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Microbiota-Derived Metabolic Factors Reduce Campylobacteriosis in Mice

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BACKGROUND & AIMS: *Campylobacter jejuni*, a prevalent foodborne bacterial pathogen, exploits the host innate response to induce colitis. Little is known about the roles of microbiota in *C jejuni*–induced intestinal inflammation. We investigated interactions between microbiota and intestinal cells during *C jejuni* infection of mice. **METHODS:** Germ-free C57BL/6 *Il10*$^{-/-}$ mice were colonized with conventional microbiota and infected with a single dose of *C jejuni* (10^9 colony-forming units/mouse) via gavage. Conventional microbiota were cultured under aerobic, microaerobic, or anaerobic conditions and orally transplanted into germ-free *Il10*$^{-/-}$ mice. Colon tissues were collected from mice and analyzed by histology, real-time polymerase chain reaction, and immunoblotting. Fecal microbiota and bile acids were analyzed with 16S sequencing and high-performance liquid chromatography with mass spectrometry, respectively. **RESULTS:** Introduction of conventional microbiota reduced *C jejuni*–induced colitis in previously germ-free *Il10*$^{-/-}$ mice, independent of fecal load of *C jejuni*, accompanied by reduced activation of mammalian target of rapamycin. Microbiota transplantation and 16S ribosomal DNA sequencing experiments showed that *Clostridium XI*, *Bifidobacterium*, and *Lactobacillus* were enriched in fecal samples from mice colonized with microbiota cultured in anaerobic conditions (which reduce colitis) compared with mice fed microbiota cultured under aerobic conditions (susceptible to colitis). Oral administration to mice of microbiota-derived secondary bile acid sodium deoxycholate, but not ursodeoxycholic acid or lithocholic acid, reduced *C jejuni*–induced colitis. Depletion of secondary bile acid–producing bacteria with antibiotics that kill anaerobic bacteria (clindamycin) promoted *C jejuni*–induced colitis in specific pathogen-free *Il10*$^{-/-}$ mice compared with the nonspecific antibiotic nalidixic acid; colitis induction by antibiotics was associated with reduced level of luminal deoxycholate. **CONCLUSIONS:** We identified a mechanism by which the microbiota controls susceptibility to *C jejuni* infection in mice, via bacteria-derived secondary bile acids.

Keywords: Metabolism; DCA; Infection; Microbiome.

Campylobacter jejuni is one of the prevalent causes of bacterial-derived diarrheal illness in developed countries, and global incidence has been on the rise this past decade.[1] Furthermore, *C jejuni* causes serious postinfection complications, including arthritis, Guillain-Barré syndrome, irritable bowel syndrome, and inflammatory bowel diseases.[1] Clinical symptoms of campylobacteriosis include abdominal cramps, watery to bloody diarrhea, fever, and gastrointestinal inflammation.[2] At the cellular level, the intestinal tract of *C jejuni*–infected patients displays infiltration of immune cells, such as neutrophils, crypt abscesses, and presence of fecal leukocytes.[3] Gnotobiotic technology applied to germ-free (GF) *Il10*$^{-/-}$ mice (129 SvEv) showed that the human clinical *C jejuni* strain 81–176 induces acute intestinal inflammation resembling key features of human campylobacteriosis (neutrophil infiltration, crypt abscesses, and bacterial invasion).[4] Subsequent studies showed that innate immunity is critical for campylobacteriosis, as the inflammatory response is similar between *Il10*$^{-/-}$ and *Il10*$^{-/-}$; *Rag2*$^{-/-}$ mice.[5] Moreover, phosphatidylinositol 3-kinase gamma (PI3Kγ) signaling mediated neutrophil migration into colonic tissues and is essential for *C jejuni*–induced colitis.[5] The mammalian target of rapamycin (mTOR), a downstream target of PI3K, has been implicated in many functions, including cell growth, proliferation, survival, and innate and adaptive immune responses.[6–8] The mTOR inhibitor rapamycin prevents and treats *C jejuni*–induced campylobacteriosis in *Il10*$^{-/-}$ mice.[9] These findings highlight the important role of PI3K/mTOR in *C jejuni*–induced colitis. However, the role of commensal gut microbiota in controlling host susceptibility to *C jejuni* infection is unknown. Interestingly, *C jejuni* colonic luminal colonization level is not associated with the bacterial ability to induce colitis,[5,9–11] suggesting a complex interaction among the pathogen, microbiota, and host response.

Abbreviations used in this paper: **BW, body weight; CONV-Biota, conventionalized microbiota; Aero-Biota, aerobic CONV-Biota; Anaero-Biota, anaerobic CONV-Biota; CA, cholic acid; CFU, colony-forming unit; DCA, deoxycholic acid; FISH, fluorescence in situ hybridization; GF, germ free; HPLC/MC, high-performance liquid chromatography/mass spectrometry; IHC, immunohistochemistry; LCA, lithocholic acid; MLN, mesenteric lymph node; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PI3K, phosphatidylinositol 3-kinase; SPF, specific pathogen-free; rDNA, ribosomal DNA; TCA, taurocholic acid; UDCA, ursodeoxycholic acid.**

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EDITOR'S NOTES

BACKGROUND AND CONTEXT

Campylobacter jejuni, a prevalent foodborne bacterial pathogen, exploits the host innate response to induce colitis. Little is known about the roles of microbiota in *C jejuni*–induced intestinal inflammation.

NEW FINDINGS

The bacterial metabolite deoxycholic acids derived from anaerobic bacteria protect the host against C. jejuni-induced colitis. The mechanism by which microbiota attenuate campylobacteriosis involved blockade of mTOR signaling.

LIMITATIONS

Further investigation is needed to identify whether microbiota-derived DCA induced expression of an mTOR inhibitor or if other mechanisms are at play.

IMPACT

These findings highlight the complex mechanism by which the microbiota controls host susceptibility to specific enteropathogen infection, and point to novel therapeutics approaches by targeting those microbial metabolites.

The intestinal microbiota exerts numerous effects on the host, especially on immune response following infection. For example, the microbiota regulates granulocytosis and neonate response to *Escherichia coli K1* and *Klebsiella pneumoniae* sepsis.[12] In addition, the microbiota is also found to enhance myelopoiesis and protect against *Listeria monocytogenes* infection.[13] At the gut level, acetate derived from *Bifidobacterium* metabolism protects the host against enterohaemorrhagic *E coli* O157:H7 infection by inhibiting its Shiga toxin translocation.[14,15] Commensal segmented filamentous bacterium induces interleukin (IL)17- and IL22-producing Th17 cells,[16] and mice with deficiency of IL22 succumb to *Citrobacter rodentium* infection.[17] Recently, microbiota transplantation has shown tremendous success against recurrent *Clostridium difficile* infection,[18] suggesting that microbial manipulation has the potential to treat infectious microorganisms. In addition, the biotransformation of secondary bile acids by *Clostridium scindens* was found to inhibit *C difficile* colonization and infection,[19] suggesting a critical role of microbial metabolites on *C difficile* pathogenesis. Intriguingly, bile acids, particularly secondary bile acid deoxycholic acid (DCA), are associated with various chronic diseases, such as metabolic diseases, intestinal inflammation, and colorectal cancers.[20–22] Whether the microbiota-derived bile acid metabolism prevents colonization of and host response to *C jejuni* is unknown.

In this study, we hypothesized that specific groups of microbiota control *C jejuni*–induced enteritis through the production of specific microbial metabolites that modulate host-derived inflammatory signaling. Our data indicate that specific anaerobic microbes and their metabolites protect *Il10*$^{-/-}$ mice against *C jejuni*–induced intestinal inflammation, through modulation of host response. These metabolites and signaling pathways represent critical events in *C jejuni*–induced intestinal inflammation and could define potential new therapeutic targets.

Materials and Methods

Mouse Experiments

Animal experiments were in accordance with the Animal Research: Reporting of In Vivo Experiments (https://www.nc3rs.org.uk/arrive-guidelines). All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Florida (201608025). Cohorts of 8- to 12-week-old age-matched male or female mice of 4 to 9 mice per group were used, and the sample size was based on previous published reports showing significant colitis with that sample size.[4,9,23] The sibling littermate mice were fed ad libitum chew diet and water in individually ventilated cages with Alpha Dri bedding. All animal procedures were performed at light cycle. GF *Il10*$^{-/-}$ mice were transferred to specific pathogen-free (SPF) conditions for 3 or 14 days. and 14-day stool was collected as conventionalized microbiota (CONV-Biota). Freshly collected stools were immediately suspended in 30% glycerol phosphate-buffered saline (PBS) stock, quantified (OD600 value of 1 was estimated as 10^8 colony-forming units [CFU]/mL), and stored at −80°C. Before oral gavage, the stool preparation was thawed, diluted, and immediately gavaged to mice at 10^8 CFU/mouse. The CONV-Biota was also cultured under aerobic (Aero-Biota), microaerobic (Microaero-Biota), or anaerobic (Anaero-Biota) conditions using Brain Hart Infusion agar plates. For *C jejuni* infection experiments, GF C57BL/6 *Il10*$^{-/-}$ mice were transferred from GF isolators to SPF housing and immediately gavaged with 10^9 *C jejuni* CFU/mouse (strain 81–176[24]) for 12 days and killed as described before.[9] For whole microbiota protection experiments, GF *Il10* $^{-/-}$ mice were orally gavaged with a single dose of CONV-Biota (10^8 CFU/mouse) for 14 days before 12-day *C jejuni* infection. For specific microbiota protection experiments, GF *Il10*$^{-/-}$ mice were gavaged with a single dose of 10^8 CFU/mouse Aero-Biota, Microaero-Biota, Anaero-Biota, or the 3 microbiota pooled. We began the 12-day *C jejuni* infection 14 days postgavage. To deplete mouse microbiota, C57BL/6 *Il10*$^{-/-}$ mice in SPF housing were given an antibiotic cocktail in drinking water[9] or clindamycin (Sigma-Aldrich, St Louis, MO) was gavaged at 67 mg/kg body weight (BW) or nalidixic acid (Sigma-Aldrich) was gavaged at 200 mg/kg BW for 7 days. One day after the antibiotic treatment, the mice were gavaged with a single dose of 10^9 *C jejuni* CFU/mouse for 21 days. To investigate the impact of bile acids on *C jejuni*–induced colitis, GF *Il10*$^{-/-}$ mice were infected as before and were gavaged daily with 30 mg/kg BW of DCA, lithocholic acid (LCA), or ursodeoxycholic acid (UDCA) (Sigma-Aldrich) for 12 days. Although we observed intestinal inflammation at different time points of *C jejuni* infection (days 4, 5, 6, and 12 postinfection) in ex-GF *Il10*$^{-/-}$ mice, we opted for the 12-day postinfection time point in this study because of the consistency in host response (severe colitis), and also because this colonization time was used to determine efficacy of therapeutic intervention in previous studies (neutrophil depletion, mTOR inhibition, and PI3Kγ blockade).[4,5,9] Mice were followed clinically for evidence of diarrhea, failure to thrive, and mortality. At the end of experiments, tissue samples from mouse colon and stool were collected for protein, RNA,