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ORIGINAL ARTICLE

Upregulation of proBDNF in the Mesenteric Lymph Nodes in Septic Mice

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Abstract

The immune status in the lymphatic system, especially mesenteric lymph nodes (MLNs), is critical to regulate the septic shock. Brain-derived neurotrophic factor (BDNF) in the enteric system has been reported to regulate enteric immunity. However, the role of its precursor, proBDNF, in the immune status of MLNs under sepsis condition is still unclear. This study aimed to characterize the expression pattern of proBDNF in MLNs after lipopolysaccharide (LPS) stimulation, and to investigate the association of pathogenesis of sepsis. LPS (20 mg/kg) was intraperitoneally injected to induce sepsis in mice. Survival curve analysis, routine blood tests, and liver and kidney function tests were performed to evaluate the severity of sepsis. QPCR and histological staining were performed to assess the mRNA levels of proinflammatory cytokines and degree of immune-inflammatory response in the MLNs. Furthermore, Western blotting, flow cytometry, and immunofluorescence were performed to examine the key molecules expression of proBDNF signaling. Intraperitoneal LPS injection significantly decreased the number of lymphocytes in blood but increased the number of T lymphocytes in MLNs. Serum alanine transaminase, aspartate transaminase, and blood urea nitrogen levels were increased in LPS-challenged mice compared to control mice. LPS administration upregulated proinflammatory cytokine gene expression and induced histological changes in the MLNs. LPS injection increased BDNF, proBDNF, and its receptor pan neutrophin receptor 75 ($p75^{NTR}$) expression in MLNs. The increased proBDNF was mainly localized on $CD3^+$ and $CD4^+$ T cells in the medulla of MLNs. LPS-induced sepsis upregulated proBDNF expression in medulla T cells of MLNs. ProBDNF upregulation may be involved in the pathogenesis of septic shock.

Keywords Brain-derived neurotrophic factor precursor · Lipopolysaccharide · Sepsis · Mesenteric lymph nodes · T cell

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Introduction

Sepsis is characterized by dysregulated immune response and severe organ dysfunctions (Manu et al. 2016) accompany with high mortality (Vincent et al. 2006, Maeder and Hunziker 2009). Lipopolysaccharide (LPS) is an endotoxin that is released from bacteria membranes. LPS exposure can induce systemic inflammatory response syndrome (Cohen 2002) by stimulating inflammatory factors release from gut, and the gut has been shown to be a source of inflammatory factors with the capability of priming neutrophils, finally driving multiple organ failure after injury (Deitch et al. 1994; Upperman et al. 1998).

The gut-associated lymphoid tissue is the largest lymphatic organ in the body and is mainly composed of mesenteric lymph nodes (MLNs) (Clark Jessica and Coopersmith 2007). MLNs accelerates endotoxin transfer from injured intestine (Deitch 2010; Gatt et al. 2010) to the lung (Langness et al. 2016) and general circulation (Zallen et al. 2000) where

endotoxin triggers dramatic inflammatory response and leads to multiple tissues and organ dysfunction (Adams et al. 2001). MLNs serve as a bridge to transport toxic factors but bypassing the portal circulation and liver to induce an inflammatory systemic response (Fanous et al. 2007). Over three decades, despite the advance on sepsis (Park et al. 2009; Wu et al. 2009), little is known about the pathophysiology of intestinal immunological barrier dysfunction in sepsis, and effective therapies are also lacking for this disorder.

Brain-derived neurotrophic factor (BDNF) is an essential survival factor for a subpopulation of sensory neurons and critical for the development and functions of enteric and central nervous systems (Park et al. 2009). In addition, BDNF is also present in non-neuronal cells such as the gastrointestinal tract even with higher levels than in brain, and is critical for the development of enteric sensory neurons (Fox and Murphy 2008; Lommatzsch et al. 1999). In the recent years, BDNF is found to play a role in gut motility (Chen et al. 2014; Coulie et al. 2000) and gut microbiota (Coulie et al. 2000; Gareau et al. 2011) by serving as trophic factor (Lucini et al. 2002) to affect the development of gut immune system (Capuron and Miller 2011). Interestingly, lymphocytes express BDNF frequently depending on cell activation status (Kerschensteiner et al. 1999; Moalem et al. 2000). For example, infection with immunodeficiency virus can substantially reduce the expression of BDNF in lymph nodes (Sloan et al. 2008). However, most work has focused on BDNF; studies investigating the precursor of BDNF (proBDNF) in the immune system are still limited.

BDNF is synthetized as a precursor form, proBDNF, and then is cleaved to generate mature form by proteolytic cleavage (Lee et al. 2001). ProBDNF exerts opposing biologic effects of BDNF by binding to its receptors, $p75^{NTR}$ and sortilin (Marler Katharine et al. 2010), which is also expressed in the intestine (Zhou et al. 2010). Our previous studies have shown that proBDNF as an inflammatory mediator activate immune cells to promote the development of inflammatory pain or spinal cord injury (Luo et al. 2016; Wong et al. 2010). However, it is unknown that the expression of proBDNF in the MLNs under septic condition.

The present study aimed to investigate the expression and its cellular localization of proBDNF in the MLNs in LPS-induced septic model mice. The characterization of proBDNF signaling in MLNs may shed some light on the role of proBDNF signaling in the pathogenesis of sepsis.

Materials and Methods

Animals The study was approved by the Medical Ethics Committee of The Second Xiangya Hospital, Central South University in Changsha, P. R. China, and followed the NIH guidelines (guide for the care and use of laboratory animals). Male C57BL/6 mice (Aged 8 weeks; weighted 20–25 g) were purchased from Laboratory Animal Co. Ltd. of Slack King (Longping Sci-tech Park, Changsha, Hunan, China). Animals were housed under identical conditions (room temperature at 26 °C, 50 ± 10% relative humidity, and 12 h light-dark cycle) and had free access to a standard rodent diet and water. The animal experiments were performed according to the guidelines for the care and use of animals established by Central South University.

Animal Model of Sepsis The mice were randomly divided into the following two groups ($n = 30$ each): the control group mice were intraperitoneally (i.p.) administered with saline solution; the LPS group mice were injected i.p. with LPS (cat no. L2880-25MG, Sigma, St. Louis, MO, USA) at a dose of 20 mg/kg once. The mice in each group had free access to food and water under pathogen-free conditions. After LPS administration, survival rate was then recorded for the next 7 days.

Measurement of Red Blood Cells and Leukocytes in Sepsis Blood was collected in EDTA-2K tubes and, white blood cell count, neutrophil count, monocyte count, and lymphocyte count were analyzed by automatic cell analyzers (ARCHITECT c 8000, Abbott Corporation, Chicago, USA).

Effect of LPS Injection on Liver Function and Kidney Function Serum ALT (alanine aminotransferase), AST (aspartate aminotransferase), and BUN (blood urea nitrogen) were determined using standard diagnostic kits on blood biochemical instruments (Sysmex xs-1000i, Sysmex Corporation, Munich, Germany).

Reverse Transcription and Quantitative Real-Time PCR Total RNA was extracted from the MLNs tissues as described previously (Luo et al. 2016) The cDNA was obtained using the reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative real-time PCR was performed with SYBR Green (Bio-Rad) on CFX96 TouchTM Deep Well Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR primers were ACCACCAT GGAGAAGGCTGG and CTCAGTGTAGCCCAGGATGC (GAPDH); CTCTGGCTTTGTCTTTCTTGTTATCTTT and AGTTGTGCAATGGCAATTCTGA (IL-6); AGGCGGTG CCTATGTCTCAG and GCTCCTCCACTTGGTGGTTT (TNF-α); and GAAATGCCACCTTTTGACAGTG and TGGATGCTCTCATCAGGACAG (IL-1β). qPCR was performed as follows: 95 °C for 3 min, and 39 cycles of 95 °C for 10 s and 60 °C for 30 s. The experiment was repeated in triplicate. Data were processed using the $2^{(-\Delta\Delta Ct)}$ method.

Hematoxylin-Eosin Staining All mice were deeply anesthetized with sevoflurane inhalation and MLNs were obtained

for hematoxylin-eosin (HE) staining as described previously (Hu et al. 2018) for infiltration of inflammatory cells and morphology analysis.

Immunofluorescence Staining Paraffin section was used for immunofluorescence labeling. Briefly, paraffin section was subjected to standard procedure for dewaxing and antigen retrieval to expose antigenic site. After that, 5 μm thick paraffin-embedded sections were permeabilized with 0.5% Triton X-100 in PBS for 20 min and blocked with non-fat 5% BSA in Tris-buffered saline for 60 min at 37 °C, incubated with primary antibody (anti-human proBDNF antibody generated by us as described previously (Luo et al. 2016) anti-CD3 antibody (cat no.14-0032-81, 1:200, eBioscience, San Diego, CA, USA), anti-CD4 antibody (cat no. 16-0041-61, 1:200, eBioscience, San Diego, CA, USA), anti-CD8 antibody (cat no .sc-7970, 1:200, Santa cruz, Dallas, TX, USA), and anti-B220 antibody (cat no. ab64100, 1:200, Abcam, Cambridge, UK) at 4 °C overnight. The sections were incubated with secondary antibody (sheep-anti-human-FITC, cat no. ab102443,1:1000, Abcam, Cambridge, UK) for proBDNF; (goat anti-mouse IgG-conjugated Cy3, cat no. ab97075, 1:1000, Abcam, Cambridge, UK), for CD3, CD4, and CD8, and (goat anti-rat IgG-conjugated 594, cat no. ab150160, 1:1000, Abcam, Cambridge, UK) for B220 at 37 °C for 1 h. The coverslips were stained with DAPI (H-1200, Vetor, California, USA) for 1 min at room temperature. Immunofluorescence images were acquired using a fluorescence microscope (Nikon ECLIPSE 80i, Nikon Corporation, Tokyo, Japan).

Western Blot MLNs were homogenized with lysis solution (CWbiotech, Beijing, China) with 1% protease inhibitor cocktails (Sigma-Aldrich, St. Louis, MO, USA) and 1% EDTA solution. After standing for 20 min, they were centrifuged at 12000 rpm for 20 min at 4 °C. Afterwards, the supernatant was denatured for future analysis. Proteins were separated using electrophoresis and transformation to polyvinylidene fluoride (PVDF) membranes. After being blocked with 5% skim milk in Tris-buffered saline (TBS) for 1 h at room temperature, the membrane was incubated with primary antibodies (anti-BDNF antibody, cat no. ab108319, 1:3000, Abcam, Cambridge, UK; anti-proBDNF antibody, cat no. P1374-200UL, 1:4000, Sigma, St. Louis, MO, USA; anti-p75 antibody, cat no. ab8874, 1:2000, Abcam, Cambridge, UK; anti-sortilin antibody, cat no. ab16640, 1:2000, Abcam, Cambridge, UK; anti-GAPDH antibody, cat no. AT0002, 1:8000, CMCTAG, Milwaukee, WI, USA) overnight at 4 °C. Membranes were thoroughly rinsed and incubated with HRP-conjugated goat anti-rabbit or goat anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, Inc., PA, USA diluted in 1:5000) for 2 h at room temperature. Finally, they were rinsed and exposed to photographic film with chemiluminescent HRP substrate (Millipore, Boston, USA). Western blotting band were analyzed by the mean gray value with NIH Image J 7.0 and standardized to GAPDH.

Flow Cytometry Analysis of Mesenteric Lymph Node Immune Cells Fresh mesenteric lymph nodes were minced and dissolved in PBS, filtered through a 40-mm cell strainer, and centrifuged at 350×*g* for 10 min at 4 °C. Cells were washed with PBS and re-suspended in 500 μl PBA (~ 2×10^7 cells/ml). The suspension (~ 2–3 × 10^6 cells/100 μl) was incubated with anti-CD3 (eBioscience, San Diego, CA, USA BV421, 1:100), CD4 (eBioscience, San Diego, CA, USA APC/Cy7, 1:100), CD8 (eBioscience, San Diego, CA, USA BV510, 1:100), CD19 (eBioscience, San Diego, CA, USA APC, 1:100) for 30 min at 4 °C, and then mixed with 1 ml PBS. Cells were then centrifugated, fixed with IC Fixation Buffer in the dark at room temperature perforated with permeabilization buffer (Invitrogen, California, CA, USA), and incubated with anti-human proBDNF (preparation method reference (Luo et al. 2016)) 0.5 μl for 30 min at 4 °C, then incubated with secondary antibody (sheep-anti-human-FITC, cat no.ab102443, 1:500, Abcam, Cambridge, UK) for 30 min at 4 °C. Cells were analyzed by using a BD FacScanto II flow cytometer. Non-specific binding of secondary antibodies was quantified, and a fluorescent signal was subtracted from values of experimental groups. Single-stained cells were used for calculations. Unstained cells and fluorescence minus one (FMO) controls were used for cytometry and gating set up.

Statistical Analysis Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using the paired Student's *t* test or one-way analysis of variance (ANOVA) followed by Bonferroni analysis where appropriate. Statistical significance was arbitrarily declared at *p*-values below 0.05. All analyses were performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA).

ResultsEffects of LPS on Mortality and Biochemistry

Mice were administered with a single dose of LPS (20 mg/kg body weight) to induce severe sepsis (Li et al. 2005) One hour after LPS injection, the mice were apathetic, with reduced activity, no water consumption, erected back hair, and sticky secretions in the eyelids. As shown in Fig. 1a, mice with sepsis had a significantly higher mortality rate. Only 41.18% of the mice survived in the septic group; their body weight decreased on day 1, approached the lowest value on day 2, and gradually returned to the normal level (Fig. 1). LPS injection also decreased the absolute number of lymphocyte count and leukocytes ($p < 0.001$), but not the number of neutrophils on day 1 later, and these counts gradually normalized on day 7 (Table 1). Serum alanine transaminase, aspartate

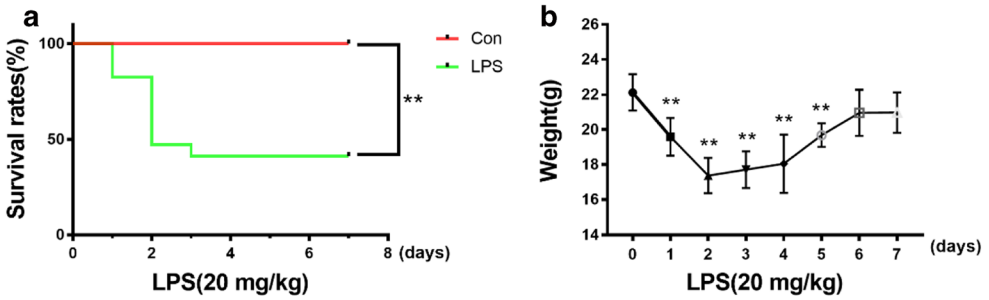

Fig. 1 Survival rate and body weight after lipopolysaccharide injection. **a** Survival rates of mice subjected to septic shock. (**$p < 0.01$, versus control, $n = 30$ per group; log-rank (Mantel-Cox) test) **b** Weight change in mice subjected to septic shock. The body weight of the mice decreased significantly from day 1 of septic shock. (**$p < 0.01$, versus day 0, repeated measures of ANOVA with Dunnett's post hoc test. Data bars represent mean ± s.e.m. values)

transaminase, and blood urea nitrogen levels increased in LPS-challenged mice compared to control mice on day 1 and day 3, and declined to normal level on day 7.

Effects of LPS on Histomorphology and Cytokine Gene Expressions of MLNs

Gross anatomy showed that MLN is enlarged rapidly after LPS injection. Hematoxylin and eosin staining revealed a loss of tissue architecture and lymphatosis in the cortex and medulla after LPS administration. Histological changes and proliferation of inflammatory and immune cells were most robust on day 1 after LPS administration (Fig. 2a). Although the WBC count and liver and kidney function returned to normal on day 7, the structural damage to MLNs was irreversible (Fig. 2a).

The proliferation was accompanied with the cytokine's expression after LPS injection. As shown in Fig. 2b, the gene expression of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 was raised dramatically after LPS injection. IL-1β and IL-6 levels increased significantly after 1 h of LPS administration ($p < 0.0001$), and TNF-α levels increased significantly after 6 h ($p < 0.0001$) in the MLNs of LPS-treated mice compared to those in the control mice. Thus, LPS injection rendered the histological changes and cytokines activation of MLNs.

Effects of LPS on the Percentage of Immune Cells in MLNs

FACS assay showed that there were around 32% $CD3^+$ T cells in the control mice. Percentage of $CD3^+$ T cells was increased to around 60% at 1 h after LPS injection and the upregulation was persistently for 7 days post-injection (Fig. 3b). Consistently, the percentage of $CD4^+$ and $CD8^+$ T cells was also increased persistently after LPS injection (Fig. 3c, d). In contrast, the percentage of $CD19^+$ B cells was downregulated, suggesting the inhibition of adaptive immunity in sepsis (Fig. 3e).

Effects of LPS on Expression Levels of proBDNF and its Receptors

Previous studies have reported that proBDNF is expressed in numerous tissues or cells, especially in the nervous system (Deinhardt and Chao 2014); however, it is unknown whether

Table 1 Changes in blood biochemical parameters after lipopolysaccharide administration

Item	Control	Day 1	Day 3	Day 7
Wbc (10^9/L)	4.04 ± 1.74	2.11 ± 1.06***	4.44 ± 1.80	3.935 ± 0.20
Neu (10^9/L)	0.32 ± 0.17	0.74 ± 0.54*	0.67 ± 0.18	0.23 ± 0.073
Lym (10^9/L)	3.07 ± 1.26	1.38 ± 0.9*	2.57 ± 1.82	1.40 ± 0.22*
Mon (10^9/L)	0.08 ± 0.008	0.23 ± 0.127	0.46 ± 0.172*	0.27 ± 0.107
AST (IU/L)	20.86 ± 1.72	32.83 ± 2.80*	25.53 ± 2.72	18.37 ± 2.21
ALT (IU/L)	68.21 ± 9.76	184.7 ± 25.84**	163.00 ± 22.39*	95.1 ± 19.27
BUN (IU/L)	9.78 ± 1.37	18.49 ± 2.46*	12.48 ± 1.44	10.34 ± 1.67

Biochemical parameters compared between lipopolysaccharide (LPS) administration on days 1, 3, 7, and control groups (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control, $n = 4$–6 per group; repeated measures of ANOVA with Dunnett's post hoc test. Data bars represent mean ± s.e.m. values)

WBC white blood cells, *Neu* neutrophils, *Lym* lymphocytes, *Mon* monocytes, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *BUN* blood urea nitrogen

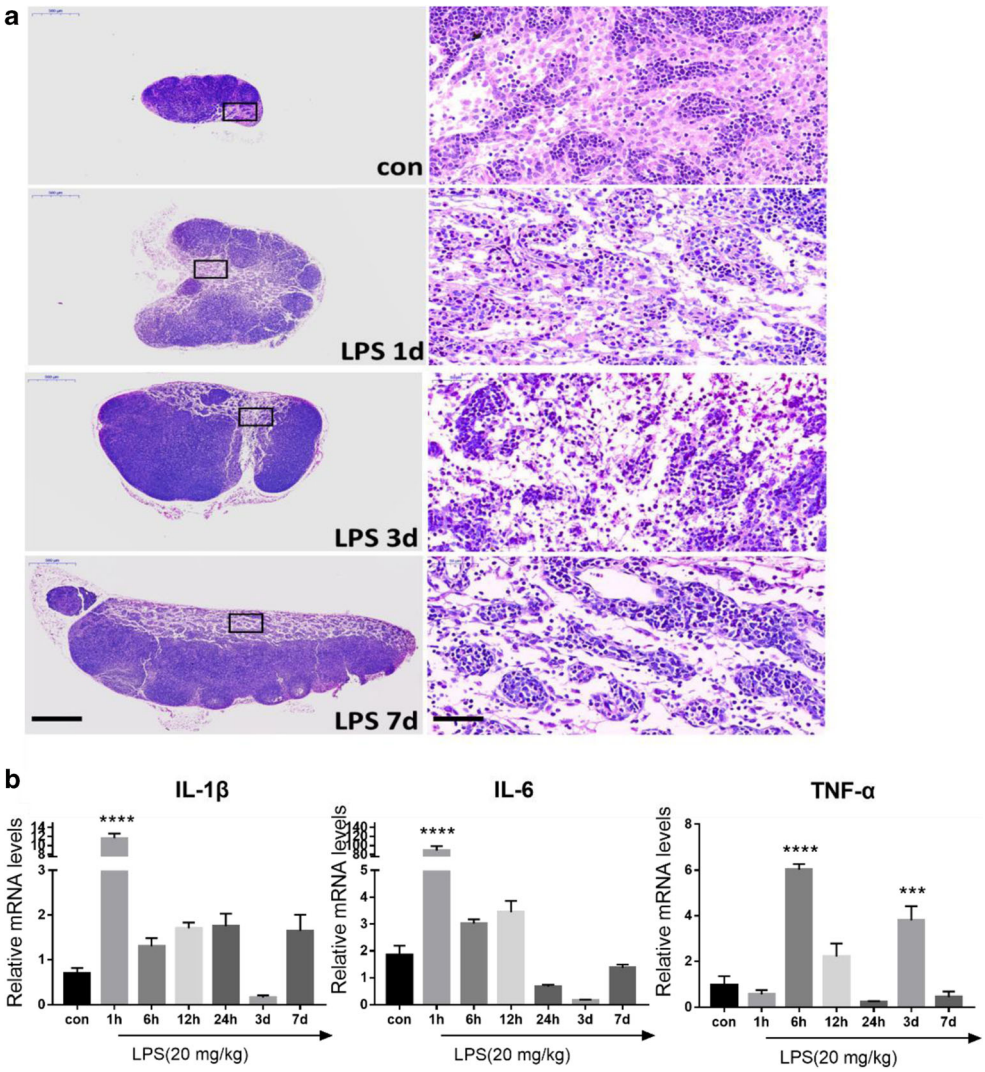

Fig. 2 Histological changes and cytokine gene expression of mesenteric lymph nodes (MLNs) in septic mice after lipopolysaccharide (LPS) injection. **a** Hematoxylin and eosin staining in the MLN at different time course after LPS injection. Scale bar, 500 μm for left column, and 50 μm for right column. **b** Time course of TNF-α, IL-1β, and IL-6 gene expression in the MLN after LPS injection. TNF tumor necrosis factor, IL interleukin

proBDNF is expressed in MLNs. To characterize the temporal expression levels of proBDNF after LPS administration, MLNs in control mice and septic mice were harvested to determine the levels of proBDNF at different time points after LPS administration. As shown in Fig. 4, proBDNF was slightly increased at 6 h ($p < 0.05$), and reached peak at 12 h ($p < 0.001$) compared with the control group; after that, proBDNF was declined at day 1, day 3, and day 7 but still higher than the baseline levels (Fig. 4c). There was no significant change of mature BDNF between the septic group and the control group in the early time points after LPS or saline injection. However, BDNF was elevated at 3 and 7 days ($p < 0.05$) when the sepsis was gradually recovered (Fig. 4a). Compared with control, $p75^{NTR}$ expression was reduced at 1 h, whereas increased at 6 h and 12 h post-injection. Interestingly, $p75^{NTR}$ expression was then sharply downregulated at day 1 and last for 7 days (Fig. 4d). In contrast, sortilin was downregulated after 1 h ($p < 0.001$) and was sustained for 7 days ($p < 0.001$) compared with the control group (Fig. 4e).

Upregulation of proBDNF in MLNs T Cells of Mice with Sepsis

BDNF can be produced by both T, B lymphocytes and monocytes in tissues subjected to inflammatory injury (Braun et al. 1999), and MLNs are mainly composed of lymphocytes (Clark and Coopersmith 2014). We investigated the expression of proBDNF in the MLNs using flow cytometry. After LPS injection, the median fluorescence intensity (MFI) of proBDNF in the $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells was significantly increased compared to control group and reached peak at 3 days ($p < 0.05$) (Fig. 5b). The results suggest that proBDNF is significantly upregulated in MLNs after LPS administration and primarily produced by T cells.

To further confirm the expression of proBDNF in T cells, double-immunofluorescence was performed (Fig. 6). The results showed that proBDNF positive staining was co-localized with $CD3^+$ T cells in the medulla of MLNs (Fig. 6a). The proBDNF immunoreactive cells were also expressed in the

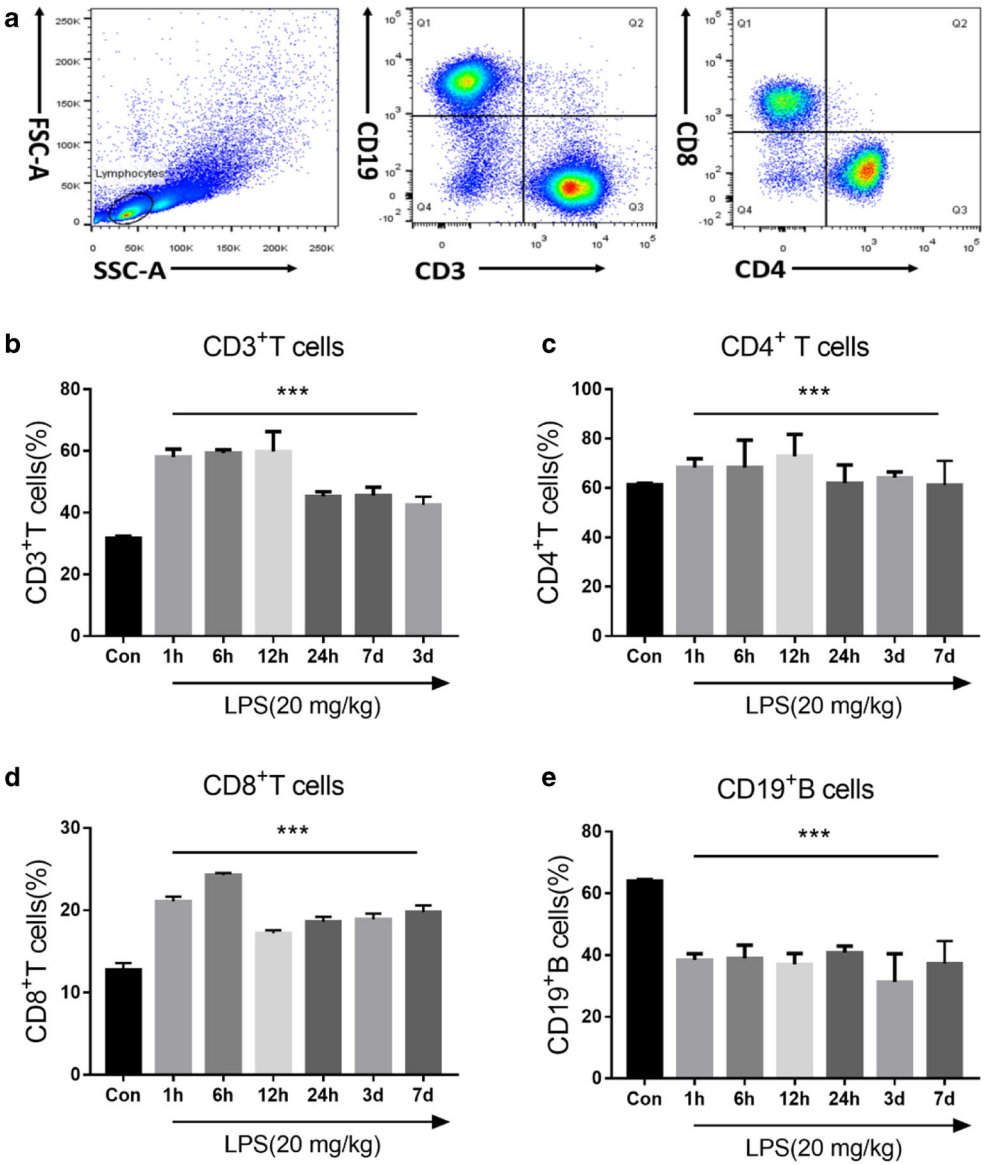

Fig. 3 Effect of LPS injection on the expression of lymphocytes in MLNs of mice. Representative images of flow cytometry for T and B lymphocytes in mouse MLNs, gating of mononuclear cells isolated from the MLNs (**a**), statistical analysis (**b**–**e**) of $CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$ cells

$CD4^+$ and $CD8^+$ T cells (Fig. 6b, c). Sparse co-localization of B220 and proBDNF can be observed in germinal center and medulla (Fig. 6d, e). Taken together, the results showed that proBDNF was upregulated in the $CD3^+$, $CD4^+$, and $CD8^+$ T cell subpopulation after LPS injection.

Discussion

The present study demonstrated that proBDNF was upregulated during the sepsis, mainly localized on $CD3^+$ and $CD4^+$ T cells in MLNs. We also found that LPS promoted the proliferation of $CD3^+$, $CD4^+$, and $CD8^+$ T cells, but inhibited $CD19^+$ B cells in the MLNs. Thus, our data extend previous studies (Kerschensteiner et al. 1999; Moalem et al. 2000) demonstrating expression of neurotrophic factors by lymphocytes is frequently dependent on cell activation.

Sepsis leads to life-threatening organ dysfunction by a host response to infection (Cohen 2002). In animals, intraperitoneal LPS injection (20 mg/kg) impaired liver and kidney functions, accompanied by a reduction in the number of leukocytes and lymphocytes on the first day, the same as in the septic patients (Hotchkiss et al. 2001). Our study showed 17.65% of mice died within the first 24 h after LPS administration, and 35.3% within the subsequent 24 h. These results indicated that LPS causes severe sepsis and multiple organ dysfunctions.

The pathogenesis of sepsis is incompletely understood, but increasing evidence points to the gut and MLNs as key mediators (Deitch 2010). In over 30% of patients with bacteremia, including those dying with clinical sepsis and multiple organ

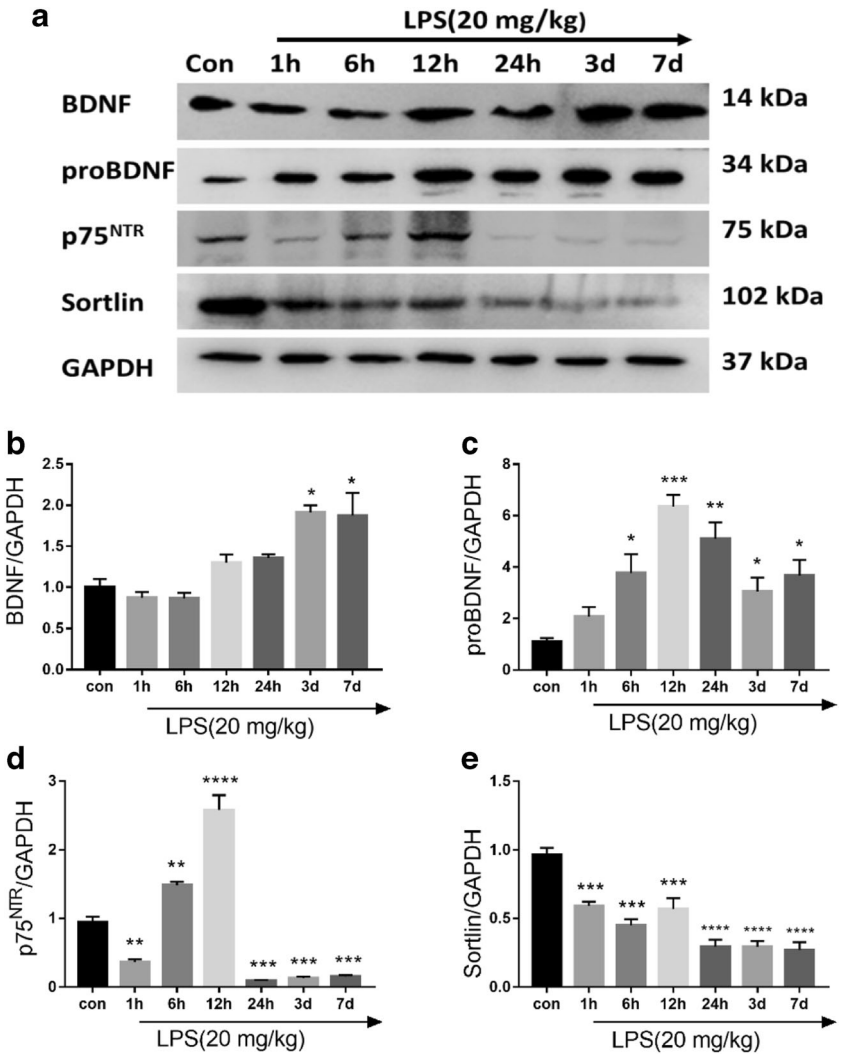

Fig. 4 Expression of BDNF, proBDNF, and its receptors in the mesenteric lymph nodes after lipopolysaccharide (LPS) injection. Representative Western blots (**a**) and their semi-quantitative analyses (**b**–**d**). (*$p <$ 0.05, **$p < 0.01$, ***$p < 0.001$, versus the control group, $n = 4$ per group; repeated measures of ANOVA with Dunnett's post hoc test. Data are presented as mean ± s.e.m. values)

dysfunction syndrome (MODS), bacteria or endotoxin were failed to be detected in the portal blood (Moore et al. 1990). Newer theories have focused on the MLNs as the inciting source for the development of systemic inflammatory response syndrome (Moore et al. 1994; Senthil et al. 2006). In the new enterolymphatic hypothesis, the mesenteric microenvironment is the "activated bed" of immune cells and can produce proinflammatory cytokines, which primarily enter the systemic circulation, resulting in sepsis (Deitch 2001). In the present study, the sepsis elevated CD3+, CD4+, and CD8+ T cells number in MLNs. The activated T cells may facilitate the transfer of endotoxin from the intestine (Deitch 2010; Gatt et al. 2010) to the lung (Langness et al. 2016) and general circulation (Adams et al. 2001). Consistently, proinflammatory cytokines, TNF-α, IL-1β, and IL-6 levels were increased significantly in MLNs at 6 h after LPS administration. Our previous study has shown that exogenous proBDNF induced the activation of proinflammatory cytokines, as accompanied by the increased phosphorylated p65, the critical component of NF-κB (Luo et al. 2016). In addition, LPS is also reported to stimulate microglial cells to synthesize and secrete nerve growth factor precursor, along the release of inflammatory cytokines (Duan et al. 2013). Thus, it is plausible to speculate that in sepsis, the upregulated proBDNF binds its receptor $p75^{NTR}$. The activated $p75^{NTR}$ then interacts with receptor-interacting protein-2 and results in NF-κB activation, which subsequently increases the gene expression of proinflammatory cytokines (Ibáñez and Simi 2012).

We assumed that the toxic intestinal factors (LPS) influence other splanchnic organs and induce a systemic response via mesenteric lymph nodes while bypassing portal and hepatic circulation at early stage. BDNF is abundantly expressed in the gastrointestinal tract (Fox and Murphy 2008; Lommatzsch et al. 1999) playing pivotal roles in gut immune system (Gareau et al. 2011.; Sudo et al. 2010.). Immunodeficiency virus infection can substantially reduce the expression of BDNF in lymph nodes (Sloan et al. 2008). proBDNF is not only an intermediate during the synthesis of BDNF but also

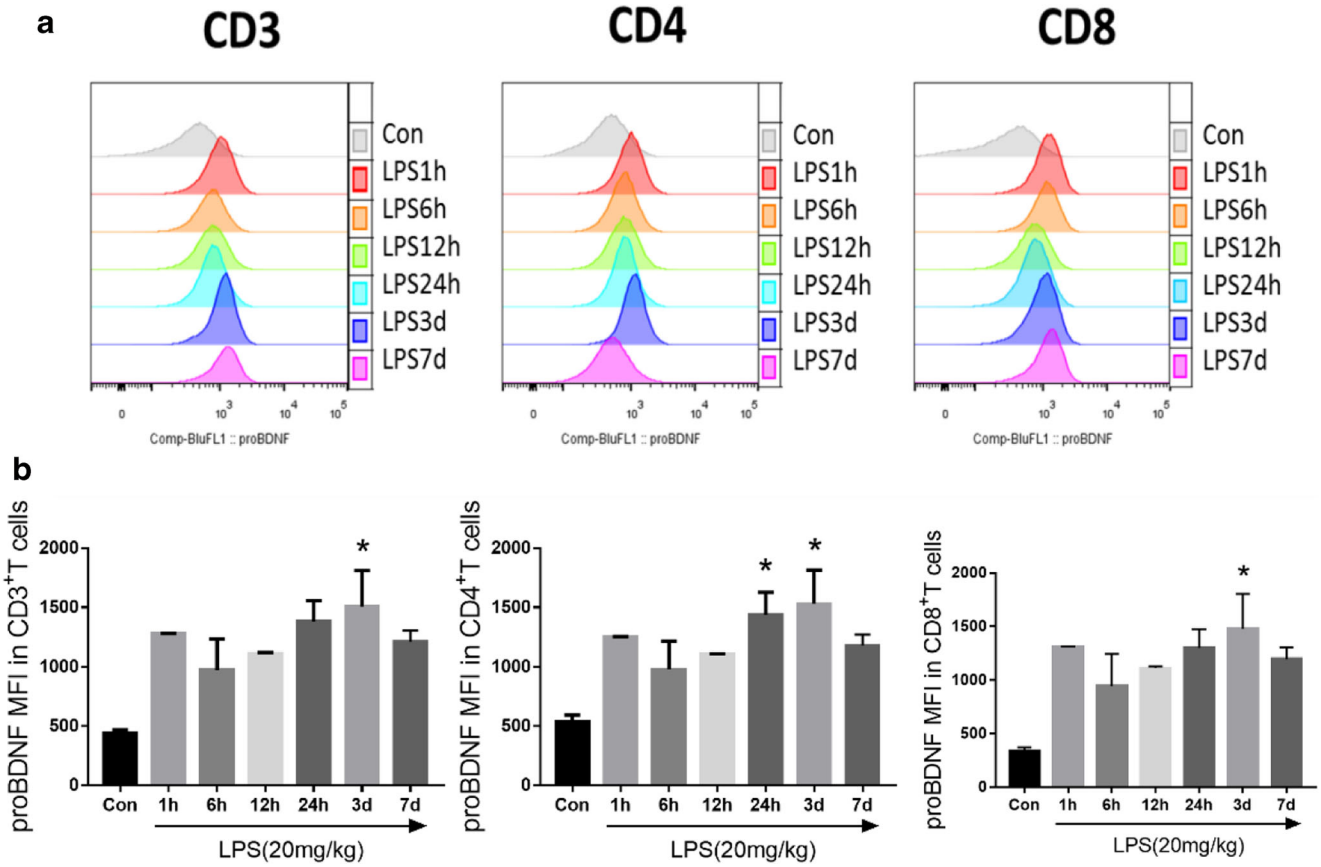

Fig. 5 Flow cytometry analysis, FITC staining of proBDNF, and the median fluorescence intensity of proBDNF in $CD3^+$, $CD4^+$, and $CD8^+$T cells in mesenteric lymph nodes (MLNs) of mice with sepsis. Upregulation of proBDNF revealed from mean fluorescence intensity indicate upregulated proBDNF level in T cells in MLNs (**a**) and its quantitative analysis (**b**). (*$p < 0.05$, versus the control group, $n = 4$ per group; repeated measures of ANOVA with Dunnett's post hoc test. Data bars represent mean ± s.e.m. values)

has an opposite biologic effects (Chen et al. 2004) by binding to its receptors, $p75^{NTR}$ and type I transmembrane protein sortilin (Koshimizu et al. 2010; Teng et al. 2005). Previous study showed that proBDNF and its receptor $p75^{NTR}$ was significantly upregulated in the hippocampal tissue of mice with sepsis encephalopathy (Ji et al. 2018). Similarly, the result from Western blots showed that proBDNF protein levels increased in MLNs from 6 h after LPS injection in septic mice. In addition, mature BDNF is elevated at 3 and 7 days ($p < 0.05$) compared with control group (Fig. 4a). The time of increased mature BDNF levels was correlated with the improvement of the disease, suggesting the endogenous BDNF may exert the protective effect during the sepsis. Supporting this assumption, supplementation of BDNF after septic shock is effective to attenuate the disease course (Zeng et al. 2017). In the present study, the expression pattern of $p75^{NTR}$ was correlated with that of proBDNF, indicating that proBDNF mainly acts on $p75^{NTR}$. In contrast, sortilin was persistently downregulated suggesting that sortilin may not be involved in sepsis. Interestingly, 1 day after LPS injection, the levels of $p75^{NTR}$ and sortlin were even lower than the normal control. This phenomenon may be similar to the feedback loop between BDNF and tropomyosin receptor kinase B (TrkB). In this case, prolonged BDNF exposure induces a negative feedback loop by depleting its receptor TrkB on the neuronal surface and resulting in long-term receptor desensitization to BDNF. Therefore, proBDNF may have a unique role in infectious diseases for the interaction of nervous system and immune system to break immunity homeostasis.

It is known that LPS can cause encephalopathy and neurodegeneration such as Parkinsonism, major depression, and other disorders (Dutta et al. 2010; Ferreira Mello et al. 2013). On the other hand, proBDNF-p75NTR signaling also induces neuronal apoptosis (Fan et al. 2008), neurite collapse (Sun et al. 2012), and inhibits the neurogenesis (Li et al. 2017). The upregulated proBDNF in the present study may be involved in the LPS-induced neurodegenerative disease (Chen et al. 2016; Tuon et al. 2014). Previous studies have shown that proBDNF in peripheral blood mononuclear cells was significantly upregulated in major depression and alcohol dependence (Zhou et al. 2013.; Zhou et al. 2017), hinting that proBDNF may involve in the immune system. Indeed, the expression of $p75^{NTR}$ co-localized with proliferating marker of T cells in the thymus, implicating its

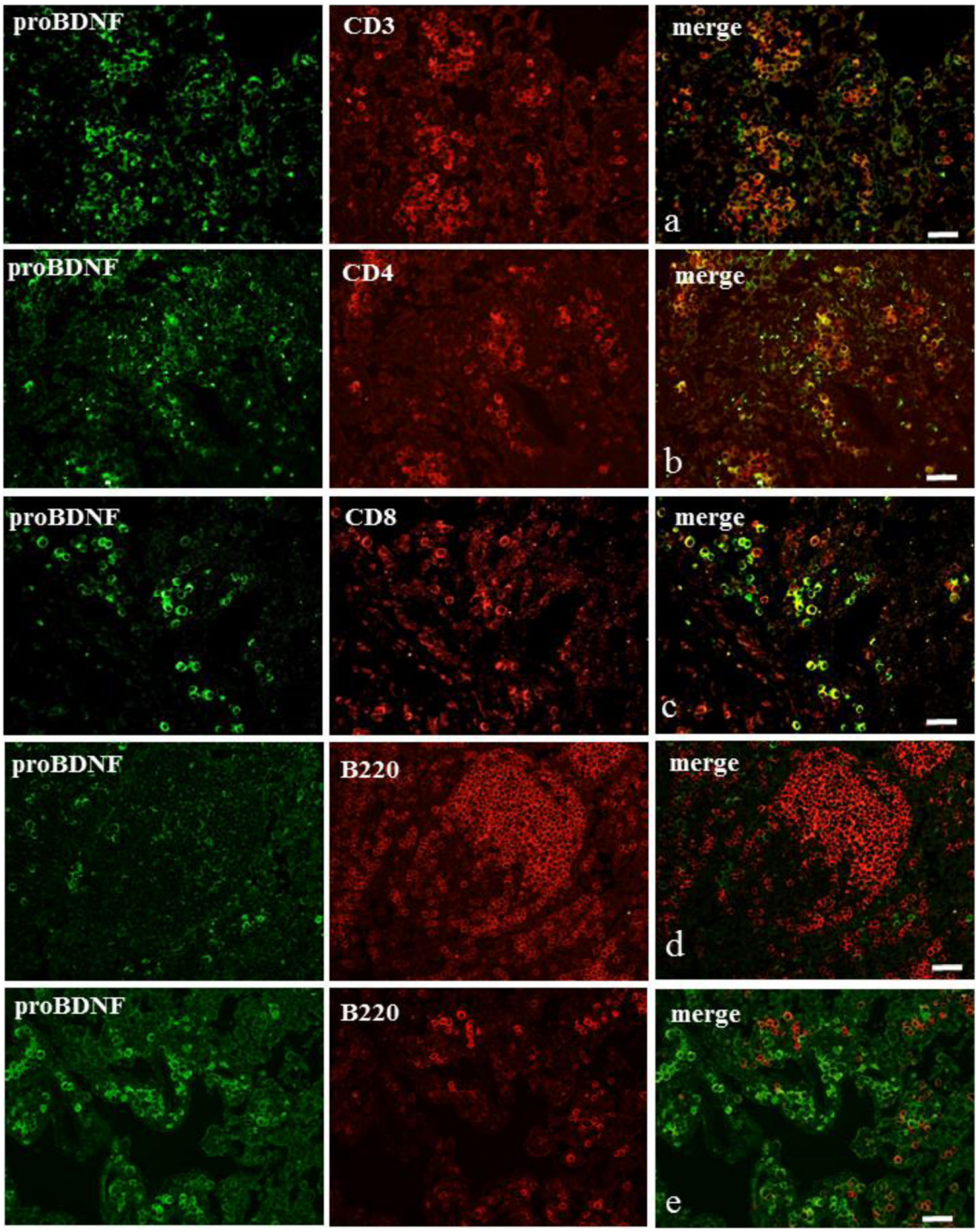

Fig. 6 Colocalization between proBDNF and T cells in the medulla (**a**–**c**), or B cells in the germinal center **(d)** and medulla (**e**) of mesenteric lymph nodes in mouse model of sepsis. Representative images of immunofluorescence staining for proBDNF (green) and CD3, CD4, CD8, and B220 (red) at 24 h after induction of sepsis. Colocalization of green and red cells is shown in yellow. Scale bar, 20 μm

involvement in lymphocyte proliferation (Berzi et al. 2008). In the present study, proBDNF expressed in T cells after LPS may be one of the mediators of the degenerative response of the central nervous system. Overall, our data strongly supports the idea of proBDNF as a mediator of the cross talk between the nervous system and immune system. The communication of the nervous and immune systems through proBDNF signaling in infection may provide novel insights into the treatment and prevention of sepsis. Further studies are required to examine the functional roles of proBDNF in sepsis, since we only analyzed proBDNF expression in MLNs of mice with sepsis.

Conclusion

In summary, this study provided direct evidence that sepsis promoted the proliferation of $CD3^+$, $CD4^+$, and $CD8^+$ T cells, but inhibits $CD19^+$ B cells in the MLNs. We further demonstrated that proBDNF was upregulated during the sepsis, which was mainly localized on $CD3^+$ and $CD4^+$ T cells in MLNs. The upregulation of proBDNF signaling and cytokines activation in the MLNs may be involved in the disease progress of sepsis.

Authors' Contributions ZW conducted the study, data collection, data analysis, and manuscript preparation; JW and FZ conducted the study, data collection, and data analysis; YL helped to do the animals-related experiments; YY and JS helped with data analysis; HL and SW helped to design and analyze the data; XFZ provided reagents, data interpretation, and revised manuscripts; ZH and RD were responsible for design and interpretation of the work, data collection, data analysis, and manuscript drafting; all authors had a final approval of the manuscript submission.

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

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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