GPER Activation Attenuates Lipopolysaccharide-Induced Acute Respiratory Distress Syndrome Sex-Dependently and Inhibits Alveolar Macrophage Activation

Cui Yang
Peking University Third Hospital
Zhukai Cong
Peking University Third Hospital
Feng Zhao
Peking University Third Hospital
Ziyuan Shen
Peking University Third Hospital
Xi Zhu (xizhuccm@163.com)
Peking University Third Hospital https://orcid.org/0000-0001-6091-8831

Research Article

Keywords: Acute respiratory distress syndrome, inflammation, GPER, alveolar macrophages

Posted Date: December 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1149082/v1

Abstract

Background: Acute respiratory distress syndrome (ARDS), a common and critical disease, is clinically characterized by uncontrolled inflammation and alveolar-capillary barrier disruption. Estrogen can reportedly alleviate ARDS caused by numerous insults in mice. Moreover, the estradiol receptors α, not β,participated in E2-induced attenuation of ARDS. But the role of another estradiol receptor, G protein-coupled estradiol receptor 1 (GPER1) in ARDS are not undertood. This study is aimed to investigate the effect of GPER activation on LPS-induced ARDS in mice.

Methods: Female mice were randomly subjected to bilateral ovarectomy (OVX) or sham surgery two weeks before lung injury. The GPER-selective agonist G1 or vehicle were intraperitoneally injected 0.5 h before intratracheal administration of LPS or phosphate-buffered saline in male and female mice. After 24 h, mice were sacrificed to collect blood, bronchoalveolar lavage fluid (BALF), and lung tissue. Histological injury and inflammatory cell infiltration in lung tissue, as well as cytokine and protein concentrations in BALF were determined. In vitro experiments were also performed on alveolar macrophages (MH-S cells) to investigate the effect of GPER activation on LPS-induced inflammatory responses.

Results: Activation of GPER by G1 administration significantly ameliorated lung pathological damage, attenuated alveolar capillary barrier destruction, inhibited recruitment of inflammatory cells into alveoli, and decreased concentrations of the pro-inflammatory factors tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) in BALF of LPS-administered male and OVX female mice, but not intact female mice. In vitro experiments demonstrated that G1 pretreatment significantly inhibited LPS-mediated increases of TNF-α, IL-6, and MIP2 in a dose-dependent manner.

Conclusions: These results demonstrated that GPER activation attenuated lung injury of male and OVX female mice by inhibiting the inflammatory response of alveolar macrophages.

Highlights

- Intratracheal instillation of LPS induced more severe damage of lung in OVX female mice compared to intact females.
- Selective activation of the non-genomic estradiol receptor, GPER-1 significantly attenuates LPS-induced ARDS in male and OVX female mice, but not intact female mice.
- GPER-1 activation attenuated LPS-induced ARDS at least in part by inhibiting alveolar macrophages secretion of TNF-α, IL-6, and MIP2.

Background

Acute respiratory distress syndrome (ARDS), an acute inflammatory lung disease caused by numerous factors, has a mortality rate of approximately 40%[1]. Recent studies indicate that uncontrolled

inflammation and disruption of the alveolar-capillary barrier contribute to the development of ARDS and its progression[2]. Current strategies to treat ARDS include fluid restriction for resuscitation and lung protection for mechanical ventilation, with no well-recognized efficient drugs thus far[3]. It is of great significance to clarify the pathogenesis of ARDS and find effective therapeutic targets. Although epidemiological results were conflicted about the sex differences in ARDS, animal researches have indicated a relative protection of females over males on ARDS[4–6]. Many factors contribute to such sex bias, sex hormones are one of them that cannot be neglected. Increasing evidence suggested that female hormones, especially estrogens, could alleviate ARDS. Administration of 17 beta-estradiol (E2) attenuated lung injury by inhibiting the production of cytokines/chemokines such as interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), ameliorating pulmonary vascular permeability[7] and increasing alveolar fluid clearance by upregulating expression of the α and γ subunits of epithelial $Na^{(+)}$ channels[8].

Although E2 can attenuate lung injury, it remains unclear which estrogen receptor (ER) types mediate these salutary effects. E2 exert function mainly through three ERs: classical receptors ER-α and ER-β act as both transcription factors to regulate protein production and direct modulators to activate secondary messengers; in contrast, G protein-coupled estrogen receptor-1 (GPER1, also known as GPR30) acts primarily in a non-genomic manner and uses existing proteins, which takes only seconds[9]. ER-α, but not ER-β, is expressed in pulmonary vessel endothelial cells and alveolar macrophages[10]. Studies using ER-knockout mice further confirmed that ER-α, but not ER-β, mediates the effects of E2 on reducing pulmonary inflammatory responses[10]. But little is known about the effects of GPER-1 in ARDS.

GPER-1 has been revealed to play critical role in immune systems[11]. Previous studies demonstrated that the GPER-selective agonist G1 effectively reduced macrophage accumulation in mice with postmenopausal atherosclerosis and inflammation was more severe in *gper*-deficient mice, suggesting that GPER may be a potential therapeutic target for the treatment of inflammation[12, 13]. Activation of GPER suppressed neuroinflammation following brain injury in male and ovariectomized (OVX) female rats, but not in intact females[14]. Thus, the anti-inflammatory effect of GPER may also have a sex bias. An ex vivo study also demonstrated that administration of G1 suppressed lipopolysaccharide (LPS)-stimulated inflammatory cytokine expression[15].

Given the crucial role of inflammation in ARDS and effective anti-inflammatory feature of GPER activation, we hypothesized that activation of GPER could inhibit inflammation of ARDS. The present study was undertaken to investigate the effect of GPER activation on LPS-induced ARDS in mice, as well as the sex dependence of any salutary effects.

Materials And Methods

Reagents and antibodies

LPS (*Escherichia coli* LPS serotype O111:B4) and sodium pentobarbital were acquired from Sigma Aldrich (St. Louis, MO, USA). G1 and corn oil were purchased from MedChemExpress (Monmouth

Junction, NJ, USA). Enzyme-linked immunosorbent assay (ELISA) kits for mouse tumor necrosis factor α (TNF-α), IL-6, and macrophage inflammatory protein 2 (MIP2) were purchased from R&D Systems (Minneapolis, MN, USA). ELISA kits for albumin and estradiol were purchased from Cloud-Clone (Wuhan, China). A Cell Counting Kit-8 (CCK-8) was purchased from Yeasen Biotechnology (Shanghai, China).

Animals and LPS-induced ARDS mouse model

Male and female BALB/c mice (22–27 g) were obtained from Vital River Laboratory Animal Technology (Beijing, China). Mouse were housed with free access to food and water under specific pathogen-free conditions in temperature-controlled rooms (25 ± 1°C) with a 12-h light/dark cycle at Peking University Health Science Center.

The ARDS model was established using a modified version of a previously reported method[16]. Briefly, 30 min after injection of G1 (5 mg/kg) dissolved in 100 μL of corn oil or corn oil alone as a control, mice were anesthetized via an intraperitoneal injection of sodium pentobarbital (20 mg/kg). Next, a small midline incision on the ventral side of the neck was performed to expose the trachea and a 22-gauge micro-syringe was inserted through the tracheal wall. Subsequently, 40 mL of sterile phosphate-buffered saline (PBS) with or without LPS (2 mg/kg) was instilled into the lumen. After instillation, mice were placed in a vertical position and rotated for about 1 min to ensure that the instillation distributed evenly within the lung. Twenty-four hours after instillation, mice were anesthetized and sacrificed by exsanguination to collect lung tissue and bronchoalveolar lavage fluid (BALF). Blood of female mice was also collected to isolate serum by centrifuging at 400 * g for 5 min at 4°C, and then stored at −80°C for later usage.

Ovariectomy and confirmation

Two weeks prior to lung injury, female mice were randomly divided into two groups that underwent bilateral OVX or sham surgery, as previously described[17]. To further confirm the success of each OVX surgery, the uterus of each mouse was harvested and weighed at the time of sacrifice to calculate the uterus ratio (uterus ratio = uterus weight/ body weight), and serum levels of 17β-estradiol were evaluated (see Additional figure 1).

Preparation of BALF and cell counts

To collect BALF, 1 mL of ice-cold PBS was infused into lung tissues and aspirated three times with a 20-gauge catheter. After collection, BALF was centrifuged at 400 * g for 5 min at 4°C. The resulting supernatant was isolated and stored at −80°C for later determination of cytokines, total protein, and albumin; whereas, the deposited cell pellet was suspended in PBS for total cell counts using a hemocytometer and differential cell counts using Wright Giemsa staining (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Histopathology

Harvested lung tissues were fixed in 4% paraformaldehyde for 48 h at room temperature, embedded in paraffin, cut into 5-mm-thick sections, and stained with hematoxylin and eosin (HE). Subsequently, slices were imaged with a Hamamatsu Nano Zoomer digital pathology microscope (Hamamatsu City, Japan).

Cell culture and treatment

The murine alveolar macrophage cell line MH-S (Bio-Rad, Hercules, CA, USA) was cultured in RPMI 1640 medium supplemented with 10% fetal calf serum in a humidified atmosphere with 5% CO_2 at 37°C. First, we measured the toxicity of G1 on MH-S cells by CCK-8 assay. MH-S cells were seeded in 96-well plates (8000 cells/well). After treatment with G1 (final concentrations of 10^{-11}M to 10^{-4}M) for 24 h, 10 μL of CCK-8 liquid was added to each well. After incubating the plates at 37°C for 2 h, the absorbance of each well was measured at 450 nm with a Varioskan Flash Multimode Reader (Thermo Fisher Scientific, Waltham, MA, USA). Second, we evaluated the viability of MH-S after exposure to both G1 and LPS. Cells were pretreated with G1 (final concentrations of 10^{-8}M to 10^{-5}M) for 30 min before LPS (10 ng/mL) or PBS administration. After 24 h, CCK-8 was added and the absorbance of each well was measured. Third, we examined the effect of G1 on inflammation by pretreating cells with G1 (final concentrations of 10^{-8}M to 10^{-5}M) for 30 min, followed by an identical volume of LPS (10 ng/mL) or PBS. After LPS stimulation for 6 h, cell culture supernatants were collected for cytokine detection.

Cytokine, albumin, and 17β-estradiol assays

Concentrations of TNF-α, IL-6, and albumin in BALF samples, as well as TNF-α, IL-6, and MIP2 in cell cultural supernatants were detected at the indicated time points using specific ELISA kits according to the manufacturers' instructions. Serum levels of 17β-estradiol were also measured using a specific ELISA kit according to the manufacturer's instructions.

Statistical analysis

All results are presented as the mean ± standard deviation (SD) of four or more repeated experiments and were analyzed using Prism 8.0 software (GraphPad Software, San Diego, CA, USA). The statistical significance of one variable (males and cell experiments) were analyzed by one-way analysis of variance (ANOVA) followed by Sidak's post hoc test for the comparison of two groups. The statistical significance of two variables (females) were analyzed by two-way ANOVA followed by Sidak's post hoc test for the comparison of two groups. Values of $p < 0.05$ were considered statistically significant. The statistical significance of 17β-estradiol and uterus ratios were analyzed by unpaired *t* test.

Results

Sex-dependent effect of G1 alleviation of LPS-mediated histopathological lung injury

HE staining was used to evaluate LPS-induced pulmonary pathological changes. As shown in Fig. 1A and Fig. 1C, the LPS-treated group exhibited infiltration of inflammatory cells and erythrocytes in alveoli,

accompanied by a thickened alveolar septum and atelectasis compared with the PBS-exposed group. Fig. 1B and Fig. 1D show the calculated lung injury scores of males (Fig. 1B; F (3,20) = 89.97, $p < 0.0001$) and females (Fig. 1D; F (1,40) = 5.699, $p = 0.0218$) according to the standard of the American Thoracic Society[18]. In parallel with HE staining, higher lung injury scores were observed in the LPS-injected versus PBS-injected groups of both male ($p < 0.0001$) and female mice (sham: $p < 0.0001$; OVX: $p < 0.0001$). In addition, lung injury was more severe in OVX females compared with intact females ($p = 0.005$). Intraperitoneal administration of the GPER agonist G1 decreased LPS-induced lung injury in males ($p < 0.0001$) and OVX females ($p = 0.0001$), but had no effect on intact females ($p = 0.9983$).

Sex-dependent effect of G1 on reducing LPS-mediated inflammatory cell infiltration of alveoli

Infiltration of inflammatory cells into alveoli was evaluated by Wright Giemsa stain. As shown in Fig. 2A and Fig. 2D, few inflammatory cells were observed in the control group, most of which were macrophages. LPS stimulation recruited large numbers of inflammatory cells, especially neutrophils, into alveoli. Consistent with Wright Giemsa staining, total numbers of inflammatory cells were higher in both males (Fig. 2B; F (3,20) = 53.39, $p < 0.0001$; PBS vs. LPS, $p < 0.0001$) and females (Fig. 2E; F (1,40) = 3.832, $p = 0.0573$; sham PBS vs. sham LPS, $p < 0.0001$; OVX PBS vs. OVX LPS, $p < 0.0001$) after LPS stimulation. As shown in Fig. 2C and Fig. 2F, similar results were observed for numbers of neutrophils in the BALF of males (Fig. 2C; F (3,20) = 50.76, $p < 0.0001$; PBS vs. LPS, $p < 0.0001$) and females (Fig. 2F; F (1,40) = 6.471, $p = 0.0149$; sham PBS vs. sham LPS, $p < 0.0001$; OVX PBS vs. OVX LPS, $p < 0.0001$). In particular, numbers of total cells and neutrophils were higher in OVX females compared with intact females (Fig. 2E, total cells: $p = 0.007$; Fig. 2F, neutrophils: $p = 0.002$). Treatment with G1 reduced LPS-induced infiltration of inflammatory cells in males (Fig. 2B, total cells: $p < 0.0001$; Fig. 2C, neutrophils: $p < 0.0001$) and OVX females (Fig. 2E, total cells: $p < 0.0001$; Fig. 2F, neutrophils: $p < 0.0001$), but not intact females (Fig. 2E, total cells: $p = 0.9993$; Fig. 2F, neutrophils: $p > 0.9999$).

Sex-dependent effect of G1 attenuation of LPS-mediated inflammatory cytokine production

As shown in Fig. 3, statistical analysis revealed significant increases of TNF-α in the BALF of male (Fig. 3A; F (3,20) = 197, $p < 0.0001$; PBS vs. LPS, $p < 0.0001$) and female (Fig. 3B; F (1,40) = 1.138, $p = 0.2925$; sham PBS vs. sham LPS, $p < 0.0001$; OVX PBS vs. OVX LPS, $p < 0.0001$) mice following LPS challenge compared with the control group. Similar results were observed for IL-6 in males (Fig. 3C; F (3,20) = 48.82, $p < 0.0001$; PBS vs. LPS, $p < 0.0001$) and females (Fig. 3D; F (1,40) = 4.45, $p = 0.0412$; sham PBS vs. sham LPS, $p = 0.0012$; OVX PBS vs. OVX LPS, $p < 0.0001$). Moreover, TNF-α (Fig. 3Bb; TNF-α, $p = 0.0246$) and IL-6 levels (Fig. 3D; IL-6, $p = 0.0015$) were significantly higher in OVX female mice compared intact female mice after LPS instillation. We also observed sex-specific changes following G1 administration. Specifically, G1 treatment significantly inhibited increases of TNF-α and IL-6 in males (Fig. 3A, TNF-α, $p < 0.0001$; Fig. 3C, IL-6, $p = 0.0002$) and OVX females (Fig. 3B, TNF-α, $p = 0.0040$; Fig. 3D,

IL-6, $p = 0.0033$). However, no such protective effect on cytokine levels was observed in G1-treated intact females (Fig. 3B, TNF-α, $p = 0.9980$; Fig. 3D, IL-6, $p = 0.9981$).

Sex-dependent effect of G1 amelioration of LPS-mediated alveolar capillary barrier disruption

Disruption of the alveolar capillary barrier, a hallmark of ARDS, leads to inflammatory cell recruitment and pulmonary edema. Total protein and albumin levels in BALF reflect alveolar capillary permeability because they reflect the levels of total protein and albumin exudated from the vasculature into alveoli. As shown in Fig. 4, compared with PBS- or G1-treated groups, LPS instillation significantly increased total protein contents the in BALF of males (Fig. 4A; $F(3,20) = 25.74$, $p < 0.0001$; PBS vs. LPS, $p < 0.0001$) and females (Fig. 4B; $F(1,40) = 0.7701$, $p = 0.3854$; sham PBS vs. sham LPS, $p < 0.0001$; OVX PBS vs. OVX LPS, $p < 0.0001$). Furthermore, total protein levels in OVX females were increased compared with sham females (Fig. 4B, $p = 0.0403$). In addition, LPS injection increased albumin concentrations in the BALF of males (Fig. 4C; $F(3,20) = 57.6$, $p < 0.0001$; PBS vs. LPS, $p < 0.0001$) and OVX females (Fig. 4D; $F(1,40) = 1.085$, $p = 0.3038$; OVX PBS vs. OVX LPS, $p < 0.0001$), but not in sham mice (Fig. 4D, $p = 0.0832$); although, a trend of elevation was observed. Additionally, no significance was observed in albumin levels between sham and OVX female mice after LPS injection (Fig. 4D, $p = 0.2817$). G1 administration significantly ameliorated increases in total protein (Fig. 4A, $p < 0.0001$) and albumin contents (Fig. 4C, $p < 0.0001$) in males, as well as total protein contents in OVX females (Fig. 4B, $p = 0.0151$).

G1 inhibited LPS-induced inflammatory responses in alveolar macrophages without decreasing their viability in vitro

Activated alveolar macrophages are the primary immune cells that initiate inflammation in ARDS caused by pulmonary insults[19]. Thus, we detected the effect of G1 on inflammatory responses in alveolar macrophages (MH-S cells) in vitro. The viability of MH-S cells after G1 administration was measured by CCK-8 assay. A slight increase of cell viability was detected at a dosage of 10^{-8}M G1 (Fig. 5A; $F(7,40) = 2.573$, $p = 0.0275$; control vs. 10^{-8}, $p = 0.0236$; 10^{-8} vs. 10^{-11}, $p = 0.0195$). As shown in Fig. 5B, G1 administration had no toxic effect below a concentration of 10^{-5}M when MH-S cells were simultaneously stimulated with LPS (10 ng/mL; $F(6,35) = 4.451$, $p = 0.0019$). Pretreatment with G1 inhibited LPS-mediated production of TNF-α (Fig. 5C; $F(6,21) = 158.6$, $p < 0.0001$), IL-6 (Fig. 5D; $F(6,21) = 67.05$, $p < 0.0001$), and CXCL2/MIP2 (Fig. 5E; $F(6,21) = 68.91$, $p < 0.0001$) in a dose-dependent manner.

Discussion

In the present study, we successfully established a mouse model of ARDS by intratracheal instillation of LPS in vivo and investigated sex differences in the effects of GPER activation on acute inflammatory responses in lung. In parallel, we used a cell line (MH-S) derived from a murine alveolar macrophage cell to explore related molecular mechanisms in vitro. Our results indicate that pretreatment with the GPER-

specific agonist G1 protected mouse lung injury in a sex-specific manner. G1 administration significantly ameliorated LPS-induced pathological lung damage and alveolar capillary barrier destruction in male and OVX female mice. Administration of G1 also inhibited the recruitment of inflammatory cells into alveoli and decreased concentrations of pro-inflammatory factors TNF-α and IL-6 in BALF of male and OVX female mice. However, such salutary effects of GPER activation were not observed in intact female mice. In vitro experiments demonstrated that G1 pretreatment significantly inhibited LPS-mediated MH-S changes in expression of TNF-α, IL-6, and MIP2.

Overactivated inflammation is believed to be the major pathophysiological driver of ARDS[20], and sex differences in the inflammatory responsiveness of lung have been reported[10, 21]. In addition, the anti-inflammatory effect of E2 was reported in an animal model of ARDS. Use of knockout mice and receptor blockers revealed that ER-α, but not ER-β, plays a key role in mediating the anti-inflammatory effects of estrogen in lung[10, 22]. The current study, for the first time, found that pretreatment with the GPER-selective agonist G1 alleviated histopathological lung injury, inhibited inflammatory cell infiltration, and decreased IL-6 and TNF-α levels in BALF of male and OVX female mice following LPS injection. However, no such anti-inflammatory effects were observed in intact female mice. These sex-specific effects of G1 administration are consistent with a study of traumatic brain injury in which G1 treatment significantly attenuated IL-1β, IL-6, and TNF-α production in males and OVX females, but induced pro-inflammatory effects in intact females[14].

In addition, we observed sex-dependent reductions of total protein and albumin concentrations in BALF, which reflect alveolar capillary barrier function during ARDS. Previous reports showed sex differences in alveolar capillary barrier disruption within the lung. In these studies, males and OVX females exhibited higher albumin contents in BALF compared with intact females, and estrogen administration reversed LPS-induced leakage of albumin into alveoli in OVX female mice[21]. In the present study, albumin levels in BALF did not differ between sham and OVX females administered LPS, although a slight trend was noticed. We considered the possibility that these differences resulted from differential dosages and routes of LPS administration, as well as differences in sample numbers. In addition, we observed a similar trend of albumin decrease following G1 administration, but no significant difference was detected between G1-treated and vehicle-treated OVX females after LPS stimulation. In a previous study, estrogen exerted a lung-protective effect on the alveolar capillary barrier in male rats exposed to paraquat or the glutamate agonist N-methyl-d-aspartate, indicating that infusion of estrogen into the pulmonary circulation significantly attenuated albumin leakage[23]. Consistent with this finding, our results also demonstrated attenuation of albumin after G1 administration in males. Few studies have attempted to identify the specific receptor mediating the beneficial effects of E2 on alveolar capillary barrier function. Here, we provide the first evidence that GPER mediates such effects.

As a crucial mediator of pulmonary inflammation during ARDS, alveolar macrophages are a promising cell target to regulate processes involved in the inflammatory response[19]. Upon injury by pulmonary insult (e.g., intratracheal LPS injection), activated macrophages recruit neutrophils into the lung by secreting chemokines and cytokines[24]. Evidence indicate that inhibiting activation of alveolar

macrophages can alleviate lung injury by inhibiting inflammatory cytokine expression and neutrophil recruitment[25]. We selected the MH-S cell line to simulate alveolar macrophage responses to G1 in vitro. Our results show that pretreatment with G1 inhibited LPS-mediated production of IL-6, TNF-α, and CXCL2/MIP2 in a dose-dependent manner. These results are consistent with previous studies in which inhibition of TNF-α and IL-6 secretion was observed in primary human macrophages and RAW 264.7 (mouse macrophage) cells via GPER signaling[26, 27].

The current study had several limitations. First, we did not explore the mechanism by which G1 is engaged. A previous study indicated a role for Akt in mediating G1-inhibited inflammation of males and OVX females with brain injury[14]. Moreover, in vitro studies demonstrated that G1 administration downregulated expression of Toll-like receptor 4 and nuclear factor κB p65 in macrophages following LPS exposure[15, 27]. Therefore, additional in vivo study is necessary to uncover the signaling pathway involved in attenuation of lung inflammation by G1. Furthermore, a binding study revealed that the anti-inflammatory activity of estrogen on human monocytes is mediated by ERa36 (a 36-kDa splice variant of ER-a) together with GPER, demonstrating crosstalk between ERa36 and GPER[28]. Thus, investigating the role of ERa36 on G1-mediated anti-inflammatory processed during ARDS is necessary. Another limitation is that we did not monitor circulating estradiol levels in female mice at the time of injury, although previous studies showed that hormonal cycling plays a crucial role in pulmonary inflammation[29]. In this study, we monitored the estradiol levels in female 24h after lung injury, which may partly represent the estradiol states in females. Nonetheless, it is still necessary to further investigate the effects of G1 in females during various estrous cycles by using cycling female mice, whose estrus cycle can be determined by vaginal smear.

Perspectives and significance

Although numerous studies have attempted to identify efficient drugs to treat ARDS owing to its high mortality, no major breakthroughs have been achieved so far[3]. Previous studies demonstrated a protective role of estrogen in ARDS, but it remains unclear which receptor mediates such salutary effects[21]. This study demonstrated a lung-protective role of the GPER-selective antagonist G1 against LPS-induced ARDS in male and OVX female mice, but not in gonadally intact females. To the best of our knowledge, this is the first report showing a sex-specific role of GPER in ARDS. Although further efforts are required to fully evaluate the efficacy and mechanisms of GPER activation following pulmonary inflammation, our findings may provide novel perspectives for gender influence in ARDS.

Declarations

Ethics approval

All experimental procedures were approved by the Animal Care and Scientific Committee of Peking University Third Hospital, China.

Consent for publication

Not applicable

Availability of data and materials

Please contact the first or corresponding author for data or materials requests.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 82172166]; the Capital's Funds for Health Improvement and Research [grant number CFH 2020-2-4094]; the Beijing Municipal Natural Science Foundation [grant number 7212130].

Author contributions

CY and XZ designed the scientific work. CY, ZkC, FZ, ZyS performed the animal and cell experiments. CY wrote the first draft of the manuscript. XZ, ZkC, FZ and ZyS reviewed and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We thank Liwen Bianji (Edanz) (www.liwenbianji.cn/) for basic language editing of a draft of this manuscript.

References

1. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, et al. Incidence and outcomes of acute lung injury. N Engl J Med. 2005;353(16):1685–93.
2. Herrero R, Sanchez G, Lorente JA. New insights into the mechanisms of pulmonary edema in acute lung injury. Ann Transl Med. 2018;6(2):32.
3. Matthay MA, McAuley DF, Ware LB. Clinical trials in acute respiratory distress syndrome: challenges and opportunities. Lancet Respir Med. 2017;5(6):524–34.
4. Moss M, Mannino DM. Race and gender differences in acute respiratory distress syndrome deaths in the United States: an analysis of multiple-cause mortality data (1979- 1996). Crit Care Med. 2002;30(8):1679–85.
5. Lemos-Filho LB, Mikkelsen ME, Martin GS, Dabbagh O, Adesanya A, Gentile N, et al. Sex, race, and the development of acute lung injury. Chest. 2013;143(4):901–9.
6. Carey MA, Card JW, Voltz JW, Germolec DR, Korach KS, Zeldin DC. The impact of sex and sex hormones on lung physiology and disease: lessons from animal studies. Am J Physiol Lung Cell Mol Physiol. 2007;293(2):L272-8.

7. Speyer CL, Rancilio NJ, McClintock SD, Crawford JD, Gao H, Sarma JV, et al. Regulatory effects of estrogen on acute lung inflammation in mice. Am J Physiol Cell Physiol. 2005;288(4):C881-90.
8. Luo L, Deng J, Wang DX, He J, Deng W. Regulation of epithelial sodium channel expression by oestradiol and progestogen in alveolar epithelial cells. Respir Physiol Neurobiol. 2015;216:52–62.
9. Barton M, Filardo EJ, Lolait SJ, Thomas P, Maggiolini M, Prossnitz ER. Twenty years of the G protein-coupled estrogen receptor GPER: Historical and personal perspectives. J Steroid Biochem Mol Biol. 2018;176:4–15.
10. Vegeto E, Cuzzocrea S, Crisafulli C, Mazzon E, Sala A, Krust A, et al. Estrogen receptor-alpha as a drug target candidate for preventing lung inflammation. Endocrinology. 2010;151(1):174–84.
11. Prossnitz ER, Hathaway HJ. What have we learned about GPER function in physiology and disease from knockout mice? J Steroid Biochem Mol Biol. 2015;153:114–26.
12. Meyer MR, Fredette NC, Howard TA, Hu C, Ramesh C, Daniel C, et al. G protein-coupled estrogen receptor protects from atherosclerosis. Sci Rep. 2014;4:7564.
13. Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, et al. Virtual and biomolecular screening converge on a selective agonist for GPR30. Nat Chem Biol. 2006;2(4):207–12.
14. Pan MX, Li J, Ma C, Fu K, Li ZQ, Wang ZF. Sex-dependent effects of GPER activation on neuroinflammation in a rat model of traumatic brain injury. Brain Behav Immun. 2020;88:421–31.
15. Okamoto M, Suzuki T, Mizukami Y, Ikeda T. The membrane-type estrogen receptor G-protein-coupled estrogen receptor suppresses lipopolysaccharide-induced interleukin 6 via inhibition of nuclear factor-kappa B pathway in murine macrophage cells. Anim Sci J. 2017;88(11):1870–9.
16. Liu L, Gao Z, Xia C, Xu Y, Ma Z, Dong C, et al. Comparative study of trans-oral and trans-tracheal intratracheal instillations in a murine model of acute lung injury. Anat Rec (Hoboken). 2012;295(9):1513–9.
17. Clipperton-Allen AE, Lee AW, Reyes A, Devidze N, Phan A, Pfaff DW, et al. Oxytocin, vasopressin and estrogen receptor gene expression in relation to social recognition in female mice. Physiol Behav. 2012;105(4):915–24.
18. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, et al. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol. 2011;44(5):725–38.
19. Huang X, Xiu H, Zhang S, Zhang G. The Role of Macrophages in the Pathogenesis of ALI/ARDS. Mediators Inflamm. 2018;2018:1264913.
20. de la Rica R, Borges M, Gonzalez-Freire M. COVID-19: In the Eye of the Cytokine Storm. Front Immunol. 2020;11:558898.
21. Card JW, Carey MA, Bradbury JA, DeGraff LM, Morgan DL, Moorman MP, et al. Gender differences in murine airway responsiveness and lipopolysaccharide-induced inflammation. J Immunol. 2006;177(1):621–30.

22. Cuzzocrea S, Bruscoli S, Crisafulli C, Mazzon E, Agostini M, Muia C, et al. Estrogen receptor antagonist fulvestrant (ICI 182,780) inhibits the anti-inflammatory effect of glucocorticoids. Mol Pharmacol. 2007;71(1):132–44.
23. Hamidi SA, Dickman KG, Berisha H, Said SI. 17beta-estradiol protects the lung against acute injury: possible mediation by vasoactive intestinal polypeptide. Endocrinology. 2011;152(12):4729–37.
24. Robbe P, Draijer C, Borg TR, Luinge M, Timens W, Wouters IM, et al. Distinct macrophage phenotypes in allergic and nonallergic lung inflammation. Am J Physiol Lung Cell Mol Physiol. 2015;308(4):L358-67.
25. Immormino RM, Lauzier DC, Nakano H, Hernandez ML, Alexis NE, Ghio AJ, et al. Neuropilin-2 regulates airway inflammatory responses to inhaled lipopolysaccharide. Am J Physiol Lung Cell Mol Physiol. 2018;315(2):L202-L11.
26. Blasko E, Haskell CA, Leung S, Gualtieri G, Halks-Miller M, Mahmoudi M, et al. Beneficial role of the GPR30 agonist G-1 in an animal model of multiple sclerosis. J Neuroimmunol. 2009;214(1-2):67–77.
27. Rettew JA, McCall SHt, Marriott I. GPR30/GPER-1 mediates rapid decreases in TLR4 expression on murine macrophages. Mol Cell Endocrinol. 2010;328(1-2):87–92.
28. Pelekanou V, Kampa M, Kiagiadaki F, Deli A, Theodoropoulos P, Agrogiannis G, et al. Estrogen anti-inflammatory activity on human monocytes is mediated through cross-talk between estrogen receptor ERalpha36 and GPR30/GPER1. J Leukoc Biol. 2016;99(2):333–47.
29. Cuzzocrea S, Mazzon E, Sautebin L, Serraino I, Dugo L, Calabro G, et al. The protective role of endogenous estrogens in carrageenan-induced lung injury in the rat. Mol Med. 2001;7(7):478–87.

Figures

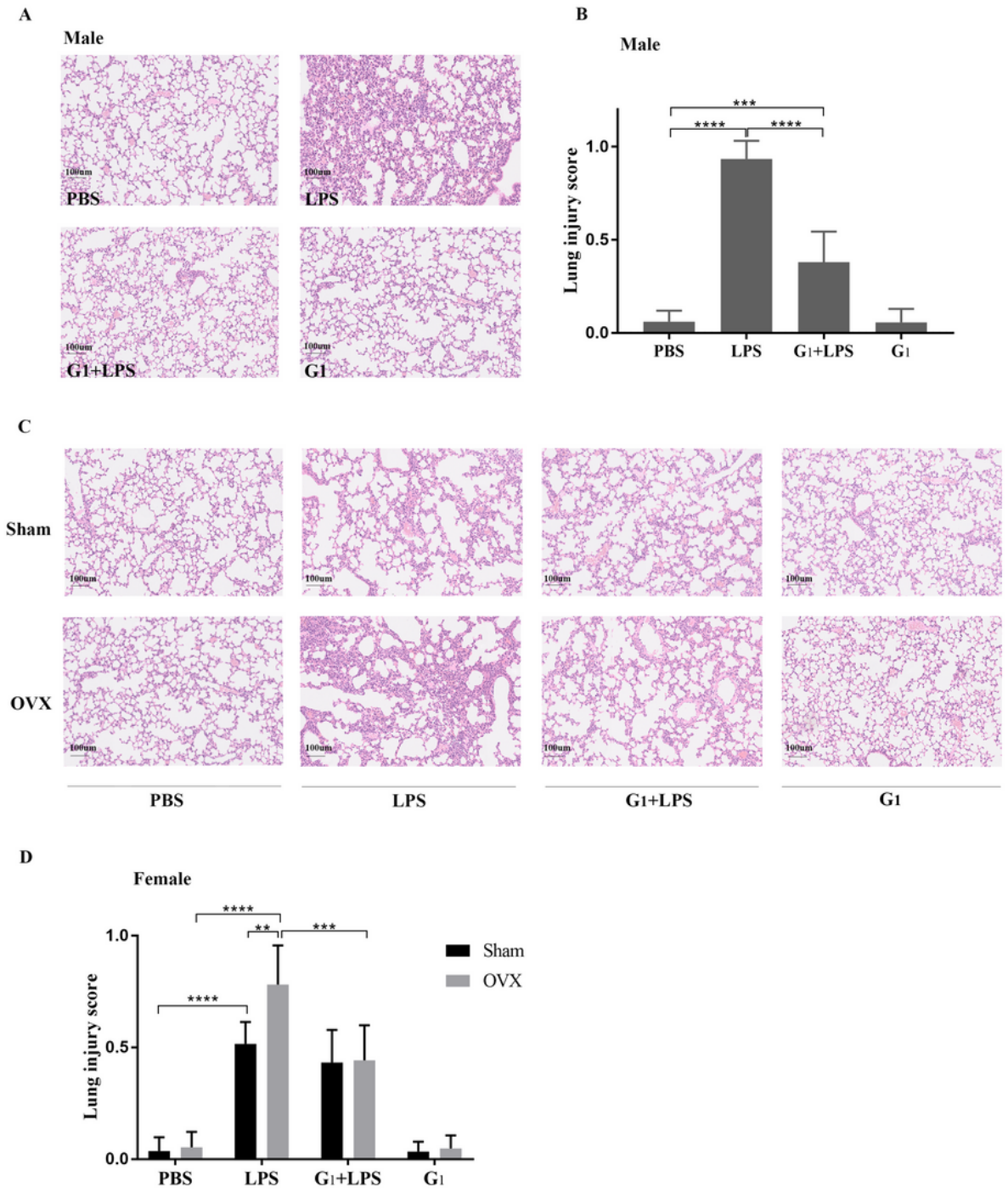

Figure 1

Sex-dependent effects of G1 on LPS-mediated injury of lung. G1 (5mg/kg in 100 μL) or corn oil was administered intraperitoneally 30 min prior to LPS injection. Mice were sacrificed at 24h after LPS administration and lung tissues was harvested to perform H&E staining. Representative images of males (A) and females (C) were showed at 200X. (B)&(D) Lung injury score of different groups. n = 6 for each group. Data were plotted as the mean ± SD for each group. Data of male group were analyzed by one-

way ANOVA and female group were analyzed by two-way ANOVA. **p<0.01, ***p < 0.001 and ****p < 0.0001.

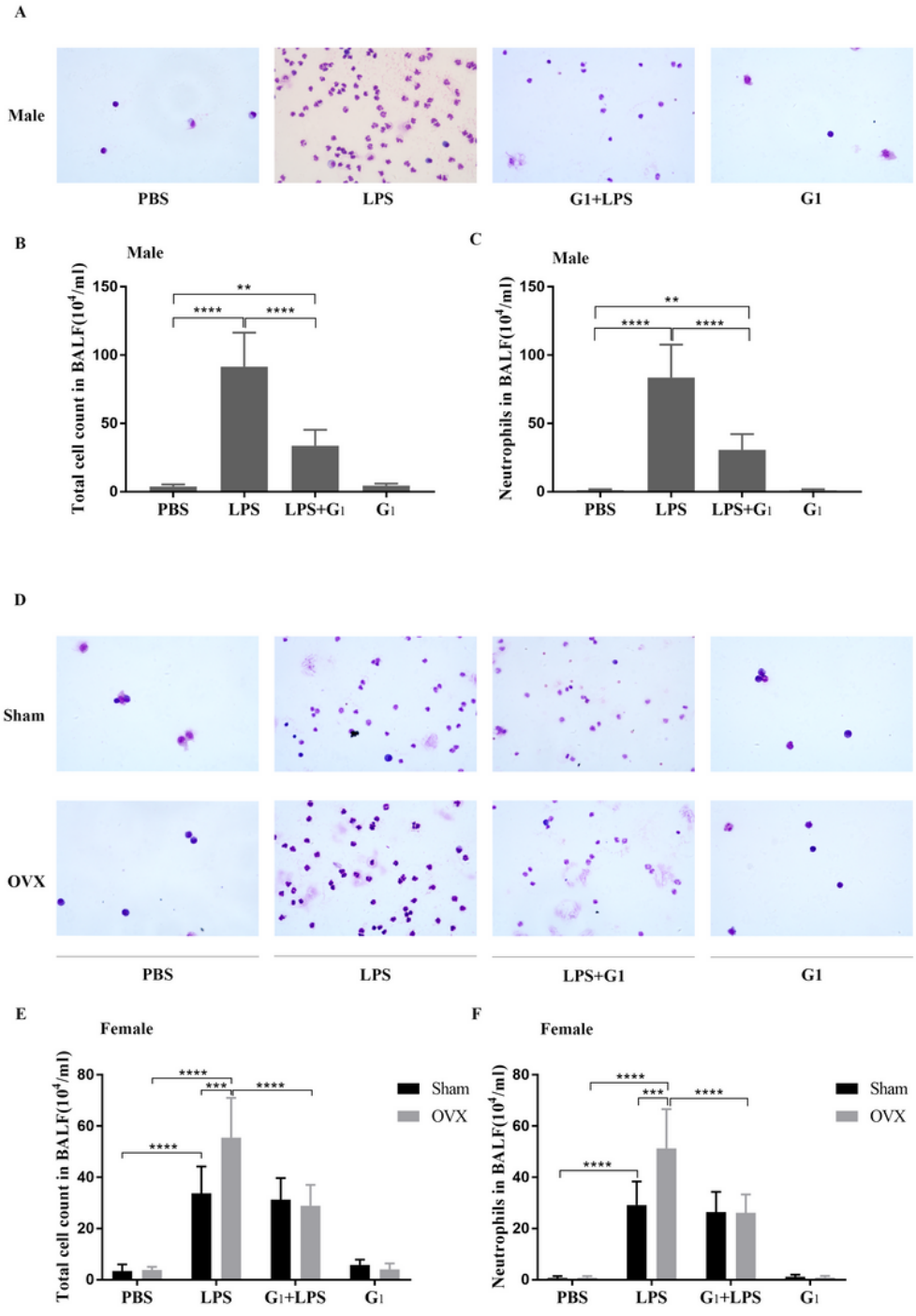

Figure 2

Sex-dependent effects of G1 on LPS-mediated inflammatory cell infiltration to the alveoli.

Bronchoalveolar lavage fluid (BALF) were collected 24h after LPS administration for total cell count using hemocytometer and for differential cell count using Wright Giemsa stain. Wright Giemsa stain of male (A) and female (D) group were showed. (B)&(C) Total cells and neutrophil count of male group in BALF. (E)& (F) Total cells and neutrophil count of female group. n = 6 for each group. Data were plotted as the mean ± SD for each group. Data of male group were analyzed by one-way ANOVA and female group were analyzed by two-way ANOVA. *$p < 0.05$, **$p<0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

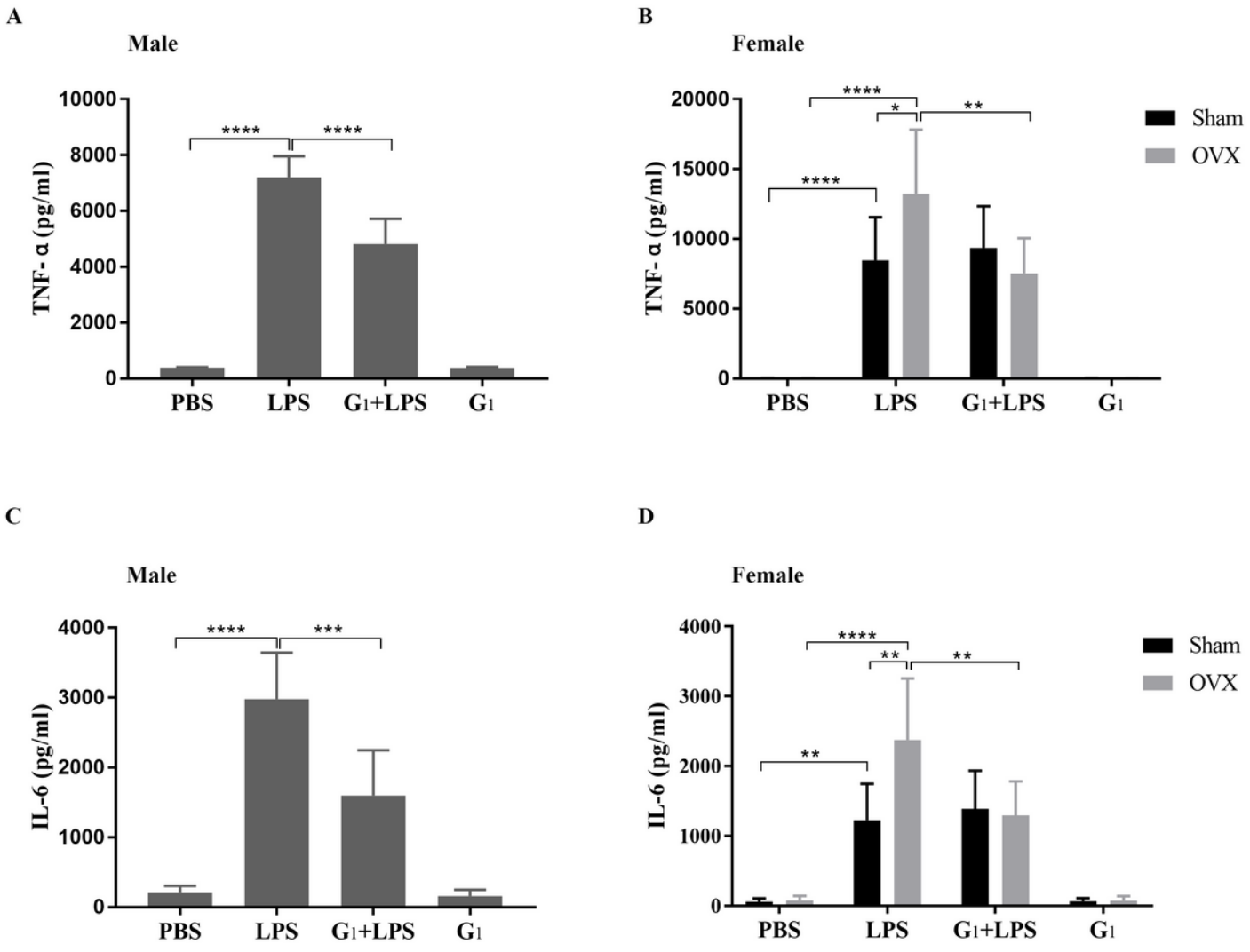

Figure 3

Sex-dependent effects of G1 on LPS-induced inflammatory cytokines in BALF.

BALF were collected 24h after LPS administration for inflammatory cytokine measurement using specific ELISA kit. The concentration of TNF-α of male and female were showed in (A) and (B) separately. (C) and (D) showed the level of IL-6 in males and females separately. n = 6 for each group. Data were plotted as the mean ± SD for each group. Data of male group were analyzed by one-way ANOVA and female group were analyzed by two-way ANOVA. *$p < 0.05$, **$p<0.01$, and ****$p < 0.0001$.

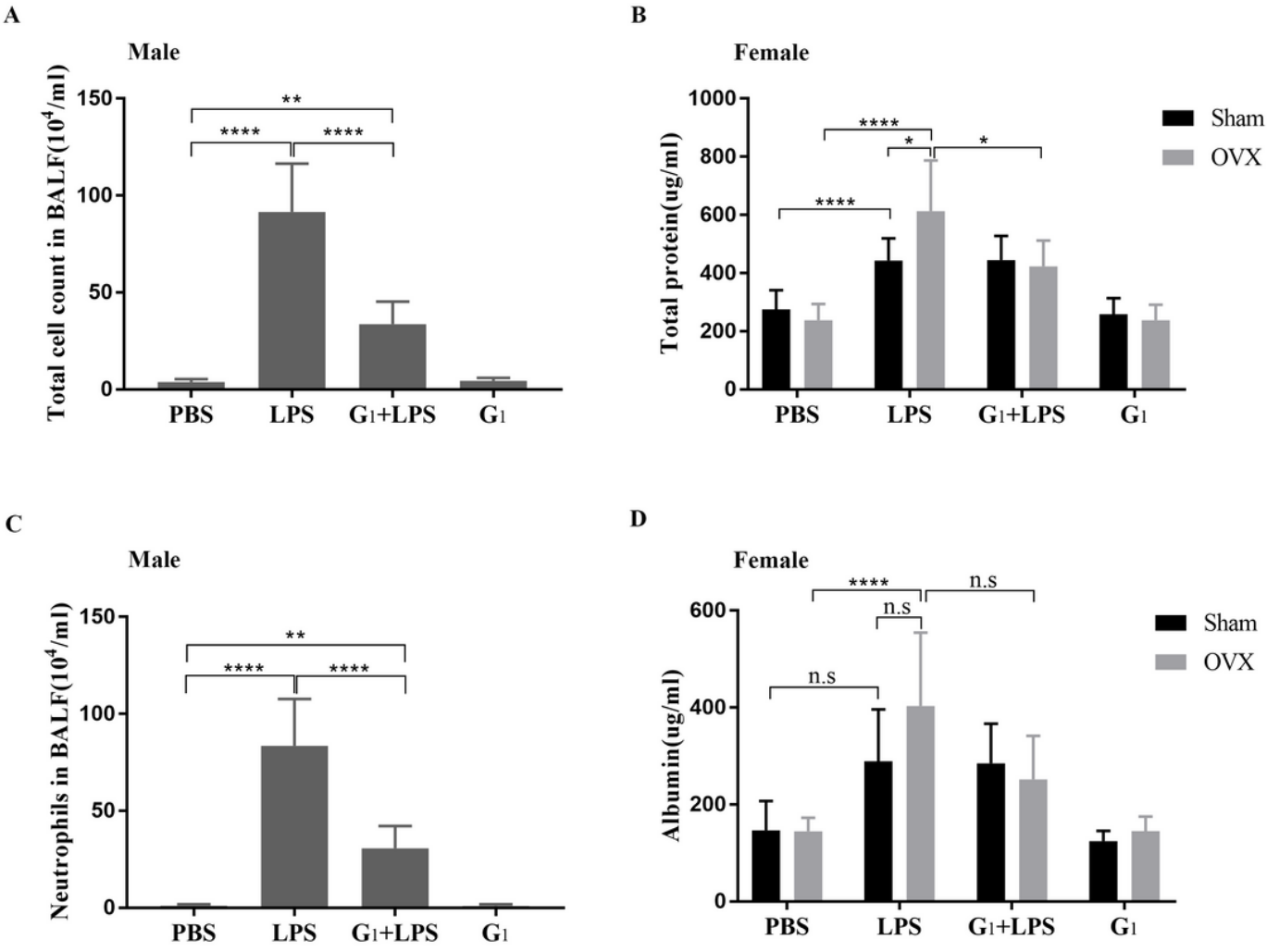

Figure 4

Sex-dependent effects of G1 on LPS-induced alveolar capillary barrier disruption.

BALF were collected 24h after LPS administration to measure the concentration of total protein and albumin. The level of total protein of male and female were showed in (A) and (B) separately. (C) and (D) showed the level of albumin in males and females separately. n = 6 for each group. Data were plotted as the mean ± SD for each group. Data of male group were analyzed by one-way ANOVA and female group were analyzed by two-way ANOVA. **p<0.01, ***p < 0.001 and ****p < 0.0001.

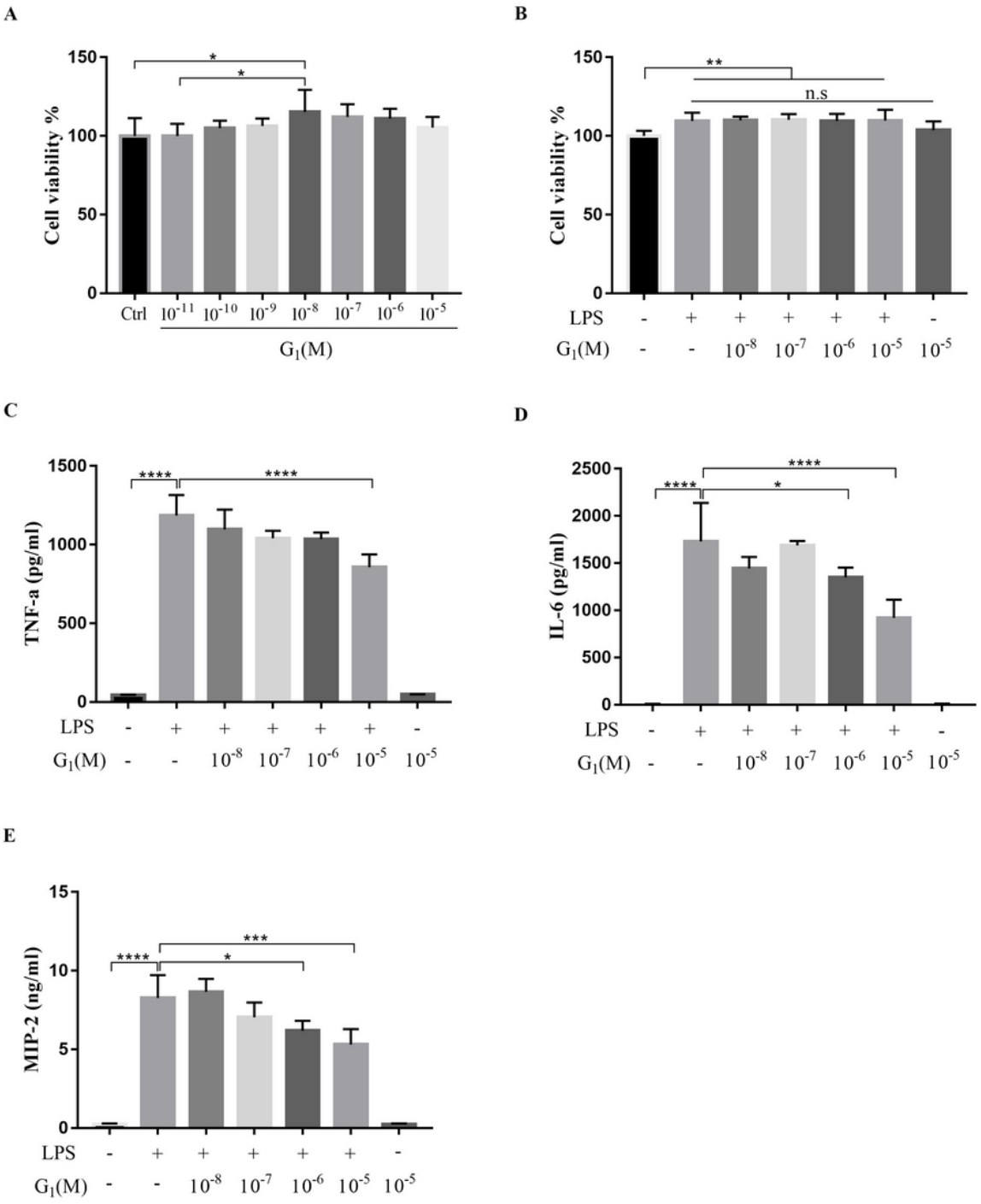

Figure 5

G1 inhibited LPS-induced inflammatory responses in alveolar macrophages without decreasing their viability in vitro. Cell viability of MH-S cells intervened with LPS or G1 or both for 24h were measured through CCK-8 assay (A-B). The level of TNF-α, IL-6 and MIP-2 were determined at 6h after LPS administration in MH-S cells (C-E). n = 4-6 for each group. Data were plotted as the mean ± SD for each group. Data were analyzed by one-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

Supplementary Files

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

- AdditionalFig.tif