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Beneficial Effects of Sodium Phenylbutyrate Administration during Infection with *Salmonella enterica* Serovar Typhimurium

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Sodium phenylbutyrate (PBA) is a derivative of the short-chain fatty acid butyrate and is approved for treatment of urea cycle disorders and progressive familial intrahepatic cholestasis type 2. Previously known functions include histone deacetylase inhibitor, endoplasmic reticulum stress inhibitor, ammonia sink, and chemical chaperone. Here, we show that PBA has a previously undiscovered protective role in host mucosal defense during infection. Administration of PBA to Taconic mice resulted in the increase of intestinal *Lactobacillales* and segmented filamentous bacteria (SFB), as well as an increase of interleukin 17 (IL-17) production by intestinal cells. This effect was not observed in Jackson Laboratory mice, which are not colonized with SFB. Because previous studies showed that IL-17 plays a protective role during infection with mucosal pathogens, we hypothesized that Taconic mice treated with PBA would be more resistant to infection with *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). By using the streptomycin-treated mouse model, we found that Taconic mice treated with PBA exhibited significantly lower *S.* Typhimurium intestinal colonization and dissemination to the reticuloendothelial system, as well as lower levels of inflammation. The lower levels of *S.* Typhimurium gut colonization and intestinal inflammation were not observed in Jackson Laboratory mice. Although PBA had no direct effect on bacterial replication, its administration reduced *S.* Typhimurium epithelial cell invasion and lowered the induction of the proinflammatory cytokine IL-23 in macrophage-like cells. These effects likely contributed to the better outcome of infection in PBA-treated mice. Overall, our results suggest that PBA induces changes in the microbiota and in the mucosal immune response that can be beneficial to the host during infection with *S.* Typhimurium and possibly other enteric pathogens.

Sodium 4-phenylbutyrate (phenylbutyric acid [PBA]) is related to the short-chain fatty acid (SCFA) butyrate, differing by the presence of a phenyl group at the 4 position. PBA is an orphan drug, originally approved for urea cycle disorders (1) and subsequently approved for progressive familial intrahepatic cholestasis type 2 (2). The compound has been used in preclinical and clinical studies as a treatment for a variety of diseases, including other urea cycle disorders (3), spinal muscular atrophy (4), homozygous β-thalassemia (5), and cancer (6). Additionally, PBA was shown to be effective in the treatment of neurodegenerative diseases, including Parkinson's disease (7).

Recent studies have revealed that PBA also plays a role in the reduction of endoplasmic reticulum (ER) stress caused by the unfolded protein response (UPR), thereby suppressing oxidative stress in several animal models (8–11), including in a mouse colitis model (12). *In vitro* studies further demonstrated that PBA can suppress the activity of nuclear factor κB (NF-κB), suggesting anti-inflammatory properties for the compound (13). Moreover, administration of PBA was shown to increase the life span of flies, which was associated with both a global increase in histone acetylation and a marked alteration of gene expression (14).

Despite the number of positive effects attributed to PBA, only a relative few studies to date have investigated whether the compound may be beneficial during infection. It was first shown that, in a rabbit model of *Shigella* infection, PBA counteracted the *Shigella*-mediated downregulation of antimicrobial protein CAP-18 (also known as cathelicidin-related antimicrobial peptide [CRAMP]), thereby leading to reduced clinical illness in the rabbit lung and gut epithelium (15, 16). In a subsequent study, the authors demonstrated that administration of PBA in combination with vitamin D_3 induced cathelicidin (called LL-37 in humans) in macrophages and lymphocytes, leading to effective intracellular killing of *Mycobacterium tuberculosis* by macrophages (17). As a result of these reports, PBA administration is now being tested in a clinical trial of adults with active pulmonary tuberculosis (17, 18). Although these studies suggest that the beneficial effect of PBA during infection is indirect, as it is dependent on the increased expression of an antimicrobial protein, a recent study by Lo et al. indicates that PBA could also have direct antimicrobial activity (19). Specifically, PBA administration was shown to inhibit the growth of *Helicobacter pylori* and *Escherichia coli*, whereas it did not influence the growth of *Bifidobacterium bifidum* and *Lactobacillus reuteri* (19).

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Altogether, PBA exerts multiple activities that can be protective for the host (reviewed in references 20 and 21). Based on these prior studies highlighting the benefit of PBA administration in several models of disease and because PBA is a drug already approved for use in humans, we sought to investigate whether PBA administration could be beneficial for the host during infection with the foodborne pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium).

S. Typhimurium is a mucosal pathogen that infects the human gastrointestinal tract and causes a severe inflammatory diarrhea, afflicting approximately 1.4 million people every year in the United States (22). Although the infection is mostly a self-limiting gastroenteritis, *S.* Typhimurium can also cause bacteremia in immunodeficient patients, children, and the elderly (22). To date, no vaccine or cure exists for *Salmonella*-induced inflammatory diarrhea, as antibiotic treatment induces prolonged fecal shedding and it is thus reserved for patients at high risk of bacteremia (23).

Both the intestinal microbiota and the host mucosal response provide colonization resistance to *S.* Typhimurium infection. Although the mechanisms by which the microbiota limits *S.* Typhimurium intestinal colonization are not completely elucidated, it has been shown that specific shifts of the microbiota confer either susceptibility or resistance to colitis (24). Once the infection is established, one arm of the host response that reduces *S.* Typhimurium dissemination is the production of proinflammatory cytokines such as interleukin 23 (IL-23), which activates intestinal T helper 17 (Th17) cells and γδ T cells to release interleukin 17 (IL-17) (25–28). In turn, IL-17 orchestrates the recruitment of neutrophils to the intestine and thereby confers protection from *S.* Typhimurium dissemination (28). A second arm of the host response is the secretion of interleukin 22 (IL-22) by innate lymphoid cells and T cells (29). The primary function of IL-22 is to induce the expression of antimicrobial proteins by intestinal epithelial cells, thereby playing a protective role during infection with many mucosal pathogens (29). Nevertheless, recent work from our group has shown that *S.* Typhimurium is resistant to at least some IL-22-mediated antimicrobial responses (30), implying that IL-17 plays a greater role than IL-22 in orchestrating the mucosal barrier to this pathogen.

Here, we employed the streptomycin-treated mouse model of *S.* Typhimurium colitis (31) to determine whether administration of PBA is beneficial to the host during infection with this pathogen. Our results demonstrate that PBA administration has a protective effect, both by modifying the microbiota and by modulating the host response.

MATERIALS AND METHODS

Bacterial strains and growth conditions. IR715 is a fully virulent, nalidixic acid (Nal)-resistant derivative of *S.* Typhimurium wild-type isolate ATCC 14028 (32). In addition to IR715, a derivative of this strain with a mutation in the invasion gene *invA* was also used (33). The strains were grown aerobically at 37°C in Luria-Bertani (LB) broth (per liter: 10 g tryptone, 5 g yeast extract, 10 g NaCl) or on LB agar plates (1.5% Difco agar), unless otherwise noted. Antibiotics were added at the following concentrations (milligrams/liter), as needed: carbenicillin (Carb), 100; nalidixic acid (Nal), 50.

Mouse experiments. C57BL/6 mice were used in our study. Mice were purchased from Taconic Farms or the Jackson Laboratory and raised under specific-pathogen-free conditions in a barrier facility at the University of California Irvine. To determine the effect of PBA administration, groups of mice were orally gavaged daily with 1,000 mg/kg (of body weight) of PBA (4-phenylbutyric acid sodium salt; Scandinavian Formulas, Inc.) in 0.1 ml of phosphate-buffered saline (PBS) solution for 7 days and, if applicable, throughout *S.* Typhimurium infection. This dose was used in previous studies, and it is comparable to the dose used in humans (17, 18). Groups of mice were orally gavaged with 0.1 ml of a 200-mg/ml streptomycin-sterile water solution 1 day prior to oral infection with 1×10^9 CFU of *S.* Typhimurium in 0.1 ml LB broth. The cecum was harvested for mRNA, protein, and histopathology at 24 to 96 h postinfection (p.i.). The colon content was collected, serially diluted, and plated on appropriate antibiotic LB agar plates to determine bacterial counts. Spleen, liver, mesenteric lymph nodes (MLN), Peyer's patches (PP), terminal ileum (TI), and colon were collected, weighed, homogenized, serially diluted, and plated on appropriate antibiotic LB agar plates to determine bacterial counts. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of California Irvine.

Isolation of colon crypts. PBA- or mock-treated C57BL/6 mice were streptomycin treated and then either infected with *S.* Typhimurium or mock infected and sacrificed at 24 to 96 h postinfection. Crypt isolation from colon and cecum was performed as described previously (34, 35).

Cell extraction from large intestine, antibody staining, and flow cytometry analysis. Large intestine (colon and cecum) was collected and kept in Iscove's modified Dulbecco's medium (IMDM; 10% fetal bovine serum [FBS], 1% antibiotic/antimycotic) at 4°C until further processing. Next, the intestine was cut open longitudinally and washed in "wash solution" (15 mM HEPES-1% antibiotic/antimycotic in 1× Hanks balanced salt solution [HBSS]; all from Invitrogen) until the supernatant appeared clear. Then, the intestine was shaken in 10 ml of 1× HBSS-15 mM HEPES-5 mM EDTA-10% FBS solution at 37°C in a water bath for 15 min. Supernatant was removed and kept on ice. Remaining tissue was cut into small pieces; run once on a GentleMACS dissociator (Miltenyi Biotech) using the program spleen04; and digested in a 10-ml mixture of collagenase (Sigma; type VII; 1 mg/ml), Liberase (20 μg/ml), and DNase (0.25 mg/ml) in IMDM for 15 min in a shaking water bath, followed by another run on the GentleMACS dissociator (program spleen04). Afterwards, both fractions were strained through a 70-μm cell strainer (BD Biosciences) and pooled, followed by cell counting using a hemocytometer. Extracted large intestinal cells (2×10^6 to 4×10^6) were used for flow cytometry staining. Briefly, unspecific binding was reduced by blocking with a CD16/32 antibody (eBioscience), and cells were stained with the viability marker eFluor780 (eBioscience), followed by extracellular staining using the following monoclonal antibodies: CD11b (clone M1/70), Ly-6G (clone 1A8), CD3 (clone 17A2), and CD4 (clone RM4-5) (all from eBioscience). Afterward, cells were fixed and permeabilized according to the manufacturer's instructions (eBioscience "Fix and Perm" kit) and stained intracellularly with an IL-17 antibody (clone eBio17B7; eBioscience). Data were acquired on an LSRII flow cytometer (BD Biosciences, San Jose, CA) and analyzed with FlowJo software (TreeStar, Ashland, OR). Mean fluorescence intensity (MFI) for IL-17 was determined as previously described (36, 37) and calculated as fold change over uninfected samples.

Analysis of microbiota. The composition of the bacterial microbiota was analyzed as previously described (35, 38, 39). Briefly, the colon content was collected from mice after 7 days of PBA administration and at 96 h postinfection and snap-frozen in liquid nitrogen. The DNA was subsequently extracted using the QIAamp DNA stool kit (Qiagen), according to the manufacturer's instructions with the following modifications. In a 1.5-ml reaction tube, a fecal sample was added to 300 mg of 0.1-mm-diameter glass beads in 700 μl of stool lysis buffer, and the tube was vortexed for 20 min and then shaken vigorously for 10 min at 25 Hz in a Retsch CryoMill (without the use of liquid nitrogen). The sample was then heated for 5 min at 95°C and centrifuged, and the supernatant was collected. Seven hundred microliters of ASL buffer was added to the pellet of glass beads and fecal material for a second round of extraction. Supernatant from this step was combined with the supernatant from the first

extraction and was processed according to the QIAamp DNA stool kit instructions. With this method, we were able to extract DNA from low-abundance species like those of *Bifidobacterium* (30). Two microliters of extracted bacterial DNA was used as a template for quantitative PCR (qPCR) with the primer pairs developed by Barman et al. (38). The 16S gene copy numbers per microliter of DNA from each sample (one fecal pellet collected from each colon) were determined using the plasmids described by Barman et al. and Winter et al. (38, 39).

Bacterial growth in medium supplemented with PBA. The *S.* Typhimurium wild type (IR715) was tested for its ability to grow under nutrient-rich conditions (LB) and nutrient-limiting conditions (M9 minimal medium; per liter: 7.5 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 0.1 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.2% glucose) supplemented with or without PBA. Inocula were prepared from overnight cultures of the strains grown aerobically in LB broth at 37°C with agitation. Absorbance ($\lambda = 600$ nm) of the overnight cultures was determined by spectrophotometry and used to calculate the volume required to obtain 10^9 cells. To prepare the LB inoculum, 10^9 cells were harvested by centrifugation, resuspended in 1 ml of LB broth, and serially diluted. To prepare M9 minimal medium inoculum, 10^9 cells were washed twice in M9 medium, resuspended in 1 ml of M9 medium, and serially diluted. LB or M9 minimal medium was inoculated with a starting culture of approximately 10^4 cells/ml. Growth was monitored by determining the number of CFU per milliliter of culture after 0, 2, 5, 8, and 24 h of incubation at 37°C with agitation. Each experiment was repeated three times.

Invasion assay. For the invasion assay, T84 colonic adenocarcinoma epithelial cells (ATCC CCL-248) were cultured in Dulbecco's modified Eagle's medium–F-12 (DMEM–F-12) with glucose (4.5 g/liter) supplemented with 10% bovine serum (Invitrogen). Approximately 2.5×10^5 cells were seeded per well in 24-well plates and were either treated with 1 mM PBA for 48 h or mock treated. Then, T84 cells were infected with the indicated *S.* Typhimurium strains at a multiplicity of infection (MOI) of 10 and incubated for 1 h at 37°C in 5% CO_2 to allow bacterial invasion. To remove extracellular bacteria, cells were washed three times with sterile PBS and were incubated with medium containing gentamicin (0.1 mg/ml) for 1 h. Next, cells were lysed with 1 ml of 1% Triton X-100, and 10-fold serial dilutions were plated on LB agar plates, containing the appropriate antibiotics, to quantify the intracellular bacteria.

Analysis of cytokine secretion by macrophages. For analysis of cytokine secretion, THP-1 monocytes (ATCC TIB-202) were cultured in RPMI 1640 supplemented with 10% bovine serum (Invitrogen). For the induction of macrophage differentiation, THP-1 cells (1×10^6 per ml) were cultured in RPMI 1640 supplemented with 10% bovine serum and 200 nM phorbol myristate acetate (PMA) for 48 h. Cells were then washed twice with sterile PBS, cultured in fresh medium with or without 1 mM PBA for 48 h, and infected with *S.* Typhimurium strains (MOI of 1) for 1 h at 37°C in 5% CO_2. To remove extracellular bacteria, cells were washed three times with sterile PBS and then incubated with medium containing 0.1 mg/ml of gentamicin for 1 h, followed by an overnight incubation with fresh medium containing 0.040 mg/ml of gentamicin. The cell culture supernatant was collected 24 h postinfection, and the levels of IL-23 and IL-1β were quantified by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (eBioscience).

Quantitative real-time PCR. Total RNA was extracted from mouse cecal tissue using Tri reagent (Molecular Research Center). Reverse transcription of 1 μg of total RNA was performed using the Transcriptor first-strand cDNA synthesis kit (Roche). Quantitative real-time PCR (qRT-PCR) for the expression of *Actb*, *Il17*, *S100a8*, *S100a9*, *Nos2*, *Reg3g*, *Cramp*, *Il1b*, *Bip*, *Grp94*, *Chop*, and $P58^{IPK}$ was performed using the LightCycler 480 SYBR green master mix on the LightCycler 480 II (Roche). Primer pair sequences are shown in Table S1 in the supplemental material. Conditions for qRT-PCR were 95°C for 5 min and then 45 cycles of 95°C for 10 s, 60°C for 10 s, and 72°C for 15 s. Gene expression was normalized to *Actb* (β-actin), and fold changes in gene expression were relative to uninfected controls and calculated using the threshold cycle ($\Delta\Delta C_T$) method.

Western blot analysis. Total protein was extracted from mouse cecum using Tri reagent, resolved by SDS-PAGE, and transferred to a polyvinylidene difluoride (PVDF) membrane. Detection of mouse α/β-tubulin was performed with primary rabbit polyclonal antibodies (Cell Signaling Technology), while detection of calprotectin was performed with polyclonal goat anti-mouse S100A8 and polyclonal goat anti-mouse S100A9 antibodies (R&D Systems). Lipocalin-2 was detected by polyclonal goat anti-mouse antibodies (R&D Systems), and myeloperoxidase (MPO) was detected using a primary polyclonal goat anti-human and mouse antibody (R&D Systems). IL-1β was detected by a polyclonal rabbit anti-mouse antibody (Cell Signaling Technologies), CRAMP was detected using a primary polyclonal rabbit anti-mouse antibody (Innovagen), and BiP was detected using a primary polyclonal rabbit anti-human and mouse antibody (Cell Signaling Technologies). As secondary antibodies, goat anti-rabbit and rabbit anti-goat conjugates to horseradish peroxidase (HRP) (Jackson ImmunoResearch) were used. After washing, bands were developed using the Immobilon Western Luminol reagent and peroxide solution (Millipore) per the manufacturer's instructions and visualized using a Fujifilm LAS 4000 imaging system. Western blot bands were quantified using Fuji ImageGauge/MultiGauge software (Fujifilm).

Histopathology. Tissue samples from cecum and liver were fixed in formalin, processed according to standard procedures for paraffin embedding, sectioned at 5 μm, and stained with hematoxylin and eosin. The pathology score of cecal and liver samples was determined by blind examinations of cecal and liver sections from a board-certified pathologist using previously published methods (31, 40). Each cecal section was evaluated for the presence of neutrophils, mononuclear infiltrate, submucosal edema, surface erosions, inflammatory exudates and cryptitis. Inflammatory changes were scored from 0 to 4 according to the following scale: 0, none; 1, low; 2, moderate; 3, high; 4, extreme. The inflammation score was calculated by adding up all of the scores obtained for each parameter and interpreted as follows: 0 to 2, within normal limit; 3 to 5, mild; 6 to 8, moderate; 8+, severe. Liver sections were evaluated by qualitative assessment scoring the relative number of liver lesions and score (size) of liver lesions and size of these microabscesses, 1 being smallest and 5 being most severe.

Statistical analysis. Statistical analysis was performed by using GraphPad Prism 6. Differences between treatment groups were determined by using an unpaired, two-tailed Student's *t* test or a Mann-Whitney-Wilcoxon test. A *P* value equal to or below 0.05 was considered statistically significant.

RESULTS

Sodium phenylbutyrate administration alters the intestinal microbiota composition and yields an increase in segmented filamentous bacteria. Both diet and dietary supplements can shape the composition of the intestinal microbiota, which in turn modulates the immune system and determines resistance or susceptibility to infection (reviewed in reference 41). As supplementation with PBA was shown to provide benefit to the host during intestinal infection with *Shigella flexneri* (16) and in a dextran sodium sulfate (DSS) colitis model (12), we first set out to investigate whether PBA supplementation alters the composition of the intestinal microbiota.

To this end, groups of C57BL/6 Taconic mice were either mock treated or treated with oral PBA daily. After 1 week, the colon content was collected and fecal bacterial DNA was extracted to analyze the major intestinal phyla (*Bacteroidetes* and *Firmicutes*) by 16S rRNA gene phylotyping, as previously described (35, 38, 39). We found that administration of PBA caused an overall trend toward an increase in bacterial density, albeit not a statistically

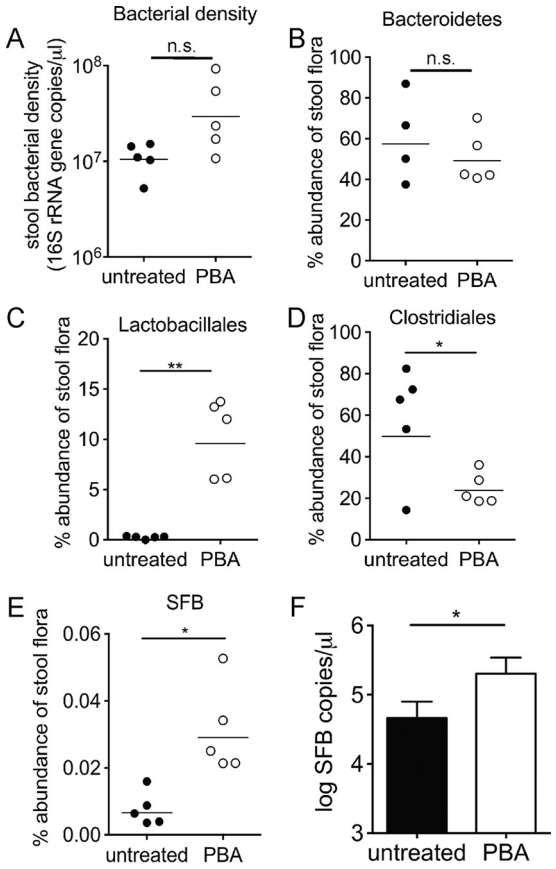

FIG 1 PBA modulates the intestinal microbiota in mice that are colonized with segmented filamentous bacteria. Analysis of the fecal microbiota using 16S rRNA gene qPCR. DNA from fecal bacteria was isolated from C57BL/6 mice (Taconic) that were either untreated or treated with sodium phenylbutyrate (PBA) via oral gavage for 7 days. (A) Bacterial density measured as 16S rRNA gene copies per microliter. (B to E) Percent abundance of *Bacteroidetes* (B), *Lactobacillales* (C), *Clostridiales* (D), and segmented filamentous bacteria (SFB) (E) in the feces of PBA-treated (white circles) and untreated (black circles) mice. (F) Copy numbers per microliter of SFB in the feces of PBA-treated (white bars) and untreated (black bars) mice. (A to E) Each circle represents one individual mouse; horizontal bars represent the geometric mean. Significant differences between PBA-treated and untreated groups are indicated by * (P value $\leq$ 0.05) or ** (P value $\leq$ 0.01). n.s., not significant. Data are representative of three replicate experiments (n = 5 mice per group).

significant one (Fig. 1A). Although no changes were detectable in the absolute or relative abundance of the phylum *Bacteroidetes* (Fig. 1B; see also Fig. S1A in the supplemental material), significant changes were observed within the phylum *Firmicutes*. Specifically, *Lactobacillales*, generally regarded as beneficial microbes, represented only 0.25% of *Eubacteria* in untreated mice but increased to approximately 9.5% in PBA-treated mice ($P \leq 0.01$) (Fig. 1C). An increase in the absolute levels of *Lactobacillales* was also observed in the 16S copy numbers, which were on average 1.23×10^4/μl in untreated mice but increased to 3.63×10^6/μl in PBA-treated mice ($P \leq 0.05$) (see Fig. S1A). In contrast to *Lactobacillales*, *Clostridiales* represented 49.8% of *Eubacteria* in untreated mice but decreased to approximately 23.8% in PBA-treated mice ($P \leq 0.05$) (Fig. 1D). Of note, the absolute levels of *Clostridiales* did not change with PBA treatment (see Fig. S1A). We also found that bacteria of the *Enterobacteriaceae* family, which are usually detected at very low levels in the absence of intestinal inflammation, were low or undetectable in both groups (data not shown).

Next, we tested whether PBA treatment would change the abundance of segmented filamentous bacteria (SFB)—commensal organisms, unculturable outside the mouse gut until recently (42)—which have been shown to specifically modulate mucosal immunity and in particular to induce the development of mucosal Th17 cells (43, 44). Th17 cells produce the cytokine interleukin 17 (IL-17), which in turn orchestrates a response that is protective against mucosal pathogens, including *Citrobacter rodentium* and *S.* Typhimurium (reviewed in reference 45). Mice obtained from some Taconic sites are colonized with SFB, develop mucosal Th17 cells, and are more resistant to *C. rodentium* infection than mice that are not colonized with SFB (e.g., mice from the Jackson Laboratory) (43). Consistent with this earlier study, the mice that we received from Taconic were also colonized with SFB. We found that both the relative abundance and the 16S copy numbers of SFB significantly increased in the feces of mice treated with PBA ($P \leq 0.05$) (Fig. 1E and F; see also Fig. S1A in the supplemental material). Collectively, these results show that PBA administration modulates the composition of the gut microbiota; in particular, PBA administration was associated with an increase in commensal organisms such as SFB, which are known to shape mucosal immunity and modulate the host response to infection by inducing Th17 cells (43, 44).

Administration of sodium phenylbutyrate increases IL-17 production by intestinal cells in mice colonized with SFB. Because we observed a significant increase in the abundance of SFB in Taconic mice treated with PBA, we investigated whether *Il17* expression was also increased in the cecum of these mice. Consistent with our prediction, we observed an approximately 21-fold increase in IL-17 mRNA expression in response to PBA treatment ($P \leq 0.05$) (Fig. 2A). Of note, the expression levels of other proinflammatory cytokines and antimicrobial proteins analyzed (including *Ifng*, *Il22*, *S100a8*, *S100a9*, *Cramp*, and *Lcn2*) were similar in untreated and PBA-treated mice (data not shown), suggesting that PBA treatment specifically modulated the expression of IL-17.

To further confirm the increase of IL-17, we next measured the relative and absolute numbers of cells that produce IL-17 in the large intestine. Although similar numbers of IL-17-producing cells were observed in both untreated and PBA-treated Taconic mice (Fig. 2B and C), IL-17$^+$ large intestinal cells isolated from PBA-treated mice produced more IL-17 on average ($P \leq 0.001$) (Fig. 2D). The observed increase in IL-17 production in mice treated with PBA was consistent with the observed increase of *Il17* mRNA expression in the cecum (Fig. 2A). Altogether, these results suggest that the increase of SFB, in conjunction with the increase of IL-17 production in the gut, could enhance resistance to infection with mucosal pathogens in PBA-treated mice.

Sodium phenylbutyrate treatment reduces *Salmonella* intestinal colonization and extraintestinal dissemination in mice colonized with SFB. As PBA treatment induced an increase in IL-17 levels in the large intestine, we next investigated whether the compound would also reduce the severity of disease in mice infected with *S.* Typhimurium. To this end, we used the streptomycin-treated mouse model, in which mice develop cecal and colonic inflammation (31, 40). Taconic mice were either treated with PBA for 1 week prior to *S.* Typhimurium infection and during the

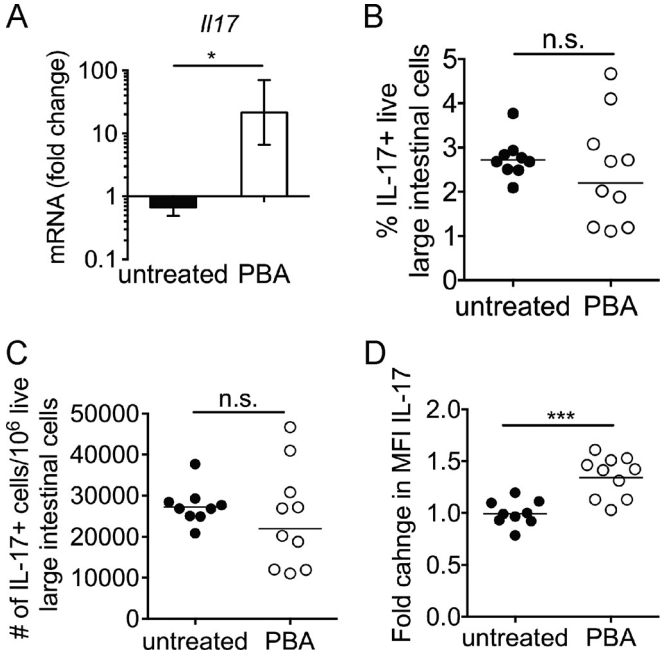

FIG 2 PBA induces an increase of IL-17 production by intestinal cells in mice that are colonized with SFB. (A) Relative *Il17* mRNA expression in the large intestine of mice treated with PBA (white bar, $n = 4$) or untreated (black bar, $n = 4$). (B to D) Enumeration of IL-17$^+$ intestinal cells by flow cytometry. Percentage (B), absolute quantification (C), and median fluorescence intensity (MFI) (D) of live IL-17$^+$ intestinal cells isolated from PBA-treated (white circles, $n = 9$) and untreated (black circles, $n = 10$) mice. Bars (A) represent the geometric mean ± standard error. Horizontal bars (B to D) represent the geometric mean. Significant differences between PBA-treated and untreated groups are indicated by * (P value ≤ 0.05) or *** (P value ≤ 0.001). n.s., not significant. Data are representative of three replicate experiments.

course of infection or left untreated (Fig. 3). At 24, 48, 72, and 96 h postinfection (p.i.), we collected fecal samples in order to assess the levels of *S.* Typhimurium colonization in the large intestinal lumen. Remarkably, at every time point during infection, PBA-treated mice showed a significantly smaller amount of *S.* Typhimurium in the colon content than did untreated mice (Fig. 3A; data for 24 h p.i. not shown), indicating that PBA administration reduced *S.* Typhimurium intestinal colonization. We also observed a reduced colonization by *S.* Typhimurium in the colon but not in the Peyer's patches or in the terminal ileum of mice treated with PBA (Fig. 3B).

As the *S.* Typhimurium intestinal burden was reduced, we next analyzed the bacterial burden in the mesenteric lymph nodes, spleen, and liver to assess whether PBA treatment also impacted *S.* Typhimurium dissemination. In these organs, we also detected lower levels of *S.* Typhimurium, suggesting that PBA administration reduces both the pathogen's intestinal colonization and its dissemination to extraintestinal sites (Fig. 3C).

Sodium phenylbutyrate reduces the severity of disease during *S.* Typhimurium infection in mice colonized with SFB. In light of the findings that *S.* Typhimurium colonization and dissemination are decreased in Taconic mice that received oral PBA, we hypothesized that the overall disease in PBA-treated mice infected with *S.* Typhimurium would be less severe. Consistent with our prediction, PBA-treated mice lost less weight than did untreated mice during the course of the infection ($P \leq$ 0.05) (Fig. 4A), indicating a better disease outcome in this group. A second measure of the severity of *Salmonella* infection is the degree of inflammation in the large intestine, which is characterized by a massive infiltrate of proinflammatory cells, especially neutrophils, and by submucosal edema and epithelial damage (31).

Blind histopathology analysis of cecal tissue from PBA-treated and untreated mice infected with *S.* Typhimurium revealed that untreated mice developed moderate to severe inflammation (average pathology score of 10), whereas PBA-treated mice exhibited lower levels of inflammation (average pathology score of 5), with some mice developing only mild inflammation (Fig. 4B to D). No abnormalities were observed in mock-infected mice, either PBA treated or untreated (Fig. 4C and D and data not shown). As we observed large differences in *S.* Typhimurium colonization between PBA-treated and untreated mice in the liver, we also examined sections of this organ. We counted fewer liver abscesses in *S.* Typhimurium-infected mice treated with PBA than in untreated mice ($P \leq 0.05$), although the sizes of the abscesses were comparable in the two groups (Fig. 4E and F).

To further confirm the reduced levels of intestinal inflammation observed by histopathology, we also isolated immune cells from the large intestine of PBA-treated and untreated mice infected with *S.* Typhimurium (Fig. 5). We found that the percentage and the absolute number of myeloid cells (defined as CD11b$^+$ cells), and in particular of neutrophils (defined as CD11b$^+$ Ly-6G$^+$ cells), were higher in untreated, infected mice than in PBA-treated, infected mice (Fig. 5A to D; see also Fig. S2A and B in the supplemental material). In contrast, the absolute number and percentage of other cells such as CD4$^+$ T cells were not affected by PBA treatment in infected mice (Fig. 5E and F; see also Fig. S2C). Altogether, these results indicated that PBA treatment reduces intestinal inflammation, the recruitment of neutrophils to the gut, and the liver pathology caused by *S.* Typhimurium infection in mice that are colonized with SFB and have Th17 cells in the gut.

Sodium phenylbutyrate reduces the expression of proinflammatory mediators in the cecum of mice colonized with SFB during *S.* Typhimurium infection. To further confirm that PBA treatment reduces intestinal inflammation in mice colonized with SFB, we measured the expression of several proinflammatory mediators in the cecum of PBA-treated and untreated Taconic mice 96 h after infection with *S.* Typhimurium (Fig. 6). Consistent with the histopathology and with the analysis of cellular infiltrate, we observed lower expression of some antimicrobial proteins and proinflammatory cytokines in mice treated with PBA and infected with *S.* Typhimurium than in untreated, infected mice (Fig. 6).

Although expression of the *Lcn2* gene, which encodes the antimicrobial protein lipocalin-2, was not significantly affected by PBA treatment at the time point analyzed (Fig. 6A), lower levels of lipocalin-2 protein were observed in PBA-treated mice infected with *S.* Typhimurium ($P \leq 0.05$) (Fig. 6B and C). Additionally, expression of the *S100a8* and *S100a9* genes, which encode the two subunits of calprotectin, an antimicrobial protein that sequesters zinc and manganese from pathogens (35, 46), was significantly lower in PBA-treated, infected mice ($P \leq 0.01$) (Fig. 6A). Similarly, we detected a trend toward lower levels of both the S100A8 and the S100A9 protein subunits of calprotectin in the cecum of PBA-treated, infected mice (Fig. 6B and C). In contrast to what was observed in rabbits infected with *Shigella* (16), PBA treatment resulted in a lower induction of the antimicrobial peptide CRAMP

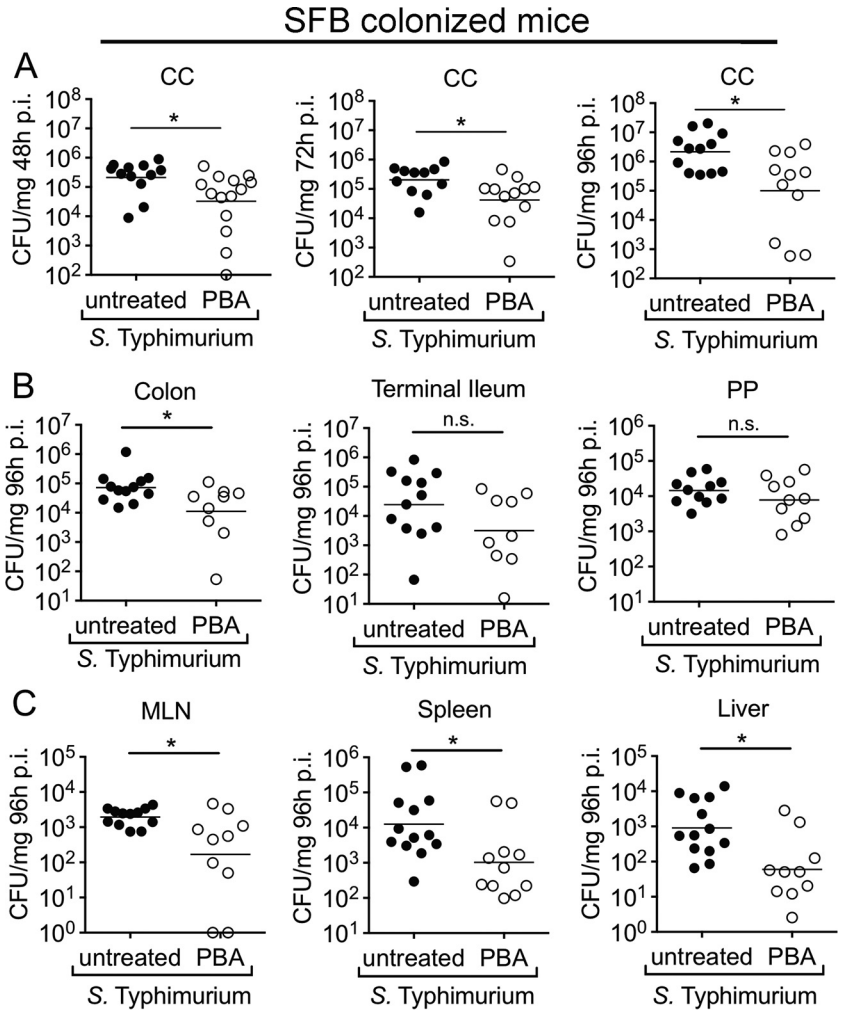

FIG 3 PBA administration reduces *S.* Typhimurium intestinal colonization and extraintestinal dissemination in mice that are colonized with SFB. *S.* Typhimurium numbers were enumerated in the colon content and in organs collected from PBA-treated (white circles, $n = 11$) or untreated (black circles, $n = 12$) mice from Taconic. (A) CFU of *S.* Typhimurium per milligram of colon content (CC) at 48 h (left), 72 h (center), and 96 h (right) postinfection (p.i.). (B) CFU of *S.* Typhimurium per milligram of colon (left), terminal ileum (center), and Peyer's patches (PP; right) collected at 96 h p.i. (C) CFU of *S.* Typhimurium per milligram of the mesenteric lymph nodes (MLN, left), spleen (center), and liver (right) at 96 h p.i. Each circle represents one mouse; horizontal bars represent the geometric mean. A significant difference is indicated by * (P value ≤ 0.05). n.s., not significant.

in the cecum of most *S.* Typhimurium-infected mice, both at the level of mRNA ($P \leq 0.01$) and at the level of protein (of note, one mouse showed increased CRAMP at the protein level) (Fig. 6A and B).

As lipocalin-2, calprotectin, and CRAMP are found in neutrophils, it is possible that the lower levels that we observed in PBA-treated, infected mice were due to the reduced neutrophil influx that we observed by histopathology and single-cell analysis (Fig. 4 and 5) and further confirmed by the trend toward lower levels of myeloperoxidase in this group (Fig. 6B and C). Also consistent with the lower levels of inflammation observed by histopathology, we found that the expression and/or production of some proinflammatory cytokines was also reduced. In particular, although PBA treatment induced an increased production of IL-17 by intestinal cells and of the baseline expression of *Il17* in uninfected mice (Fig. 2A to D), transcription of this cytokine was induced to a lesser extent in infected mice treated with PBA ($P \leq 0.05$) (Fig. 6A). Similarly, a trend toward reduction of mature IL-1β was detected in the cecum of infected mice treated with PBA in comparison to untreated mice (Fig. 6B and C). In contrast, we found that some antimicrobial genes analyzed were expressed at similar levels during infection. For instance, expression of *Reg3g*, which encodes the regenerating islet-derived protein 3 gamma (RegIIIγ), and expression of *Nos2*, which encodes inducible nitric oxide synthase (iNOS), were similarly upregulated in both untreated and PBA-treated mice infected with *S.* Typhimurium (Fig. 6A). Nevertheless, our results generally suggest that PBA treatment reduces the intestinal inflammatory response during *S.* Typhimurium infection.

Sodium phenylbutyrate treatment has limited effect on the mucosal response to *S.* Typhimurium in mice that are not colonized with SFB. To determine whether the observed beneficial effects of PBA administration during *S.* Typhimurium infection were dependent on the presence of Th17 cells and thus of SFB colonization, we administered PBA to mice from the Jackson Laboratory, which are not colonized with SFB and do not develop Th17 cells (43) (Fig. 7). We confirmed that Jackson mice exhibited

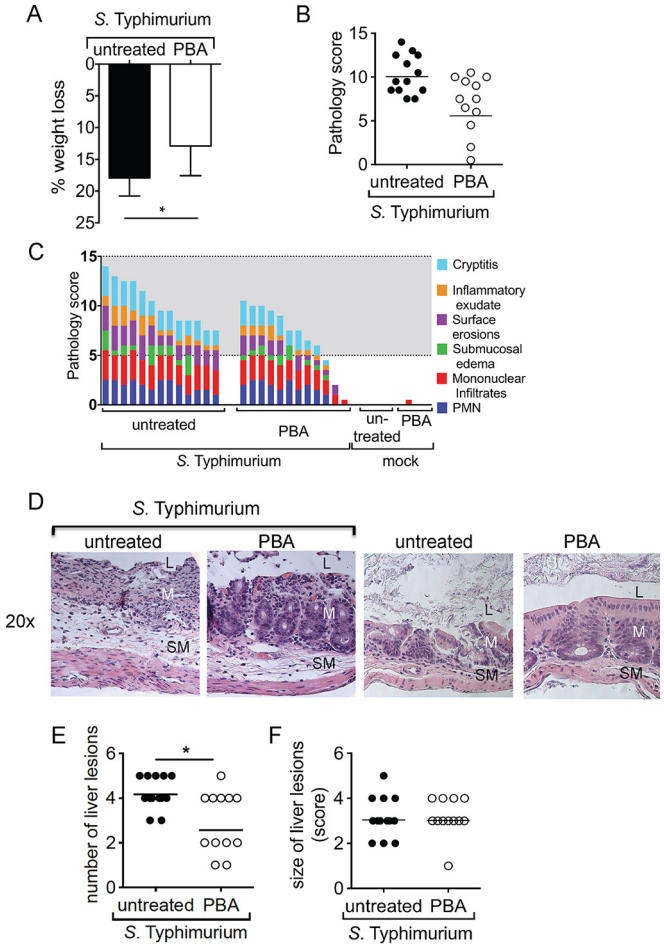

FIG 4 PBA administration reduces the severity of intestinal and liver disease caused by *S.* Typhimurium infection in mice that are colonized with SFB. Weight loss (A) and histopathology (B to F) were evaluated in mice treated with PBA ($n = 12$) or left untreated ($n = 13$). (A) Percentage of weight loss 96 h postinfection in *S.* Typhimurium-infected mice, either treated with PBA (white bar) or untreated (black bar). (B) Overall pathology score of ceca collected from *S.* Typhimurium-infected mice, either treated with PBA (white circles) or untreated (black circles). (C) Pathology score of ceca collected from mock-infected or *S.* Typhimurium-infected mice, either treated with PBA or untreated. Score criteria are shown and include the presence of polymorphonuclear cells (PMN; dark blue bars), mononuclear infiltrates (red bars), submucosal edema (green bars), surface erosions (purple bars), inflammatory exudates (orange bars), and cryptitis (light blue bars). The gray region includes overall scores indicative of moderate to severe inflammation. (D) Hematoxylin- and eosin-stained cecal sections from representative animals in each group at 96 h postinfection. Images at ×20 magnification are shown. L, lumen; M, mucosa; SM, submucosa. (E and F) Number (E) and size (F) of liver lesions from *S.* Typhimurium-infected mice either treated with PBA (white circles) or untreated (black circles). Each circle represents one mouse. Horizontal bars represent the average ± standard error. A significant difference is indicated by * (P value ≤ 0.05).

undetectable levels of SFB (see Fig. S1B in the supplemental material) and of Th17 cells when housed in our barrier facility (data not shown). In contrast to what we observed in SFB-positive mice from Taconic, PBA administration did not induce changes in the gut microbiota composition (see Fig. S1B and S3). Moreover, in contrast to what we observed in Taconic mice (Fig. 3), PBA administration did not result in a reduction of *S.* Typhimurium colonization of the colon content, the colon, or the terminal ileum of Jackson mice (Fig. 7A and B). Nevertheless, PBA administration reduced *S.* Typhimurium colonization in the Peyer's patches, mesenteric lymph nodes, and liver (Fig. 7B and C), indicating that the compound likely promotes the immune response to *S.* Typhimurium at these sites.

The absolute number and percentage of gut myeloid cells were significantly lower in Jackson mice than in Taconic mice but did not change in response to PBA administration (Fig. 5A and B; see also Fig. S2A in the supplemental material). Although the overall numbers and percentage of neutrophils recruited to the gut during *S.* Typhimurium infection were lower in Jackson mice than in Taconic mice, PBA treatment caused a further reduction in the number of neutrophils isolated from the gut (Fig. 5C and D; see also Fig. S2B). Consistent with earlier reports, few $CD4^+$ T cells were detected in the colon of Jackson mice, either untreated or PBA treated, upon infection (Fig. 5E and F; see also Fig. S2C). We also observed that PBA administration had no effect on the induction of proinflammatory cytokines or of antimicrobial proteins in Jackson mice infected with *S.* Typhimurium (see Fig. S4). Collectively, these results indicate that the beneficial effects of PBA administration were limited in the absence of SFB colonization. In particular, a significant reduction of both *S.* Typhimurium intestinal colonization and intestinal inflammation in response to PBA treatment was observed only in mice that were colonized with SFB. Moreover, we observed no differences in *S.* Typhimurium colonization or in the levels of intestinal inflammation when PBA was administered 1 day after *S.* Typhimurium infection to Taconic mice (data not shown), indicating that PBA likely exerts its beneficial effect by modulating the microbiota and the host response prior to infection.

Sodium phenylbutyrate has no effect on the ER stress response induced by *S.* Typhimurium infection in the murine large intestine. PBA has been shown to ameliorate several diseases, including cystic fibrosis, diabetes, and colitis, by reducing ER stress (8–11). In particular, two recent reports showed that PBA administration alleviates ER stress and reduces lipopolysaccharide (LPS)-induced lung inflammation (47) and colitis in mice (12). Based on these studies, we investigated whether one of the mechanisms by which PBA reduces intestinal inflammation in *Salmonella*-induced colitis was the reduction of ER stress during infection.

First, we determined whether *S.* Typhimurium infection would induce ER stress in the gut by analyzing the mRNA expression of ER stress response mediators at 96 h postinfection. As shown in Fig. 8A, we detected a significant induction of the genes *Bip* (*Hspa5*) and *Grp94*, which encode two molecular chaperones that control protein folding and are upregulated when the ER workload and the amount of misfolded protein increase (48) (Fig. 8A, black bars). In contrast, we observed no significant changes in the expression of the chaperone gene $P58^{IPK}$ (*Dnajc3*) or of the gene encoding the transcription factor CHOP. To confirm the qPCR results, we also detected the BiP chaperone protein by Western blotting in cecal tissue and we observed a 1.5- to 2-fold increase of BiP protein in mice infected with *S.* Typhimurium (Fig. 8B and C). Our results thus indicate that some ER stress markers are induced in the cecum of mice infected with *S.* Typhimurium, as was observed in mice during chemically induced colitis (12). However, in contrast to what was found in the DSS colitis model (12), PBA administration did not appear to reduce ER stress in the colon of mice infected with *Salmonella*. Indeed, we observed sim-

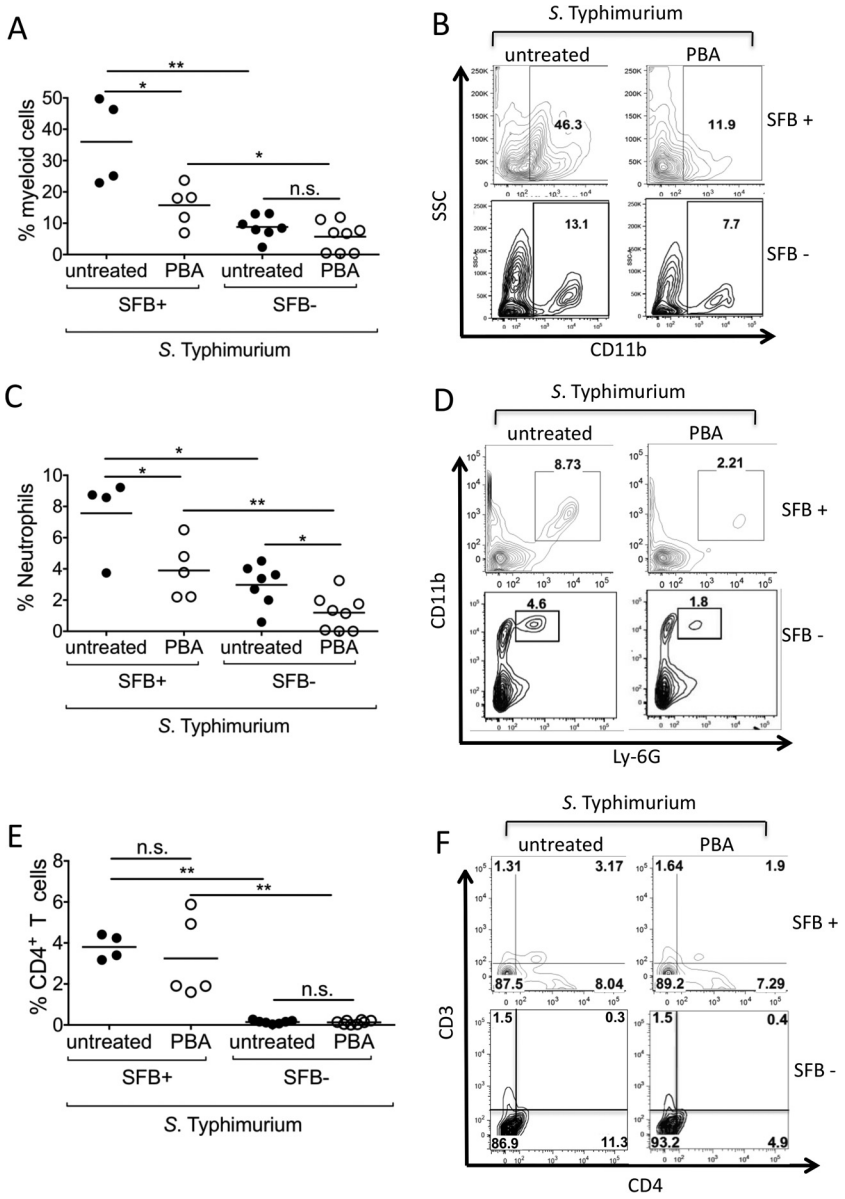

FIG 5 Single-cell analysis of immune cell types in the large intestine of *S.* Typhimurium-infected mice that were positive or negative for SFB colonization. (A, C, and E) Abundance of myeloid ($CD11b^+$) cells (A), neutrophils ($CD11b^+$ $Ly\text{-}6G^+$ cells) (C), and $CD4^+$ T cells ($CD3^+$ $CD4^+$) (E), each shown as percentages of large intestinal cells isolated from *S.* Typhimurium-infected mice that were either treated with PBA (white circles) or untreated (black circles). Gut cells were isolated from mice that were either colonized with segmented filamentous bacteria (SFB^+; $n = 4$ per group) or not (SFB^-; $n = 7$ per group). (B, D, and F) Representative flow cytometry contour plots showing myeloid ($CD11b^+$) cells (B), neutrophils ($Ly\text{-}6G^+$ $CD11b^+$) (D), and $CD4^+$ T cells ($CD3^+$ $CD4^+$) (F). SSC, side scatter. (A, C, and E) Each circle represents one mouse; horizontal bars represent the geometric mean. A significant difference is indicated by * (P value ≤ 0.05) or ** (P value ≤ 0.01). n.s., not significant.

ilar upregulation of *Bip* and *Grp94* in PBA-treated, infected mice (Fig. 8A, white bars, and D). Therefore, the observed reduction of intestinal inflammation during *S.* Typhimurium infection in PBA-treated mice does not appear to be linked to a reduction in the ER stress response.

Sodium phenylbutyrate reduces *S.* Typhimurium invasion of intestinal epithelial cells and production of IL-23 by macrophage-like cells. To gain further insight into the mechanism by which PBA treatment reduces intestinal inflammation during *S.* Typhimurium infection, we tested whether PBA has direct inhibitory effects on the replication of *S.* Typhimurium. We found that addition of PBA to either rich medium (LB broth) or minimal medium (M9 medium plus glucose) did not influence *S.* Typhimurium replication (data not shown), suggesting that a direct effect of PBA against *S.* Typhimurium *in vivo* is unlikely.

Two essential steps during *S.* Typhimurium infection are invasion of epithelial cells and replication in antigen-presenting cells, such as macrophages. We thus investigated whether PBA treatment reduces *S.* Typhimurium invasion of intestinal epithelial cells. To this end, we incubated T84 intestinal epithelial cells either with medium alone or with medium supplemented with

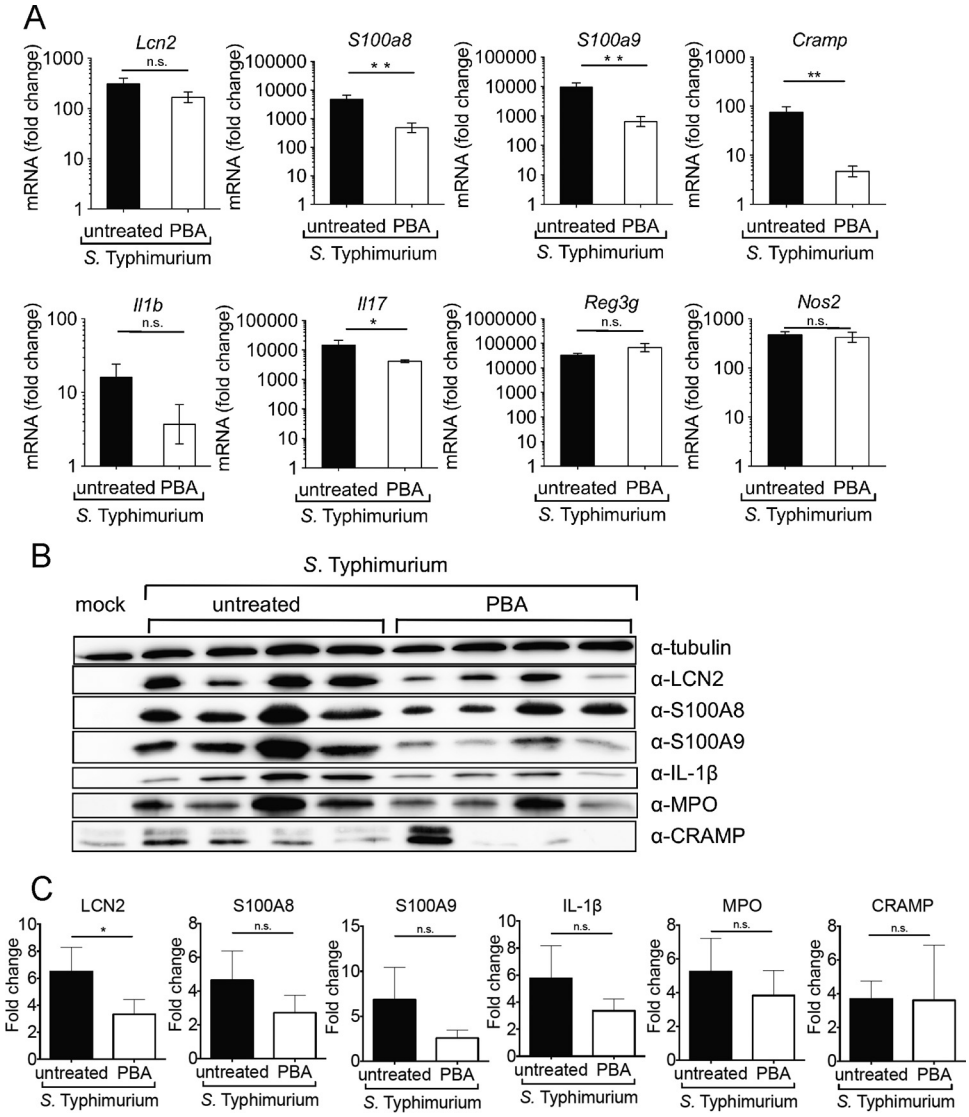

FIG 6 Expression of proinflammatory cytokines and antimicrobial proteins in the cecum of mice colonized with SFB that were infected with *S.* Typhimurium and treated with PBA or untreated. (A) Expression of *Lcn2*, *S100a8*, *S100a9*, *Cramp*, *Il1b*, *Il17*, *Reg3g*, and *Nos2* was detected by qPCR at 96 h post-infection with *S.* Typhimurium in the cecum of Taconic mice treated with PBA (white bars, $n = 4$) or untreated (black bars, $n = 4$). Data are expressed as fold increase over mock-infected mice. Bars represent the geometric mean ± standard error. (B) Tubulin (as a loading control), LCN2, S100A8, S100A9, IL-1β, MPO, and CRAMP were detected by Western blotting at 96 h postinfection in the cecum of mice treated with PBA or untreated. (C) Quantification of bands in panel B, relative to the expression of tubulin. Bars represent the averages ± standard deviations. A significant increase over mock control is indicated by * (P value ≤ 0.05) or ** (P value ≤ 0.01). n.s., not significant.

PBA overnight. Cells were then infected either with wild-type *S.* Typhimurium or with an *S.* Typhimurium mutant defective in invasion (Δ*invA*) for 1 h, followed by gentamicin treatment to kill the extracellular bacteria. We observed that preincubation of T84 cells with PBA reduces *S.* Typhimurium invasion (Fig. 9A), suggesting that this essential process may be partly hindered by PBA administration. These effects were not seen if PBA was removed from the tissue culture medium prior to infection (data not shown).

In addition to epithelial cells, macrophages are also essential for the control of *S.* Typhimurium replication and for the production of proinflammatory cytokines. We thus tested whether PBA influences *S.* Typhimurium interaction with macrophages by infecting macrophage-like THP-1 cells that were cultured in medium supplemented with PBA or were mock treated. We recovered equal numbers of intracellular *S.* Typhimurium at every time point analyzed in both PBA-treated and untreated THP-1 cells, suggesting that both *S.* Typhimurium phagocytosis and replication in macrophages are not affected by PBA administration (Fig. 9B). Despite recovering equal numbers of intracellular *S.* Typhimurium organisms, we found that PBA administration to THP-1 cells reduced the induction of the proinflammatory cytokine IL-23 (Fig. 9C) but not of IL-1β (Fig. 9D). Taken together, these results suggest that the reduced levels of inflammation observed during *S.* Typhimurium infection *in vivo* could be attributed, at least in part, to reduced epithelial invasion and to the direct immunomodulatory effect of PBA on macrophages.

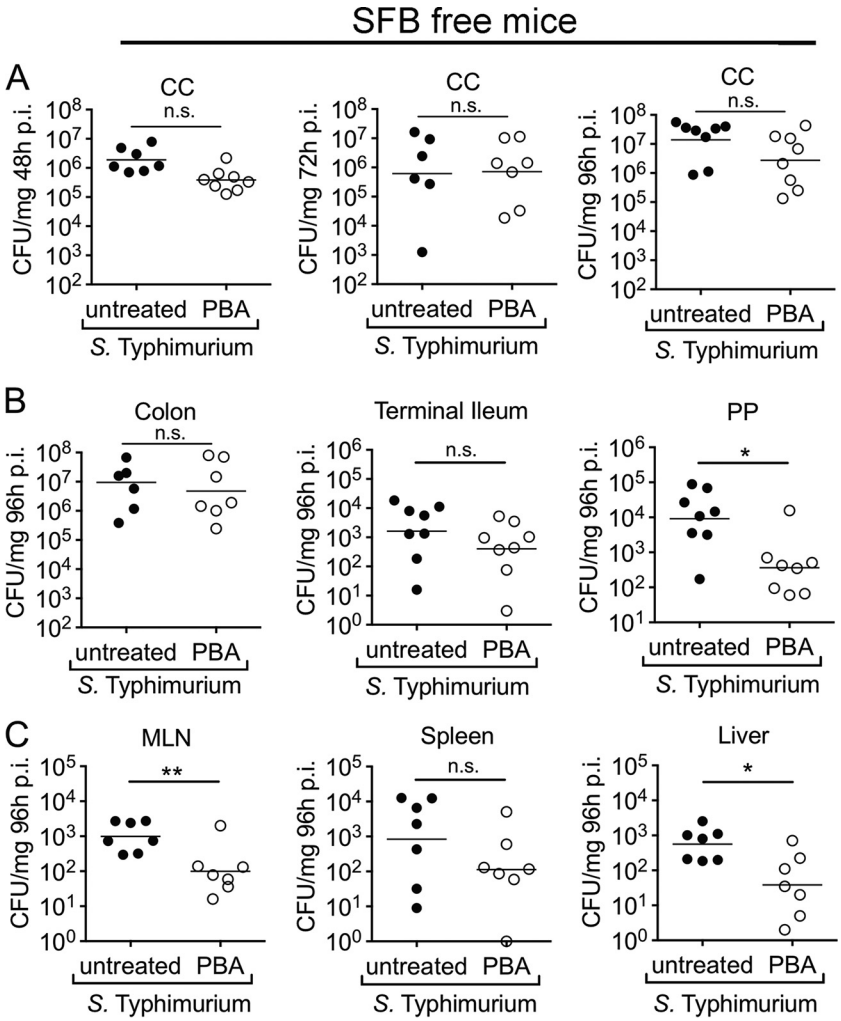

FIG 7 PBA administration has no effect on *S.* Typhimurium intestinal colonization in mice that are not colonized with SFB. *S.* Typhimurium numbers were enumerated in the colon content and in organs collected from PBA-treated (white circles, $n = 8$) or untreated (black circles, $n = 7$) mice from the Jackson Laboratory. (A) CFU of *S.* Typhimurium per milligram of colon content (CC) at 48 h (left), 72 h (center), and 96 h (right) postinfection (p.i.). (B) CFU of *S.* Typhimurium per milligram of colon (left), terminal ileum (center), and Peyer's patches (PP; right) collected at 96 h p.i. (C) CFU of *S.* Typhimurium per milligram of the mesenteric lymph nodes (MLN, left), spleen (center), and liver (right) at 96 h p.i. Each circle represents one mouse; horizontal bars represent the geometric mean. A significant difference is indicated by * (P value ≤ 0.05) or ** (P value ≤ 0.01); n.s., not significant.

DISCUSSION

The treatment of many bacterial infections relies on the use of antibiotics to directly target the causative agents. However, with the awareness that antibiotic use can be detrimental to certain infections and with antibiotic resistance on the rise, the need to develop innovative strategies to combat infections and to improve disease outcome is more important than ever. In this study, we show that the orphan drug PBA, which has been safely used in children and adults for the past 20 years to treat urea cycle disorders (1), provides several beneficial effects to the host during infection with the pathogen *S.* Typhimurium.

In mice treated with PBA, we observed a reduction in intestinal and systemic *S.* Typhimurium colonization, as well as in disease manifestation, including reduced weight loss, reduced colitis severity, and lower expression of proinflammatory cytokines. These advantageous outcomes are consistent with previous studies that illustrated the beneficial effects of PBA treatment during infection with either *Shigella* or *M. tuberculosis* (16, 17), as well as during DSS colitis (12). However, the mechanism by which PBA administration is beneficial during *S.* Typhimurium infection appears to be different than what has been described in these earlier studies.

Specifically, whereas PBA treatment led to increased expression of the antimicrobial protein CRAMP in the colon of rabbits infected with *Shigella* (16), we observed an overall reduction in the levels of CRAMP in the cecum of *S.* Typhimurium-infected mice treated with PBA (Fig. 6). Because CRAMP is highly expressed in neutrophils, our results likely reflect the reduced recruitment of these cells to the cecum of PBA-treated mice (Fig. 4 and 5). Furthermore, in contrast to what was shown in a DSS colitis model (12), PBA treatment did not decrease *S.* Typhimurium-induced ER stress in colon crypts (Fig. 8). Given the broad spectrum of beneficial effects attributed to PBA (21), it is not too surprising that the specific mode of action may vary depending on the disease models employed. Nevertheless, our study agrees with those of

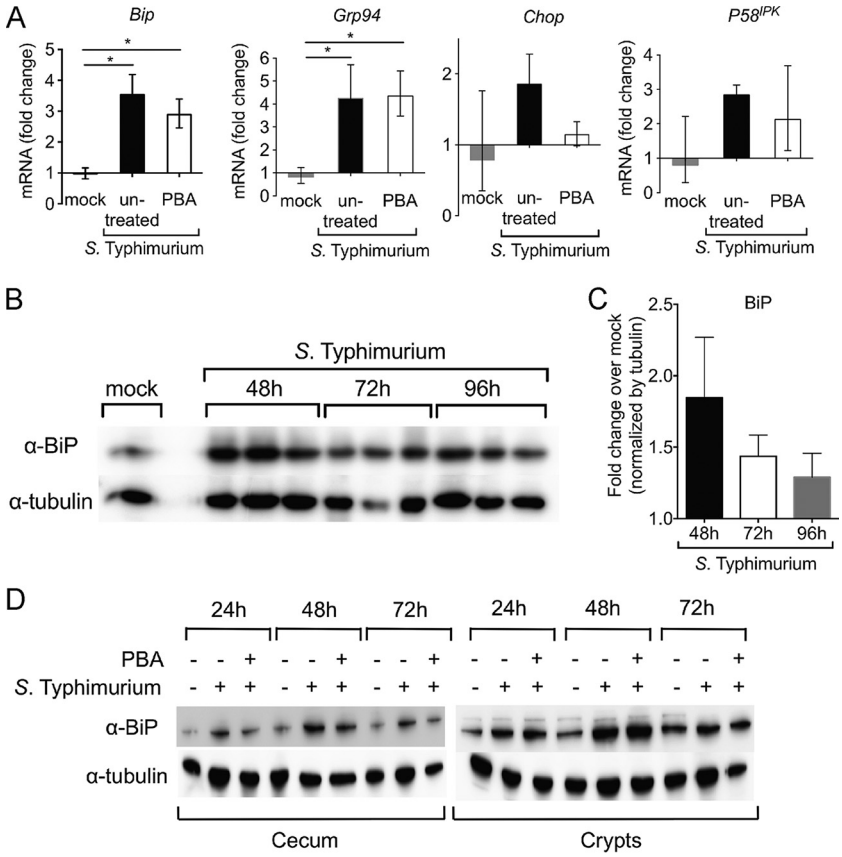

FIG 8 Induction of ER stress markers in mice infected with *S.* Typhimurium, either treated with PBA or untreated. (A) Expression of *Bip*, *Grp94*, *Chop*, and *P58*^{IPK} was detected by qPCR at 96 h postinfection with *S.* Typhimurium in the cecum of mice treated with PBA (white bars, $n = 4$) or untreated (black bars, $n = 4$). Data are expressed as fold increase over mock-infected mice. Bars represent the geometric mean ± standard error. A significant increase over mock control is indicated by * (P value ≤ 0.05). (B) Tubulin and BiP were detected by Western blotting at 48, 72, and 96 h postinfection in the cecum of mice infected with *S.* Typhimurium ($n = 3$). (C) Quantification of bands in panel B, relative to the expression of tubulin. Bars represent the mean ± standard deviation. (D) Tubulin and BiP were detected by Western blotting at 24, 48, and 72 h postinfection in the cecum and in the isolated crypts of uninfected mice, *S.* Typhimurium-infected mice with no PBA treatment, or *S.* Typhimurium-infected mice treated with PBA. One representative sample for each experimental group is shown.

Sarker et al. and Cao et al. on the benefit of PBA administration during colitis (12, 16), although different mechanisms appear to contribute to this effect.

In this study, we identified additional mechanisms that could explain some of the beneficial effects of PBA during *Salmonella* infection. First, PBA administration led to an increase of *Lactobacillales* and, most importantly, of SFB (Fig. 1 and 2), the latter being commensal organisms of the phylum *Clostridiales*, which have been heavily studied in recent years for their immunomodulatory functions (reviewed in references 49 to 52). The most notable effect of SFB is the induction of Th17 cell accumulation in the mouse gut (43). Th17 cells secrete IL-17, an important mediator of the immune response against intestinal pathogens, including *C. rodentium* and *S.* Typhimurium (45). Indeed, mice that are colonized with SFB displayed increased resistance to infection with *C. rodentium* (43). Consistent with these earlier findings, we also observed that in mice treated with PBA, the increase in the SFB abundance was associated with the increase in IL-17 production (Fig. 2). Because IL-17 orchestrates the mucosal response during *S.* Typhimurium infection (25, 28), it is likely that the increase of baseline IL-17 expression explains, at least in part, why mice treated with PBA were less susceptible. In agreement with this hypothesis, mice that were not colonized with SFB did not exhibit alteration of the microbiota, reduced *S.* Typhimurium colonization in the cecum, or reduced inflammation when treated with PBA (Fig. 7; see also Fig. S1 to S4 in the supplemental material). Although it may appear contradictory, the reduced levels of IL-17 and other proinflammatory markers 4 days after infection in PBA-treated mice can be explained by the reduced bacterial burden and the reduced levels of intestinal inflammation. PBA-induced early changes in the microbiota and in IL-17 expression could lead to reduced *S.* Typhimurium colonization and, in turn, lower levels of inflammation.

Two additional benefits of PBA treatment are a partial inhibition of *S.* Typhimurium invasion of epithelial cells and a reduced induction of the proinflammatory cytokine IL-23 in infected macrophage-like cells (Fig. 9). The modest inhibition of *S.* Typhimurium invasion is unlikely to be the sole explanation for the observed reduction in intestinal inflammation and in the pathogen's colonization, because even mice infected with noninvasive mutants develop disease (53). The inhibitory effect on the production of IL-23 and, possibly, of other proinflammatory cytokines may also help explain the reduction in intestinal inflammation observed in PBA-treated, infected mice. These findings are also in agreement with

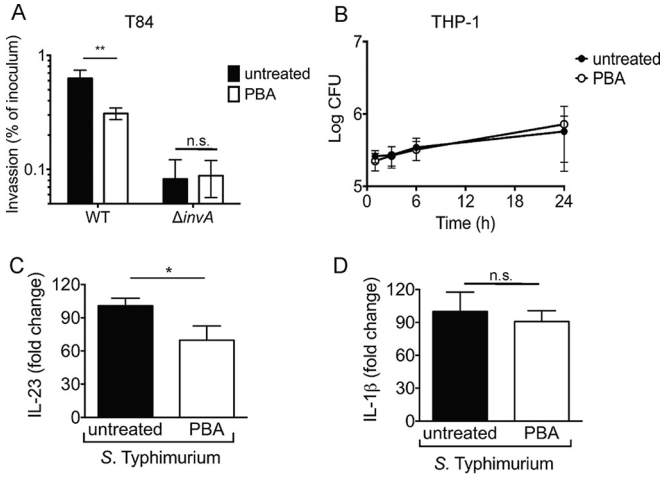

FIG 9 PBA partly inhibits *S.* Typhimurium invasion of intestinal epithelial cells and the expression of the proinflammatory cytokine IL-23 by macrophages *in vitro*. (A) Invasion of T84 epithelial cells either treated with PBA (white bars) or untreated (black bars). WT, *S.* Typhimurium wild type; Δ*invA*, *S.* Typhimurium *invA* mutant. (B) Replication of *S.* Typhimurium in macrophage-like THP-1 cells either treated with PBA (white circles) or untreated (black circles). (C and D) Fold change of IL-23 secretion (C) and of IL-1β secretion (D) in macrophage-like THP-1 cells either treated with PBA (white bars) or untreated (black bars). Bars (A, C, and D) and circles (B) represent the geometric mean ± standard error. A significant difference is indicated by * (P value ≤ 0.05) or ** (P value ≤ 0.01). n.s., not significant.

an earlier study, which showed that PBA treatment elicits an anti-inflammatory effect on mouse macrophages by inhibiting NF-κB and extracellular signal-regulated kinase (ERK) signaling pathways (13). Nevertheless, the precise activity of PBA on the expression of cytokines *in vivo* needs further elucidation.

Altogether, our study shows that PBA administration yields multiple beneficial effects, including changes of the microbiota composition, the increase of IL-17 production by gut cells, the reduction of *Salmonella* epithelial cell invasion, and a reduction of proinflammatory cytokine production, which collectively contribute to the reduction of *Salmonella* colonization, dissemination, and intestinal inflammation. Future studies are warranted to elucidate the precise mechanisms of PBA's mode of action on the host immune response and on the microbiota.

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