

# Lipopolysaccharide induces the early enhancement of mice colonic mucosal paracellular permeability mainly mediated by mast cells

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**Summary.** The alteration of intestinal mucosal barrier is considered to be the central pathophysiological process in response to gastrointestinal infections, and mucosal microstructural damage is a major factor for enhancing epithelial permeability in persistent bacterial infections. However, the mechanism involved in hyperpermeability in the early stage of acute bacterial infections is not fully understood. In the present study, fluorescein isothiocyanate-dextran across and transepithelial resistance measured in Ussing chambers were used to assess the intestinal paracellular permeability. Mast cell activation was evaluated by western blotting for the presence of tryptase released from mast cells. Serum levels of interleukin-6 were evaluated using enzyme-linked immunosorbent assay. Our results indicated that mast cells played a pivotal role in colonic mucosal hyperpermeability in wild type mice treated with lipopolysaccharide (LPS) for 2 h. And the effect of LPS was mainly dependent on mast cell degranulation, while no change in permeability was observed in the mast cell-deficient mice (*Wads<sup>-/-</sup>*) after LPS administration. No obvious changes of the mucosal structure including histomorphological architecture and expression of intercellular junction proteins were obtained in either wild type or *Wads<sup>-/-</sup>* mice after LPS stimulation by hematoxylin and eosin staining, immunofluorescence staining and western blot analysis. Furthermore, the self-

renewal of intestinal epithelia, detected by using proliferation marker 5'-bromo-2'-deoxyuridine, was not involved in increased permeability. Collectively, activation of mast cells induced by LPS mediated intestinal hyperpermeability in the initial stage, and played a crucial role in barrier dysfunction rather than mucosal microstructural damage in acute enterogenous bacterial infection.

**Key words:** Lipopolysaccharide, Degranulation, Mast cells, Intestinal permeability, Acute infection

## Introduction

Gastrointestinal (GI) disorders usually affect functions of the digestive tract, i.e., digestion, absorption, or excretion. Clinically, the usual symptoms include diarrhea, constipation, vomiting and gastroenteritis, which are mainly due to infections by various kinds of bacteria or parasitic organisms. Among them, infectious gastroenteritis, representing common entities in daily clinical practice, is especially associated with an increased risk for functional dyspepsia and irritable bowel syndrome (IBS), affecting up about one fifth of worldwide population (Wouters et al., 2016). It is well known that the intestinal mucosa comprises the largest surface area of the body and is also constantly exposed to a vast array of microbes, toxins and food antigens, thus it undertakes not only the task of immune defence but also as the central component of intestinal barrier protecting from bacteria translocation.

Bacteria and their metabolites are important factors for the occurrence and development of enterogenic infection. Lipopolysaccharide (LPS), a major cell wall component of Gram-negative bacteria, has high concentrations in the gut lumen, where many trillions of commensal bacteria reside (Guo et al., 2013; Neyen and Lemaitre, 2016). Normally, Gram-negative bacteria and LPS do not penetrate across the healthy intestinal epithelium, however, under some pathologic conditions, LPS can leak out from the GI tract into the body through raising intestinal permeability and cause a series of GI infections or multiple injuries (Benoit et al., 1998; Ge et al., 2000). Nonetheless, the involvement of LPS-induced intestinal mucosal hyperpermeability has not been fully elucidated yet.

It is well documented that mast cells (MCs) have been viewed, for the most part, as effectors of allergy and anaphylaxis in pathological conditions. In addition, it is reported that toll-like receptors (TLRs), which ligands are unique products of bacteria, such as LPS and peptidoglycan, existed on the surface of MCs (Halova et al., 2018). TLR4-mediated activation of MCs has been discussed as being of potential relevance in inflammatory bowel disease, suggesting that Gram-negative bacteria or bacterial products are crucial for MC triggering (Kobayashi et al., 2007). However, whether MCs are involved in the increase in mucosal permeability at the early stage after bacterial infections is still not entirely clear. Therefore, in the present study, we introduce *Wads*<sup>-/-</sup> mice, which have the T-to-C transition mutation in *c-kit* gene that results in Phe-to-Ser substitution at amino acid 856 (F856S) (Ruan et al., 2005). The homozygous mutant mice present a novel loss-function of *c-kit* showing white color, anemic, deaf, and sterile and thus named *Wads* mice. More importantly, the mutation of *c-kit* in the *Wads*<sup>-/-</sup> mice can lead to MC deficiency and loss of interstitial cells of Cajal in the GI tract, thereby, it is considered that the *Wads*<sup>-/-</sup> mice can be used as a good laboratory animal model for studying the function of MCs in the GI tract (Ruan et al., 2005).

The main aim of this study is to investigate the mechanism involved in colonic barrier dysfunction in the initial stage of acute bacterial infections. Our study demonstrated that LPS-induced early epithelial hyperpermeability was mainly mediated by triggering activation of MCs in the intestinal mucosa rather than disruption of epithelial microstructure. These results would provide a meaningful insight for clinical treatment of relevant disease.

## Materials and Methods

### Mice

*Wads*<sup>+/-</sup> mice on a C57BL/6 background were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). By mating *Wads*<sup>+/-</sup> parents, wild type (WT) mice and *Wads*<sup>-/-</sup> mice were

obtained, and the latter presented a novel loss-function of *c-kit* gene and MC deficiency (Ruan et al., 2005). In this study, adult WT and *Wads*<sup>-/-</sup> mice (8-10 weeks old, 18-22 g) of either gender were housed in a controlled environment under a 12 h light/dark cycle at 22±2°C, 55±5% humidity with access to food and water ad libitum. All mice were sacrificed by appropriate anesthesia and/or cervical dislocation prior to sample collection. The distal 1-cm segment of the colon, beginning 2 cm distal to the anus, was applied in all experiments. All animal procedures were carried out strictly under protocols approved by the Animal Care and Use Committee of Capital Medical University (Permit Number AEEI-2016-143, 17 October 2016). Every effort was made to minimize the number of animals used as well as their suffering.

### LPS administration

For mimicking the early stage of bacterial infection, mice were intraperitoneally injected with 10 mg/kg of LPS (from *Escherichia coli* serotype O55:B5, Cat.No.L4005, Sigma-Aldrich, USA) dissolved in sterile saline or vehicle (0.9% saline) and sacrificed 2 h after injection (Zhou et al., 2013).

### Intestinal epithelial cell proliferation

As the widely used and well-established reagent for labeling and quantifying proliferating cells, 5'-bromo-2'-deoxyuridine (BrdU) is incorporated into nuclear DNA in place of the nucleotide thymidine during DNA synthesis in S-phase of the cell cycle. Here mice were intraperitoneally injected with BrdU (30 mg/kg, Cat.No.B5002, Sigma-Aldrich, USA) or vehicle (0.9% saline) and sacrificed 2 h after injection. Subsequently, BrdU-labeled epithelial cells of the distal colon were detected by immunofluorescence staining of frozen sections. The numbers of BrdU-positive cells were counted in 10 colonic crypts per section, ten random sections were counted per mouse and 5 mice per group.

### Intestinal permeability

After sacrifice, the mouse distal colon was removed and placed in Krebs buffer, then 1-cm segment of distal colonic mucosa was mounted in dual channel Ussing chambers (U-2500, Warner Instruments, USA) and exposed 0.3 cm<sup>2</sup> of tissue to 10 mL of Krebs buffer. Krebs buffer (pH=7.4, 37°C) was gassed with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>), and contained 4.70 mM KCl, 2.52 mM CaCl<sub>2</sub>, 118.50 mM NaCl, 1.18 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.64 mM MgSO<sub>4</sub>, and 24.88 mM NaHCO<sub>3</sub>. After 15 min equilibrium period, the transepithelial resistance (TER) was measured as previously described (Forbes et al., 2008). Fluorescein isothiocyanate (FITC)-dextran (2.2 mg/mL, molecular mass 4 kDa, Sigma-Aldrich, USA) was added to the mucosal side of the chamber. Medium (220 µL) was removed from the serosal reservoir and

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replaced with fresh medium every 20 min over a period of 180 min. 220  $\mu$ L sample was added to 780  $\mu$ L Krebs buffer and FITC-dextran concentration was determined by Bio-Tek FL-500 fluorescence plate reader (Bio-Tek Instruments, USA) with an excitation wavelength of 490 nm and an emission wavelength of 530 nm.

### Toluidine blue staining

The intestinal mucosal MCs quantification and their activation status were processed by standard toluidine blue staining. Briefly, the 8- $\mu$ m frozen sections of distal colon were fixed in Carnoy's solution for 1 h at 4°C and stained with 0.5% toluidine blue in 0.5 N HCl in phosphate buffered saline (PBS, 0.01 M, pH 7.4) for 45 min at 25°C. Sections were then viewed under a light microscope (Leica DM LB2, Germany) and MCs were evaluated by characteristic metachromatic granules, and the activation was identified by the presence of extruded granules close to the cell surface or staining less than half of the cytoplasm with toluidine blue staining. MC count was performed as the number of toluidine blue-positive cells per section, ten random sections were counted per mouse and 8 mice per group.

### Hematoxylin and eosin staining

Distal colon tissues were fixed in 4% paraformaldehyde, dehydrated in rising concentrations of ethanol, embedded in paraffin, and sectioned (5  $\mu$ m). The sections were stained with hematoxylin and eosin according to the standard procedure. Images were acquired with a light microscope.

### Immunofluorescence staining

Frozen sections (8  $\mu$ m) were fixed in 4% paraformaldehyde for 20 min. Non-specific binding was blocked with 1% bovine serum albumin (BSA, Sigma-Aldrich, USA) for 1 h. Then sections were incubated with primary antibodies including anti-claudin-1 (Cat.No.Ab15098, Abcam, USA, 1:200), anti-occludin (Cat.No.71-1500, Invitrogen, USA, 1:400), anti-E-cadherin (Cat.No.3195, CST, USA, 1:200) and anti-BrdU (Cat.No.MCA2060, AbD Serotec, UK, 1:300) overnight at 4°C, respectively. After washing with PBS, sections were incubated with the corresponding secondary antibodies for 1 h at 25°C. Negative-control sections were incubated in solutions lacking the primary antibody. Images were acquired with a fluorescence microscope (Nikon 90i, Japan) and analyzed using Image-Pro Plus 6.0 software (USA).

### Enzyme-linked immunosorbent assay (ELISA) measurement

For measurement of interleukin (IL)-6 serum concentrations, blood from retro-orbital plexus of mice

was collected 2 h after LPS stimulation, centrifuged at 3000 r/min for 15 min to separate serum, the serum pipetted into an extra cup and stored at -80°C. IL-6 levels were determined using Quantikine ELISA kit (Cat.No.M6000B, R&D Systems, USA) following the manufacturer's instructions. The absorbance at 450 nm was measured using a microplate reader (SpectraMax i3, Molecular Devices, USA). The results were expressed as pg/mL derived from standard curves.

### Western blot

Total proteins were extracted from the colonic mucosal tissues using RIPA lysis buffer (Cat.No.C1053, Applygen, China) containing protease inhibitor cocktail (Cat.No.P8340, Sigma-Aldrich, USA) and phosphatase inhibitor cocktail (Cat.No.P5726, Sigma-Aldrich, USA). The protein concentrations were determined by NanoDrop 2000c spectrophotometer (Thermo Scientific, USA) using BCA protein assay kit (Cat.No.P1511, Applygen, China). Equal amounts (20-40  $\mu$ g) of total protein from each sample were electrophoresed on an SDS-PAGE and transferred to a nitrocellulose membrane. After blocking with Tris-buffered saline containing 0.05% Tween-20 (TBST) and 5% non-fat dry milk or 5% BSA for 1 h, membranes were incubated with primary antibodies including anti-claudin-1 (Cat.No.Ab15098, Abcam, USA, 1:1000), anti-occludin (Cat.No.71-1500, Invitrogen, USA, 1:400), anti-E-cadherin (Cat.No.3195, CST, USA, 1:1000) and anti-mast cell tryptase (Cat.No.sc-32889, Santa Cruz, 1:800) overnight at 4°C, then washed in TBST, followed by incubating with horseradish peroxidase-conjugated secondary antibodies for 1 h. The proteins were detected using enhanced chemiluminescence (Thermo Scientific, USA) and viewed in Fusion FX Vilber Lourmat (France). The housekeeping gene  $\beta$ -actin (Cat.No.sc-47778, Santa Cruz, USA, 1:2000) was used as an internal control.

### Statistics

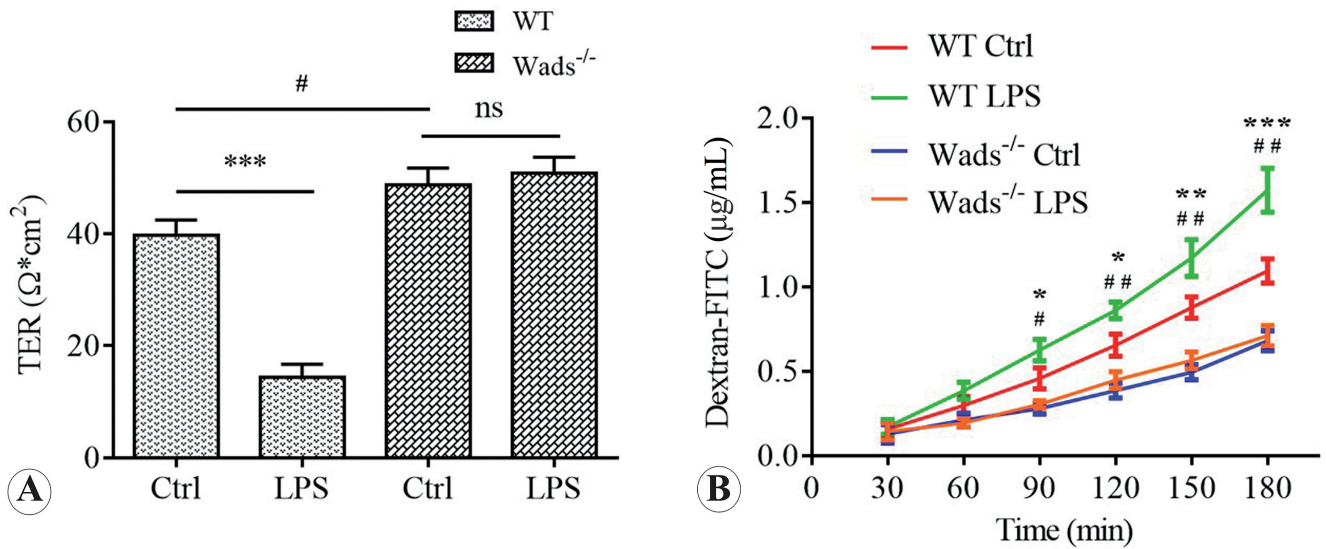
Obtained data were compared using ANOVA analysis for multiple groups and Student's *t*-test for two groups with SPSS 23.0 software (IBM Corporation, USA). Results were presented as mean  $\pm$  SEM. A *P* value <0.05 was considered to be statistically significant.

## Results

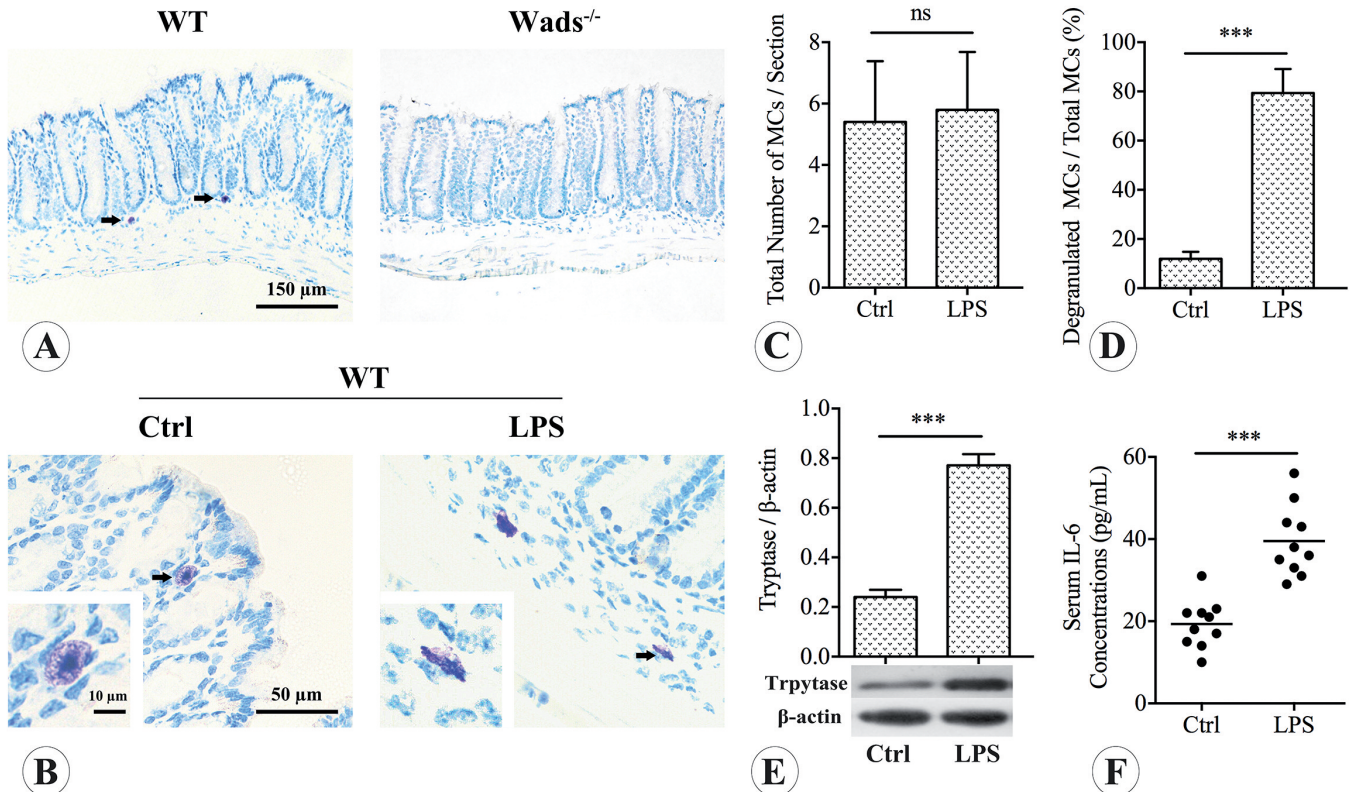
### LPS induces mucosal barrier dysfunction in WT mice but not in *Wads*<sup>-/-</sup> mice

First, we compared TER of *ex vivo* distal colon and paracellular permeability by luminal-to-serosal flux of FITC-dextran across colon segments from normal and LPS-treated WT mice to ascertain the exact influence of LPS on homeostatic intestinal barrier function during

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**Fig. 1.** LPS-induced alterations of intestinal TER and permeability in WT and Wads<sup>-/-</sup> mice. Segments of the distal colon from mice were mounted in Ussing chambers and LPS-stimulated alterations in TER were measured (A). *Ex vivo* intestinal permeability was calculated as luminal-to-serosal flux of FITC-dextran (B). Values represent mean±SEM; n=8 mice per group. Statistical significance is: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001, WT Ctrl compared with WT LPS group; # P<0.05, ## P<0.01, WT Ctrl compared with Wads<sup>-/-</sup> Ctrl group; ns P>0.05. Ctrl, control.



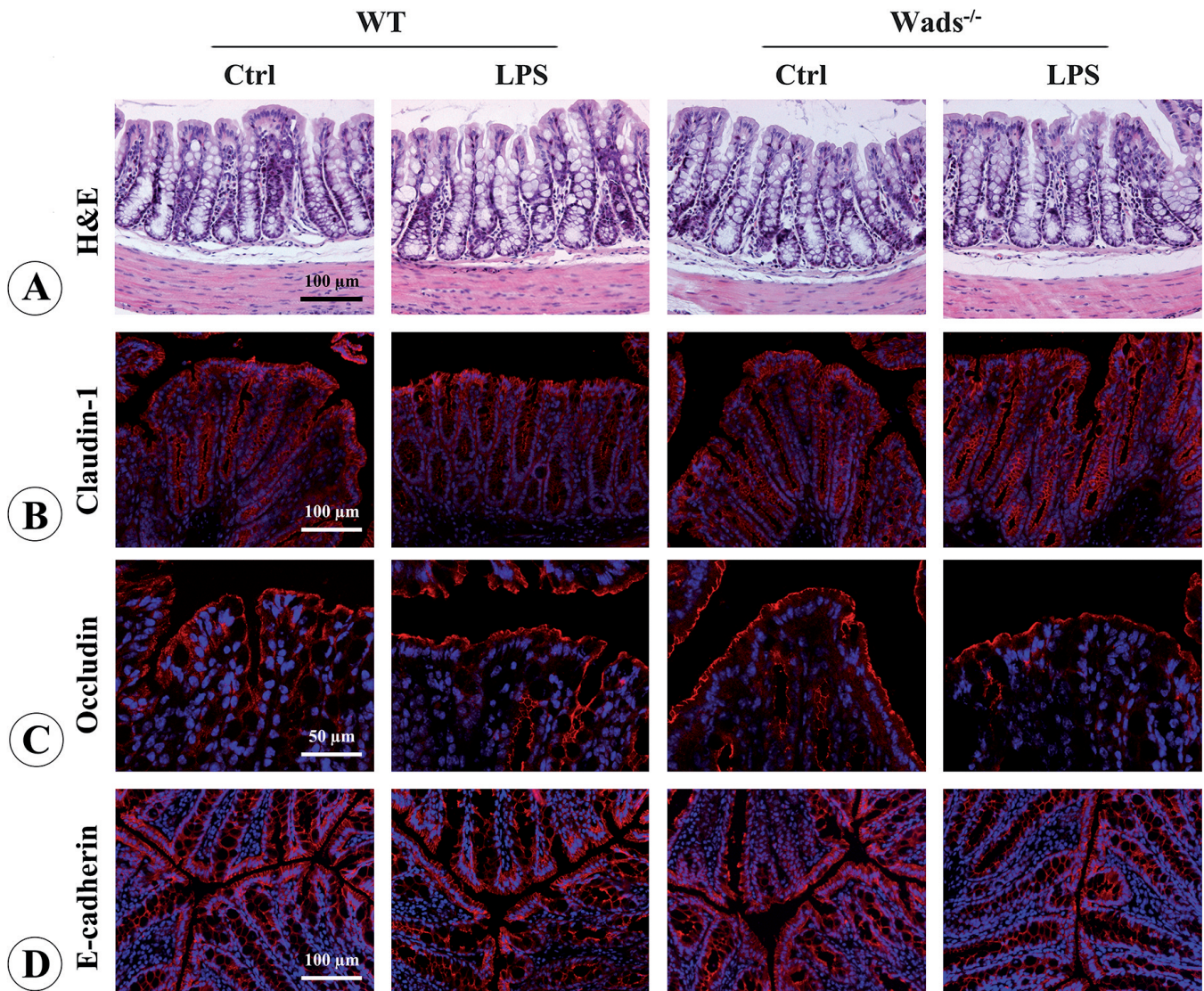
**Fig. 2.** Effect of LPS stimulation on mucosal MC degranulation in the distal colon of WT mice. No MCs were observed in Wads<sup>-/-</sup> mice colon tissue (A). Toluidine blue-positive MCs in normal WT mice were filled with red-purple granules, whereas MCs in the LPS-stimulated colon were degranulated which indicated by loss of granularity and free purple granules in the surrounding tissue (B). Statistical analysis indicated no significant difference in total number of MCs between normal and LPS-treated groups (C), while increased number of degranulated MCs was observed after LPS injection (D). The expression of tryptase protein in the colonic mucosa using western blot analysis (E) and serum IL-6 level by ELISA test (F) in LPS-stimulated WT mice were significantly increased compared with control group. Values represent mean±SEM. Statistical significance is: \*\*\* P<0.001, ns P>0.05. MC: mast cell; Ctrl: control.

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early stage of stimulation. Clearly, the TER was distinctly decreased (Fig. 1A), and the permeability was remarkably increased in WT mice after LPS injection, which variational trend was opposite to TER (Fig. 1B). Notably, TER was increased (Fig. 1A) and the FITC-dextran concentration was greatly decreased (Fig. 1B) in *Wads*<sup>-/-</sup> mice compared with WT mice. However, there were no significant differences between normal and LPS-treated *Wads*<sup>-/-</sup> mice in either TER or FITC-dextran across of distal colon. These results suggested that LPS increased permeability of intestinal mucosa in the initial stage, which was mainly dependent on the MCs.

### LPS-induced mucosal hyperpermeability relies on MC activation

Toluidine blue staining was used to examine the MC population on colon sections from WT and *Wads*<sup>-/-</sup> mice, and as expected, no MCs were seen in *Wads*<sup>-/-</sup> mice colon (Fig. 2A). To evaluate the involvement of MCs mediated distal colonic barrier function during LPS stimulation, we then performed toluidine blue staining and western blot analysis to reveal the activation of MCs in the colonic mucosa from WT mice treated with or without LPS. The majority of MCs were round or oval in



**Fig. 3.** No intestinal microarchitecture changes in normal and LPS-stimulated WT and *Wads*<sup>-/-</sup> mice. Epithelial microstructure stained with hematoxylin and eosin was not influenced by LPS (A). Immunofluorescence staining of the colonic mucosa from WT and *Wads*<sup>-/-</sup> mice indicated that there were no significant differences in expression and distribution of claudin-1 (B), occludin (C), and E-cadherin (D) between four groups; nuclei stained by DAPI (blue). Ctrl: control.

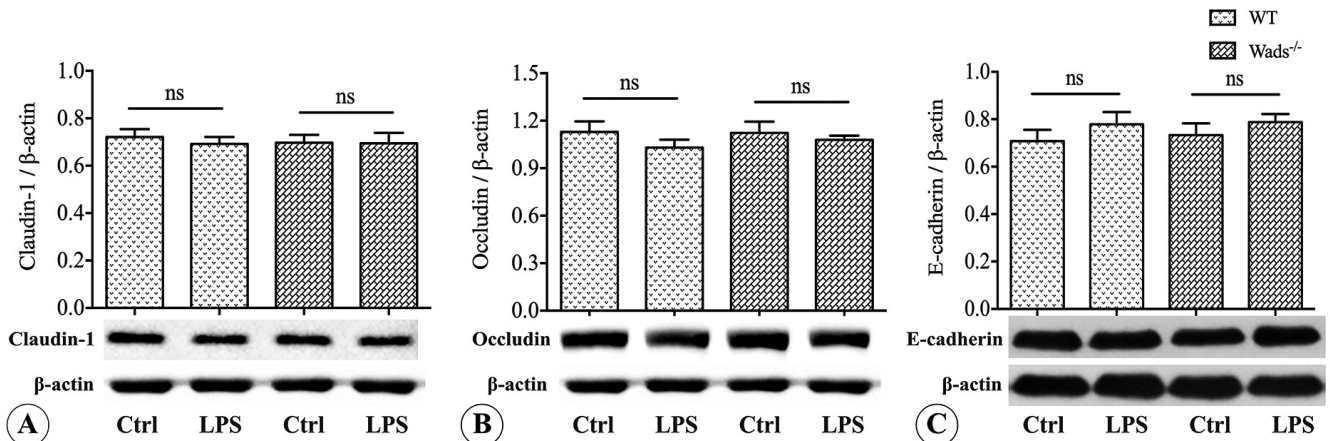
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shape, full of heterogeneous granules and with few or no degranulation in normal WT mice, whereas LPS-treated mice showed distinct degranulation of MCs, which actively released their intracellular contents compared to that of LPS non-treated mice (Fig. 2B). Statistical analysis (Fig. 2C,D) revealed that the ratio of number of degranulated MCs to that of total toluidine blue-positive cells was 79.40% after LPS stimulation, much higher than that in the control group (11.95%), but the total number of MCs remained unchanged before and after LPS intraperitoneal injection. The activation of MCs was further evaluated by measuring tryptase released by MCs, and the expression of tryptase from colonic mucosa was significantly enhanced in LPS-treated mice compared with normal mice (Fig. 2E). We also detected an increased serum concentration of IL-6 after LPS

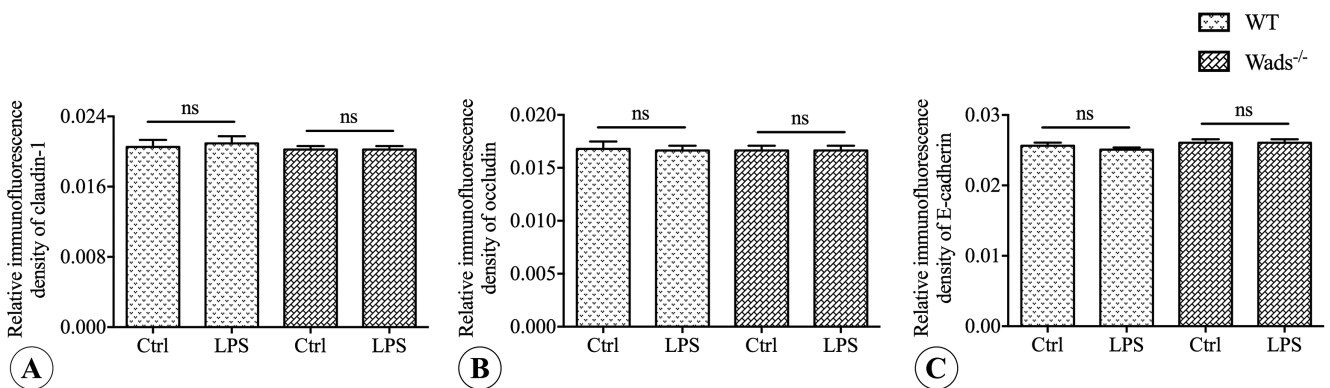
stimulation by ELISA (Fig. 2F), indicating inflammation in the body.

*No detected alterations of mucosal microstructure are observed in LPS-treated mice*

To clarify whether difference of intestinal permeability between WT and *Wads*<sup>-/-</sup> mice was partly due to abnormal epithelial microstructure, we examined the intestinal architecture using hematoxylin and eosin staining (Fig. 3A), and expression of the intercellular tight junction proteins, such as claudin-1 and occludin, together with the adherens junction protein E-cadherin, by immunofluorescence (Fig. 3B-D) and western blotting (Fig. 4). Obviously, the epithelial microstructure was intact and there was no active inflammation,



**Fig. 4.** Western blot analysis showing expressions of junctional proteins in the colonic mucosa of WT and *Wads*<sup>-/-</sup> mice. Densitometric analysis of protein expressions normalized to  $\beta$ -actin. No significant differences in claudin-1 (A), occludin (B) and E-cadherin (C) expression were seen between the four groups. Values represent mean $\pm$ SEM of three independent experiments; n=8 mice per group. Statistical significance is: ns P>0.05. Ctrl: control.



**Fig. 5.** Relative immunofluorescence density of claudin-1, occludin and E-cadherin of the colonic mucosa from WT and *Wads*<sup>-/-</sup> mice. The results of fluorescence assay indicated that there were no significant differences in expressions of claudin-1 (A), occludin (B), and E-cadherin (C) between the four groups. Values represent mean $\pm$ SEM. Statistical significance is: ns P>0.05. Ctrl: control.

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epithelial damage or ulceration in WT mice and *Wads*<sup>-/-</sup> mice. Importantly, expressions of claudin-1, occludin and E-cadherin in *Wads*<sup>-/-</sup> mice were comparable to that observed in WT mice (Fig. 5), along with normal distribution which localized at lateral boundaries of epithelia. Furthermore, no noticeable alterations of colonic mucosal architecture and cell-cell junction proteins expression were found in WT and *Wads*<sup>-/-</sup> mice despite up to 2 h of LPS stimulation. Our results indicated that LPS increased intestinal permeability without altering epithelial microstructure, further verifying that MCs played a vital role in mediating intestinal mucosal permeability.

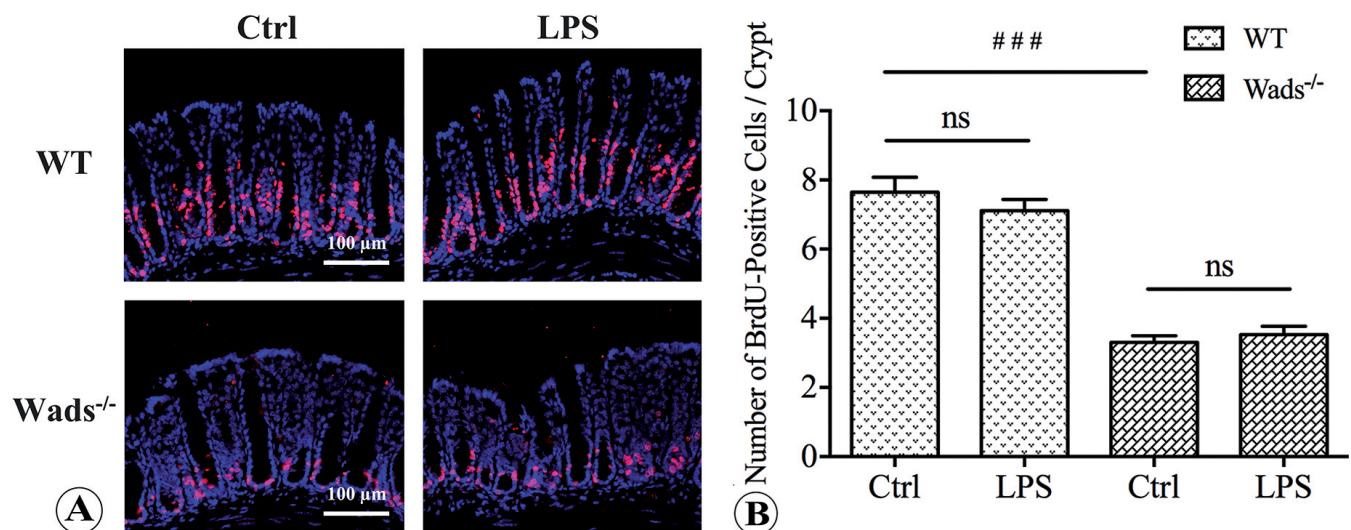
### *Proliferation of intestinal epithelial cells did not contribute to the LPS-induced hyperpermeability*

The stem cell factor (SCF)/c-kit signal pathway is considered to play key roles in cell proliferation and migration. Considering the most striking difference between WT mice and *Wads*<sup>-/-</sup> mice, the latter of which lost the c-kit function, proliferation of intestinal epithelial cells was assessed by intraperitoneal injection with BrdU in WT and *Wads*<sup>-/-</sup> mice. Compared with WT mice, a meaning lower proliferative activity of distal colonic crypt cells was observed in *Wads*<sup>-/-</sup> mice (Fig. 6A). It was worth noting that the number of BrdU-positive intestinal epithelial cells was unchanged in LPS-treated mice in both groups compared with normal mice (Fig. 6B). The discovery raised a possibility that the epithelial renewal did not directly contribute to the LPS-induced increase of the intestinal mucosal permeability.

## Discussion

Direct host-microbe interactions are very relevant in the GI tract, where the trillions of microbiotas belonged to vast of different species inhabit. Among them, the bacteria represent a major stimulus to the development of immune system and many other physiological functions, and some of them, as enteric pathogens, can also cause acute GI illness such as nausea, vomiting, hemorrhagic colitis or diarrhea (Kverka and Tlaskalová-Hogenová, 2017). Noticeably, acute diarrheal illnesses often result in dehydration and electrolyte imbalance, even infectious shock, and increase the risk of death in the clinic worldwide (Perez-Lopez et al., 2016). Therefore, clarifying the mechanism of intestinal mucosal hyperpermeability in acute bacterial infection would be conducive to the timely treatment of clinically relevant diseases. In the present study, mice became fatigued, less dynamic, hair ruffled and was accompanied by liquid stools within 2 h after LPS administration, while control group defecated normally.

In general, in order to prevent bacteria and harmful substances entering the body, the intestinal mucosal barrier integrity played an essential role in preventing enterogenous infection and bacterial translocation (König et al., 2016; Perez-Lopez et al., 2016; Odenwald and Turner, 2017). The dysfunction of the intestinal mucosal barrier is often considered to be closely involved in the pathophysiological processes of several GI diseases including infections with intestinal pathogens, inflammatory bowel disease, IBS and celiac disease (König et al., 2016). It has been reported that



**Fig. 6.** Effects of LPS on intestinal epithelial cells turnover. Immunofluorescence staining for BrdU-positive cells showed the proliferation of colonic epithelium in WT and *Wads*<sup>-/-</sup> mice (A). *Wads*<sup>-/-</sup> mice had a slower basal renewal rate of intestinal epithelial cells compared with WT mice. But in either WT or *Wads*<sup>-/-</sup> mice, no difference was found in cell proliferation between normal and LPS-treated mice (B). Values represent mean±SEM; Statistical significance is: ### P < 0.001, WT Ctrl compared with *Wads*<sup>-/-</sup> Ctrl group; ns P > 0.05. Ctrl: control.

various mediators are able to affect intestinal barrier function and integrity, thereby enhancing permeability and eventually contributing to the onset of local and systemic diseases. Intestinal epithelial permeability is composed of transcellular permeability, which is the main route for nutrient absorption, while the paracellular pathway is mostly effective in regulating water absorption and transport in response to numerous stimuli, including microbial pathogens and antigenic molecules (Suzuki, 2013; Van Spaendonk et al., 2017). The current study monitored two indicators of paracellular transport, TER and permeability of the tracer, FITC-dextran, to evaluate intestinal barrier function. It was found that TER was obviously reduced, and FITC-dextran was more permeable in the distal colonic mucosa of WT mice 2 h after LPS treatment, which indicated the dysfunction of mucosal barrier in the early period of LPS stimulation.

It is well known that the intercellular junctions, including tight junction, adherens junction and desmosome, exist on the lateral surface of epithelia from an apical to basal direction (Odenwald and Turner, 2017). Among them, the tight junction can mechanically link adjacent epithelial cells and seal the intercellular space, and have a crucial role in paracellular permeability by conferring selectivity to the flow of ions, small molecules and solutes (Bischoff et al., 2014). Generally, occludin, claudin family and zonula occludens mainly constitute tight junction, which linked to the actin cytoskeleton to regulate permeability function (König et al., 2016). Its disruption and dynamics, influenced by inflammatory mediators such as IL-6 and tumor necrosis factor- $\alpha$  (Al-Sadi et al., 2013; Garcia-Hernandez et al., 2017) as well as allergic reaction (Kale et al., 2017), often lead to increased epithelial permeability, which further facilitated the translocation of pathogens into the body. And that event seems to be partially dependent on MCs. Bednarska and co-workers showed that short-time exposure to *Salmonella typhimurium* in colonic biopsies from IBS patients which mounted in Ussing chambers induced a lower TER and decreased occludin expression compared with healthy controls, and this effect was reversed by blocking MCs with ketotifen (Bednarska et al., 2017). In addition, adherens junctions contribute to epithelial barrier function by stabilizing tight junctions. Deletion of adherens junction proteins has also been proved to participate in intestinal barrier dysfunction, in which epithelial hyperpermeability and the pathogens' invasion often appear (Vanheel et al., 2014; He et al., 2017). Here, to evaluate the involvement of cell junctions in the early stage of LPS-mediated intestinal epithelial dysfunction, the expression and distribution of claudin-1, occludin and E-cadherin in the colonic epithelia were analyzed. However, the localizations and expression levels of three proteins in LPS-treated groups were comparable to control groups in both WT and *Wads*<sup>-/-</sup> mice. It was suggested that the colonic mucosal hyperpermeability in the early-stage of LPS administration was not dependent

on the disruption of cell junctions but other factors must be involved. Interestingly, serum IL-6 levels in peripheral blood were markedly higher after LPS injection. This result illustrated that the factors like IL-6, which could impact tight junctions, showed enhanced expression prior to the onset of the barrier damage, providing the possibility of early intervention of therapeutic time-window for patients with acute bacterial infections. Moreover, the rise in serum IL-6 indicated an inflammatory state of the body after LPS treatment, further hinting towards colonic barrier dysfunction and intestinal hyperpermeability caused by LPS-induced MCs degranulation.

Furthermore, it was reported that chronic psychological stress induced an increase in permeability to macromolecules and altered interactions of bacteria with intestinal epithelium in the terminal ileum of rats, and the proliferation of enterocytes in the crypts was increased, believing that intestinal epithelial renewal played a protective effect on chronic stress-induced mucosal hyperpermeability (Söderholm et al., 2002; Boudry et al., 2007). Nevertheless, our results indicated that proliferating enterocyte labeled by BrdU did not remarkable affect the permeability before and after LPS stimulation in both WT and *Wads*<sup>-/-</sup> mice, suggesting that epithelial renewal might have less impact on early enhancement of permeability after LPS treatment. In another study on stress-induced barrier disruption, water avoidance stress-induced permeability increase in rat ileum could be inhibited by pretreatment with MC stabilizer, indicating the role of MCs in both stress and intestinal permeability (Keita et al., 2013). Intriguingly, MC-deficient *Wads*<sup>-/-</sup> mice had the lower permeability in either normal or LPS-stimulated mucosa than WT mice, meanwhile, no remarkable changes of TER and FITC-dextran across were observed in *Wads*<sup>-/-</sup> mice after LPS stimulation, adding to the evidence that the presence of MCs was relative to intestinal mucosal permeability.

In fact, there are large numbers of MCs in the normal GI tract, which are preferentially located at mucosa where the host and the environment interact with each other (Bischoff, 2016). Previous studies on MCs often focused on allergy diseases and proved that MCs played an important role in their pathogenesis, while recent studies indicated that MCs also triggered mucosal hyperpermeability through releasing its contents. For instance, MC-derived histamine could alter the intestinal permeability and enhanced risk of bacteremia during malaria/non-typhoidal *Salmonella* serotypes co-infection (Potts et al., 2016); In an *in vitro* Caco-2/HMC-1 co-culture model, tryptase released from MCs disrupted the epithelial integrity shown by a decrease in TER, an increase of FITC-dextran flux and a decrease in the expression of claudin-1 and ZO-1, which effects were prevented by tryptase inhibition (Wilcz-Villega et al., 2013). In our study, a significant decrease of TER and increase in leakage of FITC-dextran were obtained from WT mice after LPS treatment, indicating that MCs performed a vital function on epithelial permeability



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during the early-stage of LPS stimulation, because no changes of junctional proteins were detected in WT mice. To further detail the contribution of MCs, we assessed the influence of LPS on the activation and numbers of colonic mucosal MCs in WT mice. Besides toluidine blue staining, levels of tryptase, a characteristic marker of MC activation, sharply showed degranulation of MCs in LPS-treated group compared with untreated group. However, no changes in number of total MCs between the two groups were observed. Taken together, the activity of MCs, rather than an increasing number, was essential and indispensable in LPS-induced intestinal barrier dysfunction.

In conclusion, the present study confirmed that MCs in the intestinal mucosa, as a pivotal initiator of early responses to LPS, directly contributed to the early enhancement of colonic mucosal permeability by their degranulation. Moreover, *Wads<sup>-/-</sup>* mice did not show any significant changes in TER and permeability 2 h after LPS stimulation, which further supported that MCs played a crucial role in promoting mucosal hyperpermeability in the acute enterogenous bacterial infections.

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**Conflicts of Interest.** The authors declare no conflicts of interest.

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## References

- Al-Sadi R., Guo S., Ye D. and Ma T.Y. (2013). TNF- $\alpha$  modulation of intestinal epithelial tight junction barrier is regulated by ERK1/2 activation of Elk-1. *Am. J. Pathol.* 183, 1871-1884.
- Bednarska O., Walter S.A., Casado-Bedmar M., Ström M., Salvo-Romero E., Vicario M., Mayer E.A. and Keita Å.V. (2017). Vasoactive intestinal polypeptide and mast cells regulate increased passage of colonic bacteria in patients with irritable bowel syndrome. *Gastroenterology* 153, 948-960.
- Benoit R., Rowe S., Watkins S.C., Boyle P., Garrett M., Alber S., Wiener J., Rowe M.I. and Ford H.R. (1998). Pure endotoxin does not pass across the intestinal epithelium *in vitro*. *Shock* 10, 43-48.
- Bischoff S.C. (2016). Mast cells in gastrointestinal disorders. *Eur. J. Pharmacol.* 778, 139-145.
- Bischoff S.C., Barbara G., Buurman W., Ockhuizen T., Schulzke J.D., Serino M., Tilg H., Watson A. and Wells J.M. (2014). Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol.* 14, 189.
- Boudry G., Jury J., Yang P.C. and Perdue M.H. (2007). Chronic psychological stress alters epithelial cell turn-over in rat ileum. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292, G1228-G1232.
- Forbes E.E., Groschwitz K., Abonia J.P., Brandt E.B., Cohen E., Blanchard C., Ahrens R., Seidu L., McKenzie A., Strait R., Finkelman F.D., Foster P.S., Matthaei K.I., Rothenberg M.E. and Hogan S.P. (2008). IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J. Exp. Med.* 205, 897-913.
- Garcia-Hernandez V., Quiros M. and Nusrat A. (2017). Intestinal epithelial claudins: expression and regulation in homeostasis and inflammation. *Ann. NY Acad. Sci.* 1397, 66-79.
- Ge Y., Ezzell R.M. and Warren H.S. (2000). Localization of endotoxin in the rat intestinal epithelium. *J. Infect. Dis.* 182, 873-881.
- Guo S., Al-Sadi R., Said H.M. and Ma T.Y. (2013). Lipopolysaccharide causes an increase in intestinal tight junction permeability *in vitro* and *in vivo* by inducing enterocyte membrane expression and localization of TLR-4 and CD14. *Am. J. Pathol.* 182, 375-387.
- Halova I., Rönnerberg E., Draberova L., Vliagoftis H., Nilsson G.P. and Draber P. (2018). Changing the threshold-Signals and mechanisms of mast cell priming. *Immunol. Rev.* 282, 73-86.
- He C., Yu T., Shi Y., Ma C., Yang W., Fang L., Sun M., Wu W., Xiao F., Guo F., Chen M., Yang H., Qian J., Cong Y. and Liu Z. (2017). MicroRNA 301A promotes intestinal inflammation and colitis-associated cancer development by inhibiting BTG1. *Gastroenterology* 152, 1434-1448.
- Kale S.L., Agrawal K., Gaur S.N. and Arora N. (2017). Cockroach protease allergen induces allergic airway inflammation via epithelial cell activation. *Sci. Rep.* 7, 42341.
- Keita A.V., Carlsson A.H., Cigéhn M., Ericson A.C., McKay D.M. and Söderholm J.D. (2013). Vasoactive intestinal polypeptide regulates barrier function via mast cells in human intestinal follicle-associated epithelium and during stress in rats. *Neurogastroenterol. Motil.* 25, e406-e417.
- Kobayashi R., Okamura S., Ohno T., Saito H., Mori M., Ra C. and Okayama Y. (2007). Hyperexpression of Fc $\gamma$ RI and Toll-like receptor 4 in the intestinal mast cells of Crohn's disease patients. *Clin. Immunol.* 125, 149-158.
- König J., Wells J., Cani P.D., García-Ródenas C.L., MacDonald T., Mercenier A., Whyte J., Troost F. and Brummer R.J. (2016). Human intestinal barrier function in health and disease. *Clin. Transl. Gastroenterol.* 7, e196.
- Kverka M. and Tlaskalová-Hogenová H. (2017). Intestinal microbiota: facts and fiction. *Dig. Dis.* 35, 139-147.
- Neyen C. and Lemaître B. (2016). Sensing Gram-negative bacteria: a phylogenetic perspective. *Curr. Opin. Immunol.* 38, 8-17.
- Odenwald M.A. and Turner J.R. (2017). The intestinal epithelial barrier: a therapeutic target? *Nat. Rev. Gastroenterol. Hepatol.* 14, 9-21.
- Perez-Lopez A., Behnsen J., Nuccio S.P. and Raffatellu M. (2016). Mucosal immunity to pathogenic intestinal bacteria. *Nat. Rev. Immunol.* 16, 135-148.
- Potts R.A., Tiffany C.M., Pakpour N., Lokken K.L., Tiffany C.R., Cheung K., Tsois R.M. and Luckhart S. (2016). Mast cells and histamine alter intestinal permeability during malaria parasite infection. *Immunobiology* 221, 468-474.
- Ruan H.B., Zhang N. and Gao X. (2005). Identification of a novel point mutation of mouse proto-oncogene *c-kit* through N-ethyl-N-nitrosourea mutagenesis. *Genetics* 169, 819-831.
- Söderholm J.D., Yang P.C., Ceponis P., Vohra A., Riddell R., Sherman P.M. and Perdue M.H. (2002). Chronic stress induces mast cell-dependent bacterial adherence and initiates mucosal inflammation in rat intestine. *Gastroenterology* 123, 1099-1108.
- Suzuki T. (2013). Regulation of intestinal epithelial permeability by tight junctions. *Cell. Mol. Life Sci.* 70, 631-659.
- Vanheel H., Vicario M., Vanuytsel T., Van Oudenhove L., Martinez C., Keita Å.V., Pardon N., Santos J., Söderholm J.D., Tack J. and Farré R. (2014). Impaired duodenal mucosal integrity and low-grade

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- inflammation in functional dyspepsia. *Gut* 63, 262-271.
- Van Spaendonk H., Ceuleers H., Witters L., Patteet E., Joossens J., Augustyns K., Lambeir A.M., De Meester I., De Man J.G. and De Winter B.Y. (2017) Regulation of intestinal permeability: The role of proteases. *World J. Gastroenterol.* 23, 2106-2123.
- Wilcz-Villega E.M., McClean S. and O'Sullivan M.A. (2013). Mast cell tryptase reduces junctional adhesion molecule-A (JAM-A) expression in intestinal epithelial cells: implications for the mechanisms of barrier dysfunction in irritable bowel syndrome. *Am. J. Gastroenterol.* 108, 1140-1151.
- Wouters M.M., Vicario M. and Santos J. (2016). The role of mast cells in functional GI disorders. *Gut* 65, 155-168.
- Zhou H., Liang H., Li Z.F., Xiang H., Liu W. and Li J.G. (2013). Vagus nerve stimulation attenuates intestinal epithelial tight junctions disruption in endotoxemic mice through  $\alpha 7$  nicotinic acetylcholine receptors. *Shock* 40, 144-151.

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