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ORIGINAL ARTICLE

Sodium Butyrate Ameliorates Intestinal Injury and Improves Survival in a Rat Model of Cecal Ligation and Puncture-Induced Sepsis

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Abstract— Sepsis is a life-threatening condition with a high rate of mortality. Unfortunately, very few therapies can improve outcomes in patients with sepsis. Butyrate, which is the most potent histone deacetylase (HDAC) inhibitor among short-chain fatty acids, exerts anti-inflammatory effects in a variety of inflammatory diseases. Butyrate might thus be valuable in the treatment of sepsis, in which inhibition of overwhelming cytokine release is vitally important. Sepsis was induced in 7- to 8-week-old Sprague-Dawley rats by cecal ligation and puncture (CLP) with a 21-g double-puncture technique. Rats received an intravenous injection of normal saline (vehicle) or sodium butyrate (200 mg/kg) after CLP and were sacrificed 12 h later. Hematoxylin and eosin staining was performed to observe the intestinal mucosal morphology. RT-PCR and ELISA were used to determine the intestinal inflammatory response *in vivo*. Intestinal permeability was evaluated by measuring fluorescein isothiocyanate dextran (FD-4) absorption *in vivo*, and tight junction protein expression was examined by western blot. NF-κB p65 activities were assessed by western blot and immunohistochemistry. Sodium butyrate treatment improved the survival rate of CLP rats and alleviated sepsis-induced intestinal mucosal injury. Proinflammatory cytokine expression was lower in butyrate-treated rats than in the vehicle group. FD-4 leakage from the intestinal tract was reduced, and the expression levels of the tight junction proteins claudin-1 and ZO-1 were also restored in rats that received sodium butyrate treatment. These effects were associated with less NF-κB p65 nuclear translocation, whereas the expression of Iκ-Bα was not affected or even increased. Sodium butyrate mitigates the inflammatory response and maintains intestinal barrier function in polymicrobial sepsis partly through inhibition of NF-κB activation and may serve as a novel therapy for sepsis.

KEY WORDS: butyrate; NF-kappa B; sepsis; intestinal injury.

INTRODUCTION

Sepsis is a life-threatening disorder with a high rate of mortality. Unfortunately, few clinical studies have managed to improve outcomes in patients with sepsis. Despite significant advances in our understanding of the pathophysiology of sepsis, its treatment is still limited to antibiotics, aggressive fluid resuscitation, vasopressor administration, and supportive care [1], and no targeted therapeutics for sepsis are approved

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Abbreviations: CLP, Cecal ligation and puncture; NaB, Sodium butyrate; NF-κB, Nuclear factor-kappa B

for use in patients [2]. Because few methods can improve outcomes in patients with sepsis, it is imperative that other therapeutic options be explored.

In human sepsis, the intestine is the first organ to be targeted. Intestinal dysfunction often leads to augmented gut permeability, resulting in the translocation of intestinal microbes and/or their products. The gut also becomes a proinflammatory organ that promotes deleterious effects in distant organs, even without the need for systemic bacterial translocation [3]. Thus, by acting as a motor that both drives and perpetuates multiple organ dysfunctions [4], the gut plays a pivotal role in the pathophysiology of sepsis. Thus, one promising therapeutic strategy is urgently needed to diminish or turn off the "motor" of organ failure in sepsis.

Butyrate has recently emerged as a potent anti-inflammatory agent that reduces the production of proinflammatory cytokines, both *in vivo* and *in vitro*. It is a product of the bacterial fermentation of dietary fiber, which plays an important physiological role in maintaining the health and integrity of the colonic mucosa [5]. The available data suggest that butyrate can suppress the LPS- and cytokine-stimulated production of proinflammatory mediators such as TNF-α, IL-6, and NO by immune cells [6]. In addition, it exerts a protective effect in different rodent animal models against inflammatory-related injuries [7–11]. Because the pathogenesis of sepsis also depends on the activation of cytokine responses, butyrate might also be protective in sepsis. It has already been shown that sodium butyrate (NaB) can inhibit the lethality of severe sepsis in rats and reduce multiple organ damage associated with severe sepsis [12, 13]. However, whether NaB also protects against sepsis-induced intestinal injury remains uninvestigated. Thus, we decided that it would be valuable to study the effect of NaB on the intestinal mucosa in the setting of sepsis.

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats weighing 200–220 g obtained from Changsheng Biotechnology (Changchun, China) were housed individually under standard conditions (12-h light/dark cycles with a room temperature of 22–24 °C). Sixty-six rats were randomly divided into a sham operation group, vehicle-treated group (referred to as the CLP group), and NaB-treated group (referred to as the CLP + NaB group; $n = 22$ per group). Rats were housed for 3 days before manipulation and fasted overnight before the surgical procedure. The sepsis model was induced *via* the cecal ligation and puncture (CLP) method as previously described [14]. In short, the peritoneal cavity was opened under isoflurane inhalation anesthesia. The cecum was eviscerated, and one-third of the distal cecum was ligated using a 3–0 suture and punctured through and through (two holes) with a 21-gauge needle. The punctured cecum was squeezed to expel a small amount of fecal material and returned to the peritoneal cavity. The abdominal incision was closed in two layers with 4–0 silk suture. Animals were resuscitated *via* subcutaneous injection of 1 ml saline immediately after the operation. Sham-operated animals were handled in the same manner, except that the cecum was not ligated or punctured.

In the NaB treatment group, rats were intravenously injected with 200 mg/kg NaB (Absin, Shanghai, China) 1 h after the CLP operation and 8 h later. Equal volumes of normal saline were administered to the sham operation and vehicle-treated (CLP) groups. To determine the effect of NaB on mortality from CLP-induced sepsis, survival after CLP was assessed four times a day for at least 7 days and the cumulative survival curve was plotted using the Kaplan-Meier method. All experiments were performed in accordance with National Institutes of Health guidelines and with the approval of the local ethics committee of China Medical University.

Histological Investigation and Immunofluorescence Staining

Ileal samples were removed and placed in 4% buffered formaldehyde, dehydrated, embedded in paraffin, and sectioned into 4-μm-thick sections. Hematoxylin and eosin staining was then performed. Intestinal injuries were evaluated using Chiu's scoring system [15]. Two observers who were unaware of the group assignments performed all of the damage assessments.

For immunofluorescence staining, frozen sections were obtained and mounted on slides, which were then washed with PBS and blocked with 5% normal goat serum for 1 h at room temperature. Primary antibodies against ZO-1 and claudin-1 (1:100; Wanleibio, Shenyang, China) were added to the slides and incubated overnight at 4 °C in a humidity chamber. After washing, the sections were incubated with FITC-conjugated secondary IgG antibodies for 4 h at room temperature. The slides were again washed extensively and stained with DAPI for 10 min. Staining was visualized under a Nikon 300 microscope. The expression of NF-κB p65 in formalin-fixed and paraffin-embedded ileal tissues was assessed by immunohistochemistry. Sections were incubated with 1:100 diluted specific anti-rat NF-κB p65 antibodies (Proteintech, Chicago, USA).

Quantitative Polymerase Chain Reaction Analysis of RelA, IL-1β, TNF-α, IL-6, and iNOS

Total RNA was extracted from the intestine using a TRIzol Reagent Kit (Takara, Japan) according to the manufacturer's instructions, and the quality and concentration of the RNA were verified by spectrophotometry with a NanoDrop™ apparatus (Implen, Germany). cDNA was generated from 1 μg of total RNA in a 20-μl reaction volume using a PrimeScript™ RT Reagent Kit (Takara). RelA (p65), IL-1β, TNF-α, IL-6, and iNOS mRNA levels were quantified by real-time quantitative (q) reverse-transcriptase polymerase chain reaction (RT-PCR) based on SYBR green labeling with a Light Cycler 480 real-time system from Roche, Co. Ltd. (Switzerland). The specific primers (Shenggong, Shanghai) used to amplify the mRNAs are listed in Table 1; β-actin was used as an internal control. Melting curve analysis and real-time qRT-PCR data were analyzed using the $2^{-\triangle\triangle Ct}$ method.

Evaluation of Cytokines in Intestinal Tissues

The levels of TNF-α and IL-6 in ileal tissues were tested using ELISA kits (SAB, Maryland, USA) according to the manufacturer's instructions. Briefly, 100 μl of the homogenate supernatant was added to each well, which had been coated with the corresponding primary antibody, and then 100 μl of the biotin conjugate was added. After the addition of 100 μl of streptavidin-HRP and the substrate, the absorbance was measured at 450 nm. Then, the levels of TNF-α and IL-6 were calculated by standard curves that were acquired according to the instructions.

Measurement of Intestinal Permeability *In Vivo*

Intestinal permeability to FITC-dextran (FD-4; molecular weight, 4000 Da; Sigma-Aldrich, USA) was assessed using the method of Martin et al. [16]. Briefly, an animal was anesthetized using an intraperitoneal injection of sodium pentobarbital (40 mg/kg). Then, a 2-cm, median laparotomy incision was made and a 10-cm segment of the distal ileum, with preserved superior mesenteric vessels, was dissected 3 cm proximal to the cecum. The two ends of the isolated intestinal segment were ligated. One milliliter of FD-4 was injected into the lumen, and the 10-cm intestinal segment was carefully returned to the abdomen, covered, and protected with gauze soaked in warm saline. After 30 min, a blood sample was taken through cardiac puncture and serum was isolated using centrifugation at 3000×*g* for 10 min at 4 °C. Serial dilutions of FD-4 were made to generate a standard curve, and the serum concentrations of FD-4 were determined using a fluorescence microplate reader (ELX808IU; Bio-Tek, USA) with an excitation wavelength of 492 nm and emission wavelength of 518 nm. Serum concentrations of FD-4 in the CLP and NaB-treated groups were normalized to the FD-4 concentration of the sham operation group.

Western Blot

Ileal tissues were homogenized in RIPA lysis buffer and a proteinase inhibitor cocktail (1:100; Beyotime, Haimen, China). Lysates were clarified by centrifugation at 13000×*g* for 30 min at 4 °C. The supernatant was collected, and protein concentrations were quantified using the BCA assay. Cytoplasmic and nuclear extracts were prepared using the Minute™ cytoplasmic and nuclear extraction kit (Invent Biotechnologies, Inc., Eden Prairie, MN, USA) according to the manufacturer's instructions. The sample was denatured at 100 °C for 5 min, separated by 10% SDS-PAGE gel, and then transferred to a PVDF membrane at 100 V for 1 h. The membrane was blocked with 5% nonfat milk dissolved in Tris-buffered saline Tween-20 (TBST) overnight at 4 °C. Western blot analysis was performed with a specific primary antibody against NF-κB p65 (Proteintech), ZO-1, claudin-1, Iκ-Bα, histone protein 3 (1:500; Wanleibio), and β-actin (1:1000; Absin). Histone protein 3 was used as an internal control of nuclear extracts, and β-actin was used as an internal control of cytosolic extracts and of total protein extracts for the tissue. The results were expressed as the relative density to β-actin or histone protein 3 and then normalized to the mean value of the control group.

Table 1. Specific Primers Used to Amplify Target Genes in RT-PCR

	Forward (5′-3′)	Reverse (3′-5′)
NF-κB	TCGCCACCGGATTGAAGAAA	CTCGGGAAGGCACAGCAATA
TNF-α	GCATGATCCGAGATGTGGAACTGG	CGCCACGAGCAGGAATGAGAAG
IL-1β	AAATGCCTCGTGCTGTCTGA	AGGCCACAGGGATTTTGTCG
IL-6	AGGAGTGGCTAAGGACCAAGACC	TGCCGAGTAGACCTCATAGTGACC
iNOS	GGTGAGGGGACTGGACTTTTA	GTGACTTTGTGCTTCTGCACC

Statistical Analysis

Data are expressed as means ± SEM. For survival, Kaplan-Meier plots were used and assessed using a log-rank test. Statistical analysis was performed by one-way ANOVA of repeated experiments followed by Bonferroni's multiple group comparison with Prism 7 (GraphPad). A p value < 0.05 was considered statistically significant.

RESULTS

NaB Alleviates Gut Injury and Improves Survival in CLP Rats

To determine the effects of NaB on CLP-related intestinal injury, we evaluated the histology of ileal specimens stained with hematoxylin and eosin (Fig. 1a–i). The ileal tissues from sham operation rats showed minimal inflammation. The ileal tissues from vehicle-treated rats showed severe inflammatory cell infiltrate, disruption of intestinal villi, and diffuse intestinal wall thickening after CLP. Compared with vehicle-treated rats, NaB-treated rats showed less inflammatory cell infiltrate and alleviated villus injury and gut wall edema in the ileum. Thus, the histological changes induced by CLP in the gut were effectively mitigated by NaB treatment. The histologic damage to the intestinal mucosa induced by CLP was also assessed based on Chiu's grading scale, which is shown in Fig. 1j.

Survival was evaluated using Kaplan-Meier survival curves. As shown in Fig. 2, a decline in the survival rate was found in all CLP groups. Rats treated with NaB had significantly higher survival than CLP rats (22% *vs.* 50% mortality, $p < 0.05$).

NaB Reduces Proinflammatory Cytokine Production

Because TNF-α and IL-6 are considered prominent components of the cytokine network during sepsis, we used ELISA to determine the levels of TNF-α and IL-6 in intestinal tissue to further evaluate the role of the overwhelming inflammation in the small intestine. The concentrations of the two proinflammatory factors were increased in the vehicle-treated group, whereas butyrate markedly inhibited the CLP-induced elevation of both proinflammatory cytokines ($p < 0.01$; Fig. 3).

NaB Treatment Restrains Proinflammatory Cytokine mRNA Expression

To test whether the butyrate-mediated inhibition of cytokine production occurred at the transcriptional level, we determined the mRNA levels of cytokines using RT-PCR. We found that there was a significant increase in the mRNA levels of RelA (p65), IL-6, TNF-α, and IL-1β in CLP-challenged rats, which was downregulated after butyrate treatment (Fig. 4). All of these results suggest that butyrate treatment restrained the CLP-stimulated expression of cytokine mRNAs in the small intestine.

NaB Treatment Protects Against Intestinal Barrier Disruption During CLP-Induced Sepsis

Because the above results showed a reduced inflammatory response after NaB treatment, intestinal barrier function was evaluated in the groups to investigate its role in the development of the polymicrobial sepsis induced by CLP. As shown in Fig. 5c, rats in the CLP group had an approximately threefold increase in serum FD-4 compared with the sham and NaB-treated groups ($p < 0.05$). We then examined the localization and expression of the tight junction proteins claudin-1 and ZO-1 by immunofluorescence and western blotting. Claudin-1 was mainly immunolocalized at the cell apices and the lateral cell membrane in the intestine epithelium. In contrast, ZO-1 was immunolocalized to the cytosol. The expression of both tight junction proteins was restored in CLP rats treated with NaB. However, no significant changes in the localization of the two tight junction proteins were observed (Fig. 5a and b). The expression levels of claudin-1 and ZO-1 were also restored in NaB-treated rats on western blot analysis (Fig. 5d and e). These findings indicate that intravenous administration of NaB suppresses damage to the intestinal mucosal barrier during sepsis.

NaB Treatment Suppresses NF-κB Activation in Ileal Tissues from CLP Rats

As a transcription factor, NF-κB regulates the expression of a variety of genes involved in inflammation and immunity and is also considered to be a central regulator of inflammation. Accordingly, we evaluated NF-κB p65 activation to investigate the mechanism of action of butyrate by assessing p65 levels in nuclear, cytosolic, and total protein extracts. As shown by immunohistochemistry (Fig. 6a–f), NaB-treated rats exhibited decreased nuclear accumulation of p65 in ileal tissue compared with vehicle-treated rats. These results were supported by western blot analysis (Fig. 6g and h), with the nuclear levels of p65 significantly increased after CLP treatment. In contrast, this increase in p65 nuclear accumulation was effectively suppressed by butyrate treatment. The nuclear/cytosolic ratios of p65 were 0.51 ± 0.23, 3.83 ± 1.91, and $0.53 \pm$

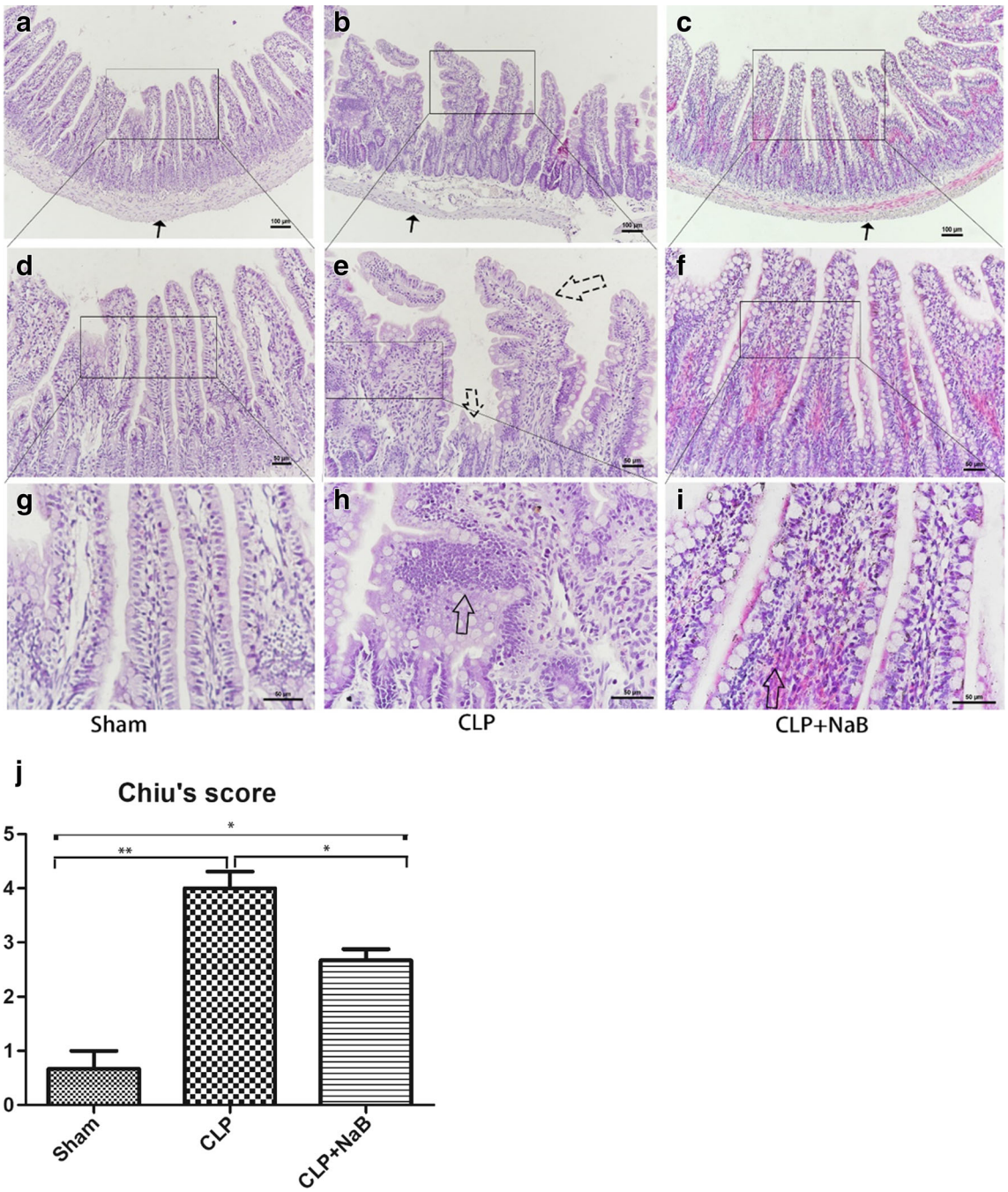

Fig. 1. Effects of NaB on intestinal histology. **a**–**i** Histological sections of the ileum harvested 12 h after CLP application in rats with and without NaB treatment. The ileal specimens were stained with hematoxylin and eosin. The sham operation group showed normal intestinal mucosa, whereas tissues from the vehicle-treated group showed ground neutrophil infiltration (open arrow), mucosa edema (solid black arrow), and villus shrinkage (dotted arrow) compared with the sham operation. However, histological changes were markedly ameliorated after NaB treatment (magnification × 100, × 200, and × 400 in the upper, middle, and bottom panels). **j** Chiu's score of intestinal specimens in each group. The vehicle group had a significantly higher score than the sham group, which was significantly reduced by butyrate treatment (*$p < 0.05$, **$p < 0.01$).

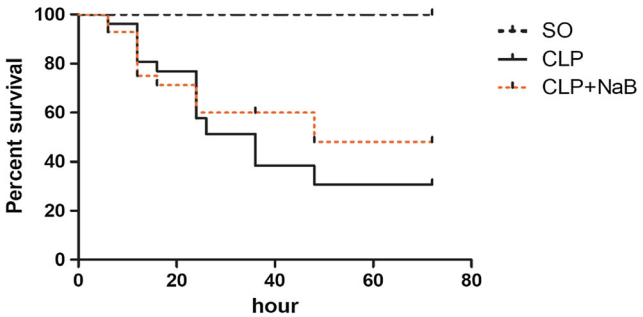

Fig. 2. NaB treatment protects against CLP-induced lethality. Septic rats were treated with NaB and observed. A significant difference in survival was seen between the vehicle- and NaB-treated groups at 72 h ($n = 10$ rats per group, $p < 0.05$).

0.37 in the sham operation, CLP + vehicle, and CLP + NaB groups, respectively ($p < 0.01$). Because NF-κB is tightly regulated by Iκ-Bα, Iκ-Bα was also examined in the protein extracts. Both cytosolic and nuclear protein extracts showed a notable increase in the Iκ-Bα concentration, resulting in an overall increase in Iκ-Bα in NaB-treated rats.

DISCUSSION

The gut, which has long been hypothesized to be the "motor" of multiple organ dysfunction syndrome, plays a pivotal role in the pathological process of sepsis [17]. However, the role of butyrate in the small intestine during sepsis has rarely been investigated. Previous studies showed that butyrate exerted a protective effect on multiple organ damage in sepsis, with butyrate lowering the mortality rate [12]. In addition, butyrate attenuates the inflammatory response and reduces lung and heart injury in a mouse endotoxin-induced sepsis model [18, 19]. In this study, we used the CLP model that closely mimics the progression and characteristics of human sepsis [20] to investigate the effects of NaB on sepsis-induced intestinal injury in ileal tissues. We found that intravenous injection of NaB attenuated the production of proinflammatory cytokines and protected against intestinal barrier disruption. Consistent with previous studies, the mortality rate related to the CLP procedure was also lowered. In addition, the protective effects of NaB were significantly correlated with its ability to inhibit NF-κB activation. Specifically, NaB was applied after the onset of sepsis, suggesting a promising potential clinical application. These findings suggest that inhibition of NF-κB activation by NaB after the onset of sepsis might be an option for suppressing the systemic inflammatory response and downstream organ injury caused by microbial infection.

The release of large amounts of inflammatory mediators including cytokines and chemokines caused by overactivation of the innate immune system is often regarded as

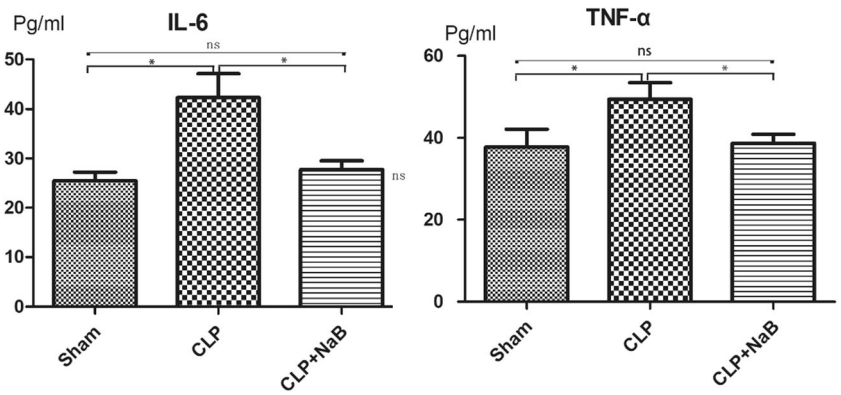

Fig. 3. Effects of butyrate on IL-6 and TNF-α levels in ileal tissues. Butyrate treatment reduced IL-6 and TNF-α production in the ileal tissue of the CLP rats. Tissue homogenates were prepared to determine the levels of IL-6 and TNF-α by ELISA. The proteins in the intestinal samples were quantitated using the BCA assay to normalize the cytokine levels in the gut tissue (*$p < 0.05$).

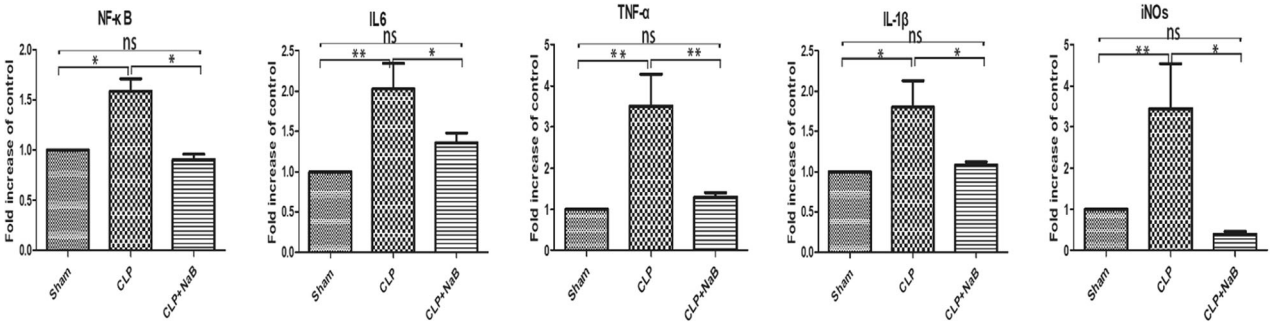

Fig. 4. Butyrate treatment decreases proinflammatory cytokine mRNA expression in the ileum of rats subjected to CLP. The total RNA in ileal tissues was extracted to detect the mRNA levels of NF-κB (p65), TNF-α, IL-6, IL-1β, and iNOS by qPCR. β-actin was used as the internal control. The target mRNAs in the sham group were normalized to 1 to analyze the cytokine concentrations in the ileal tissues. $*p < 0.05$, $**p < 0.05$; $n \geq 6$ rats per group.

a "cytokine storm" that contributes to septic shock and even multiple organ failure. The levels of cytokines such as IL-6 and TNF-α are significantly increased during the early phase of sepsis in septic patients [21] and in animal models of sepsis [22]. Of the proinflammatory cytokines inhibited by butyrate, TNF-α acts as an activator of NF-κB [23], a transcription factor that is considered to be critically involved in the pathogenesis of sepsis [24]. Lowered TNF-α production could result in reduced NF-κB pathway activation, leading to even less proinflammatory cytokine production. In the present work, butyrate decreased IL-6 and TNF-α levels in ileal homogenates and resulted in reduced neutrophil infiltration and intestinal mucosal injury after CLP. Consistent with the decreased cytokine production observed in our study, the mRNA levels of several cytokines were also declined in the small intestine during sepsis, suggesting that NaB may regulate the inflammatory response at the transcriptional level. Thus, it is reasonable to speculate that, by inhibiting proinflammatory production, butyrate injection helps to block the vicious "cytokine storm" cycle that frequently occurs during severe sepsis.

Sepsis is associated with disturbed gut barrier function, which facilitates the translocation of pathogens and their toxins into the systemic circulation [25]. Although butyrate is known to enhance the barrier function of the colonic mucosa, its role in the small intestine is far from being understood. In our study, claudin-1 and ZO-1 expression levels were significantly increased after NaB treatment; the permeability of the small intestine to large molecules was consequently reduced as well. Some proinflammatory cytokines such as IL-6 and TNF-α have been found to contribute to the disruption of intestinal epithelial barrier function [26]. Hence, maintenance of epithelial integrity in the NaB-treated rats might be attributed to inhibition of proinflammatory cytokine secretion in the small intestine.

In septic patients, increased nuclear NF-κB expression is associated with higher rates of mortality and worse clinical outcome [24]. The NF-κB transcription factor is part of a family that comprises several subunits that form homo- and heterodimers. The primary level of regulation of NF-κB activity is through its retention in the cytoplasm *via* its interaction with Iκ-Bα. In response to various stimuli, the Iκ-B is dislocated, and the activated transcription factor translocates to the nucleus, where it induces a large number of target genes that most commonly trigger the release of cytokines and chemokines and recruit inflammatory cells [27]. In our study, the nuclear translocation of NF-κB p65 coupled with the secretion of proinflammatory cytokines was observed to be increased in the ileum of septic rats, which was effectively reduced by butyrate treatment at 12 h after sepsis induction. The results suggest that butyrate is capable of ameliorating the early phase of sepsis-related inflammatory responses mediated by NF-κB-induced proinflammatory cytokine release.

The suppressive effect of butyrate on NF-κB nuclear translocation might be mediated by its inhibitory effects on histone deacetylase 3 (HDAC3) [28, 29]. The strength and duration of NF-κB activity are regulated by its post-translational modification, such as (de)acetylation [30], and butyrate is the most potent short-chain fatty acid inhibitor of HDAC [31]. The available evidence suggests that the acetylation of specific lysine sites could reduce the ability of p65 to bind to κB DNA, which facilitates its removal from κB DNA and consequently promotes Iκ-Bα-mediated export of p65 from the nucleus to the cytoplasm [32–34]. Our results appear to be in line with this theory.

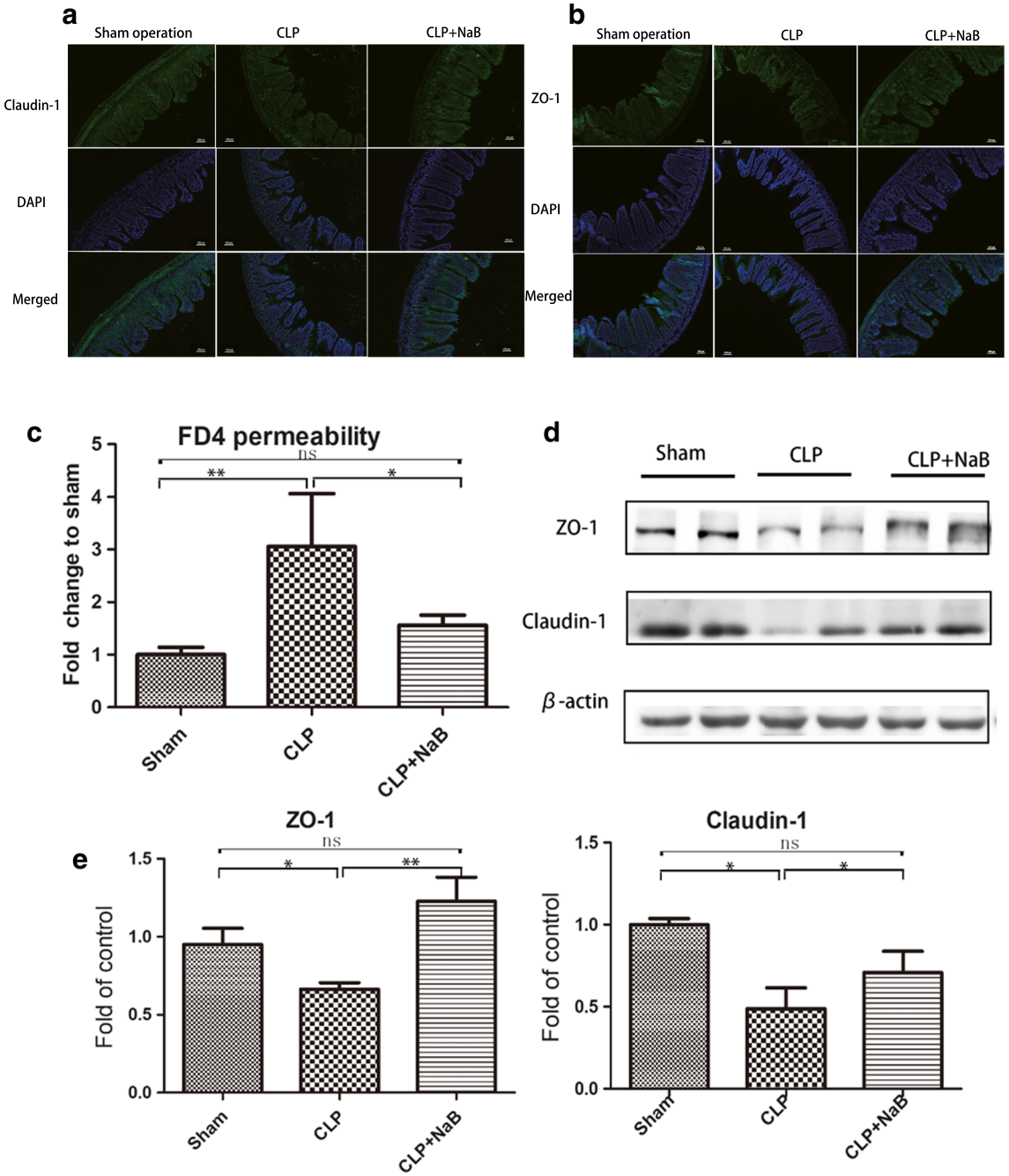

Fig. 5. Butyrate administration protects against intestinal barrier disruption in CLP-treated septic rats. Localization of claudin-1 (**a**), ZO-1 (**b**), and DAPI within ileal sections assessed by immunofluorescence from septic rats and controls. Tight junction proteins (green) and DAPI (blue); merged tight junction proteins and DAPI are presented. Scale bar = 100 μm. Original magnification, × 100. The effects of NaB treatment on intestinal permeability to FD-4 (**c**). CLP significantly enhanced the intestinal permeability to FD-4, whereas NaB treatment mitigated intestinal permeability to FD-4. Data were normalized to those of the sham operation group. $n = 6$ rats per group. The protein levels of claudin-1 and ZO-1 in the intestinal mucosa were measured by western blot (**d**) and quantified by densitometry (**e**). $N = 6$ rats per group. Data are presented as mean ± SEM of three independent experiments ($^{*}p < 0.05$, $^{**}p < 0.01$).

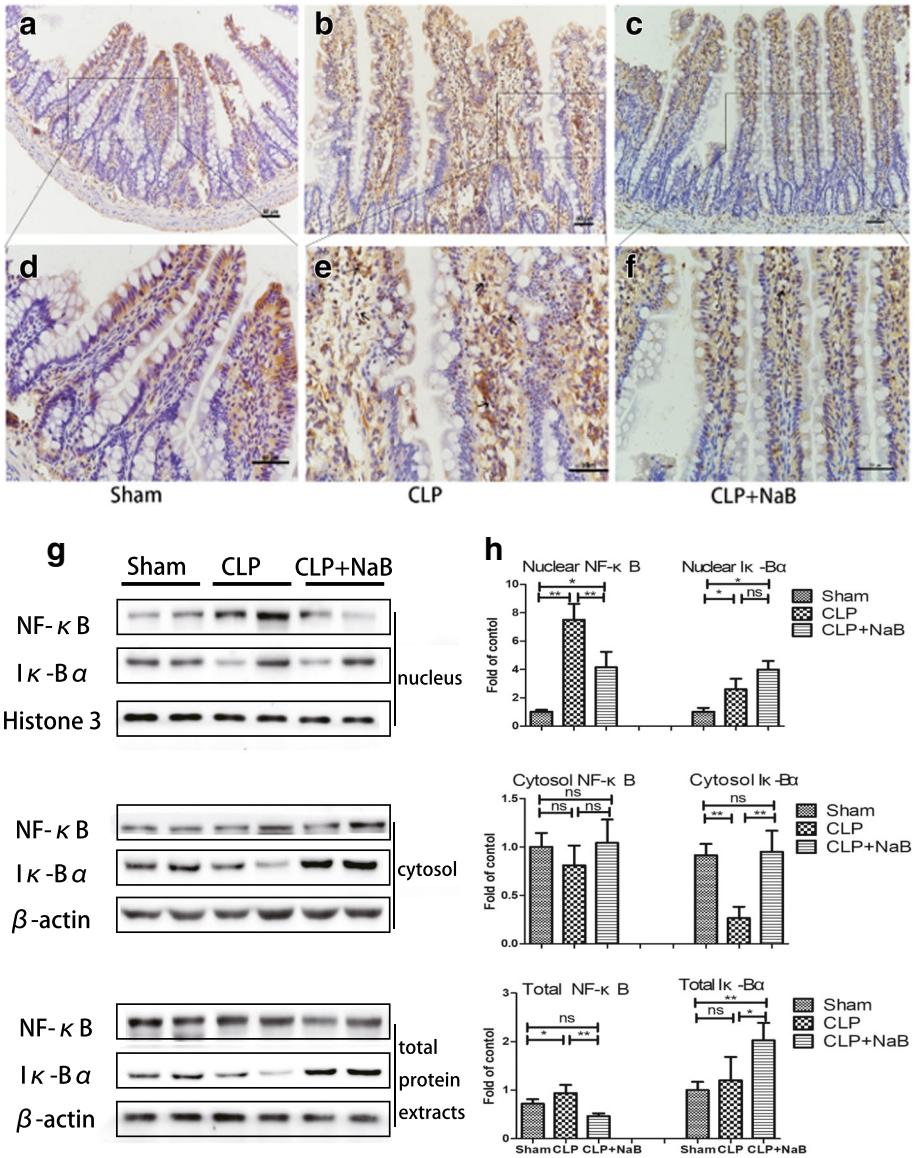

Fig. 6. Treatment with NaB effectively inhibits NF-κB nuclear translocation in the small intestine of CLP rats. Treatment with NaB reduced p65 nuclear translocation (black arrow), as shown by immunohistochemistry (**a**–**c**, × 200; **d**–**f**, × 400). Western blotting was performed to detect RelA (p65) and Iκ-Bα in nuclear, cytoplasmic, and total protein extracts from ileal tissue using specific antibodies. p65 was reduced in the nuclear compartment in the ileum of rats treated with NaB compared with vehicle-treated (CLP) rats. The overall p65 expression in the total protein extracts was reduced in parallel with the changes in p65 that occurred in the nucleus. Iκ-Bα levels in the small intestine were unaffected or even increased in the intestinal extracts. The quantitative changes in the levels of p65 and Iκ-Bα are indicated to the right of the panels (**h**). The results represent three independent experiments ($n = 6$ rats per group; $^{*}p < 0.05$, $^{**}p < 0.01$).

With the potential to effectively inhibit HDACs, butyrate reduced p65 nuclear accumulation, leading to decreased transcription of a series of proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β and even NF-κB itself. Importantly, it seems that short-term inhibition of NF-κB does not compromise a host's bacterial defense [35]. Therefore, this evidence provides a rationale for the medical application of butyrate in the treatment of sepsis.

The resynthesis of Iκ-B proteins induced by activated NF-κB is one of the well-accepted mechanisms for termination of the NF-κB response. The Iκ-Bα gene is one of the target genes activated by NF-κB [36]. Newly

synthesized Iκ-Bα then enters the nucleus, removes NF-κB from the DNA, and relocalizes it to the cytosol. Interestingly, because the expression of Iκ-Bα is also induced by activation of NF-κB, in contrast to the reduced cytokine expression, the expression level of Iκ-Bα in cytoplasmic extracts was found to be upregulated after NaB treatment in our study. Similar findings were observed for another drug targeting NF-κB in the treatment of sepsis-induced lung injury [37]. The increased Iκ-Bα expression may also be attributed to NF-κB acetylation. Acetylation of p65 at some specific sites has been shown to increase its binding to κB enhancers [23, 38] and to elevate NF-κB gene expression [39]. Thus, acetylation at different sites of p65 mediated by butyrate through its HDAC inhibitory activity might result in contrasting effects on its gene transcription. Nevertheless, this association needs further investigation.

HDAC inhibition may also have proinflammatory effects. Thus, the role of HDAC inhibition in inflammatory diseases appears to be double-edged. The effects of HDAC inhibition on NF-κB activation might also be dependent on the cell type, stimulus, and diseases status [40].

The present study has some limitations to be acknowledged. First, we only measured selected cytokines and explored limited pathways. Many more mechanisms are likely to be involved in the pathophysiology of sepsis. Thus, whether the use of NaB affects other septic molecular pathways needs further investigation. The dosage of NaB for rats was selected according to the results of toxicology and pharmacokinetic studies of the drug [41], as well as previous studies [12, 13, 42]. In our study, NaB was administered again 8 h after the first dose to compensate for its short *in vivo* plasma half-life [41]. Thus, a more comprehensive assessment is warranted using a variety of sepsis models to better define the optimal timing and degree of NF-κB inhibition to minimize the gut injury caused by sepsis while preserving host defense.

CONCLUSION

In summary, the results of our study demonstrated that butyrate, when given after sepsis induction, could inhibit NF-κB activation and thereby regulate the secretion of inflammatory cytokines and mitigate the detrimental effects of sepsis on barrier function in the small intestine of septic rats. The findings presented in this study justify further optimization and investigation of butyrate as a HDAC inhibitor with the aim of developing it as an anti-inflammatory drug for application in sepsis.

COMPLIANCE WITH ETHICAL STANDARDS

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

REFERENCES

1. Dellinger, R.P., M.M. Levy, A. Rhodes, et al. 2013. Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Medicine* 39: 165–228.
2. Hotchkiss, R.S., G. Monneret, and D. Payen. 2013. Sepsis-induced immunosuppression: From cellular dysfunctions to immunotherapy. *Nature Reviews. Immunology* 13: 862–874.
3. Assimakopoulos, S.F., C. Triantos, K. Thomopoulos, F. Fligou, I. Maroulis, M. Marangos, and C.A. Gogos. 2018. Gut-origin sepsis in the critically ill patient: Pathophysiology and treatment. *Infection.* 46: 751–760.
4. Klingensmith, N.J., and C.M. Coopersmith. 2016. The gut as the motor of multiple organ dysfunction in critical illness. *Critical Care Clinics* 32: 203–212.
5. Scheppach, W. 1994. Effects of short chain fatty acids on gut morphology and function. *Gut.* 35: S35–S38.
6. Vinolo, M.A., H.G. Rodrigues, R.T. Nachbar, et al. 2011. Regulation of inflammation by short chain fatty acids. *Nutrients.* 3: 858–876.
7. Vieira, E.L., A.J. Leonel, A.P. Sad, et al. 2012. Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. *The Journal of Nutritional Biochemistry* 23: 430–436.
8. Sun, J., F. Wang, H. Li, et al. 2015. Neuroprotective effect of sodium butyrate against cerebral ischemia/reperfusion injury in mice. *BioMed Research International* 2015: 395895.
9. Qiao, Y.L., J.M. Qian, F.R. Wang, Z.Y. Ma, and Q.W. Wang. 2014. Butyrate protects liver against ischemia reperfusion injury by inhibiting nuclear factor kappa B activation in Kupffer cells. *The Journal of Surgical Research* 187: 653–659.
10. Correa-Oliveira, R., J.L. Fachi, A. Vieira, et al. 2016. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunology.* 5: e73.
11. Liu, J., F. Wang, H. Luo, A. Liu, K. Li, C. Li, and Y. Jiang. 2016. Protective effect of butyrate against ethanol-induced gastric ulcers in mice by promoting the anti-inflammatory, anti-oxidant and mucosal defense mechanisms. *International Immunopharmacology* 30: 179–187.
12. Zhang, L.T., Y.M. Yao, J.Q. Lu, X.J. Yan, Y. Yu, and Z.Y. Sheng. 2007. Sodium butyrate prevents lethality of severe sepsis in rats. *Shock.* 27: 672–677.
13. Zhang, L., S. Jin, C. Wang, R. Jiang, and J. Wan. 2010. Histone deacetylase inhibitors attenuate acute lung injury during cecal ligation and puncture-induced polymicrobial sepsis. *World Journal of Surgery* 34: 1676–1683.
14. Mishra, S.K., and S. Choudhury. 2018. Experimental protocol for cecal ligation and puncture model of polymicrobial sepsis and assessment of vascular functions in mice. *Methods in Molecular Biology* 1717: 161–187.

15. Chiu, C.J., A.H. McArdle, R. Brown, et al. 1970. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Archives of Surgery* 101: 478–483.
16. Kao, N.R., A. Xenocostas, D.K. Driman, et al. 2011. Recombinant human erythropoietin improves gut barrier function in a hemorrhagic shock and resuscitation rat model. *The Journal of Trauma* 71: S456–S461.
17. Meng, M., N.J. Klingensmith, and C.M. Coopersmith. 2017. New insights into the gut as the driver of critical illness and organ failure. *Current Opinion in Critical Care* 23: 143–148.
18. Ni, Y.F., J. Wang, X.L. Yan, et al. 2010. Histone deacetylase inhibitor, butyrate, attenuates lipopolysaccharide-induced acute lung injury in mice. *Respiratory Research* 11.
19. Wang, F., Z. Jin, K. Shen, T. Weng, Z. Chen, J. Feng, Z. Zhang, J. Liu, X. Zhang, and M. Chu. 2017. Butyrate pretreatment attenuates heart depression in a mice model of endotoxin-induced sepsis via anti-inflammation and anti-oxidation. *The American Journal of Emergency Medicine* 35: 402–409.
20. Dejager, L., I. Pinheiro, E. Dejonckheere, and C. Libert. 2011. Cecal ligation and puncture: The gold standard model for polymicrobial sepsis? *Trends in Microbiology* 19: 198–208.
21. Gentile, L.F., A.G. Cuenca, E.L. Vanzant, P.A. Efron, B. McKinley, F. Moore, and L.L. Moldawer. 2013. Is there value in plasma cytokine measurements in patients with severe trauma and sepsis? *Methods.* 61: 3–9.
22. Aksoy, A.N., A. Toker, M. Celik, et al. 2014. The effect of progesterone on systemic inflammation and oxidative stress in the rat model of sepsis. *Indian Journal of Pharmacology* 46: 622–626.
23. Huang, B., X.D. Yang, A. Lamb, and L.F. Chen. 2010. Posttranslational modifications of NF-kappaB: Another layer of regulation for NF-kappaB signaling pathway. *Cellular Signalling* 22: 1282–1290.
24. Liu, S.F., and A.B. Malik. 2006. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 290: L622–L645.
25. Li, Q., Q. Zhang, C. Wang, X. Liu, N. Li, and J. Li. 2009. Disruption of tight junctions during polymicrobial sepsis in vivo. *The Journal of Pathology* 218: 210–221.
26. Lechuga, S., and A.I. Ivanov. 2017. Disruption of the epithelial barrier during intestinal inflammation: Quest for new molecules and mechanisms. *Biochimica et Biophysica Acta, Molecular Cell Research* 1864: 1183–1194.
27. Berkes, J., V.K. Viswanathan, S.D. Savkovic, and G. Hecht. 2003. Intestinal epithelial responses to enteric pathogens: Effects on the tight junction barrier, ion transport, and inflammation. *Gut.* 52: 439–451.
28. Singh, N., M. Thangaraju, P.D. Prasad, P.M. Martin, N.A. Lambert, T. Boettger, S. Offermanns, and V. Ganapathy. 2010. Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases. *The Journal of Biological Chemistry* 285: 27601–27608.
29. Park, J., M. Kim, S.G. Kang, A.H. Jannasch, B. Cooper, J. Patterson, and C.H. Kim. 2015. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunology* 8: 80–93.
30. Ito, K. 2007. Impact of post-translational modifications of proteins on the inflammatory process. *Biochemical Society Transactions* 35: 281–283.
31. Flint, H.J., K.P. Scott, P. Louis, and S.H. Duncan. 2012. The role of the gut microbiota in nutrition and health. *Nature Reviews. Gastroenterology & Hepatology* 9: 577–589.
32. Kiernan, R., V. Bres, R.W. Ng, et al. 2003. Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. *The Journal of Biological Chemistry* 278: 2758–2766.
33. Ziesche, E., D. Kettner-Buhrow, A. Weber, et al. 2013. The coactivator role of histone deacetylase 3 in IL-1-signaling involves deacetylation of p65 NF-kappaB. *Nucleic Acids Research* 41: 90–109.
34. Leus, N.G., M.R. Zwinderman, and F.J. Dekker. 2016. Histone deacetylase 3 (HDAC 3) as emerging drug target in NF-kappaB-mediated inflammation. *Current Opinion in Chemical Biology* 33: 160–168.
35. Li, H., W. Han, V. Polosukhin, et al. 2013. NF-kappaB inhibition after cecal ligation and puncture reduces sepsis-associated lung injury without altering bacterial host defense. *Mediators of Inflammation* 503213: 2013.
36. Chen, L.F., and W.C. Greene. 2004. Shaping the nuclear action of NF-kappaB. *Nature Reviews. Molecular Cell Biology* 5: 392–401.
37. Kaplan, J., M. Nowell, R. Chima, and B. Zingarelli. 2014. Pioglitazone reduces inflammation through inhibition of NF-kappaB in polymicrobial sepsis. *Innate Immunity* 20: 519–528.
38. Ghizzoni, M., H.J. Haisma, H. Maarsingh, and F.J. Dekker. 2011. Histone acetyltransferases are crucial regulators in NF-kappaB mediated inflammation. *Drug Discovery Today* 16: 504–511.
39. Spange, S., T. Wagner, T. Heinzel, and O.H. Krämer. 2009. Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *The International Journal of Biochemistry & Cell Biology* 41: 185–198.
40. Choo, Q.Y., P.C. Ho, and H.S. Lin. 2008. Histone deacetylase inhibitors: New hope for rheumatoid arthritis? *Current Pharmaceutical Design* 14: 803–820.
41. Egorin, M.J., Z.M. Yuan, D.L. Sentz, K. Plaisance, and J.L. Eiseman. 1999. Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats. *Cancer Chemotherapy and Pharmacology* 43: 445–453.
42. Qiao, Y., J. Qian, Q. Lu, Y. Tian, Q. Chen, and Y. Zhang. 2015. Protective effects of butyrate on intestinal ischemia-reperfusion injury in rats. *The Journal of Surgical Research* 197: 324–330.