

CAN MICROBIOTA TRANSPLANTATION ABROGATE MURINE COLONIZATION RESISTANCE AGAINST *CAMPYLOBACTER JEJUNI*?

M. M. Heimesaat*, R. Plickert, A. Fischer, U. B. Göbel and S. Bereswill

Department of Microbiology and Hygiene, Charité, University Medicine Berlin, Berlin, Germany

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Enterocolitis caused by *Campylobacter jejuni* represents an important socioeconomic burden worldwide. The host-specific intestinal microbiota is essential for maintaining colonization resistance (CR) against *C. jejuni* in conventional mice. Notably, CR is abrogated by shifts of the intestinal microbiota towards overgrowth with commensal *E. coli* during acute ileitis. Thus, we investigated whether oral transplantation (TX) of ileal microbiota derived from *C. jejuni* susceptible mice with acute ileitis overcomes CR of healthy conventional animals. Four days following ileitis microbiota TX or ileitis induction and right before *C. jejuni* infection, mice displayed comparable loads of main intestinal bacterial groups as shown by culture. Eight days following ileitis induction, but not ileal microbiota TX, however, *C. jejuni* could readily colonize the gastrointestinal tract of conventional mice and also translocate to extra-intestinal tissue sites such as mesenteric lymph nodes, spleen, liver, and blood within 4 days following oral infection. Of note, *C. jejuni* did not further deteriorate histopathology following ileitis induction. Lack of *C. jejuni* colonization in TX mice was accompanied by a decrease of commensal *E. coli* loads in the feces 4 days following *C. jejuni* infection. In summary, oral ileal microbiota TX from susceptible donors is not sufficient to abrogate murine CR against *C. jejuni*.

Keywords: *Campylobacter jejuni*, colonization resistance, fecal transplantation, microbiota, intestinal inflammation, acute ileitis, *Toxoplasma gondii*, bacterial translocation, susceptibility to infection, *E. coli*, pathogen–commensal interaction, pathogen–host interaction

Introduction

Campylobacter (C.) jejuni is a very common bacterial pathogen causing gastroenteritis worldwide [1, 2]. The zoonotic pathogen is transmitted from animals to humans via the foodchain by contaminated meat, milk products, or surface water [2, 3]. *C. jejuni* colonizes the distal parts of the gastrointestinal (GI) tract and subsequently induces an influx of inflammatory cells leading to ulcerations and crypt abscesses [3–6]. Following an incubation period of approximately 2 to 5 days, infected patients suffering from campylobacteriosis present with fever, abdominal cramps, and watery or even bloody diarrhea [5, 6]. Whereas in most cases the disease is self-limited, rarely chronic post-infectious sequelae of campylobacteriosis, such as Guillan–Barré syndrome or reactive polyarthropathy might arise [2, 7].

In vivo studies of molecular mechanisms underlying *C. jejuni*-induced immunopathology are hampered by the

lack of suitable vertebrate models [8]. Conventional laboratory mice, for instance, can, if at all, only sporadically be colonized by the pathogen [1, 2]. Thus, significant immunopathology mimicking human campylobacteriosis cannot be investigated. This is due to the colonization resistance (CR) against *C. jejuni* exerted by adult mice, a phenomenon caused by the age-specific composition of the conventional intestinal microbiota harbored by mice older than 6 weeks of age, which are commonly used in experimental settings [2]. We have recently shown that this host-specific CR can be overcome by modifying the intestinal microbiota [9]. Conventionally colonized control animals had expelled the pathogen within 48 h following even high dose *C. jejuni* infection on three consecutive days, whereas the GI tract of gnotobiotic mice lacking any bacteria following antibiotic treatment could be stably colonized by *C. jejuni*. The pathogen subsequently induced cellular immune and inflammatory responses in the distal intestine [9]. Strikingly, gnotobiotic mice perorally transplanted with com-

*Corresponding author: Markus M. Heimesaat; Charité-University Medicine Berlin, CC5, Department of Microbiology and Hygiene, Campus Benjamin Franklin, Hindenburgdamm 27, D-12203 Berlin, Germany; Phone: +49-30-8445-2194; Fax: +49-30-450-524-902; E-mail: markus.heimesaat@charite.de

plex human, but not conventional murine microbiota could be infected by *C. jejuni* and displayed immunohistopathological features mimicking human campylobacteriosis [9]. These findings highlight the host specific microbiota composition as pivotal prerequisite in susceptibility to enteropathogens. Furthermore, we clearly showed that murine CR against *C. jejuni* was abrogated under conditions of acute intestinal inflammation. For instance, mice suffering from acute ileitis or colitis (induced by *Toxoplasma (T.) gondii* or IL-10 gene deficiency, respectively) could be stably infected with *C. jejuni* [10–12]. Given that a common feature of acute intestinal inflammation is a tremendous intestinal overgrowth with commensal *E. coli* [13–16], we artificially elevated the intestinal *E. coli* load of conventionally colonized mice (lacking intestinal inflammation) by adding live *E. coli* into the drinking water, and rendered mice susceptible to *C. jejuni* infection as long as the commensal *E. coli* burden was kept to supra-physiological levels [10].

In the present study, we investigated whether the CR against *C. jejuni* displayed by conventionally colonized mice without intestinal inflammation can be overcome by a single peroral fecal transplantation (TX) of the complex microbiota derived from mice suffering from acute ileitis. We further studied the quantitative microbiota and potential histopathological changes in the intestines of TX mice following *C. jejuni* infection.

Materials and Methods

Ethics statement

All animal experiments were conducted according to the European Guidelines for animal welfare (2010/63/EU) with approval of the commission for animal experiments headed by the “Landesamt für Gesundheit und Soziales” (LaGeSo, Berlin, Germany). Animal welfare was monitored twice daily by assessment of clinical conditions.

Mice

All animals were bred and maintained in the facilities of the “Forschungsinstitut für Experimentelle Medizin” (FEM, Charité-Universitätsmedizin Berlin, Berlin, Germany) under specific pathogen-free (SPF) conditions. Female age matched C57BL/6j wildtype mice between 10 and 12 weeks of age were used.

Acute ileitis induction

For induction of acute ileitis, mice were infected perorally with 100 *T. gondii* cysts (ME49 strain) from homogenized brains of intraperitoneally infected NMRI mice in a volume of 0.3 mL phosphate-buffered saline

(PBS, pH 7.4) by gavage, as described previously [10, 13, 17].

Ileitis microbiota transplantation

Fresh samples (free of enteropathogenic bacteria, parasites, and viruses) were collected from the ileal lumen of ten mice suffering from severe *T. gondii* induced ileitis at day 8 p.i., pooled and dissolved in an equal volume of sterile PBS, and then immediately used for oral re-colonization (transplantation; TX) in a final volume of 0.3 mL by gavage. Right before TX the total as well as the bacterial loads of distinct communities (*E. coli*, *Enterococcus* spp., *Lactobacillus* spp., *Bacteroides/Prevotella* spp., *Clostridium/Eubacterium* spp.) were quantified in serious dilutions by cultivation on appropriate solid media as described previously. In addition, colonization efficiency was investigated by cultural analyses of fecal samples at day 4 and day 8 following TX. Between independent experiments, counts of bacterial groups in suspensions used for fecal TX varied less than 0.5 log orders of magnitude.

Campylobacter jejuni infection

Mice were infected with 10⁹ viable colony forming units (CFU) of *C. jejuni* 43431 ATCC strain by gavage in a total volume of 0.3 mL PBS on three consecutive days starting 4 days following fecal TX or induction of acute ileitis as described earlier in detail [9].

Sampling procedures

Mice were sacrificed by isofluran treatment (Abbott, Germany). Cardiac blood and tissue samples from spleen, liver, mesenteric lymph nodes (MLNs), and luminal contents from the gastrointestinal tract (stomach, duodenum, terminal ileum and colon) were removed under sterile conditions. Ileal samples from each mouse were collected in parallel for microbiological and histopathological analyses. For the latter, ileal samples were immediately fixed in 5% formalin and embedded in paraffin, sections (5 µm) were stained with HE and subjected to a standardized scoring system by two blinded independent investigators (R.P. and M.M.H.) [9, 13, 18].

Quantitative analysis of *C. jejuni* translocation

Live *C. jejuni* were detected in MLNs, spleen, liver, and cardiac blood by culture as described earlier [9]. In brief, tissues were homogenized in sterile PBS and analyzed in dilution series on karmali agar (Oxoid, Wesel, Germany) in a microaerophilic atmosphere at 37 °C for at least 48 h. Cardiac blood (0.2 mL) was immediately streaked out on karmali agar plates.

Analysis of the intestinal microbiota

Cultural analyses and biochemical identification of luminal bacterial communities from stomach, duodenum, ileum, and colon were performed as previously described [13–15].

Statistical analysis

Mean values, medians, standard deviations, and levels of significance were determined using appropriate tests as indicated (two-tailed Student's *t*-test, Mann–Whitney *U* test). Two-sided probability (*p*) values ≤ 0.05 were considered significant. All experiments were repeated at least twice.

Results

Oral transplantation of luminal ileal microbiota derived from mice suffering from acute ileitis into conventional mice

We have recently shown that physiological CR against *C. jejuni* infection displayed by adult mice harboring a conventional intestinal microbiota (without intestinal inflammation) is abrogated under inflammatory conditions of the intestinal tract such as acute ileitis [10] accompanied by a tremendous overgrowth of the intestinal microbiota with commensal *E. coli* [13, 14, 18]. We were therefore hypothesizing that oral transplantation of the complex ileal microbiota derived from mice suffering from acute ileitis (i.e. susceptible donor) into conventionally colonized mice (and thus resistant acceptor) might render them sus-

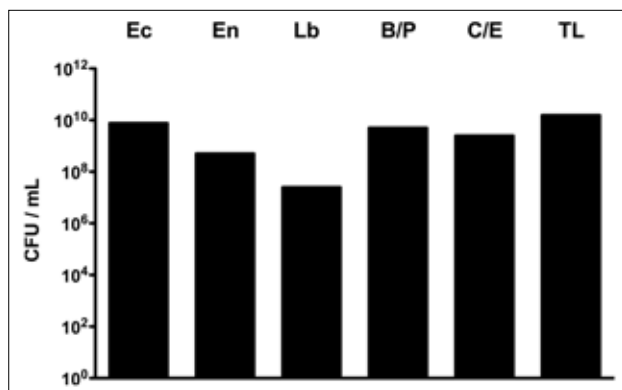


Fig. 1. Bacterial groups within suspension for oral ileitis microbiota transplantation. Luminal ileal contents derived from ten mice with acute *T. gondii*-induced ileitis were pooled for oral ileal microbiota transplantation as described in Materials and methods. Prior gavage main bacterial groups within the suspension such as *E. coli* (Ec), *Enterococcus* spp. (En), *Lactobacillus* spp. (Lb), *Bacteroides/Prevotella* spp. (B/P), *Clostridium/Eubacterium* spp. (C/E), and total bacterial loads (TL) were determined by detection of colony forming units (CFU) per mL on appropriate culture media (see Materials and methods)

ceptible to *C. jejuni* infection. To address this question, C57BL/6 wildtype mice were perorally infected with 100 cysts of *T. gondii* ME49 strain in order to induce acute ileitis, sacrificed at day 8 post-infection (p.i.) and fresh luminal ileal content prepared for subsequent oral TX. The quantitative and qualitative bacterial yield of the suspension with “ileitis microbiota” is shown in Fig. 1: Conventional C57BL/6 wildtype mice were challenged perorally with a total load of more than 10^{10} CFU live bacteria per mL suspension, predominantly consisting of Gram-negative species such as *E. coli* and obligate anaerobic *Bacteroides/Prevotella* spp. Gram-positive bacterial groups, such as enterococci, lactobacilli, and anaerobic *Clostridium/Eubacterium* spp., however, were up to 100 times less abundant.

Next, we quantitatively assessed the intestinal colonization efficiency of bacterial species following peroral ileal microbiota TX by culture. Four days following peroral challenge, TX mice harbored approximately 10^7 CFU *E. coli*, 10^6 CFU *Enterococcus* spp., 10^9 CFU *Lactobacillus* spp., *Bacteroides/Prevotella* spp. and *Clostridium/Eubacterium* spp. per g feces (Fig. 2; TX: open circles; *n* = 10). Notably, right before oral *C. jejuni* infection, the bacterial loads of the respective species in TX mice and in animals at day 4 following acute ileitis induction by *T. gondii* displaying beginning histopathological changes in their ileal mucosa (Fig. 2; ILE: closed circles; *n* = 10) were comparable, except for 1.5 orders of magnitude higher intestinal *Enterococcus* spp. counts in the latter group (*p* < 0.05).

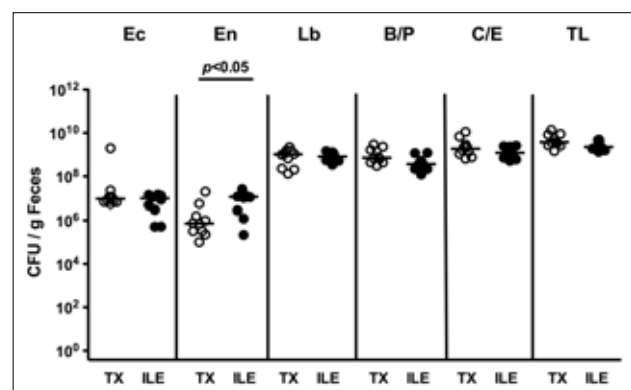


Fig. 2. Fecal microbiota composition in conventionally colonized mice following oral ileitis microbiota transplantation right before *C. jejuni* infection. Four days following oral transplantation of ileitis microbiota (TX, open circles; *n* = 10 mice) into conventional mice and right before oral *C. jejuni* ATCC 43431 infection, fecal samples were obtained to quantitatively assess *E. coli* (Ec), *Enterococcus* spp. (En), *Lactobacillus* spp. (Lb), *Bacteroides/Prevotella* spp. (B/P), *Clostridium/Eubacterium* spp. (C/E), as well as total bacterial loads (TL) by detection of colony forming units (CFU) per mL on appropriate culture media (see Materials and methods) and compared to conventional mice with beginning acute ileitis 4 days following *T. gondii* infection (ILE, solid circles; *n* = 10). Medians (black bars) and levels of significance (*p*-values) as determined by the Mann–Whitney *U* test are indicated. Data shown are representative for three independent experiments

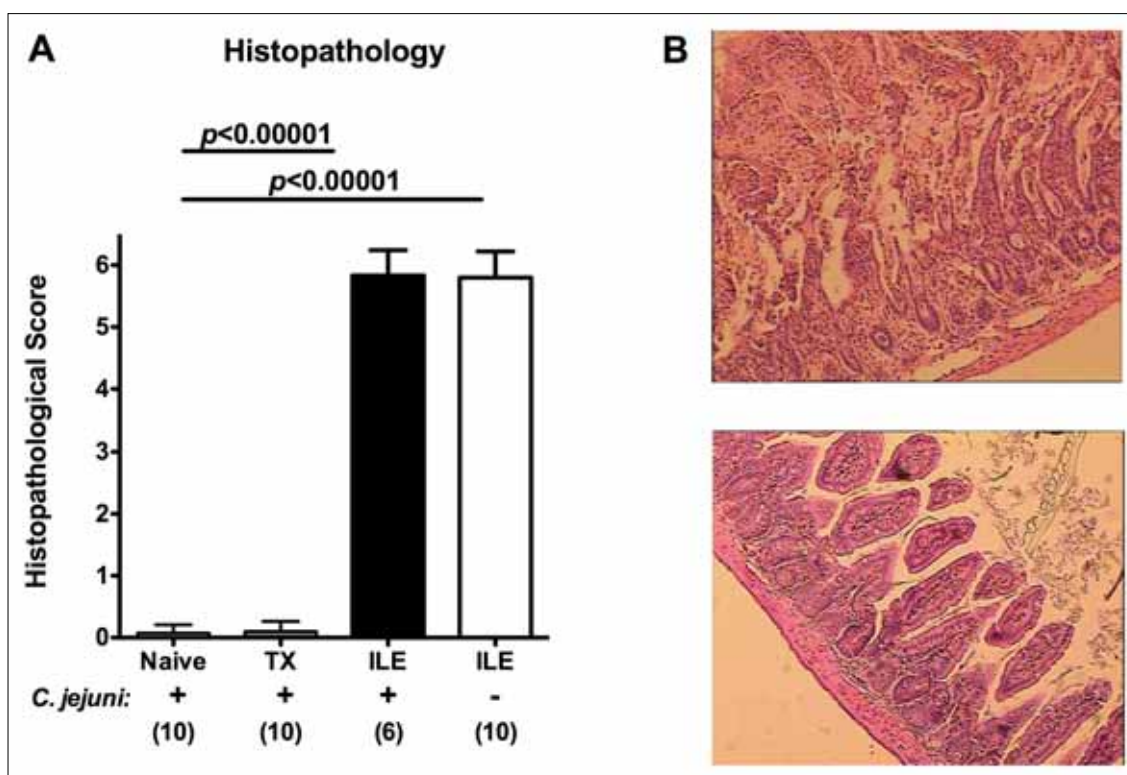


Fig. 3. Ileal histopathology in *C. jejuni* infected mice following oral ileitis microbiota transplantation or induction of acute ileitis. (A) Luminal ileal contents derived from ten mice with acute *T. gondii*-induced ileitis were pooled for oral ileitis microbiota transplantation into mice harbouring a conventional microbiota by gavage as described in Materials and methods (TX). Four days following transplantation, TX mice were perorally infected with *C. jejuni* strain ATCC 43431 (as indicated on x-axis: + *C. jejuni*) and compared to conventional mice without TX (Naive). In addition, conventionally colonized animals were orally infected with *T. gondii* in order to induce acute ileitis. At day 4 following ileitis induction (the time point when beginning histopathological changes of the ileal mucosa can be detected; ILE) mice were orally infected with *C. jejuni* strain ATCC 43431 (as indicated on x-axis: + *C. jejuni*; black bar) and compared to mice without *C. jejuni* infection (as indicated on x-axis: - *C. jejuni*; white bar). Histopathological changes of the ileal mucosa were assessed in ileum paraffin sections at day 8 following ileitis induction applying a standardized histopathological score. Numbers of analyzed animals are given in parentheses. Means, standard deviations and levels of significance (*p*-values) determined by Student's *t*-test are indicated. Data shown are representative for three independent experiments. (B) Representative photomicrographs of HE-stained ileal paraffin sections ($\times 200$ magnification) of mice following acute *T. gondii*-induced ileitis irrespective of *C. jejuni* infection (top). Micrograph shows severe necrosis affecting all layers and entirely disturbed villus structures. Conventional mice with transplanted ileitis microbiota (TX) displayed an intact ileal mucosa 4 days following *C. jejuni* comparable to non-transplanted conventional (Naive) mice (bottom)

Thus, conventional mice with transplanted ileitis microbiota as well as mice with subtle ileal mucosal histopathology following ileitis induction displayed relatively comparable commensal bacterial species loads in their feces right before *C. jejuni* infection.

Transplantation of ileal microbiota derived from mice with acute ileitis does not abrogate CR against C. jejuni

We next infected with ileitis microbiota transplanted mice as well as animals 4 days following ileitis induction perorally with 10^9 CFU *C. jejuni* ATCC 43431 strain for three consecutive days, respectively. Whereas 2 days following *C. jejuni* infection neither TX mice nor naive conventionally colonized control animals displayed histopathological changes within their ileal mucosa (Fig. 3A, B bottom), mice with preceding ileitis induction 4 days prior *C. je-*

juni infection were severely compromised due to acute transmural small intestinal inflammation as indicated by histopathological scores of 5.7 ± 0.5 (Fig. 3A) and severe ileal necroses (Fig. 3B, top). Of note, *C. jejuni* infection did not further deteriorate *T. gondii*-induced acute ileitis as indicated by comparable histopathological scores versus uninfected mice with acute ileitis (Fig. 3A).

Lack of C. jejuni colonization and translocation in conventionally colonized mice following ileitis microbiota transplantation

We next assessed the pathogen loads in the GI tract following *C. jejuni* infection. Whereas TX mice had expelled the pathogen within the first 2 days following the latest infection, animals with *T. gondii*-induced ileitis were stably infected by *C. jejuni* (Fig. 4; $p < 0.0001$). Furthermore,

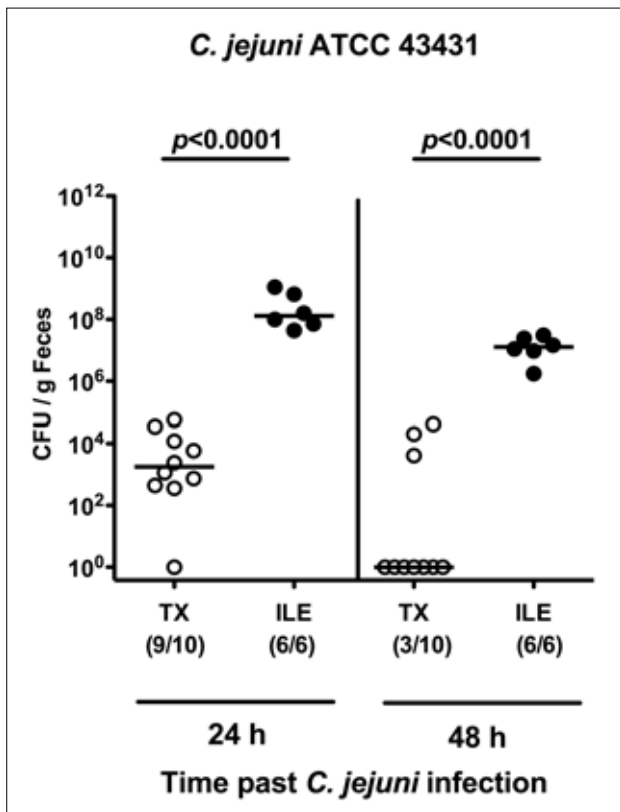


Fig. 4. Kinetic analysis of *C. jejuni* colonization in conventional mice following oral ileitis microbiota transplantation. Mice harboring a conventional intestinal microbiota were orally infected with *T. gondii* in order to induce ileitis (ILE; filled circles). At day 4 following ileitis induction (the time point when beginning histopathological changes of the ileal mucosa can be detected) mice were orally infected with *C. jejuni* strain ATCC 43431 as described in Materials and methods. In addition, conventionally colonized mice were subjected to oral transplantation of luminal ileum flora derived from mice with acute ileitis (TX; open symbols) and after 4 days orally infected with *C. jejuni* strain ATCC 43431. The pathogen densities in the feces were quantified 24 and 48 h following infection by cultural analysis of live *C. jejuni* (CFU, colony forming units) per g feces. Numbers of animals harboring *C. jejuni* out of the total number of analyzed animals are given in parentheses. Medians (black bars) and levels of significance (p -values) determined by Mann–Whitney U test are indicated. Data shown are representative for three independent experiments

2 days following infection, live *C. jejuni* could be cultured at high levels from proximal and distal parts of the intestinal tract in virtually all mice suffering from acute ileitis with maximum loads in the inflamed ileum of up to 10^{10} CFU/g luminal content (Fig. 5A), whereas in only 20–30% of ileal microbiota transplanted animals *C. jejuni* could be detected at low numbers in the duodenal, ileal, and colonic lumen (Fig. 5A). Moreover, *C. jejuni* did not translocate to extra-intestinal tissue sites in transplanted animals, but could be cultured from mesenteric lymph nodes (MLNs; 100%), spleens (66.7%), livers (66.7%), and even cardiac blood (83.3%) of mice with acute ileitis (Fig. 5B).

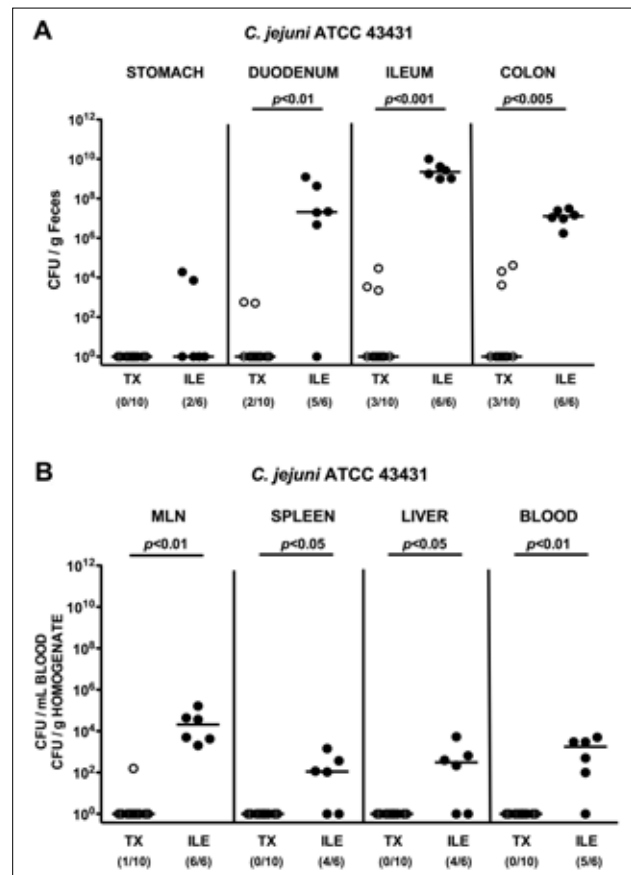


Fig. 5. *C. jejuni* colonization along the gastrointestinal tract and translocation in mice following oral ileitis microbiota transplantation. Conventional mice were orally infected with *T. gondii* in order to induce ileitis (ILE; filled circles). At day 4 following ileitis induction (the time point when beginning histopathological changes of the ileal mucosa become overt) mice were orally infected with *C. jejuni* strain ATCC 43431 as described in Materials and methods. In addition, conventional mice were subjected to oral transplantation of luminal ileum flora derived from mice with acute ileitis (TX; open symbols) and also orally infected with *C. jejuni* strain ATCC 43431 after 4 days of TX. The pathogen densities along the gastrointestinal tract (stomach, duodenum, ileum, and colon; A) as well as in homogenates of mesenteric lymph nodes (MLN), spleen, liver and cardiac blood (B) were determined 48 h following infection by cultural analysis of live *C. jejuni* (CFU, colony forming units). Numbers of animals harboring *C. jejuni* out of the total number of analyzed animals are given in parentheses. Medians (black bars) and levels of significance (p -values) as determined by Mann–Whitney U test are indicated. Data shown are representative for three independent experiments

Microbiota changes following ileal microbiota transplantation, induction of acute ileitis and C. jejuni infection

Given that following *C. jejuni* infection, the pathogen was expelled in with ileitis microbiota-transplanted animals, but not in conventional mice with induced acute ileitis, we were wondering whether this might be due to changes in the fecal microbiota composition in the course of the *C. jejuni* and/or *T. gondii* infection. To address this question, we

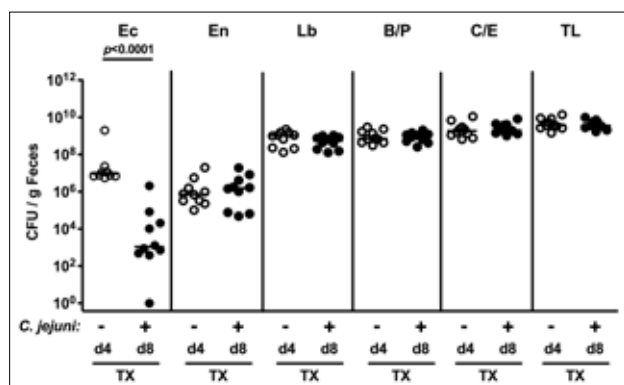


Fig. 6. Fecal microbiota composition of conventional mice following oral ileitis microbiota transplantation and *C. jejuni* infection. Four days (d4, open circles; $n = 10$ mice) following oral ileitis microbiota transplantation of conventional mice (TX) and right before oral *C. jejuni* ATCC 43431 infection ($- C. jejuni$) as well as 4 days following *C. jejuni* ATCC 43431 infection and thus 8 days after TX ($+ C. jejuni$, d8, closed circles; $n = 10$ mice), fecal samples were obtained to quantitatively assess *E. coli* (Ec), *Enterococcus* spp. (En), *Lactobacillus* spp. (Lb), *Bacteroides/Prevotella* spp. (B/P), *Clostridium/Eubacterium* spp. (C/E), as well as total bacterial loads (TL) by detection of colony forming units (CFU) per mL on appropriate culture media (see Materials and methods). Medians (black bars) and levels of significance (p -values) as determined by the Mann–Whitney U test are indicated. Data shown are representative for three independent experiments

performed quantitative cultural analyses of fecal samples derived from mice 4 days after TX and another 4 days following *C. jejuni* infection (Fig. 6). Whereas total bacterial loads as well as counts of *Enterococcus* spp., *Lactobacillus* spp., *Bacteroides/Prevotella* spp., and *Clostridium/Eubacterium* spp. remained virtually unchanged, intestinal *E. coli* loads dropped by four orders of magnitude within 4 days upon *C. jejuni* infection (Fig. 6).

Interestingly, *C. jejuni* infected mice with acute ileitis displayed slightly lower fecal *E. coli* and subtle higher *Lactobacillus* spp. loads of less than one order of magnitude as compared to uninfected control mice 8 days following acute ileitis induction (Fig. 7; $p < 0.01$). Numbers of the remaining bacterial groups as well as the total bacterial load were comparable (Fig. 7).

Taken together, murine CR against *C. jejuni* is abrogated by ileitis induction, but not by ileal microbiota transplantation. In mice suffering from acute ileitis, *C. jejuni* readily colonized the GI tract and translocated to extra-intestinal tissue sites due to barrier defects set by the preceding *T. gondii* infection. The lack of *C. jejuni* colonization in TX mice was accompanied by a marked decrease of commensal *E. coli* loads in the feces 4 days following *C. jejuni* infection.

Discussion

Reliable experimental data regarding intestinal colonization capacities of *C. jejuni*, mechanisms of pathogen–host

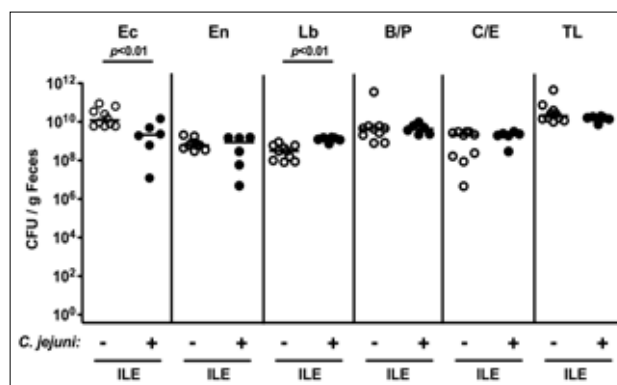


Fig. 7. Fecal microbiota composition of conventional mice following acute ileitis induction and *C. jejuni* infection. Four days following acute ileitis induction by *T. gondii* infection (ILE), conventional mice were orally infected with *C. jejuni* ATCC 43431 ($+ C. jejuni$; closed circles, $n = 6$) and fecal samples obtained 4 days thereafter (8 days following ileitis induction) to quantitatively assess *E. coli* (Ec), *Enterococcus* spp. (En), *Lactobacillus* spp. (Lb), *Bacteroides/Prevotella* spp. (B/P), *Clostridium/Eubacterium* spp. (C/E), as well as total bacterial loads (TL) by detection of colony forming units (CFU) per mL on appropriate culture media (see Materials and methods) and compared to mice at day 8 following ileitis induction without *C. jejuni* infection ($- C. jejuni$; open circles, $n = 10$). Medians (black bars) and levels of significance (p -values) as determined by the Mann–Whitney U test are indicated. Data shown are representative for three independent experiments

interactions, and immunopathological responses in the course of *C. jejuni* infections in vertebrates are scarce. Adult mice without intestinal inflammation harboring a conventional intestinal microbiota display CR against *C. jejuni* infection and are therefore not suitable as valid vertebrate model for the study of *C. jejuni* infection *in vivo* [9]. In the past, we have successfully overcome these limitations by modifying the commensal intestinal microbiota [9, 10, 19]. Given that mice suffering from acute ileitis following oral *T. gondii* infection display a tremendous overgrowth of their intestinal microbiota with commensal *E. coli* [13, 14, 18], we were wondering whether oral transplantation of the complex ileal microbiota derived from mice with acute ileitis (i.e. susceptible donor) into conventionally colonized animals (i.e. resistant acceptor) might result in abrogation of CR against *C. jejuni*. Interestingly, TX mice expelled the pathogen from their GI tract within 2 days upon infection as seen in conventionally colonized adult mice from previous studies [9, 10, 12, 20], whereas conventional mice suffering from acute ileitis harbored *C. jejuni* in their small and large intestines at high levels. Of note, live translocated *C. jejuni* could be cultured from extra-intestinal locations such as MLNs, spleen, liver, and even blood pointing toward bacteremia and sepsis. Interestingly, *C. jejuni* infection did not further deteriorate ileal histopathology following acute ileitis induction. Furthermore, *C. jejuni*-infected mice with acute ileitis harbored slightly lower *E. coli* and higher *Lactobacillus* spp. loads (one order of magnitude difference; $p < 0.05$). Whether this might be attributed to

direct pathogen–commensal interaction or considered an epiphenomenon without biological impact needs to be further unraveled.

When quantitatively comparing main intestinal bacterial groups harbored by mice right before *C. jejuni* infection, TX mice displayed slightly lower *Enterococcus* spp. numbers (approximately 1.5 log) as compared to animals with induced ileitis following *T. gondii* infection, whereas *E. coli* numbers were comparable at approximately 10^7 CFU per g feces. The total bacterial loads as well as the burden of *Lactobacillus* spp. and obligate anaerobic species did not differ between ileitis microbiota transplanted and ileitis-induced mice upon *C. jejuni* infection. From previous experiments, where we artificially raised the intestinal *E. coli* load by adding the commensal into the drinking water, we know, however, that stable *C. jejuni* colonization in the GI tract of conventional mice is facilitated when the intestinal commensal *E. coli* loads are kept above a certain threshold of at least 10^8 CFU per g feces [10]. As soon as the *E. coli* load goes below this threshold, however, e.g. after withdrawal of the *E. coli* suspension, then *C. jejuni* becomes expelled from the GI tract within a few days [10]. This might have been the case in the present study given that at the time point of *C. jejuni* infection, the intestinal *E. coli* burden was slightly (one order of magnitude) below this threshold. In addition, 4 days following *C. jejuni* infection, *E. coli* loads in TX mice had further dropped by approximately four orders of magnitude to 10^3 CFU per g feces whereas the loads of the other main bacterial groups had remained stable. This raises the question whether the microbiota transplantation procedure in our study (e.g. with just one single oral gavage of luminal ileal content derived from mice with acute ileitis) was sufficient to guarantee a stable and long-term quantitative and qualitative establishment of the transplanted species within the acceptor mice where the conventional microbiota competes for nutrients and niches.

As shown in our recolonization experiments of gnotobiotic mice generated by antibiotic treatment, we need to take into consideration that CR against *C. jejuni* is caused by a multi-faceted interplay of the complex microbiota composition, physiological intraluminal milieu of the intestine or so far unknown factors. CR can thus not simply be attributed to absolute loads or relative distribution of single bacterial species [9, 19].

In a few studies, TX of fecal microbiota has been successfully performed in animals already populated with intestinal microbiota without compromising CR by antibiotic treatment before [21]. These studies had made use of both gavage and cohousing, given that rodents are coprophagous and thus continuously reinoculate themselves with microbiota by eating feces from their littermates or themselves [22–27]. In humans, fecal microbiota transplantation has been taken into consideration as a potential treatment option in severe cases of antibiotic-associated diarrhea, i.e. pseudomembranous colitis caused by *Clostridium difficile* toxin, inflammatory bowel diseases, such as ulcerative colitis, irritable bowel syn-

drome and constipation, or diabetes and obesity applying fecal samples from healthy donors via rectal enemas or nasogastric tubes [28–35].

Taken together, in the presented study, a single oral transplantation of microbiota derived from susceptible mice with acute ileitis into conventional resistant mice is not sufficient to abrogate the physiological CR against *C. jejuni*. Mice harboring ileitis microbiota plus displaying beginning histopathologic changes of the ileum mucosa following *T. gondii* infection, however, were highly susceptible to *C. jejuni* infection.

In conclusion, future studies need to be undertaken to further unravel the interplay between intestinal pathogens, intestinal microbiota and intraluminal milieu, as well as the innate and adaptive immune system.

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