AKADÉMIAI KIADÓ

European Journal of Microbiology and Immunology

13 (2023) 2, 45–56

DOI:
10.1556/1886.2023.00024

ORIGINAL RESEARCH PAPER

Not only for Christmas: Prophylactic oral application of *trans*-cinnamaldehyde alleviates acute murine campylobacteriosis

MARKUS M. HEIMESAAT*, SORAYA MOUSAVI, DENNIS WESCHKA and STEFAN BERESWILL

Gastrointestinal Microbiology Research Group, Institute of Microbiology, Infectious Diseases and Immunology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, 12203 Berlin, Germany

Received: August 7, 2023 • Accepted: August 21, 2023
Published online: September 1, 2023

ABSTRACT

The prevalence of *Campylobacter jejuni* infections is increasing worldwide and responsible for significant morbidities and socioeconomic expenses. The rise in antimicrobial resistance of *C. jejuni* underscores the urge for evaluating antibiotics-independent compounds as therapeutic and preventive treatment options of human campylobacteriosis. Given its well-known anti-microbial and immune-modulatory properties we here surveyed the disease-modifying effects of *trans*-cinnamaldehyde pretreatment in experimental campylobacteriosis. Therefore, secondary abiotic IL-10$^{-/-}$ mice were orally challenged with *trans*-cinnamaldehyde starting 7 days prior *C. jejuni* infection. Whereas gastrointestinal colonization properties of the enteropathogens remained unaffected, *trans*-cinnamaldehyde pretreatment did not only improve clinical signs in infected mice, but also alleviated colonic epithelial cell apoptosis on day 6 post-infection. Furthermore, *trans*-cinnamaldehyde application resulted in less pronounced T cell responses in the colon that were accompanied by dampened proinflammatory mediator secretion in distinct intestinal compartments. Notably, the immune-modulatory effects of *trans*-cinnamaldehyde were not restricted to the intestinal tract but could also be observed in extra-intestinal organs such as the liver and kidneys. In conclusion, our preclinical placebo-controlled intervention study provides first evidence that due to its immune-modulatory effects, *trans*-cinnamaldehyde constitutes a promising prophylactic option to alleviate campylobacteriosis.

KEYWORDS

trans-Cinnamaldehyde, enteropathogenic infection, *Campylobacter jejuni*, immune-modulatory effects, secondary abiotic IL-10$^{-/-}$ mice, experimental campylobacteriosis model, host-pathogen interaction, preclinical placebo-controlled intervention study, natural antibiotics-independent compounds

INTRODUCTION

Campylobacter jejuni are highly motile rod-shaped bacteria and can be commonly found as commensal residents in the intestinal tract of warm-blooded vertebrates, including livestock such as poultry, that usually do not develop *C. jejuni* induced clinical signs [1, 2]. Humans, however, can become infected by *C. jejuni* upon ingestion of contaminated meat products or surface water and develop campylobacteriosis after an incubation period of 2–5 days [3]. The clinical course of the disease and the severity of symptoms depend on the arsenal of virulence factors expressed by the enteropathogen on one side, and by the immunological fitness of the infected host on the other [4]. Campylobacteriosis patients can present with general malaise, nausea and vomiting, abdominal pain, fever, and watery to bloody diarrhea with mucous discharge, for instance [3, 5]. Whereas symptomatic measures such as non-steroidal analgesic and antipyretic medications, rehydration and electrolyte substitution are indicated in

*Corresponding author.
Gastrointestinal Microbiology Research Group, Institute of Microbiology, Infectious Diseases and Immunology, Charité - Universitätsmedizin Berlin, Hindenburgdamm 30, D-12203 Berlin, Germany.
Tel.: +49 30 450524318.
E-mail: markus.heimesaat@charite.de

AKJournals

immune-competent individuals, antibiotics such as macrolides and fluoroquinolones might be prescribed in severe and invasive disease affecting multimorbid immune-compromised patients with bacteremia [6]. *C. jejuni* infected individuals usually fully recover from enteritis within two weeks post-infection (p.i.) [3, 7]. In rare cases, however, post-infectious autoimmune diseases such as the Guillain-Barré syndrome (GBS), reactive arthritis (RA), irritable bowel syndrome (IBS), and chronic inflammatory bowel diseases (IBD) can occur with a latency of weeks to months following the initial enteritis [8–12]. Notably, the risk for the development of such post-infectious collateral damages of *C. jejuni* induced disease directly correlates with the severity of the initial enteritis event [13]. The *C. jejuni* cell wall constituent lipo-oligosaccharide (LOS) could be identified as the main immunopathological factor determining the severity of campylobacteriosis with distinct sialylated LOS variants being responsible for particularly severe and invasive diseases [13]. It would be therefore highly appreciable to develop alternative antibiotics-independent treatment options for human campylobacteriosis. Patients would not only benefit from direct disease-alleviating effects but also, in consequence, from a reduced risk for the development of post-infectious sequelae later-on.

Cinnamaldehyde constitutes a biologically active constituent of cinnamon being part of the Lauraceae family with approximately 250 *Cinnamomum* species [14] that is isolated from the bark essential oil of the ancient tropic plant and gives cinnamon its odor and flavor [15, 16]. For long, cinnamaldehyde has been used not only as culinary spice, but also as phytomedical compound in traditional medicine given its various health-promoting and disease-alleviating properties. For instance, cinnamaldehyde is known to exert anti-oxidant [17] and anti-inflammatory [18], but also anti-diabetic and lipid-lowering [19] and even anti-tumor [20] effects. Furthermore, cinnamaldehyde was shown to act neuro-protective in Parkinson's and Alzheimer's diseases [21]. Previous studies reported that cinnamaldehyde could exert anti-bacterial effects *in vitro* [22], that were also directed against food-borne pathogens such as *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *C. jejuni*, for instance [23, 24]. Regarding the molecular mechanisms underlying the anti-bacterial effects of cinnamaldehyde directed against *E. coli* a previous study revealed that cinnamaldehyde-derived metabolites interacted with distinct bacterial cell membrane components inducing oxidative stress and ultimately cell death upon diffusion into the cell [25]. Furthermore, cinnamaldehyde was shown to modify the lipid profile of the bacterial membrane and to inhibit cell division [26].

Given this plethora of health-promoting properties, we tested *trans*-cinnamaldehyde for its disease-alleviating including anti-bacterial and immune-modulatory (i.e., anti-inflammatory) effects in secondary abiotic IL-10$^{-/-}$ mice as valid *C. jejuni* infection and inflammation *in vivo* model [27, 28]. The preceding murine gut microbiota depletion upon antibiotic treatment helped to overcome the physiological colonization resistance and hence, enabled the enteropathogens to stably colonize the gastrointestinal tract of the secondary abiotic IL-10$^{-/-}$ mice [27–29]. Notably, conventional wildtype mice are approximately 10,000-times more resistant to Toll-like receptor-4 (TLR-4) ligands such as lipo-polysaccharide (LPS) and LOS preventing them from severe *C. jejuni* induced intestinal inflammation [30]. The *il10* gene deficiency, however, rendered the orally challenged mice susceptible to the enteropathogenic LOS. In consequence, *C. jejuni* infection of IL-10$^{-/-}$ mice has been shown to result in pronounced proinflammatory immune responses not only in the intestinal tract but also in extra-intestinal and even systemic compartments and to mount in severe enteritis with bloody diarrhea and wasting symptoms within less than a week p.i. [27, 28]. Our previous studies addressing the disease-alleviating effects of urolithin-A [31], of activated charcoal [32], of the iron-chelating compound deferoxamine [33], of essential oils derived from garlic [34], cardamom [35], cumin [36], clove [37], coriander, and lemon [38], of phenolic compounds such as carvacrol [39], resveratrol [40], and curcumin [41], of organic acids including butyrate, benzoate, caprylate, and sorbate [42, 43], and furthermore, of the vitamins ascorbate [44] and vitamin D [45] have underlined the suitability of the here applied secondary abiotic IL-10$^{-/-}$ mice as preclinical setting to test antibiotics-independent therapeutic and preventive measures in the combat of campylobacteriosis. In the present placebo-controlled intervention trail we therefore surveyed the disease-alleviating effects of prophylactic oral *trans*-cinnamaldehyde application in secondary abiotic IL-10$^{-/-}$ mice starting a week prior oral *C. jejuni* infection. At the end of the experiment on day 6 p.i., we assessed i.) the gastrointestinal pathogen loads, ii.) the clinical outcome, and iii.) the intestinal as well as iv.) the extra-intestinal proinflammatory immune responses in diseased mice.

MATERIAL AND METHODS

Secondary abiotic IL-10$^{-/-}$ mice

IL-10$^{-/-}$ mice (C57BL/6j background) were bred and maintained in the Forschungsinstitute für Experimentelle Medizin, Charité – Universitätsmedizin Berlin, Germany. Mice were housed in cages including filter tops within an experimental semi-barrier under standard conditions (i.e., 22–24 °C room temperature, 55 ± 15% humidity, 12 h light/12 h dark cycle) and had free access to autoclaved water (*ad libitum*) and standard chow (food pellets: ssniff R/M-H, V1534-300, Sniff, Soest, Germany). To eradicate the commensal gut microbiota, 3-week-old female and male mice were transferred to sterile cages (maximum of 3–4 animals per cage) immediately after weaning and received an antibiotic treatment with ampicillin plus sulbactam (2 g L^{-1} plus 1 g L^{-1}, respectively; Dr. Friedrich Eberth Arzneimittel, Ursensollen, Germany) added to the drinking water (*ad libitum*) as reported recently [28]. The antibiotic solution was replaced by autoclaved tap water immediately before start of the cinnamaldehyde prophylaxis (i.e., on day −7). Mice were kept and handled under sterile conditions throughout the experiment.

Cinnamaldehyde pre-treatment and *C. jejuni* infection

Starting 7 days prior *C. jejuni* infection, secondary abiotic mice were pre-treated with *trans*-cinnamaldehyde (purchased from Sigma-Aldrich, Munich, Germany) added to the autoclaved drinking water (final concentration of 1,500 mg L^{-1}; *ad libitum*). Given a mean murine body weight of 25 g and an approximal daily drinking volume of 5 mL, mice were subjected to the daily *trans*-cinnamaldehyde dose of 30 mg kg^{-1} body weight. Mice from the placebo cohort received autoclaved tap water instead. For enteropathogenic infection, *C. jejuni* strain 81-176 was thawed from frozen stocks and grown on karmali agar plates (Oxoid, Wesel, Germany) under microaerophilic conditions at 37 °C for at least 48 h as described earlier [46]. Age- and sex-matched mice (3-month-old littermates) were infected with 10^9 colony forming units (CFU) of the pathogen on days 0 and 1 by oral gavage (total volume of 0.3 mL).

Gastrointestinal *C. jejuni* cell numbers

After *C. jejuni* infection, the pathogen loads were determined in fecal samples daily, and upon necropsy on day 6 p.i. in luminal samples from the stomach, duodenum, ileum, and colon by culture as described previously [46]. In brief, intraluminal gastrointestinal samples were homogenized in sterile phosphate-buffered saline (PBS, Thermo Fisher Scientific, Waltham, MA, USA) with a sterile pestle and serial dilutions plated onto karmali agar (Oxoid, Wesel, Germany) and incubated under microaerophilic conditions in a jar at 37 °C for at least 48 h (CampyGas Packs; Oxoid, Wesel, Germany). The detection limit of viable pathogens was 100 CFU per g.

Clinical conditions

Immediately before and after infection, we quantitatively surveyed the daily clinical outcome of mice by using a cumulative clinical score (maximum 12 points), addressing the abundance of blood in feces (0: no blood; 2: microscopic detection of blood by the Guajac method using Haemoccult, Beckman Coulter/PCD, Krefeld, Germany; 4: macroscopic blood visible), the stool consistency (0: formed feces; 2: pasty feces; 4: liquid feces) and the clinical aspect (i.e., wasting symptoms; 0: normal; 1: ruffled fur; 2: less locomotion; 3: isolation; 4: severely compromised locomotion, pre-final aspect) as described earlier [47].

Sampling procedures

On day 6 p.i., mice were sacrificed by CO_2 asphyxiation. *Ex vivo* biopsies from mesenteric lymph nodes (MLN), the colon, ileum, liver, kidneys, and spleen as well as luminal samples from stomach, duodenum, ileum, and colon were derived under aseptic conditions. From each mouse colonic samples were collected in parallel for subsequent microbiological, immunohistopathological, and immunological analyses.

Histopathology

Histopathological analyses were performed in colonic *ex vivo* biopsies that had been immediately fixed in 5% formalin and embedded in paraffin. Sections (5 µm) were stained with hematoxylin and eosin (H&E), examined by light microscopy (100-times magnification), and histopathological changes in the large intestines quantitatively assessed with histopathological scores [48]: Score 0, intact epithelium, no inflammatory cell infiltrates. Score 1, minimal inflammatory cell infiltrates in the mucosa with intact epithelium. Score 2, mild inflammatory cell infiltrates in the mucosa and submucosa with mild hyperplasia and mild goblet cell loss. Score 3, moderate inflammatory cell infiltrates in the mucosa and submucosa with moderate goblet cell loss. Score 4, marked inflammatory cell infiltration into the mucosa and submucosa with marked goblet cell loss, multiple crypt abscesses, and crypt loss.

In situ immunohistochemistry

Quantitative *in situ* immunohistochemical analyses were performed in colonic *ex vivo* biopsies following immediate fixation in 5% formalin and embedding in paraffin as reported previously [49, 50]. In brief, to detect apoptotic epithelial cells, macrophages and monocytes, neutrophils, T lymphocytes, regulatory T cells, and B lymphocytes, colonic paraffin sections (5 µm) were stained with primary antibodies against cleaved caspase-3 (Asp175, Cell Signaling, Beverly, MA, USA; 1:200), F4/80 (no. 14-4801, clone BM8, eBioscience, San Diego, CA, USA; 1:50), MPO7 (No. A0398, Dako, Glostrup, Denmark, 1:500), CD3 (no. N1580, Dako, Glostrup, Denmark; 1:10), FOXP3 (clone FJK-165, no. 14-5773, eBioscience, San Diego, CA, USA; 1:100), and B220 (no. 14-0452-81, eBioscience, San Diego, CA, USA; 1:200), respectively. Positively stained cells were quantitated by a blinded independent investigator applying light microscopy. The average number of respective positively stained cells in each sample was determined within at least six high power fields (HPF, 0.287 mm^2, 400-times magnification).

Proinflammatory mediator secretion

Intestinal *ex vivo* biopsies collected from MLN (3 nodes) as well as from the colon and ileum (longitudinally cut strips of approximately 1 cm^2), the liver (approximately 1 cm^3), the kidney (one half after the longitudinal cut), and the spleen (one third) were washed in sterile PBS (Thermo Fisher Scientific, Waltham, MA, USA) and transferred to 24-flat-bottom well culture plates (Thermo Fisher Scientific, Waltham, MA, USA) containing 500 µL serum-free RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with penicillin (100 µg mL^{-1}) and streptomycin (100 µg mL^{-1}; Biochrom, Berlin, Germany). After an 18-h incubation period at 37 °C, respective culture supernatants and serum samples were tested for interleukin-6 (IL-6), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) by the Mouse Inflammation Cytometric Bead Assay (CBA; BD Biosciences, Heidelberg, Germany) in a BD FACSCanto II flow cytometer (BD Biosciences, Heidelberg, Germany). Nitric oxide concentrations were determined by the Griess reaction as stated earlier [51].

Statistical analyses

Medians and significance levels were calculated using GraphPad Prism (version 8; San Diego, CA, USA). Normalization of data was assessed by the Anderson-Darling test. The Mann-Whitney test was applied for pairwise comparisons of not normally distributed data. For multiple comparisons, the one-way ANOVA with Tukey post-correction (for normally distributed data) and the Kruskal-Wallis test with Dunn's post-correction (for not normally distributed data) were performed. Definite outliers were identified by the Grubb's and the Rout tests (α = 0.001). Two-sided probability (p) values $\leq$0.05 were considered significant. Data were pooled from four independent experiments.

Ethics statement

All animal experiments were carried out according to the European animal welfare guidelines (2010/63/EU) following approval by the commission for animal experiments ("Landesamt für Gesundheit und Soziales", LaGeSo, Berlin; registration number G0104/19). The clinical conditions of mice were monitored daily.

RESULTS

Gastrointestinal *C. jejuni* cell numbers following cinnamaldehyde pretreatment of infected secondary abiotic IL-10$^{-/-}$ mice

We first addressed whether peroral cinnamaldehyde pretreatment of *C. jejuni* infected secondary abiotic IL-10$^{-/-}$ mice affected gastrointestinal colonization properties of the enteropathogen. Whereas from day 2 until day 5 p.i., *C. jejuni* numbers were comparable in feces of cinnamaldehyde and placebo treated mice (not significant (n.s.); Fig. 1), this was also the case when analyzing the bacterial loads luminal samples collected from the stomach, the duodenum, the ileum, and the colon of both cohorts (n.s.; Fig. 2) indicating that cinnamaldehyde pretreatment did not interfere with *C. jejuni* colonization alongside the murine gastrointestinal tract.

Macroscopic and microscopic inflammatory outcomes following cinnamaldehyde pretreatment of *C. jejuni* infected mice

We next surveyed the clinical outcome of *C. jejuni* infected mice upon cinnamaldehyde pretreatment. Therefore, the clinical signs were quantitated in mice by applying a clinical scoring system that addressed key features of acute campylobacteriosis such as wasting symptoms and bloody diarrhea. On day 6 p.i., when placebo control mice were suffering from acute *C. jejuni* induced disease, the clinical signs were milder in cinnamaldehyde treated mice as indicated by lower clinical scores in the latter versus the former ($P < 0.01$; Fig. 3A). We further quantitatively assessed microscopic inflammatory sequelae of *C. jejuni* infection. When applying histopathological scores for inflammatory changes in H&E-stained colonic paraffin sections we found severe histopathological sequelae of infection in mice from both cohorts as indicated by maximum median scores of 4 ($P < 0.001$ versus naive; Fig. 3B). Since apoptosis is considered a reliable parameter for intestinal inflammatory grading [46], we assessed apoptotic colonic epithelia cell numbers by quantitative *in situ* immunohistochemistry. *C. jejuni* infection resulted in marked increases in cleaved caspase-3^{+} colonic epithelial cells ($P < 0.01$–0.001), which were, however,

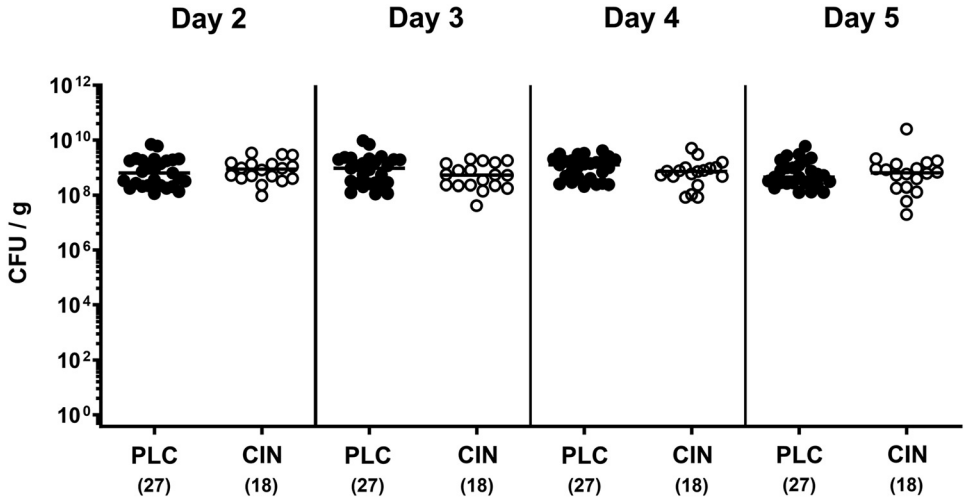

Fig. 1. **Fecal *C. jejuni* loads over time following *C. jejuni* infection of secondary abiotic IL-10$^{-/-}$ mice that had been pretreated with cinnamaldehyde**. Secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1. Starting 7 days prior infection, mice received either placebo (PLC, black circles) or *trans*-cinnamaldehyde (CIN; white circles) via the drinking water. *C. jejuni* cell numbers were assessed in fecal samples at defined time points post-infection (as indicated) by culture and expressed as colony-forming units per gram (CFU/g). Medians (black bar) and the total numbers of analyzed animals (in parentheses) are given. Data were pooled from four independent experiments

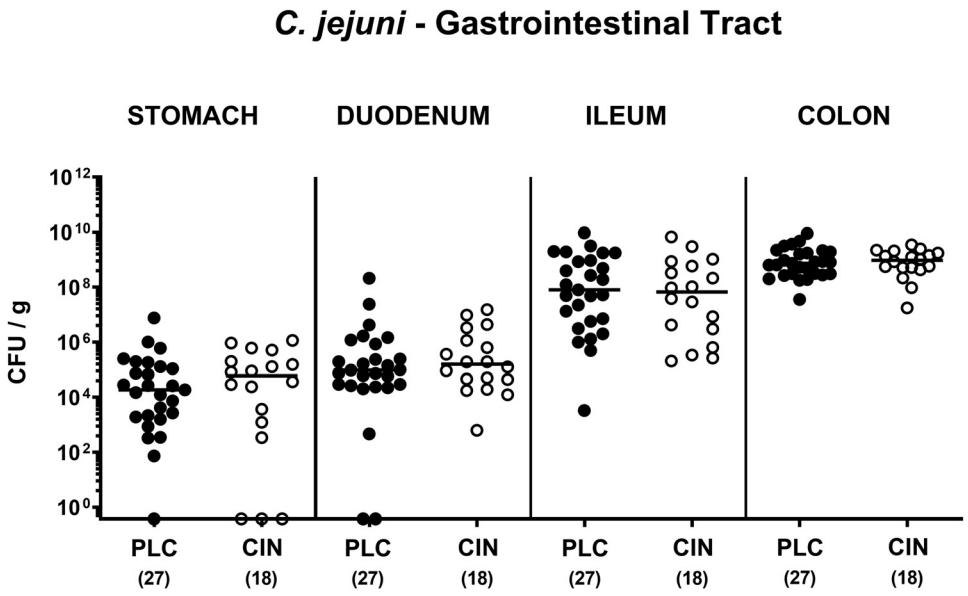

Fig. 2. **Gastrointestinal *C. jejuni* cell numbers following cinnamaldehyde pretreatment of infected secondary abiotic IL-10$^{-/-}$ mice.** Secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1. Starting 7 days prior infection, mice received either placebo (PLC, black circles) or *trans*-cinnamaldehyde (CIN; white circles) via the drinking water. On day 6 post-infection, the *C. jejuni* cell numbers were assessed in luminal samples taken from distinct gastrointestinal compartments (as indicated) by culture and expressed as colony-forming units per gram (CFU/g). Medians (black bar) and the total numbers of analyzed animals (in parentheses) are given. Data were pooled from four independent experiments

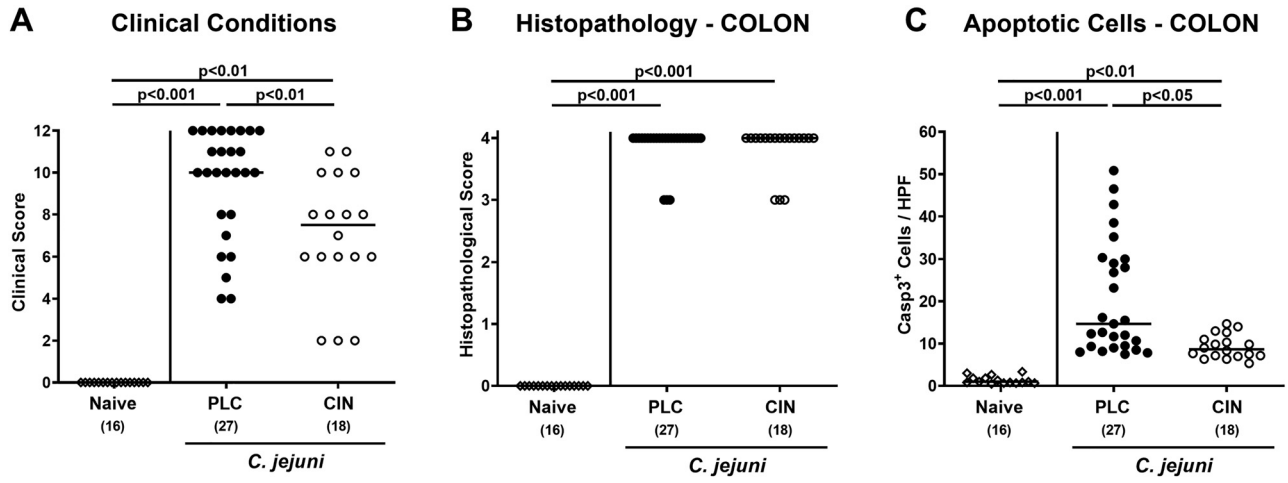

Fig. 3. **Macroscopic and microscopic inflammatory outcomes following cinnamaldehyde pretreatment of *C. jejuni* infected secondary abiotic IL-10$^{-/-}$ mice.** Secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1. Starting 7 days prior infection, mice received either placebo (PLC, black circles) or *trans*-cinnamaldehyde (CIN; white circles) via the drinking water. On day 6 post-infection, **(A)** the overall clinical conditions of mice were quantitatively determined by using a cumulative clinical scoring system (see methods). To assess microscopic inflammatory signs of disease, **(B)** histopathological changes were quantified in hematoxylin and eosin (H&E) stained colonic paraffin sections by using histopathological scores (see methods). In addition, **(C)** the average numbers of apoptotic colonic epithelial cells were assessed microscopically from six high power fields (HPF, 400-times magnification) per animal in paraffin sections positive for cleaved caspase-3 (Casp3^{+}). Naive mice (open diamonds) served as untreated and uninfected control animals. Medians (black bar), significance levels (P values) as determined by the Kruskal-Wallis test and Dunn's post-correction, and the total number of analyzed mice (in parentheses) are given. Data were pooled from four independent experiments

less pronounced upon cinnamaldehyde as compared to placebo treatment ($P < 0.05$; Fig. 3C). Hence, cinnamaldehyde pretreatment did not only improve clinical signs in *C. jejuni* infected mice, but also alleviated colonic epithelial apoptosis.

Large intestinal immune cell responses following cinnamaldehyde pretreatment of *C. jejuni* infected mice

We then asked whether cinnamaldehyde treatment dampened *C. jejuni* induced innate and adaptive immune responses

in the colon of infected mice. Therefore, colonic paraffin sections were subjected to *in situ* immunohistochemistry. Whereas *C. jejuni* infection was accompanied by pronounced increases in F4/80$^+$ macrophages and monocytes, in MPO7$^+$ neutrophils, in CD3$^+$ T lymphocytes, in FOXP3$^+$ regulatory T cells, and in B220$^+$ B lymphocytes in the colonic mucosa and lamina propria ($P < 0.01$–0.001; Fig. 4), cinnamaldehyde treated mice displayed lower large intestinal T lymphocyte numbers as compared to placebo counterparts on day 6 p.i. ($P < 0.01$; Fig. 4B). Hence, cinnamaldehyde pretreatment resulted in less pronounced pathogen-induced T cell responses in the colon of *C. jejuni* infected mice.

Proinflammatory mediator secretion in distinct intestinal compartments following cinnamaldehyde pretreatment of *C. jejuni* infected mice

We next tested whether cinnamaldehyde pretreatment dampened *C. jejuni* induced proinflammatory mediator secretion in distinct intestinal compartments. On day 6 following infection of mice from both cohorts, increased nitric oxide concentrations were measured in MLN draining the infected intestines ($P < 0.001$; Fig. 5A). In case of IL-6 and IFN-γ, however, *C. jejuni* induced increases could only be detected in the MLN of placebo ($P < 0.001$ and $P < 0.01$, respectively), but not cinnamaldehyde treated mice (Fig. 5B and C). In the colon of mice from either treatment cohort, comparably increased nitric oxide, IL-6, and IFN-γ concentrations could be assessed on day 6 p.i. ($P < 0.001$ versus naive; Fig. 5D–F), whereas this also held true for the latter two cytokines measured in ileal *ex vivo* biopsies ($P < 0.05$–0.001 versus naive; Fig. 5H and I). Of note, nitric oxide concentrations were exclusively elevated in the ileum of mice from the placebo control group on day 6 p.i. ($P < 0.01$ versus naive; Fig. 5G). Hence, cinnamaldehyde pretreatment dampened *C. jejuni* induced proinflammatory mediator secretion in distinct parts of the intestinal tract.

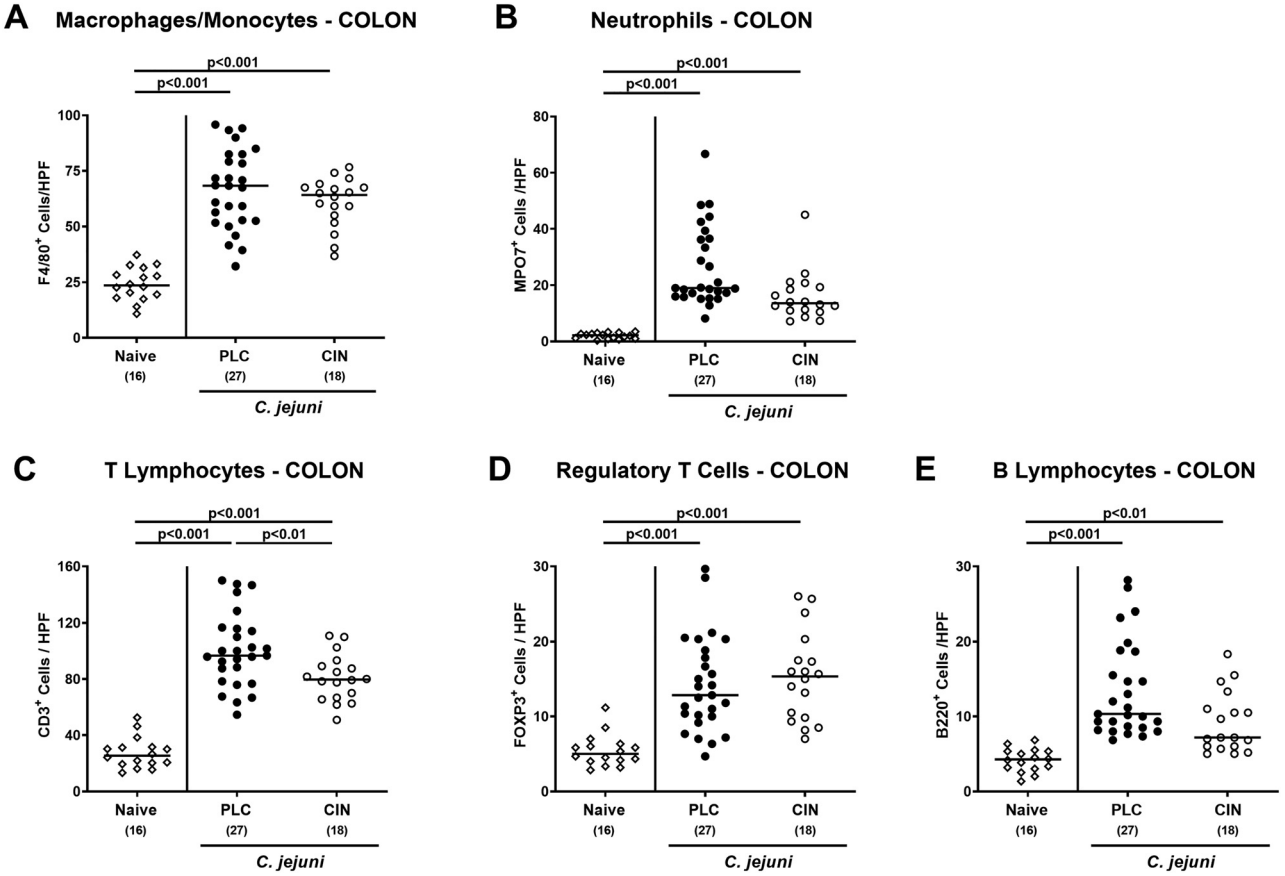

Fig. 4. **Large intestinal immune cell responses following cinnamaldehyde pretreatment of *C. jejuni* infected secondary abiotic IL-10$^{-/-}$ mice**. Secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1. Starting 7 days prior infection, mice received either placebo (PLC, black circles) or *trans*-cinnamaldehyde (CIN; white circles) via the drinking water. On day 6 post-infection, the average numbers of **(A)** macrophages and monocytes (F4/80$^+$), **(B)** neutrophils (MPO7$^+$), **(C)** T lymphocytes (CD3$^+$), **(D)** regulatory T cells (FOXP3$^+$), and **(E)** B lymphocytes (B220$^+$) per animal were determined in immunohistochemically stained colonic paraffin sections from six high power fields (HPF, 400-times magnification). Naive mice (open diamonds) served as untreated and uninfected control animals. Medians (black bar), significance levels (P values) as determined by the one-sided ANOVA test with Tukey post-correction or the Kruskal-Wallis test and Dunn's post-correction, and the total number of analyzed mice (in parentheses) are given. Data were pooled from four independent experiments

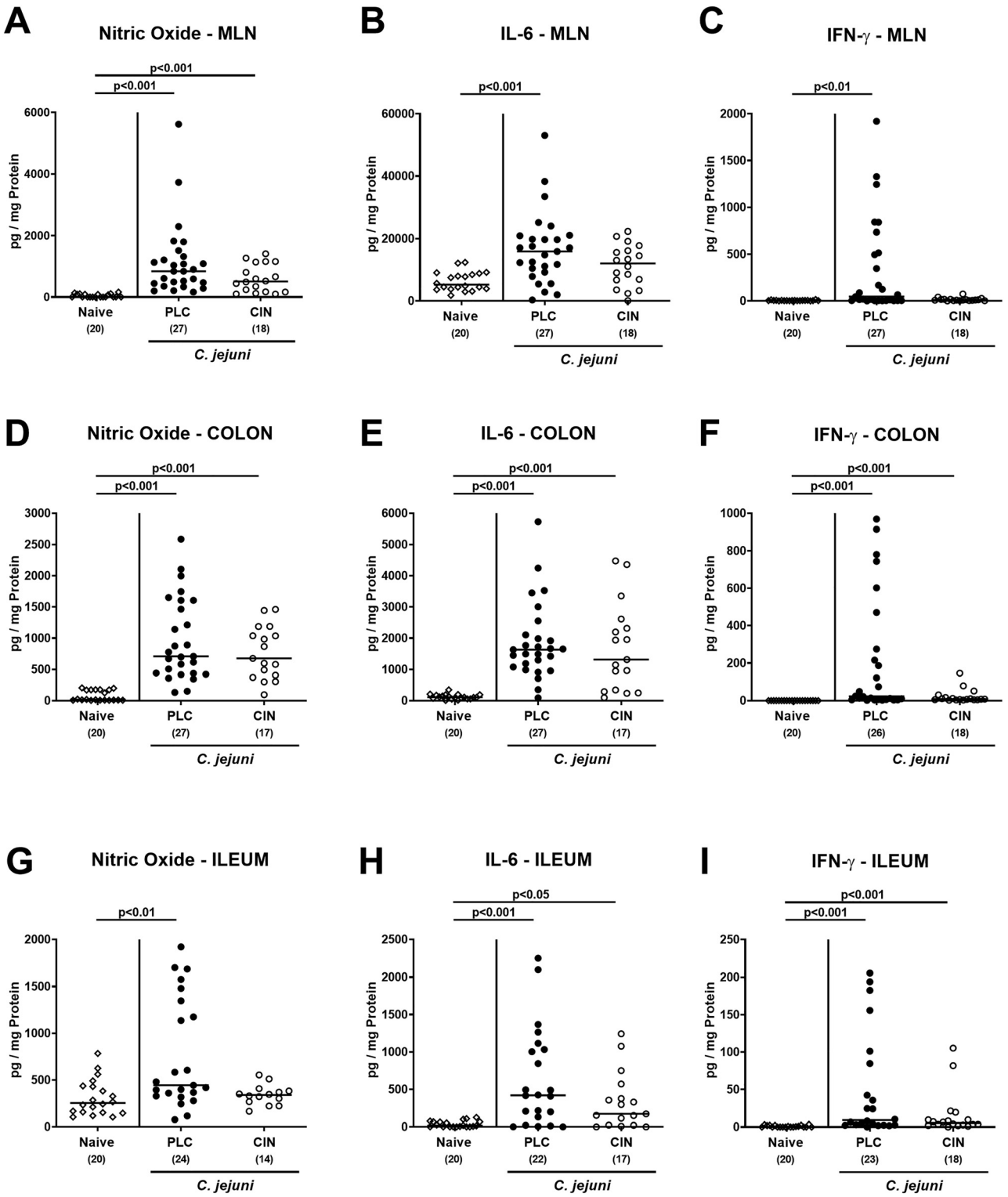

Fig. 5. **Proinflammatory mediator secretion in distinct intestinal compartments following cinnamaldehyde pretreatment of *C. jejuni* infected secondary abiotic IL-10$^{-/-}$ mice**. Secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1. Starting 7 days prior infection, mice received either placebo (PLC, black circles) or *trans*-cinnamaldehyde (CIN; white circles) via the drinking water. On day 6 post-infection, secretion of proinflammatory mediators such as **(A,D,G)** nitric oxide, (**B,E,H**) IL-6, and **(C,F,I)** IFN-γ were measured in distinct intestinal compartments including mesenteric lymph nodes (MLN; **A–C**), colon (**D–F**), and ileum (**G–I**). Naive mice (open diamonds) served as untreated and uninfected control animals. Medians (black bar), significance levels (*P* values) as determined by the Kruskal-Wallis test and Dunn's post-correction or by the one-sided ANOVA test with Tukey post-correction, and the total number of analyzed mice (in parentheses) are given. Definite outliers were identified by the Grubb's and the Rout tests. Data were pooled from four independent experiments

Proinflammatory mediator secretion in distinct extra-intestinal compartments following cinnamaldehyde pretreatment of *C. jejuni* infected mice

We further tested whether cinnamaldehyde treatment could dampen proinflammatory mediator secretion also beyond the intestinal tract. In fact, *C. jejuni* infection resulted in increased hepatic TNF-α and renal IL-6 concentrations in placebo as opposed to cinnamaldehyde treated mice ($P < 0.05$ and 0.01, respectively; Fig. 6A and B), whereas nitric oxide concentrations were comparably elevated in the spleen of mice from both treatment groups on day 6 p.i. ($P < 0.001$ versus naive; Fig. 6C). Hence, cinnamaldehyde pretreatment dampened *C. jejuni* induced proinflammatory mediator secretion in distinct organs beyond the intestinal tract.

DISCUSSION

In our here presented preclinical placebo-controlled intervention trial, the prophylactic oral application of synthetic *trans*-cinnamaldehyde starting a week prior *C. jejuni* infection of secondary abiotic IL-10$^{-/-}$ mice i.) did not affect gastrointestinal pathogen loads but resulted in ii.) alleviated campylobacteriosis symptoms that was accompanied by iii.) less pronounced apoptosis of colonic epithelial cells, iv.) less distinct accumulation of T lymphocytes in the large intestinal mucosa and lamina propria and furthermore, v.) dampening of *C. jejuni* induced proinflammatory mediator secretion in intestinal as well as extra-intestinal compartments to basal levels.

The missing anti-*Campylobacter* effect of oral cinnamaldehyde is somewhat surprising given that the concentration of the applied cinnamaldehyde solution (i.e., 1,500 mg L^{-1}) was exceeding the measured minimal inhibitory concentration (MIC) of 256 mg L^{-1} by more than 5-times. A possible explanation might be that the concentrations of the biologically active cinnamaldehyde molecules within the intestinal lumen were much lower due to dilution with the secretory intestinal fluids. Nevertheless, the mice from the verum cohort benefited from preventive cinnamaldehyde application given an improved clinical outcome with alleviated enteritis symptoms and less pronounced colonic apoptosis. In support, cinnamaldehyde has been shown to prevent oxidative stress-induced apoptotic cell responses in myoblasts [52], as well as in adrenal pheochromocytoma cells *in vitro* [53], which also held true for anti-apoptotic and in turn, neuro-protective effects observed in an *in vitro* Parkinson's disease model [54]. Given comparable intestinal *C. jejuni* loads in the verum and placebo cohorts of our study, the disease-alleviating effects of cinnamaldehyde were rather the consequence of its immune-modulatory modes of action. In fact, the *C. jejuni* induced increases in colonic T lymphocytes were less pronounced upon cinnamaldehyde prevention that was accompanied by attenuated proinflammatory mediator secretion in distinct compartment of the intestinal tract. In the MLN of infected mice from the cinnamaldehyde cohort, for instance, basal IL-6 and IFN-γ concentrations were measured which also held true for ileal nitric oxide concentrations obtained from cinnamaldehyde treated mice on day 6 p.i. that did not differ from those in naive (i.e., non-infected and untreated) counterparts. In support, the anti-oxidant and anti-inflammatory effects of

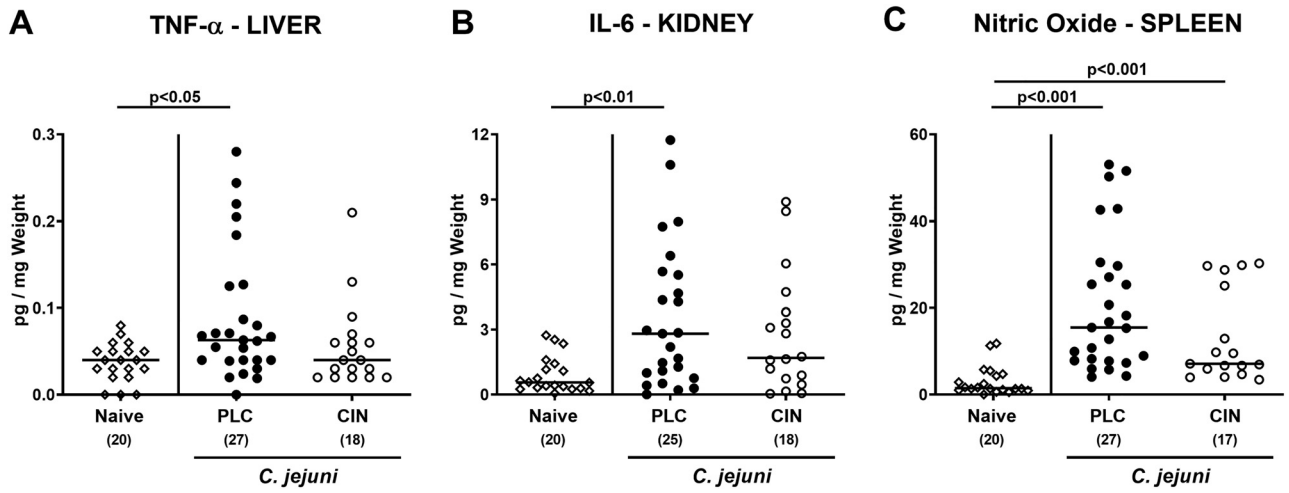

Fig. 6. **Proinflammatory mediator secretion in distinct extra-intestinal compartments following cinnamaldehyde pretreatment of *C. jejuni* infected secondary abiotic IL-10$^{-/-}$ mice**. Secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1. Starting 7 days prior infection, mice received either placebo (PLC, black circles) or *trans*-cinnamaldehyde (CIN; white circles) via the drinking water. On day 6 post-infection, extra-intestinal proinflammatory mediator secretion such as **(A)** hepatic TNF-α, **(B)** renal IL-6, and **(C)** splenic nitric oxide were assessed in respective *ex vivo* biopsies. Naive mice (open diamonds) served as untreated and uninfected control animals. Medians (black bar), significance levels (P values) as determined by the Kruskal-Wallis test and Dunn's post-correction, and the total number of analyzed mice (in parentheses) are given. Definite outliers were identified by the Grubb's test. Data were pooled from four independent experiments

exogenous cinnamaldehyde have been previously shown to be due to suppression of inducible nitric oxide synthase (iNOS) expression [55] and of nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) activation [18]. Furthermore, the compound could effectively inhibit the secretion of proinflammatory cytokines from LPS-stimulated macrophages and monocytes [56]. *In vivo*, cinnamaldehyde ameliorated chronic dextran sulfate sodium (DSS) induced ulcerative colitis in mice [57], and reduced IL-6 concentrations measured in activated macrophages during DSS colitis [58]. Furthermore, cinnamaldehyde alleviated experimental necrotizing enterocolitis (i.e., *Cronobacter sakazakii* induced intestinal inflammation in newborn mice) that was accompanied by suppressed apoptosis and dampened intestinal secretion of the proinflammatory cytokines TNF-α and IL-6 [59].

The disease-ameliorating effects of cinnamaldehyde were not restricted to the intestinal tract but could also be observed beyond given extra-intestinal mediator secretion that did not exceed basal levels in *C. jejuni* infected mice from the cinnamaldehyde cohort as opposed to the placebo counterparts as shown for hepatic TNF-α and renal IL-6 concentrations. In support, recent studies revealed that exogenous cinnamaldehyde protected from acetaminophen-induced liver toxicity by alleviating oxidative stress and hepatic proinflammatory cytokine secretion [60] and reduced inflammatory including oxidative stress and apoptotic cell responses in the liver of *Salmonella* Typhimurium infected mice [61]. In the kidneys, cinnamaldehyde application alleviated renal inflammation in mice by inhibition of the NLRP3 inflammasome and related signaling pathways [62].

Since *C. jejuni*-LOS constitutes the main enteropathogenic molecule inducing enterocolitis upon oral infection [63] it is tempting to speculate that an inhibitory effect of the here applied cinnamaldehyde on the LOS induced TLR-4 mediated pathway was the most likely underlying mechanism for the better clinical outcomes observed in treated mice. This hypothesis is supported by both, *in vitro* and *in vivo* studies showing that cinnamaldehyde suppressed TLR-4 activation upon inhibition of receptor oligomerization [64] and that the compound exerted potent systemic anti-inflammatory effects in endotoxin-induced inflammation in mice [65]. Furthermore, Li and colleagues reported in a recent study that oral application of cinnamon essential oil enriched with cinnamaldehyde to mice with acute DSS colitis alleviated intestinal disease that was paralleled by down-regulated TLR-4 mRNA expression in the colon and attenuated secretion of TNF-α and IL-6 in the serum [66], underscoring our results obtained from dampened extra-intestinal cytokines to basal levels in cinnamaldehyde treated mice. Hence, there is increasing evidence that distinct bioactive compounds including cinnamaldehyde modulate TLR-4 mediated inflammation and thereby, open novel avenues for future therapeutic and preventive measures of TLR-4 driven diseases including campylobacteriosis [67].

When considering safety aspects, both, the United States Food and Drug Administration (FDA), and Flavour and Extract Manufacturer's Association (FEMA), have categorized *trans*-cinnamaldehyde as "Generally Recognized as Safe" (GRAS), whereas the Council of Europe has granted an "A status" (i.e., it may be used in foodstuffs). On this basis, *trans*-cinnamaldehyde can be viewed as a safe food and flavor additive [68].

CONCLUSION

Our preclinical placebo-controlled intervention study provides first evidence that due to its immune-modulatory effects, cinnamaldehyde constitutes a promising antibiotics-independent prophylactic option to alleviate campylobacteriosis in infected mice and men. Since the severity of the initial enteritis event is known to correlate with the risk for post-infectious sequelae in human campylobacteriosis [13], the prevention and treatment of *C. jejuni* induced inflammation by natural compounds such as cinnamaldehyde may protect from the development of subsequent severe auto-immune diseases including GBS, RA, and IBD. Further non-toxic natural compounds with known anti-inflammatory and/or even anti-pathogenic effects should be therefore tested to alleviate or even prevent from food-borne enteropathogenic diseases including campylobacteriosis.

Conflict of interests: MMH and SB are Editorial Board members. Therefore, the submission was handled by a different member of the editorial board, and they did not take part in the review process in any capacity.

Funding: This work was supported from the German Federal Ministries of Education and Research (BMBF) in frame of the zoonoses research consortium PAC-*Campylobacter* to MMH and SB (IP7/01KI1725D and 01KI2007D) and from the Federal Ministry for Economic Affairs and Energy following a resolution of the German National Parliament, Deutscher Bundestag to MMH and SB (ZIM, ZF4117908 AJ8).

The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Authors' contributions: MMH: Designed and performed experiments, analyzed data, critically discussed results, wrote the paper.

SM: Performed experiments, analyzed data, critically discussed results, edited the paper.

DW: performed experiments

SB: Provided advice in experimental design, critically discussed results, edited the paper.

ACKNOWLEDGMENTS

We thank Alexandra Bittroff-Leben, Ines Puschendorf, Ulrike Fiebiger, Sumaya Abdul-Rahman, Gernot Reifenberger, and

the staff of the animal research facility at Charité - University Medicine Berlin for excellent technical assistance and animal breeding.

REFERENCES

1. Linz B, Sharafutdinov I, Tegtmeyer N, Backert S. Evolution and role of proteases in *Campylobacter jejuni* lifestyle and pathogenesis. Biomolecules. 2023;13(2):323.
2. Bolton DJ. Campylobacter virulence and survival factors. Food Microbiol. 2015;48:99–108.
3. Backert S, Tegtmeyer N, Cróinín TÓ, Boehm M, Heimesaat MM. Chapter 1 - human campylobacteriosis. In: Klein G, editor. Campylobacter. Academic Press; 2017. p. 1–25.
4. Heimesaat MM, Backert S, Alter T, Bereswill S. Molecular targets in *Campylobacter* infections. Biomolecules. 2023;13(3):409.
5. Kist M, Bereswill S. Campylobacter jejuni. Contrib Microbiol. 2001; 8:150–65.
6. Mouftah SF, Cobo-Díaz JF, Álvarez-Ordóñez A, Elserafy M, Saif NA, Sadat A, et al. High-throughput sequencing reveals genetic determinants associated with antibiotic resistance in *Campylobacter* spp. from farm-to-fork. PloS One. 2021;16(6):e0253797.
7. O Cróinín T, Backert S. Host epithelial cell invasion by *Campylobacter jejuni:* trigger or zipper mechanism? Front Cell Infect Microbiol. 2012;2:25.
8. Heimesaat MM, Backert S, Alter T, Bereswill S. Human campylobacteriosis-A serious infectious threat in a one health perspective. Curr Top Microbiol Immunol. 2021;431:1–23.
9. Rees JH, Soudain SE, Gregson NA, Hughes RA. *Campylobacter jejuni* infection and Guillain–Barré syndrome. New Engl J Med. 1995;333(21):1374–9.
10. Hannu T, Mattila L, Rautelin H, Pelkonen P, Lahdenne P, Siitonen A, et al. *Campylobacter*-triggered reactive arthritis: a population-based study. Rheumatology. 2002;41(3):312–8.
11. Peters S, Pascoe B, Wu Z, Bayliss SC, Zeng X, Edwinson A, et al. *Campylobacter jejuni* genotypes are associated with post-infection irritable bowel syndrome in humans. Commun Biol. 2021;4(1): 1015.
12. Kaakoush NO, Mitchell HM, Man SM. Role of emerging *Campylobacter* species in inflammatory bowel diseases. Inflamm Bowel Dis. 2014;20(11):2189–97.
13. Mortensen NP, Kuijf ML, Ang CW, Schiellerup P, Krogfelt KA, Jacobs BC, et al. Sialylation of *Campylobacter jejuni* lipo-oligosaccharides is associated with severe gastro-enteritis and reactive arthritis. Microbes Infect. 2009;11(12):988–94.
14. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evid Based Complement Alternat Med. 2014;2014:642942.
15. Ranasinghe P, Pigera S, Premakumara GA, Galappaththy P, Constantine GR, Katulanda P. Medicinal properties of 'true' cinnamon (*Cinnamomum zeylanicum*): a systematic review. BMC Complement Altern Med. 2013;13:275.
16. Kawatra P, Rajagopalan R. Cinnamon: mystic powers of a minute ingredient. Pharmacognosy Res. 2015;7(Suppl 1):S1–6.
17. Singh G, Maurya S, DeLampasona MP, Catalan CA. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem Toxicol. 2007;45(9):1650–61.
18. Kim DH, Kim CH, Kim MS, Kim JY, Jung KJ, Chung JH, et al. Suppression of age-related inflammatory NF-kappaB activation by cinnamaldehyde. Biogerontology. 2007;8(5):545–54.
19. Kumar S, Vasudeva N, Sharma S. GC-MS analysis and screening of antidiabetic, antioxidant and hypolipidemic potential of *Cinnamomum tamala* oil in streptozotocin induced diabetes mellitus in rats. Cardiovasc Diabetol. 2012;11:95.
20. Ka H, Park HJ, Jung HJ, Choi JW, Cho KS, Ha J, et al. Cinnamaldehyde induces apoptosis by ROS-mediated mitochondrial permeability transition in human promyelocytic leukemia HL-60 cells. Cancer Lett. 2003;196(2):143–52.
21. Brahmachari S, Jana A, Pahan K. Sodium benzoate, a metabolite of cinnamon and a food additive, reduces microglial and astroglial inflammatory responses. J Immunol. 2009;183(9):5917–27.
22. Chang ST, Chen PF, Chang SC. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. J Ethnopharmacol. 2001;77(1):123–7.
23. Friedman M, Henika PR, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica*. J Food Prot. 2002;65(10):1545–60.
24. Friedman M. Chemistry, antimicrobial mechanisms, and antibiotic activities of cinnamaldehyde against pathogenic bacteria in animal feeds and human foods. J Agric Food Chem. 2017;65(48):10406–23.
25. Mousavi F, Bojko B, Bessonneau V, Pawliszyn J. Cinnamaldehyde characterization as an antibacterial agent toward *E. coli* metabolic profile using 96-blade solid-phase microextraction coupled to liquid chromatography-mass spectrometry. J Proteome Res. 2016;15(3): 963–75.
26. Vasconcelos NG, Croda J, Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: a review. Microb Pathogenesis. 2018;120:198–203.
27. Haag L-M, Fischer A, Otto B, Plickert R, Kühl AA, Göbel UB, et al. *Campylobacter jejuni* induces acute enterocolitis in gnotobiotic IL-10−/− mice via Toll-like-receptor-2 and-4 signaling. PloS one. 2012;7(7):e40761.
28. Heimesaat MM, Mousavi S, Bandick R, Bereswill S. *Campylobacter jejuni* infection induces acute enterocolitis in IL-10-/-mice pretreated with ampicillin plus sulbactam. Eur J Microbiol Immunol. 2022.
29. Herzog MK-M, Cazzaniga M, Peters A, Shayya N, Beldi L, Hapfelmeier S, et al. Mouse models for bacterial enteropathogen infections: insights into the role of colonization resistance. Gut microbes. 2023;15(1):2172667.
30. Warren HS, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L, Tesini B, et al. Resilience to bacterial infection: difference between species could be due to proteins in serum. J Infect Dis. 2010;201(2): 223–32.
31. Mousavi S, Weschka D, Bereswill S, Heimesaat MM. Preclinical evaluation of oral urolithin-A for the treatment of acute campylobacteriosis in *Campylobacter jejuni* infected microbiota-depleted IL-10-/-mice. Pathogens. 2021;10(1):7.
32. Bereswill S, Mousavi S, Weschka D, Heimesaat MM. Disease-alleviating effects of peroral activated charcoal treatment in acute murine campylobacteriosis. Microorganisms. 2021;9(7):1424.

33. Bereswill S, Mousavi S, Weschka D, Buczkowski A, Schmidt S, Heimesaat MM. Iron deprivation by oral deferoxamine application alleviates acute campylobacteriosis in a clinical murine *Campylobacter jejuni* infection model. Biomolecules. 2022;13(1):71.
34. Heimesaat MM, Mousavi S, Weschka D, Bereswill S. Garlic essential oil as promising option for the treatment of acute campylobacteriosis—results from a preclinical placebo-controlled intervention study. Microorganisms. 2021;9(6):1140.
35. Heimesaat MM, Mousavi S, Weschka D, Bereswill S. Anti-pathogenic and immune-modulatory effects of peroral treatment with cardamom essential oil in acute murine campylobacteriosis. Microorganisms. 2021;9(1):169.
36. Mousavi S, Weschka D, Bereswill S, Heimesaat MM. Immune-modulatory effects upon oral application of cumin-essential-oil to mice suffering from acute campylobacteriosis. Pathogens. 2021;10(7).
37. Bereswill S, Mousavi S, Weschka D, Buczkowski A, Schmidt S, Heimesaat MM. Peroral clove essential oil treatment ameliorates acute campylobacteriosis-results from a preclinical murine intervention study. Microorganisms. 2021;9(4).
38. Mousavi S, Weschka D, Bereswill S, Heimesaat MM. Disease alleviating effects following prophylactic lemon and coriander essential oil treatment in mice with acute campylobacteriosis. Front Microbiol. 2023;14:1154407.
39. Mousavi S, Schmidt A-M, Escher U, Kittler S, Kehrenberg C, Thunhorst E, et al. Carvacrol ameliorates acute campylobacteriosis in a clinical murine infection model. Gut Pathog. 2020;12(1):2.
40. Heimesaat MM, Mousavi S, Escher U, Lobo de Sá FD, Peh E, Schulzke J-D, et al. Resveratrol alleviates acute *Campylobacter jejuni* induced enterocolitis in a preclinical murine intervention study. Microorganisms. 2020;8(12):1858.
41. Lobo de Sa FD, Butkevych E, Nattramilarasu PK, Fromm A, Mousavi S, Moos V, et al. Curcumin mitigates immune-induced epithelial barrier dysfunction by *Campylobacter jejuni*. Int J Mol Sci. 2019;20(19).
42. Du K, Foote MS, Mousavi S, Buczkowski A, Schmidt S, Bereswill S, et al. Less pronounced immunopathological responses following oral butyrate treatment of *Campylobacter jejuni*-infected mice. Microorganisms. 2022;10(10):1953.
43. Du K, Foote MS, Mousavi S, Buczkowski A, Schmidt S, Peh E, et al. Combination of organic acids benzoate, butyrate, caprylate, and sorbate provides a novel antibiotics-independent treatment option in the combat of acute campylobacteriosis. Front Microbiol. 2023;14.
44. Mousavi S, Escher U, Thunhorst E, Kittler S, Kehrenberg C, Bereswill S, et al. Vitamin C alleviates acute enterocolitis in *Campylobacter jejuni* infected mice. Scientific Rep. 2020;10(1):2921.
45. Mousavi S, Lobo de Sa FD, Schulzke JD, Bucker R, Bereswill S, Heimesaat MM. Vitamin D in acute campylobacteriosis-results from an intervention study applying a clinical *Campylobacter jejuni* induced enterocolitis model. Front Immunol. 2019;10:2094.
46. Bereswill S, Fischer A, Plickert R, Haag LM, Otto B, Kuhl AA, et al. Novel murine infection models provide deep insights into the "menage a trois" of *Campylobacter jejuni*, microbiota and host innate immunity. PloS One. 2011;6(6):e20953.
47. Heimesaat MM, Alutis M, Grundmann U, Fischer A, Tegtmeyer N, Böhm M, et al. The role of serine protease HtrA in acute ulcerative enterocolitis and extra-intestinal immune responses during *Campylobacter jejuni* infection of gnotobiotic IL-10 deficient mice. Front Cell Infect Microbiol. 2014;4:77.
48. Erben U, Loddenkemper C, Doerfel K, Spieckermann S, Haller D, Heimesaat MM, et al. A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int J Clin Exp Pathol. 2014;7(8):4557–76.
49. Heimesaat MM, Giladi E, Kühl AA, Bereswill S, Gozes I. The octapetide NAP alleviates intestinal and extra-intestinal anti-inflammatory sequelae of acute experimental colitis. Peptides. 2018;101:1–9.
50. Heimesaat MM, Fischer A, Jahn HK, Niebergall J, Freudenberg M, Blaut M, et al. Exacerbation of murine ileitis by Toll-like receptor 4 mediated sensing of lipopolysaccharide from commensal *Escherichia coli*. Gut. 2007;56(7):941–8.
51. Heimesaat MM, Bereswill S, Fischer A, Fuchs D, Struck D, Niebergall J, et al. Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with *Toxoplasma gondii*. J Immunol. 2006;177(12):8785–95.
52. Choi YH. Trans-cinnamaldehyde protects C2C12 myoblasts from DNA damage, mitochondrial dysfunction and apoptosis caused by oxidative stress through inhibiting ROS production. Genes Genomics. 2021;43:303–12.
53. Lv C, Yuan X, Zeng HW, Liu RH, Zhang WD. Protective effect of cinnamaldehyde against glutamate-induced oxidative stress and apoptosis in PC12 cells. Eur J Pharmacol. 2017;815:487–94.
54. Ramazani E, YazdFazeli M, Emami SA, Mohtashami L, Javadi B, Asili J, et al. Protective effects of Cinnamomum verum, Cinnamomum cassia and cinnamaldehyde against 6-OHDA-induced apoptosis in PC12 cells. Mol Biol Rep. 2020;47(4):2437–45.
55. Lee HS, Kim BS, Kim MK. Suppression effect of *Cinnamomum cassia* bark-derived component on nitric oxide synthase. J Agric Food Chem. 2002;50(26):7700–3.
56. Chao LK, Hua KF, Hsu HY, Cheng SS, Lin IF, Chen CJ, et al. Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes/macrophages through suppression of intracellular signaling. Food Chem Toxicol. 2008;46(1):220–31.
57. Qu S-l, Chen L, Wen X-s, Zuo J-p, Wang X-y, Lu Z-j, et al. Suppression of Th17 cell differentiation via sphingosine-1-phosphate receptor 2 by cinnamaldehyde can ameliorate ulcerative colitis. Biomed Pharmacother. 2021;134:111116.
58. Qu S, Shen Y, Wang M, Wang X, Yang Y. Suppression of miR-21 and miR-155 of macrophage by cinnamaldehyde ameliorates ulcerative colitis. Int Immunopharmacol. 2019;67:22–34.
59. Yang G, Jin T, Yin S, Guo D, Zhang C, Xia X, et al. trans-Cinnamaldehyde mitigated intestinal inflammation induced by *Cronobacter sakazakii* in newborn mice. Food Funct. 2019;10(5):2986–96.
60. Hussain S, Ashafaq M, Alshahrani S, Siddiqui R, Ahmed RA, Khuwaja G, et al. Cinnamon oil against acetaminophen-induced acute liver toxicity by attenuating inflammation, oxidative stress and apoptosis. Toxicol Rep. 2020;7:1296–304.
61. Wang R, Li S, Jia H, Si X, Lei Y, Lyu J, et al. Protective effects of cinnamaldehyde on the inflammatory response, oxidative stress, and apoptosis in liver of *Salmonella* Typhimurium-challenged mice. Molecules (Basel, Switzerland). 2021;26(8):2309.
62. Ka S-M, Chao LK, Lin J-C, Chen S-T, Li W-T, Lin C-N, et al. A low toxicity synthetic cinnamaldehyde derivative ameliorates renal inflammation in mice by inhibiting NLRP3 inflammasome and its related signaling pathways. Free Radic Biol Med. 2016;91:10–24.
63. Mousavi S, Bereswill S, Heimesaat MM. Novel clinical *Campylobacter jejuni* infection models based on sensitization of mice to

lipooligosaccharide, a major bacterial factor triggering innate immune responses in human campylobacteriosis. Microorganisms. 2020;8(4).
64. Youn HS, Lee JK, Choi YJ, Saitoh SI, Miyake K, Hwang DH, et al. Cinnamaldehyde suppresses toll-like receptor 4 activation mediated through the inhibition of receptor oligomerization. Biochem Pharmacol. 2008;75(2):494–502.
65. Lee S-C, Wang S-Y, Li C-C, Liu C-T. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice. J Food Drug Anal. 2018;26(1):211–20.
66. Li AL, Ni WW, Zhang QM, Li Y, Zhang X, Wu HY, et al. Effect of cinnamon essential oil on gut microbiota in the mouse model of dextran sodium sulfate-induced colitis. Microbiol Immunol. 2020; 64(1):23–32.
67. Coutinho-Wolino KS, Almeida PP, Mafra D, Stockler-Pinto MB. Bioactive compounds modulating Toll-like 4 receptor (TLR4)-mediated inflammation: pathways involved and future perspectives. Nutr Res. 2022;107:96–116.
68. Doyle AA, Stephens JC. A review of cinnamaldehyde and its derivatives as antibacterial agents. Fitoterapia. 2019;139:104405.