Lipopolysaccharide induced intestinal epithelial injury: a novel organoids-based model for sepsis *in vitro*

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Abstract

Background: Advances in organoid culture technology have provided a greater understanding of disease pathogenesis, which has been rarely studied in sepsis before. We aim to establish a suitable organoids-based intestinal injury model for sepsis.

Methods: Stable passaged organoids were constructed and pre-treated with lipopolysaccharide (LPS) to mimic sepsis-induced intestinal injury. The LPS-induced sepsis model was used as a reference. We used quantitative real-time polymerase chain reaction to evaluate the RNA levels of inflammatory factors and antimicrobial peptides. Enzyme-linked immunosorbent assay was used to evaluate the protein levels, hematoxylin and eosin staining was used to evaluate the pathology of the small intestine of mice, and immunohistochemistry and immunofluorescence were used to evaluate the intestinal epithelial barrier function. Perkin Elmer Operetta™ was used to obtain high-resolution images of three-dimensional organoids.

Results: An LPS concentration >150 μg/mL after 24 h was identified to cause organoid growth restriction. The fluorescence intensity of zonula occludens-1 and occludins at LPS concentrations >100 μg/mL decreased significantly after 24 h. After LPS stimulation for 8 h, the RNA expression levels of interleukin (IL)-1α, tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, IL-6, and regenerating islet-derived protein 3 alpha, beta, and gamma increased. These results resembled those of intestinal epithelial layer alterations in a mouse sepsis model. For IL-10, the RNA expression level increased only when the LPS level >200 μg/mL for 24 h.

Conclusions: This study provides the primary intestinal *in vitro* model to study the effects of LPS-induced intestinal injury resembling sepsis. This model provides a platform for immune associated mechanism exploration and effective drug screening.

Keywords: Organoids; Sepsis; Lipopolysaccharide; Barrier function; Inflammatory factors

Introduction

Sepsis is a complex and dynamic syndrome, with a wide heterogeneity among patients. In 2017, an estimated 11.0 million sepsis-related deaths were reported, representing 19.7% of global deaths.[1] Thus, a better understanding of the fundamental processes involved in the complex pathology of sepsis is essential for addressing the high prevalence and mortality rates. The intestine plays a central role in the pathophysiological sequence of events that lead sepsis to multiple organ dysfunction.[2] However, the therapy for sepsis-induced acute gastrointestinal injury (AGI) is rare, which mainly focus on early enteral nutrition and acid inhibitor to protect the gastrointestinal mucosa.[3] In-hospital mortality of patients with sepsis and AGI is still around 21.9% despite the best efforts.[4] Novel evaluation indicators and effective therapeutic targets are necessary to be investigated to improve the clinical outcomes.

Intestinal barrier, which is mainly composed of crypts and villi in the intestinal epithelium, is compromised in sepsis, leading to production of endotoxin and bacterial translocation. Advances in organoid culture technology have provided a greater understanding of disease pathogenesis and have enabled the modeling of various diseases *in vitro*. Intestinal organoids are three-dimensional (3D) self-organizing epithelial structures *in vitro*, consisting of intestinal stem cells and their differentiated epithelial cells, such as enteroendocrine cells, Paneth cells, tuft cells, and M cells.[5] Organoids can retain the characteristics and physiological features of the intestinal

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epithelium and can be cultured for over one year, while retaining its genetic stability.[5-7]

However, studies on many enteric pathogens lacked a good intestinal injury model of sepsis. Human blood endotoxin levels are released by microorganisms during growth and destruction processes and induce a pro-inflammatory immune response, which can reach up to 500 pg/mL.[8]

Lipopolysaccharide (LPS) is the classical endotoxin from the Gram-negative bacterial membrane that is formed mainly due to the differences in the O-antigen and lipid A, which determine the antigenicity and toxicity of endotoxins. LPS administration has typically been used to model the acute inflammatory response associated with sepsis *in vivo* and *in vitro*.[9-13]

A limited number of approaches to LPS co-cultures have been developed to mimic pathogenic bacterial infections.[14] For example, there was a preliminary exploration of LPS administration in neonatal mice intestinal organoid that induced intestinal inflammation and disrupted the tight junctions.[15] The systemic evaluation of LPS-induced *in vitro* platforms to study intestinal inflammation is not available. Thus, this study aimed to explore different time points and concentrations of LPS to establish a suitable *in vitro* organoids-based intestinal injury model for sepsis.

Methods

Animals

Protocols for animal studies were approved by the Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine Animal Policy and Welfare Committee and followed the institutional and national guidelines for the care and use of animals. Male C57BL/6 mice weighing 18 to 22 g were purchased from the Shanghai SLAC Laboratory Animal Centre (Shanghai, China, Certificate No. 20170005050539) and housed and bred under the specific pathogen-free conditions of Shanghai Kingbio Biosciences Inc. (Shanghai, China).

Crypts isolation and 3D organoid culture

The protocol used for the separation of intestinal crypts and 3D organoid culture has been described previously.[16] Briefly, primary small intestine crypts were isolated from 8- to 10-week-old C57BL/6J mice following a published procedure. Advanced Dulbecco's modified Eagle medium/Nutrient Mixture F12 medium (500 μL/well; Invitrogen, CA, USA) supplemented with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (10 mmol/L final concerntion; Invitrogen), GlutaMAX (1 × final concerntion; Invitrogen), Pen/Strep (1 × final; Invitrogen), N2 Supplement (1 mmol/L final concerntion; Invitrogen), B27 Supplement (1 × final concerntion; Invitrogen), Y-27632 (10 μmol/L final concerntion; Sigma, Darmstadt, Germany), and N-acetylcysteine (1 mmol/L final concerntion; Sigma-Aldrich, Darmstadt, Germany) named as basal liquid medium. Matrigel (Becton Dickinson Bioscience, NJ, USA) used for the 3D units supportment was mixed with the growth factors, R-spondin 1 (1 μg/mL final concerntion; Invitrogen), Noggin (100 ng/mL final concerntion), and epidermal growth factor (EGF) (50 ng/mL final concerntion). Crypts were resuspended in an appropriate volume of the pre-mixed Matrigel, yielding approximately 4000 to 8000 crypts/mL. Fifty microliters of the crypts-Matrigel mixture were carefully pipetted into the center of each well of a 24-well plate, incubated for 20 min at 37°C for solidification. Basal liquid medium was added to cover the matrigel for nourish supportment. The organoids were cultured in a 37°C, 5% carbon dioxide incubator. Complete liquid medium, such as basal liquid medium supplemented with growth factors R-spondin 1, Noggin, and EGF, was used for the refreshment every 3 days. The microscopic morphology can be used to estimate the success of culture [Supplementary Figure 1, http://links.lww.com/CM9/B200].[16,17] Small-intestine crypts were plated in the Matrigel and closeing-up as circles, undergoing extensive budding with a structure of crypt-villus. Organoids can be passaged 7 to 10 days after seeding, and the ones used in the present study were from the same passage.

LPS treatments

For the *in vivo* experiments, C57BL/6J mice (8–10-week-old, male) were pre-treated with 10 mg/kg of LPS (Sigma, Darmstadt, Germany; intraperitoneal, i.p.) at 12, 24, and 48 h (four mice per time point), respectively. The control group mice were injected with phosphate-buffered saline (PBS) (vehicle; i.p.), and the small intestines were collected 12 h after injection. The mice were euthanized, and the small intestines were isolated for hematoxylin and eosin (HE) staining, and immunohistochemical staining. Small intestinal epithelial layers (including villus and crypts) were isolated as described previously,[16] and used for subsequent RNA isolation.

For the *in vitro* experiments, the intestinal organoids that had been passaged for 2 days were treated with LPS by direct addition to the liquid medium. The organoids area before and after the concentrations of 0, 25, 50, 100, 150, 200, and 400 μg/mL LPS stimulation for 24 h was evaluated. Optical microscope was used to capture the bright-field image. The concentrations of 0, 25, 50, 100, 200, and 400 μg/mL LPS stimulation for 8 and 24 h were selected for analysis. The liquid medium was collected at 8 and 24 h after adding LPS for secreted tumor necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) protein analysis. For the gene expression analysis, the organoids in Matrigel were dissolved in ice-cold Dulbecco's PBS. Two wells were pooled, and the cells were centrifuged for subsequent RNA isolation.

Assessment of organoids intestinal epithelial injury

For assessment of organoids intestinal epithelial injury, we referred to previous studies *in vivo*.[18] Comprehensive criteria containing four domains were used for evaluating intestinal epithelial injury, including epithelial growth restriction, compromised epithelial barrier function, elevated inflammatory responses, and elevated production of antimicrobial peptides.

Quantitative real-time polymerase chain reaction (qPCR)

The total RNA was extracted using the TaKaRa MiniBEST Universal RNA Extraction Kit (Takara, Tokyo, Japan, No. 9767) according to the manufacturer's instructions. The quality of RNA was tested by the absorbance ratio of $A_{260/230}$ and $A_{260/280}$ using an ultramicro spectrophotometer (Denovix, Wilmington, USA). One microgram of RNA per sample was reverse transcribed using an Oligo (dT) primer and PrimeScript RT Enzyme Mix (Takara). qPCR was performed using the SYBR Premix Ex Taq Kit (Takara) and an ABI 7500 System (Applied Biosystems, MA, USA). Each reaction included an initial denaturation step at 95°C for 30 s, followed by 40 cycles of amplification at 95°C for 5 s and annealing at 60°C for 34 s. The primers used in the experiments are listed in Supplementary Table 1, http://links.lww.com/CM9/B201. All assays were performed in triplicate. Relative quantification of RNA levels was performed using the comparative cycle threshold method with β-actin as the endogenous control.

HE staining

Small intestines of mouse were excised and inflated with 4% paraformaldehyde. Then, the tissue was embedded in paraffin, sectioned, and mounted onto 4 μm-thick slices. Slices were stained with HE and assessed by a pathologist for the presence of histopathological features of intestinal injury.

Immunohistochemistry

The small intestinal tissue sections were dewaxed, hydrated, and transferred to sodium citrate buffer for heat-induced antigen retrieval. The sections were then incubated with 3% hydrogen peroxide for 25 min at 25°C. The sections were blocked and incubated overnight at 4°C, with a 1:500 dilution of zonula occludens-1 (ZO-1) antibody (Affinity Biosciences, Jiangsu, China), a 1:500 dilution of occludin antibody (Affinity Biosciences, China), or a 1:500 dilution of claudin-1 antibody (Affinity Biosciences, China).

The slides were then incubated with biotinylated goat anti-rabbit secondary antibodies (Beyotime Biotechnology, Shanghai, China) for 30 min at 37°C, according to the manufacturer's instructions. The immunoreactivities were visualized using 3,3′-diaminobenzidine tetrahydrochloride solution (Sigma-Aldrich), followed by staining of the nucleus with hematoxylin, dehydrating, and mounting. Then, the sections were analyzed under a light microscope.

Immunofluorescence

After our tests, 10 μL Matrigel/organoid suspension inoculating into the well of 96-well chamber (PerkinElmer, MA, USA) was suitable. The organoids were no more than 20 in a single well. After LPS stimulation with different times and concerntions, matrigel-embedded organoids were subjected to whole mount staining in the 96-well chamber. The cells were fixed for 1 h in 10% formalin. Autofluorescence was quenched by incubation with ammonium chloride for half an hour. Permeabilization and blocking were performed for 1 h with 0.5% Triton-X100 and 10% fetal bovine serum in Dulbecco's PBS. Organoids were stained with occludin, ZO-1, claudin-1 (Affinity Biosciences, China), or rabbit anti-Ki-67 (Abcam, Cambridge, UK). Unconjugated antibodies were visualized in a second step using goat anti-rabbit DyLight 488 antibody (Beyotime Biotechnology, China). Primary and secondary antibodies were diluted in 0.1% Triton-X100, 5% fetal bovine serum in Dulbecco's PBS, and incubated overnight at 4°C. The new Opera Phenix™ high-content imaging system (PerkinElmer) that resembled to confocal imaging, realizing a high-throughput scanning of organoids with 7 nm per layer, were used to provide high-resolution images of the whole 3D cell-culture models.[18] Microscopy data were captured using a 20 × air immersion objective lens on an Opera Phenix instrument. Harmony, version 4.1 (PerkinElmer), which drives the Opera Phenix instruments, was used for reconstructing image and quantitative calculation, including the surface area and immunofluorescence intensity of organoids. The overall fluorescence expression calculation enabled the results more accurate and objective.

Enzyme-linked immunosorbent assay (ELISA)

The organoid culture supernatant was collected and stored at −80°C. The protein levels of TNF-α (MultiSciences, Hangzhou, China) and GM-CSF (MultiSciences) were determined using specific ELISA kits, according to the manufacturer's instructions.

Statistical analysis

The organoids area was evaluated with Image J (Image J software, National Institutes of Health, USA). The qPCR data were first analyzed using StepOne software (Life Technologies, MA, USA). Relative quantification results were then input into GraphPad Prism (GraphPad Software, CA, USA). Data were presented as mean ± standard deviation and compared using Student's *t*-tests. The cytokine levels measured by ELISA were analyzed using Excel (Microsoft). The results were also input into GraphPad Prism and analyzed using Student's *t*-test or non-parametric tests (Kolmogorov–Smirnov test). All statistical tests were two-sided, and $P < 0.05$ were considered with statistical significance.

Results

Organoid growth restricted by high-concentration LPS

Organoids exhibit a typical intestine phenotype with a crypt-villus structure, successful crypt isolation and organoids culture were shown [Supplementary Figure 1, http://links.lww.com/CM9/B200]. To mimic sepsis-like intestinal injury *ex vivo*, different LPS concentrations were administered [Figure 1A]. Organoid growth restriction was identified with high concentration of LPS, and 150 μg/mL or higher was identified as the effective concentration ($t = 2.763$, $P = 0.012$; Figure 1B, 1C).

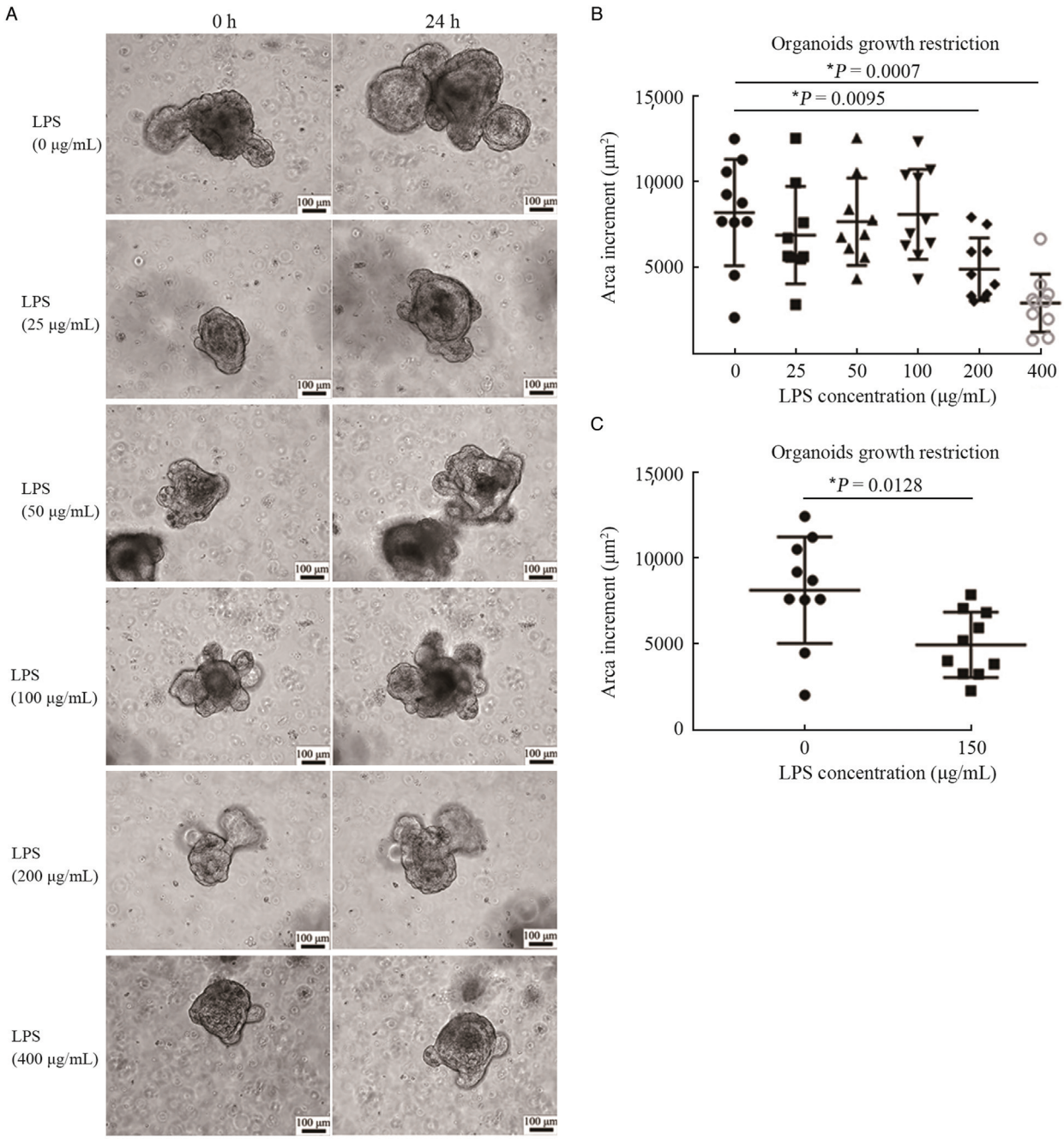

Figure 1: The 24 h growth status of organoids cocultured with LPS of different concentrations. (A) Bright-field image of organoids. The 24 h organoid growth status was restricted with higher LPS concentration. (B) The 24 h organoid growth restriction with different LPS concentrations was compared using area increaments. (C) LPS concentration of 150 μg/mL was successfully identified as an effective concentration. Data are presented as means ± standard deviation, compared with LPS 0 μg/mL group using Student's *t*-tests. LPS: Lipopolysaccharide.

Intestinal epithelial injury induced by LPS in vitro

Immunofluorescence analysis showed that the expression of the tight junction markers ZO-1, occludins, and claudin-1 decreased significantly after exposure to LPS [Figure 2A–D]. With or without LPS stimulation, the expression of the cell proliferation marker Ki-67 was not different markedly [Figure 2E]. The fluorescence intensity of ZO-1 and occludins in groups with LPS concentrations >100 μg/mL and cocultured for 24 h showed a significant difference compared with that in the control groups [Figure 2F,G]. Moreover, the fluorescence intensity of claudin-1 was decreased only at the highest LPS concentration (400 μg/mL) after 24 h [Figure 2H]. Each layer of the organoid morphology for immunofluorescence was presented in Supplementary Figures 2–5, http://links.lww.com/CM9/B200.

Inflammatory environment stimulated by LPS

After 8 h or 24 h of LPS stimulation, the expression of different genes involved in inflammatory cytokines

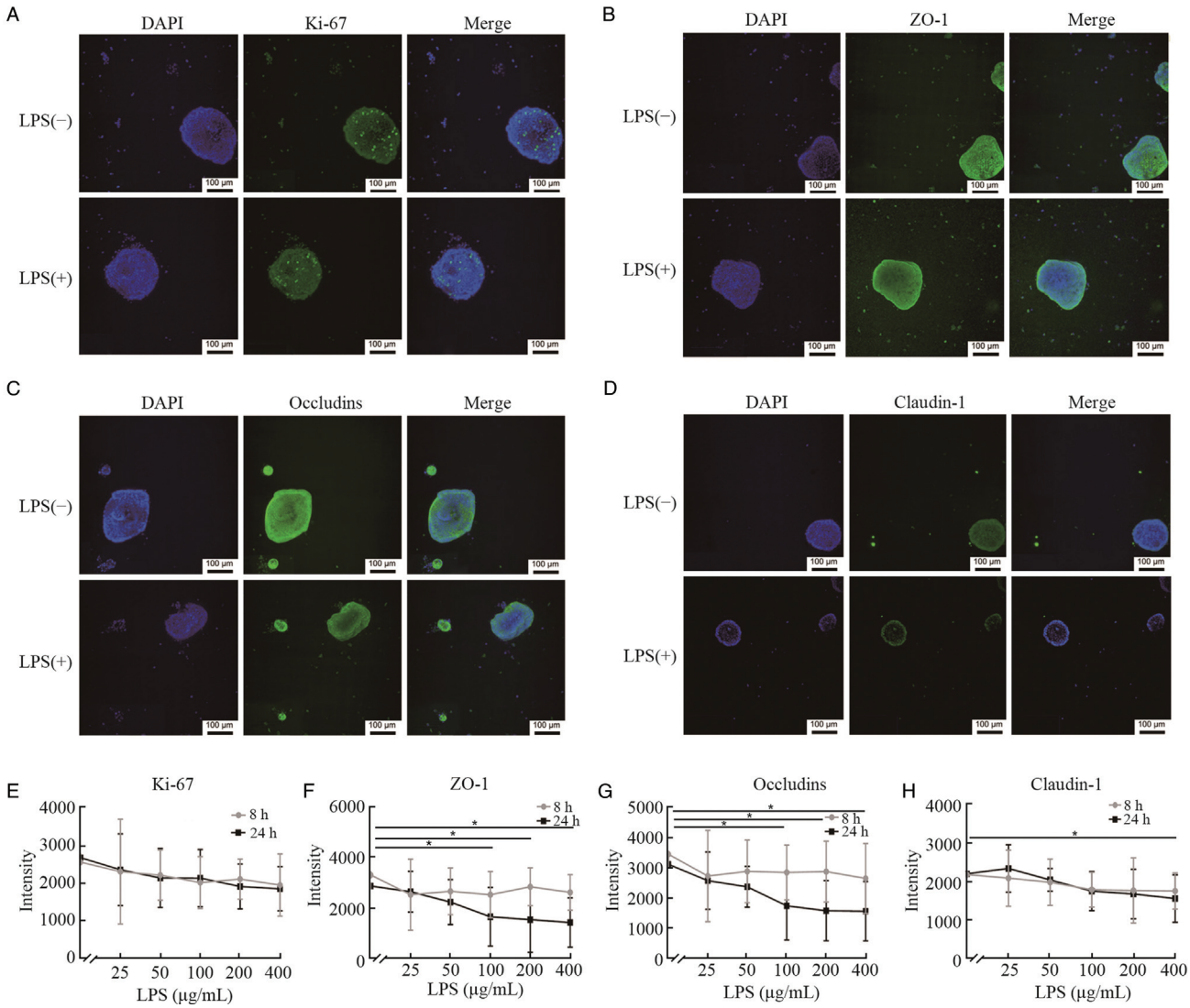

Figure 2: The immunofluorescence of organoids with or without LPS stimulation. The images were captured by Opera Phenix™ high-content imaging system and compressed by Harmony. (A) The expression of Ki-67, LPS 100 μg/mL, 24 h. (B) The expression of ZO-1, LPS 100 μg/mL, 24 h. (C) The expression of occludins, LPS 100 μg/mL, 24 h. (D) The expression of claudin-1, LPS 100 μg/mL, 24 h. (E) Quantitative analysis of Ki-67 with different concentrations of LPS stimulated for 8 h or 24 h. (F) Quantitative analysis of ZO-1 with different concentrations of LPS stimulated for 8 h or 24 h. (G) Quantitative analysis of occludins with different concentrations of LPS stimulated for 8 h or 24 h. (H) Quantitative analysis of claudin-1 with different concentrations of LPS stimulated for 8 h or 24 h. Data are presented as means ± SD, compared with LPS 0 μg/mL 8 h or 24 h group accordingly using Student's *t*-tests. * is used for 24 h. SD: Standard deviation; LPS: Lipopolysaccharide; ZO-1: Zonula occludens-1.

(interleukin [IL]-1α, IL-10, IL-6, TNF-α, and GM-CSF) and antimicrobial peptides (regenerating islet-derived protein 3 [Reg 3] alpha [Reg 3α], beta [Reg 3β], and gamma [Reg 3γ]) were analyzed [Figure 3A]. For 8 h stimulation, the messenger RNA expression levels of IL-1α (LPS, 400 μg/mL; $t = 3.476$, $P = 0.0132$), TNF-α (LPS, 100 μg/mL; $t = 5.125$, $P = 0.0022$), GM-CSF (LPS, 100 μg/mL; $t = 3.785$, $P = 0.0091$), IL-6 (LPS, 100 μg/mL; $t = 3.886$, $P = 0.0081$), Reg 3α (LPS, 100 μg/mL; $D = 1$, $P = 0.0286$), Reg 3β (LPS, 100 μg/mL; $t = 2.548$, $P = 0.0436$), and Reg 3γ (LPS, 50 μg/mL; $t = 3.52$, $P = 0.0125$) were increased significantly. After 24 h of LPS stimulation, the RNA expression levels of IL1-α, IL-6, GM-CSF, and Reg 3α and Reg 3β showed no significant differences in all organoids, whereas the RNA expression level of TNF-α was increased for LPS >100 μg/mL, and the RNA expression level of IL-10 was increased for LPS >200 μg/mL as well as for Reg 3γ (LPS 100 μg/mL). For LPS concentrations of 100 μg/mL and 400 μg/mL after 8 h [Figure 3B] and 50 μg/mL, 200 μg/mL, and 400 μg/mL after 24 h [Figure 3C], the protein levels of TNF-α were increased significantly. For GM-CSF, after LPS stimulation for 8 h [Figure 3B] and LPS stimulation at a concentration ≥50 μg/mL for 24 h, the protein levels increased significantly [Figure 3C].

LPS-induced mouse sepsis-associated intestinal epithelial injury

Mice that were pre-treated with LPS for 12 and 24 h showed signs of intestinal injury, which were mainly characterized by severe villus atrophy, massive crypt loss, and increased cellular infiltration of the lamina propria [Figure 4A]. After 48 h of LPS pre-treatment, the intestinal injury recovered [Figure 4A]. Quantification analysis of immunohistochemistry showed the protein level of ZO-1

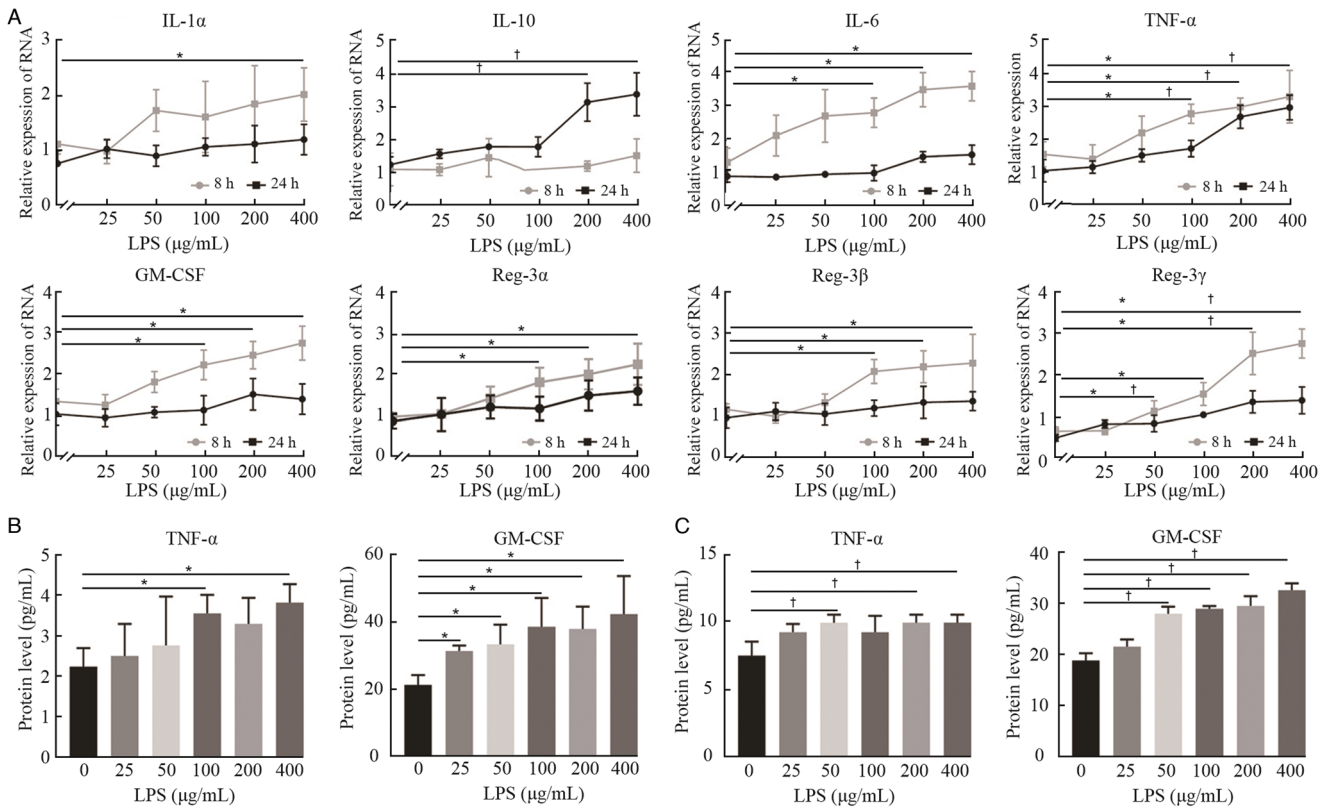

Figure 3: RNA levels of inflammatory cytokines and antimicrobial peptides, protein level of inflammatory cytokines. (A) The organoids RNA levels of inflammatory cytokines (IL1-α, IL-10, IL-6, TNF-α, and GM-CSF) and antimicrobial peptides (Reg 3α, Reg 3β, and Reg 3γ) with different concentrations of LPS stimulated for 8 h or 24 h. (B) The protein level of TNF-α and GM-CSF with different concentrations of LPS stimulated for 8 h. (C) The protein level of TNF-α and GM-CSF with different concentrations of LPS stimulated for 24 h. Data are presented as means ± standard deviation, compared with LPS 0 μg/mL 8 h or 24 h group accordingly using Student's *t*-tests. * is used for 8 h and † is used for 24 h. GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; LPS: Lipopolysaccharide; TNF-α: Tumor necrosis factor alpha.

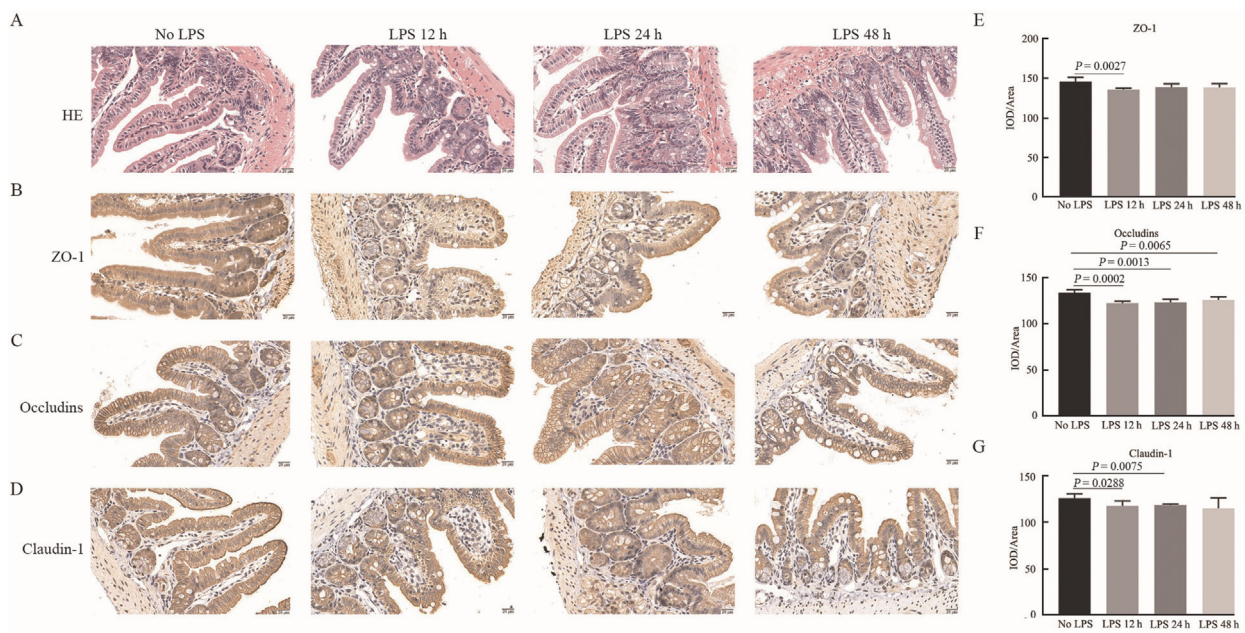

Figure 4: Intestinal pathology of mice sepsis model and control. (A) HE for mouse intestinal. Immunohistochemistry showed the ZO-1(B), occludins (C), claudin-1 (D) expression of mouse intestinal. Quantification of ZO-1 (E), occludins (F), claudin-1 (G) staining by digital image analysis, the ocular fields (80 × original magnification) per specimen were assessed as mean IOD/Area, data are presented as mean ± standard deviation ($n = 5$). HE: Hematoxylin and eosin; IOD: Integrated option density; ZO-1: Zonula occludens-1.

decreased significantly after 12 h of LPS pre-treatment, occludins decreased significantly after 12, 24, and 48 h of LPS pre-treatment, and claudin-1 decreased significantly after 24 and 48 h of LPS pre-treatment [Figure 4B–G]. The expression of inflammatory cytokines IL-1α and antimicrobial peptide Reg 3γ increased significantly after 24 h or 48 h of LPS pre-treatment [Supplementary Figure 6, http://links.lww.com/CM9/B326]. The expression of inflammatory cytokines IL-10 only increased significantly after 48 h of LPS pre-treatment ($D = 1$, $P = 0.0286$). The expression of IL-6, GM-CSF, Reg 3β, and Reg 3γ increased significantly after 12 h or 24 h of LPS pre-treatment [Supplementary Figure 6, http://links.lww.com/CM9/B326]. The expression of TNF-α increased significantly after 12 h ($t = 7.59$, $P = 0.0003$), 24 h ($t = 7.62$, $P = 0.0003$), and 48 h ($t = 7.62$, $P = 0.0003$) of LPS pre-treatment [Supplementary Figure 2, http://links.lww.com/CM9/B200].

Discussion

This study provides the primary intestinal *in vitro* system to study the effects of LPS-induced intestinal injury resembling sepsis, which would be a valuable tool to study epithelial barrier defects in various injury and disease contexts. In this study, the intestinal injury model induced by LPS *in vivo* showed highly consistent biological characteristics with our organoids based internal epithelial injury model *in vitro*. For that, LPS stimulation in organoids could induce growth restriction [Figure 1], epithelial barrier defection that the protein levels of tight junctions including ZO-1 and occludins [Figure 2B,C] was decreased, inflammatory factor (IL-1α, TNF-α, GM-CSF, IL-10, and IL-6) and antimicrobial peptides (Reg 3α, Reg 3β, and Reg 3γ; Figures 4 and Supplementary Figure 6, http://links.lww.com/CM9/B326) were increased.

According to previous studies,[16,17] the major criteria for a successful intestinal crypts derived organoids model were dynamic growth process and crypt-villus structure, and all of which were observed in our experiments, indicating the organoids model were successfully established. Unlike intestinal epithelial cell culture, organoids including enterocytes, goblet cells, enteroendocrine cells, paneth cells, tuft cells, M cells, and the expression of ZO-1 and occludins could simulate the internal environment of intestine better [Figure 2]. Further, the expression of antimicrobial peptides such as Reg 3α, Reg 3β, and Reg 3γ [Figure 3] can only be evaluated in the organoids model rather than the intestinal epithelial cell culture, because antimicrobial peptides was predominantly secreted by paneth cell.[19] After confirmation the success of organoids, the intestinal epithelial injury was induced by LPS in the organoids-based model *in vitro*. The criteria for the organoids-based intestinal epithelial injury model, including the epithelial growth restriction, compromised epithelial barrier function, elevated inflammatory responses, and elevated production of antimicrobial peptides were all observed, suggesting the intestinal epithelial injury model based on organoids was also successfully built.

Compared with former studies relate to organoids based-intestinal epithelial injury, our study has following strength. First, it systematically evaluated the biological features of organoids based-intestinal epithelial injury induced by LPS. Although previous studies have reported similar organoids based model, these studies were mainly focused on the inflammation responses induced by LPS,[20-22] rather than a complete picture of biological features of intestinal epithelial injury in organoids based model. Second, antimicrobial peptides were evaluated in organoids based-intestinal epithelial injury. Antimicrobial peptides, such as Reg 3α, Reg 3β, and Reg 3γ that are important executor of intestinal epithelial innate immunity,[23] may provide directions for the treatment of sepsis associated intestinal injury and more research for that in this model could be achieved. Finally, we improved a whole amount immunofluorescence staining method allowing inoculating organoids into the well of 96-well chamber and realizing whole layer high-throughput imaging. Previous studies use only 80 organoids in a single well of 8-well chamber for whole amount immunofluorescence staining.[16] Combining immunofluorescence in 96-well chamber with the new Opera Phenix™ high-content imaging system achieved a high-throughput screening and higher quantitative measure accuracy for the detection of whole organoids. This method can be used for high-throughput drug screening.

Our study also has some limitations. First, the digestion and absorption function of organoids have not been investigated in this study. This is because the digestion and absorption function are largely dependent on the complete structure of gastrointestinal tract, which cannot be fully mimicked by organoids. Second, the potential mechanism and underlying pathways by which LPS induced epithelial injury in organoids model have not yet been fully elucidated in this study and require further investigation.

In conclusion, we have successfully established an organoid based epithelial injury model *in vitro* and systematically evaluated the biological features of this model. This organoids based model provides a platform for uncovering the potential mechanisms of sepsis-associated intestinal epithelial injury and for screening therapeutic drugs (anti-TNF-α,[24,25] anti-IL-6,[26] and anti-GM-CSF[27]).

We have successfully established an organoids based epithelial injury model *in vitro* and systematically evaluated the biological features of this model. This organoids based model provides a platform for uncovering the potential mechanisms of sepsis-associated intestinal epithelial injury and for screening therapeutic drugs.

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Conflicts of interest

None.

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