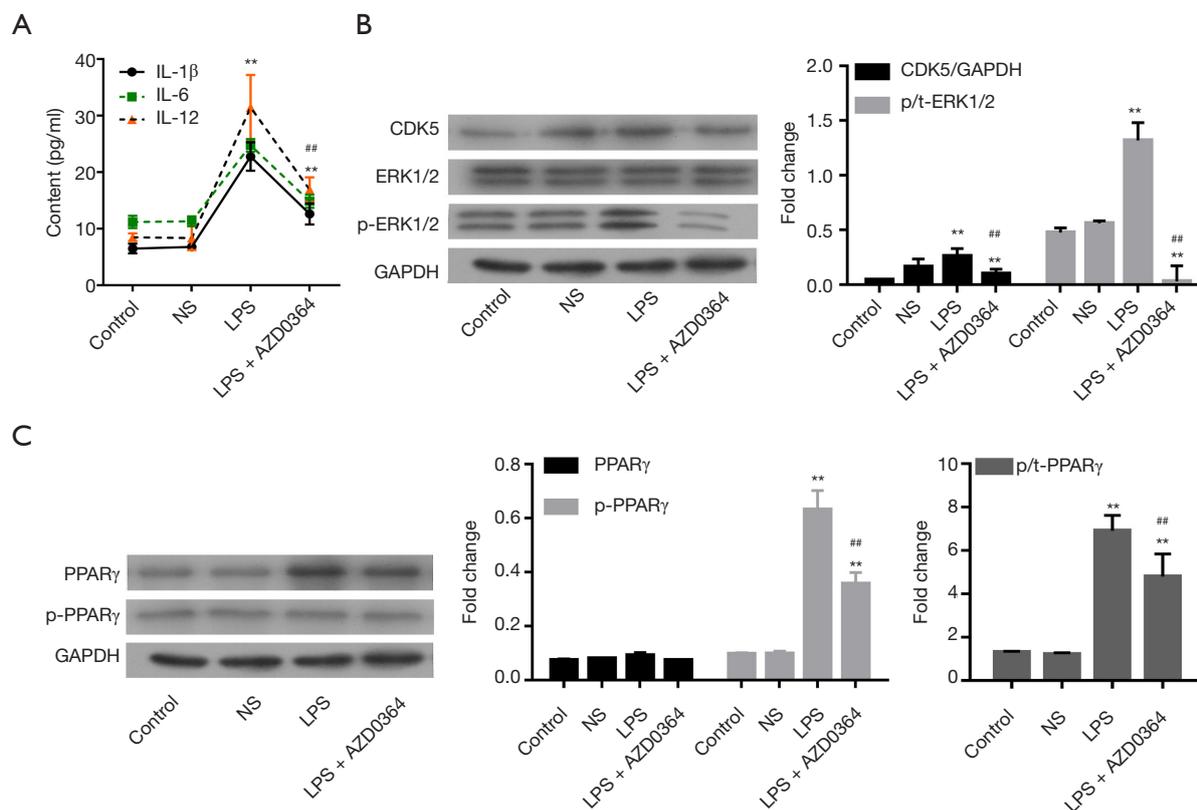
To investigate the relationship of CDK5 with ERK1/2 and PPAR $\gamma$  in LPS-induced inflammation, we knocked down the expression of CDK5 in HPMC cells with its siRNAs (siRNA-266/809). As shown in Figure 5A, the expression of CDK5 was significantly inhibited in siRNA-transfected cells. Our subsequent results showed that siRNA-809 inhibited IL-1 $\beta$ , IL-6, and IL-12 production ( $P < 0.01$ , Figure 5B), CDK5 expression ( $P < 0.01$ , Figure 5C), and PPAR $\gamma$  phosphorylation ( $P < 0.01$ , Figure 5D), but had no effect on ERK1/2 phosphorylation (Figure 5C). These results indicated that CDK5 acted as downstream of ERK1/2 and

upstream of PPAR $\gamma$  during LPS-induced inflammation and that an ERK1/2-CDK5-PPAR $\gamma$  axis was implicated in this process.

#### **Discussion**

The present study demonstrated that LPS induced inflammation in HPMCs in a dose- and time-dependent manner and that an axis involving ERK1/2, CDK5, and PPAR $\gamma$  was implicated in this process. Our results demonstrated the crucial role of an ERK/CDK5 axis in controlling LPS-induced inflammation via the modulation of PPAR $\gamma$  phosphorylation in HPMCs.

Peritonitis is an inflammatory condition which is commonly caused by spontaneous bacterial and fungal infections (4-7). Bacterial LPS typically causes inflammation via stimulating the production of proinflammatory cytokines such as IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  (5). This axis is mediated or controlled by the TLR2, PPAR $\gamma$ ,

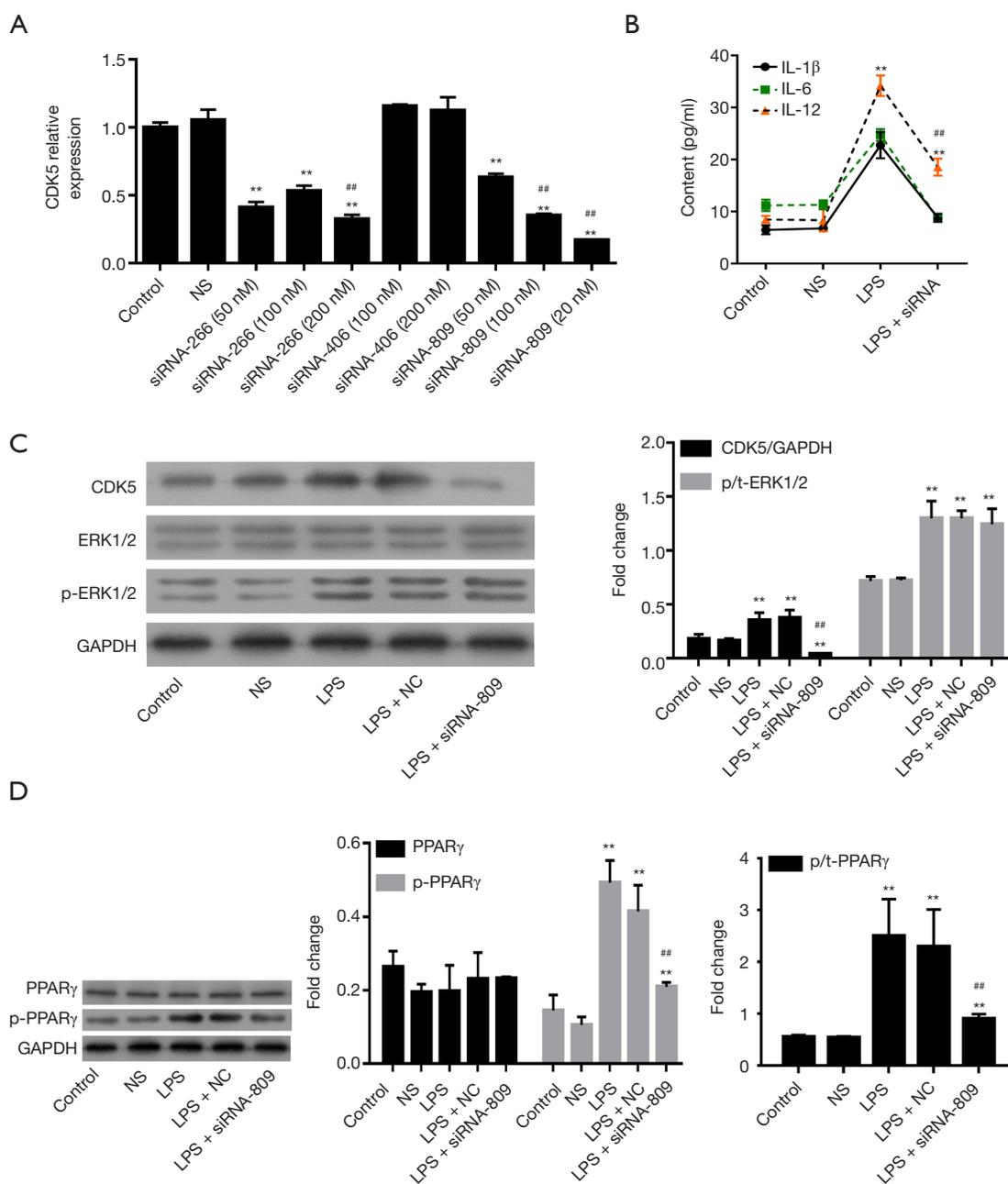


**Figure 4** ERK1/2 inhibition prevents LPS-induced inflammation. (A) ELISA assay to determine IL-1 $\beta$ , IL-6, and IL-12 content under the indicated conditions. (B) Western blot analysis of the expression levels of CDK5, ERK1/2, and phosphorylated ERK1/2 under the indicated conditions, along with quantitative analysis of CDK5/GAPDH and p-ERK1/2/total ERK1/2. (C) Western blot analysis of the expression levels of PPAR $\gamma$  and phosphorylated PPAR $\gamma$  under the indicated conditions, along with quantitative analysis of PPAR $\gamma$ /GAPDH, p-PPAR $\gamma$ /GAPDH, and p-PPAR $\gamma$ /PPAR $\gamma$ . \*\* $P < 0.01$  vs. control. ## $P < 0.01$  vs. the LPS. ERK1/2, extracellular signal-regulated kinases 1/2; ELISA, enzyme-linked immunosorbent assay; CDK5, cyclin-dependent kinase 5; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ .

ERK1/2, and NF- $\kappa$ B pathways (5,8-10,14). The inhibition of LPS-induced production of TNF- $\alpha$  and IL-6, and inflammatory responses has been shown to have a potent anti-inflammatory effect (20,21). In rat model of peritonitis, the activation of PPAR $\gamma$  was found to ameliorate LPS-induced peritoneal deterioration (8). The activation of the ERK1/2 and NF- $\kappa$ B pathways is associated with LPS-induced mastitis and acute respiratory distress syndrome via the promotion of proinflammatory cytokine production (11,13,14). PPAR $\gamma$  activation contributes to reducing NF- $\kappa$ B and ERK1/2-mediated secretion of proinflammatory cytokines (22-25). In our present study, we demonstrated an association of LPS-induced inflammation with CDK5-mediated phosphorylation and activation of ERK1/2.

By treating HPMCs with different concentrations of LPS, we found that treatment with 1  $\mu$ g/mL LPS for

12 hours was the optimal experimental design *in vitro*. At 12 hours, 1  $\mu$ g/mL LPS treatment induced the most severe inflammatory status in HPMCs of any treatment time. Overdose of LPS or an long treatment time decreased the production of the inflammatory cytokines IL-1 $\beta$ , IL-6, and IL-12, as well as the levels of CDK5 expression and PPAR $\gamma$  phosphorylation (Figures 2 and 3). Of note, it is possible that CDK5 and PPAR $\gamma$  functioned in the early stage of LPS-induced inflammation and contributed to the subsequent production of inflammatory cytokines (Figure 2). Previous studies have reported that PPAR $\gamma$  is an anti-inflammatory ligand (8,9). Together with its agonists, PPAR $\gamma$  inhibits the production of monocytic inflammatory cytokines, including IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , thus preventing inflammatory injury (26,27). However, some researchers have arrived at a different conclusion, reporting that PPAR $\gamma$  does not inhibit LPS-



**Figure 5** CDK5 siRNA prevents LPS-induced inflammation. (A) The relative expression levels of CKD5 mRNA in HPMCs transfected with different siRNAs. (B) ELISA assay to determine IL-1 $\beta$ , IL-6, and IL-12 content under the indicated treatments. (C) Western blot analysis of the expression levels of CDK5, ERK1/2, and phosphorylated ERK1/2 under the indicated conditions, along with quantitative analysis of p-ERK1/2/total ERK1/2. (D) Western blot analysis of the expressions of PPAR $\gamma$  and phosphorylated PPAR $\gamma$  under the indicated conditions, along with quantitative analysis of p-PPAR $\gamma$ /PPAR $\gamma$ . \*\* $P < 0.01$  vs. control. ## $P < 0.01$  vs. the LPS (B,C,D) or siRNA-266 (100 nM), and siRNA-809 (50 nM). CDK5, cyclin-dependent kinase 5; LPS, lipopolysaccharide; ERK1/2, extracellular signal-regulated kinases 1/2; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ .

induced IL-6 or TNF- $\alpha$  production in macrophages (28). Therefore, it is possible that the progression of LPS-induced inflammation is promoted by PPAR $\gamma$  phosphorylation.

Our study demonstrated that LPS-induced production of IL-1 $\beta$ , IL-6, and IL-12, promoted ERK1/2 activation, and increased the levels CDK5 expression and PPAR $\gamma$  phosphorylation. The administration of the ERK1/2 inhibitor AZD0364 suppressed the expression of ERK1/2, CDK5, and p-PPAR $\gamma$ , therefore preventing LPS-induced inflammation. The knockdown of CDK5 by its siRNA suppressed p-PPAR $\gamma$  and LPS-induced inflammation, but did not affect ERK1/2 activation (Figures 4 and 5). These results indicated that an ERK1/2-CDK5-PPAR $\gamma$  axis was involved in LPS-induced inflammation.

The involvement of the ERK1/2 pathway in LPS-induced IL-6 production has been revealed previously by Yu *et al.* (14), who showed that LPS activated the ERK1/2 pathway to induce IL-6 mRNA expression (14). It has also been reported that IL-6-mediated proliferation and maintenance of stemness in mesenchymal stem cells require the activation of ERK1/2 (29). However, the direct participation of PPAR $\gamma$  in ERK1/2-mediated IL-6 expression has not been evidenced before. Our results demonstrated that the inhibition of ERK1/2 or knockdown of CDK5 prevented the LPS-induced production of the inflammatory cytokines IL-1 $\beta$ , IL-6, and IL-12, partly through the regulation of PPAR $\gamma$  phosphorylation.

## Conclusions

Our study has demonstrated that LPS-induced inflammation in HPMCs could be prevented by inhibiting an ERK1/2-CDK5-PPAR $\gamma$  axis. Further study should be carried out to validate the essentiality of PPAR $\gamma$  in ERK1/2/CDK5 axis-modulated inflammation.

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## Footnote

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**Data Sharing Statement:** Available at <http://dx.doi.org/10.21037/atm-21-1623>

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/atm-21-1623>). The authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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