Human microbiota associated IL-10−/− mice: A valuable enterocolitis model to dissect the interactions of Campylobacter jejuni with host immunity and gut microbiota

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Received: December 1, 2022 ● Accepted: December 14, 2022
Published online: January 12, 2023

ABSTRACT

Secondary abiotic (SAB) IL-10−/− mice constitute a valuable Campylobacter jejuni-induced enterocolitis model. Given that the host-specific gut microbiota plays a key role in susceptibility of the vertebrate host towards or resistance against enteropathogenic infection, we surveyed immunopathological sequelae of C. jejuni infection in human microbiota associated (hma) and SAB IL-10−/− mice. Following oral challenge, C. jejuni readily colonized the gastrointestinal tract of hma and SAB mice, but with lower numbers in the former versus the latter. Whereas hma mice were clinically less severely compromised, both, macroscopic and microscopic inflammatory sequelae of C. jejuni infection including histopathological and apoptotic cell responses in the colon of IL-10−/− mice were comparably pronounced in the presence and absence of a human gut microbiota at day 6 post-infection. Furthermore, C. jejuni infection of hma and SAB mice resulted in similarly enhanced immune cell responses in the colon and in differential pro-inflammatory mediator secretion in the intestinal tract, which also held true for extra-intestinal including systemic compartments. Notably, C. jejuni infection of hma mice was associated with distinct gut microbiota shifts. In conclusion, hma IL-10−/− mice represent a reliable C. jejuni-induced enterocolitis model to dissect the interactions of the enteropathogen, vertebrate host immunity and human gut microbiota.

KEYWORDS

Campylobacter jejuni, enteropathogenic infection, acute campylobacteriosis model, microbiota-depleted mice, secondary abiotic IL-10−/− mice, human gut microbiota associated IL-10−/− mice, host-pathogen interaction, gut microbiota shifts

INTRODUCTION

Human infections with the zoonotic enteropathogen Campylobacter jejuni are responsible for tremendous health care and socioeconomic burdens all around the globe [1]. In fact, prevalence rates of campylobacteriosis have been progressively increasing not only in high-, but also in middle- and low-income countries to date [2, 3]. The spirally curved and highly motile Gram-negative bacteria reside as commensal members in the intestinal tract of many farm animals without causing clinical signs despite colonization at high loads [4]. Humans usually become infected upon ingestion of contaminated surface water, unpasteurized milk, and raw or undercooked meat products especially of poultry origin, for instance [5]. Infection doses as low as a few hundred live bacteria are sufficient to induce campylobacteriosis symptoms within a few days upon oral ingestion, whereas the severity...
of *C. jejuni* induced disease depends on the arsenal of virulence factors expressed by the enteropathogen on one side and on the immune status of the infected human host on the other [6]. Campylobacteriosis patients commonly complain about abdominal cramps, watery or even bloody diarrhea with mucous discharge, nausea, vomiting, headache, and fever [7, 8]. The acute stage of *C. jejuni* induced acute enterocolitis is characterized by accumulation of immune cells such as neutrophilic granulocytes, macrophages, monocytes and T lymphocytes in the infected colonic mucosa and lamina propria, by crypt abscesses, colonic epithelial cell apoptosis and ulcerations resulting in a malabsorption syndrome [9–12]. Diseased patients are usually treated symptomatically with rehydration, electrolyte substitution and pain-relieving measures, whereas antibiotics are exclusively indicated in severely compromised patients with immune-compromising comorbidities but are otherwise even contraindicated [7, 8]. Usually, the campylobacteriosis symptoms resolve within 14 days post-infection (p.i.) without residues. In rare occasions, however, post-infectious autoimmune morbidities might occur within weeks or even months after the primary infectious event and cause neurological morbidities such as Guillain–Barré syndrome, reactive arthritis, and chronic inflammatory diseases of the intestinal tract [1, 7, 8]. Despite the clinical and socioeconomical importance of campylobacteriosis, the molecular mechanisms underlying the interactions between the enteropathogen and the gut microbiota and the immune system on the host side are only insufficiently understood.

Particularly the host-specific commensal gut microbiota plays a key role in susceptibility of the vertebrate host towards or resistance against enteropathogenic infection [13, 14]. Conventional laboratory mice, for instance, are usually protected even from high-dose *C. jejuni* infection due to the physiological colonization resistance exerted by the murine gut microbiota composition. Following microbiota depletion upon broad-spectrum antibiotic treatment, however, secondary abiotic (SAB) mice can be readily colonized by the pathogen upon oral challenge, which is also the case when associating the SAB mice with a complex human as opposed to murine gut microbiota [13–15]. Since mice are more than 10,000-fold more resistant to lipo-poly saccharide (LPS) and lipo-oligosaccharide (LOS) representing cell wall constituents of Gram-negative bacteria including *C. jejuni* [16], wildtype mice do not exert overt clinical signs of acute campylobacteriosis even after high-dose oral challenge [15]. This can be accomplished, however, by deletion of the murine interleukin-10 gene encoding for the anti-inflammatory cytokine interleukin (IL)-10 and rendering mice susceptible to *C. jejuni* LOS [17]. In fact, within 6 days following oral *C. jejuni* challenge, the enteropathogen was shown to stably establish alongside the gastrointestinal tract of SAB IL-10 deficient (IL-10<−/−>) mice and induce immunopathological key features of severe human campylobacteriosis such as acute enterocolitis with concomitant pro-inflammatory immune responses also in extra-intestinal and even in systemic compartments [18, 19]. In order to additionally unravel the role of the human gut microbiota within the orchestrated enteropathogen-host interactions, we here analyzed the feasibility of the human microbiota associated (hma) IL-10<−/−> mouse infection model. Therefore, we pretreated conventionally raised IL-10<−/−> mice with ampicillin plus sulbactam in order to deplete the murine gut microbiota and subjected the SAB IL-10<−/−> mice to oral transplantation of a fecal microbiota from human donors. The engrafted (and with respect to the gut microbiota “humanized”) IL-10<−/−> mice were then orally challenged with *C. jejuni* and compared to infected SAB counterparts without human microbiota regarding i.) gastrointestinal pathogen loads, ii.) clinical outcome, iii.) macroscopic and microscopic inflammatory sequelae, iv.) intestinal and extra-intestinal including systemic pro-inflammatory immune responses, v.) enteropathogenic translocation and vi.) changes in human gut microbiota compositions upon oral *C. jejuni* infection.

**MATERIAL AND METHODS**

**Mice**

IL-10<−/−> mice (C57BL/6 background) were bred and maintained in the Forschungsinstitute für Experimentelle Medizin, Charité – Universitätsmedizin Berlin, Germany. Mice were housed in cages equipped with 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 *ad libitum* access to autoclaved water and standard chow (food pellets: ssniff R/M-H, V1534-300, Sniff, Soest, Germany). In order to deplete the commensal gut microbiota and generate SAB mice, 3-week-old female and male litter mate IL-10<−/−> mice were transferred to sterile cages (maximum of 3–4 animals per cage) and treated with ampicillin plus sulbactam (2 g/L; Dr. Friedrich Eberth Arzneimittel, Ursensollen, Germany) added to the autoclaved tap water (*ad libitum*) as reported recently [20]. Two days before associating mice with human fecal microbiota, the antibiotic compound was withdrawn and replaced by autoclaved tap water (*ad libitum*) to assure antibiotic washout.

**Human fecal microbiota transplantation**

SAB IL-10<−/−> mice were subjected to a human fecal microbiota transplantation (hFMT) starting one week before *C. jejuni* infection (namely, on days −7, −6 and −5) as described earlier [15, 21]. Briefly, human fecal samples obtained from 5 healthy individuals (all samples free of enteropathogenic bacteria, viruses, and parasites) were thawed, resuspended in sterile phosphate-buffered saline (PBS, Thermo Fisher Scientific, Waltham, MA, USA) and pooled before oral application to mice via gavage (0.3 mL volume). The bacterial microbiota compositions of the human fecal donor suspensions are shown in Fig. 1.
Gut microbiota analyses

Cultural analyses of the human fecal donor solutions and of the murine fecal samples were performed as described previously [15, 22]. For molecular analysis of the gut microbiota composition additionally assessing fastidious and uncultivable bacteria, DNA from fecal and colonic luminal samples was extracted as described previously [22–24]. In brief, DNA extracts and plasmids were quantified using Quant-iT PicoGreen reagent (Invitrogen, Paisley, UK) and adjusted to 1 ng per μl. Then, abundance of the main bacterial groups of the gut microbiota was assessed by the quantitative real time polymerase chain reaction (qRT-PCR) with group-specific 16S rRNA gene primers (Tib MolBiol, Berlin, Germany) as described previously [22–24]. The number of 16S rRNA gene copies/μg DNA of each sample was determined and frequencies of respective bacterial groups calculated proportionally to the eubacterial (V3) amplicon.

C. jejuni infection

C. jejuni strain 81–176 was thawed from frozen stocks and grown on selective karmali agar plates (Oxoid, Wesel, Germany). Age- and sex-matched hma and SAB IL-10+/−/− mice (3-month-old littermates) were infected perorally with 10⁹ colony-forming units (CFU) of the pathogen on days 0 and 1 by gavage (0.3 mL total volume).

Clinical conditions

The clinical outcome in infected mice was quantitatively surveyed 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 previously [19].

Sampling procedures

On day 6 p.i., mice were sacrificed by CO2 asphyxiation. Cardiac blood, ex vivo biopsies from mesenteric lymph nodes (MLN), the colon, liver, kidneys, lungs, 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 and immunohistopathological analyses.

Gastrointestinal C. jejuni loads and bacterial translocation

Six days following C. jejuni infection, the pathogen loads were determined in samples from the stomach, duodenum, ileum, and colon and furthermore, in ex vivo biopsies taken from the MLN, liver, kidneys, lungs, and spleen by culture as described earlier [15]. In brief, respective samples were homogenized in sterile PBS (Thermo Fisher Scientific, Waltham, MA, USA) with a sterile pestle, serial dilutions plated onto karmali agar (Oxoid, Wesel, Germany) and incubated under microaerophilic conditions for at least 48 h and 37 °C. The detection limit of viable pathogens was 100 CFU per g (CFU/g). In order to assess systemic spread of C. jejuni, thioglycollate enrichments broths (BD Bioscience, Heidelberg, Germany) were inoculated with approximately 200 μL of cardiac blood upon necropsy, incubated at 37 °C for one week and streaked onto karmali agar (Oxoid, Wesel, Germany) for further cultivation of C. jejuni [15, 25]. The bacterial translocation frequencies were calculated by
the percentage of *C. jejuni* culture-positive samples out of the total number of analyzed samples taken from respective organ of mice (in %).

**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 × magnification), and histopathological changes in the large intestines quantitatively assessed with histopathological scores [26]: 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 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 [27, 28]. 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 mm²; 400 × magnification).

**Pro-inflammatory mediators**

Intestinal *ex vivo* biopsies collected from the MLN (3 nodes) and the colon (longitudinally cut strips of approximately 1 cm²) were washed in PBS (Thermo Fisher Scientific, Waltham, MA, USA) and additionally, extra-intestinal explants from the liver (approximately 1 cm³) and kidney (one half after longitudinal cut) were 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; Biochrom, Berlin, Germany) and streptomycin (100 μg/mL; Biochrom, Berlin, Germany). After an 18-h incubation period at 37 °C, respective culture supernatants and serum samples were tested for interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), IL-6, and monocyte chemoattractant protein-1 (MCP-1) by the Mouse Inflammation Cytometric Bead Assay (CBA; BD Biosciences, Heidelberg, Germany) on a BD FACSCanto II flow cytometer (BD Biosciences, Heidelberg, Germany). Nitric oxide concentrations were determined by the Griess reaction as stated earlier [22].

**Statistical analyses**

Medians and significance levels were calculated using GraphPad Prism (version 9; San Diego, CA, USA). Normalization of data was assessed by the Anderson-Darling test. The Student’s *t* test and Mann-Whitney test were used for pairwise comparisons of normally and not normally distributed data, respectively. For multiple comparisons, the one-sided 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 applied. Two-sided probability (*P*) values ≤0.05 were considered significant. The Grubb’s test was used to identify definite outliers (α = 0.001). Data were pooled from five 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 pathogen loads following *C. jejuni* infection of hma versus SAB IL-10−/− mice**

We first performed a comparative survey of pathogen loads in hma and SAB IL-10−/− mice over time post oral *C. jejuni* challenges on days 0 and 1 by cultivation of fecal samples. Our cultural analyses revealed that mice from both groups harbored high median *C. jejuni* loads of between 10⁸ and 10⁹ viable bacteria per gram fecal sample as early as 24 h after the latest pathogen challenge (Fig. 2). In hma IL-10−/− mice, however, slightly lower median *C. jejuni* numbers of less than 0.5 to 1.0 order of magnitude were detected in fecal samples taken on days 2, 3, 4 and 6 p.i. if compared to SAB counterparts (*P* < 0.05–0.001; Fig. 2). At the end of the observation period, lower pathogen loads could be assessed alongside the gastrointestinal tract of hma as compared to SAB infected mice, as indicated by lower *C. jejuni* counts in luminal samples taken from the stomach, duodenum, ileum, and colon of the former versus the latter on day 6 p.i. (*P* < 0.05–0.001; Fig. 3). Of note, the differences in pathogen loads were most pronounced in the stomach, but rather subtle in the colon, given that hma mice harbored only
approximately one order of magnitude lower median C. jejuni numbers in their large intestinal lumen as compared to SAB mice ($P < 0.05$; Fig. 3). Hence, following oral pathogen challenge, hma IL-10$^{-/-}$ mice harbored slightly lower C. jejuni numbers in their distal intestines when compared to infected SAB counterparts.

**Changes in gut microbiota composition during C. jejuni infection of hma IL-10$^{-/-}$ mice**

We then asked whether the course of C. jejuni infection affected the commensal bacterial microbiota composition in hma IL-10$^{-/-}$ mice. Therefore, we performed a comprehensive quantitative survey of the fecal bacterial microbiota composition during C. jejuni infection of hma IL-10$^{-/-}$ mice.
applying both, cultural and culture-independent (i.e., 16S rRNA based) molecular methods (Fig. 4). Our analyses revealed lower total bacterial burdens, \((P < 0.001)\) as well as lower fecal loads of lactobacilli \((P < 0.05–0.01)\), bifidobacteria \((P < 0.001)\), Bacteroides/Prevotella species \((P < 0.001)\), Clostridium/Eubacterium species \((P < 0.001)\) and of Clostridium coccoides and leptum groups in fecal samples taken on day 6 p.i. as compared to day 0. Conversely, higher intestinal enterobacterial genes numbers \((P < 0.01)\) were determined in the former versus the latter, however (Fig. 4). Of note, the intestinal abundance of distinct bacterial groups such as lactobacilli and bifidobacteria but also of enterobacteria varied considerably immediately before and after infection of the hma mice as indicated by high standard deviations in respective bacterial numbers and additionally held true for C. coccoides and leptum groups and for Bacteroides/Prevotella species at the end of the observation period. Importantly, in fecal samples of individual hma mice taken immediately before infection, distinct bacteria such as enterobacteria, lactobacilli and bifidobacteria were below the detection limit pointing towards incomplete engraftment following hFMT. Hence, C. jejuni infection of hma IL-10\(^{−/−}\) mice was accompanied by distinct commensal bacterial gut microbiota shifts.

**Fig. 4.** Changes in fecal microbiota composition during C. jejuni infection of human microbiota associated IL-10\(^{−/−}\) mice. Human microbiota associated (hma; squares) IL-10\(^{−/−}\) mice were perorally infected with C. jejuni strain 81-176 on day (d) 0 and d1. Immediately before (d0, white squares) and on d6 post-infection (grey squares), the commensal fecal microbiota compositions were surveyed by (A) culture (expressed as colony-forming units per gram, CFU/g) and by (B) culture-independent, molecular methods (expressed as gene copies per ng DNA; see methods). The box plots indicating the 25th and 75th percentiles of the median (black bar within box), the total range, the significance levels \((P\) values) determined by the Mann-Whitney test, and the numbers of included mice (in parentheses) are indicated. TL, total load; EB, enterobacteria; EC, enterococci; LB, lactobacilli; BB, bifidobacteria; BP, Bacteroides/Prevotella species; CE, Clostridium/Eubacterium species; CC, Clostridium coccoides group; CL, Clostridium leptum group.
Clinical outcome following C. jejuni infection of hma versus SAB IL-10⁻/⁻ mice

We next addressed whether the abundance of a complex human gut microbiota in the murine gastrointestinal tract of IL-10⁻/⁻ mice interfered with the clinical outcome following oral C. jejuni infection. To address this, we quantitatively surveyed the clinical conditions of mice over time p.i. by applying a clinical scoring system assessing abundance of fecal blood, diarrhea and wasting symptoms. As early as 24 h after the latest pathogen challenge, mice from the hma cohort were clinically less severely compromised as compared to infected SAB mice given lower scores for the overall clinical outcomes in the former versus the latter (P < 0.05–0.001; Fig. 5A). SAB mice were particularly more distinctly suffering from fecal blood between days 2 and 6 p.i. (P < 0.05–0.001; Fig. 5B), which also held true for the severity of diarrhea (from day 4 until day 6 p.i.; P < 0.001; Fig. 5C) and wasting symptoms (on days 4 and 6 p.i.; P < 0.001; Fig. 5D). Of note, especially at the late stage of infection (i.e., days 5 and 6 p.i.), the severity of symptoms varied considerably within the hma cohort, given that infected mice exhibited the whole range of clinical scores indicative for almost absent, for mild, for moderate, and also for severe clinical signs of campylobacteriosis (Fig. 5).

Hence, hma IL-10⁻/⁻ mice were clinically less severely compromised by C. jejuni infection as compared to SAB counterparts and exhibited a broad variation in disease manifestation ranging from rather mild to severe disease.

Macroscopic and microscopic inflammatory changes in the colon following C. jejuni infection of hma versus SAB IL-10⁻/⁻ mice

We further tested whether the observed differences in clinical outcomes in C. jejuni infected hma and SAB mice were mirrored by different macroscopic and microscopic inflammatory changes within the infected large intestines. Given that inflammatory conditions of the gastrointestinal tract are associated with shortening of the affected intestinal part [19, 22], we measured the colonic lengths upon necropsy of mice. In fact, C. jejuni infection was associated with shorter colonic lengths at day 6 p.i. (P < 0.001 versus naïve; Fig. 6A), whereas no differences could be assessed in mice from the hma and SAB groups (n.s.; Fig. 6A). Furthermore, we assessed microscopic sequelae of C. jejuni infection and quantitated the histopathological changes in the large intestinal mucosal tissues with a histopathological scoring scheme [26]. When compared to naïve control animals, the histopathological scores in mice from both cohorts were

Fig. 5. Clinical outcome following C. jejuni infection of human microbiota associated versus secondary abiotic IL-10⁻/⁻ mice. Human microbiota associated (hma; squares) and secondary abiotic (SAB, circles) IL-10⁻/⁻ mice were perorally infected with C. jejuni strain 81-176 on day (d) 0 and d1. The clinical outcome was quantitatively surveyed over time post-infection as indicated by applying a clinical scoring scheme for the (A) overall outcome, (B) abundance of fecal blood, (C) diarrheal and (D) wasting symptoms (see methods). Median (black bars), the significance levels (P values) determined by the Student’s t test or Mann-Whitney test, and the numbers of included mice (in parentheses) are indicated.
considerably elevated on day 6 p.i. \((P < 0.001; \text{Fig. 6B})\) indicative for severe pathogen-induced microscopic changes such as marked inflammatory cell infiltration into both, mucosa and submucosa, marked goblet cell loss, abundance of multiple crypt abscesses and even crypt losses. Whereas the histopathological scores were comparable in infected hma and SAB IL-10\(^{−/−}\) mice (n.s.; Fig. 6B), a relatively high standard deviation of histopathological sequelae ranging from moderate to severe alterations could be observed in the colon of the former at day 6 p.i.

Since apoptosis is well known as reliable parameter for the grading of intestinal inflammatory tissue damage as seen in acute campylobacteriosis [15], we quantitated pathogen-induced apoptotic cell responses in the colonic epithelia by \textit{in situ} immunohistochemical staining of large intestinal paraffin sections with an antibody directed against cleaved caspase-3. On day 6 p.i., multifold, but comparably increased numbers of apoptotic colonic epithelial cells could be assessed in mice from both cohorts \((P < 0.001 \text{ versus naive; Fig. 6C})\). Hence, both, macroscopic and microscopic inflammatory sequelae of \textit{C. jejuni} infection including histopathological and apoptotic cell responses in the colon of IL-10\(^{−/−}\) mice were comparably pronounced in the presence and absence of a human gut microbiota.

**Innate and adaptive immune cell responses in the colon following \textit{C. jejuni} infection of hma versus SAB IL-10\(^{−/−}\) mice**

Next, we quantitatively assessed pathogen-induced immune cell responses in the large intestines by \textit{in situ} immunohistochemistry and therefore, stained colonic paraffin sections with antibodies directed against distinct innate and adaptive immune cell subsets. \textit{C. jejuni} infection resulted in enhanced innate immune cell responses in the large intestines of mice from the hma and SAB cohorts as indicated by comparably elevated numbers of F4/80\(^{+}\) macrophages and monocytes and of MPO\(^{+}\) neutrophilic granulocytes in the colonic mucosa and lamina propria of infected mice \((P < 0.001 \text{ versus naive; Fig. 7A and B})\). This held also true for adaptive immune cell subsets given that multifold increased numbers of CD3\(^{+}\) T lymphocytes, of FOXP3\(^{+}\) regulatory T cells and of B220\(^{+}\) B lymphocytes were determined in the large intestines taken from hma and SAB IL-10\(^{−/−}\) mice on day 6 p.i. \((P < 0.01–0.001; \text{Fig. 7C–E})\). Furthermore, under naïve conditions B lymphocyte numbers were slightly higher in hma as compared to SAB IL-10\(^{−/−}\) mice \((P < 0.05; \text{Fig. 7E})\). Hence, \textit{C. jejuni} infection of hma and SAB mice resulted in similarly enhanced innate and adaptive immune cell responses in the colon.

**Intestinal pro-inflammatory mediator secretion in the colon following \textit{C. jejuni} infection of hma versus SAB IL-10\(^{−/−}\) mice**

We further assessed pro-inflammatory mediator secretion in distinct parts of the intestinal tract. On day 6 p.i. of both, hma and SAB IL-10\(^{−/−}\) mice, increased IFN-\(\gamma\) and TNF-\(\alpha\) concentrations were measured in \textit{ex vivo} biopsies derived from the colon and MLN, respectively \((P < 0.01–0.001 \text{ versus naive; Fig. 8A and D})\). Furthermore, \textit{C. jejuni} infected SAB, but not hma mice displayed elevated nitric oxide and TNF-\(\alpha\) concentrations in their colon \((P < 0.001 \text{ versus naive; Fig. 8B and C})\). Hence, \textit{C. jejuni} infection of hma and SAB IL-10\(^{−/−}\) mice resulted in differential pro-inflammatory mediator responses in the intestinal tract.
Extra-intestinal pro-inflammatory mediator secretion following *C. jejuni* infection of hma versus SAB IL-10−/− mice

We further asked whether the differential pathogen-induced pro-inflammatory mediator secretion in hma and SAB IL-10−/− mice also held true for extra-intestinal compartments. On day 6 p.i. increased IFN-γ and TNF-α concentrations were measured in liver and kidney explants derived from both, hma and SAB mice (P < 0.001 versus naive; Fig. 9), whereas less pronounced hepatic TNF-α and renal IFN-γ secretion could be assessed in the former as compared to the latter (P < 0.05; Fig. 9B and C). Hence, *C. jejuni* infection of IL-10−/− mice with and without a human gut microbiota resulted in enhanced pro-inflammatory cytokine secretion from extra-intestinal tissue sites.

Systemic pro-inflammatory mediator secretion following *C. jejuni* infection of hma versus SAB IL-10−/− mice

Next, we tested for systemic pro-inflammatory mediator secretion in *C. jejuni* infected IL-10−/− mice with and without a human gut microbiota. *C. jejuni* infection of mice from both cohorts was associated with enhanced secretion of IFN-γ, TNF-α and IL-6 in serum samples (P < 0.01–0.001; Fig. 10A–C). However, TNF-α concentrations were lower in the sera taken from hma as compared to SAB mice on day 6 p.i. (P < 0.05; Fig. 10B). Furthermore, infected SAB (P < 0.001 versus naive), but not hma IL-10−/− mice (n.s. versus naive); displayed elevated MCP-1 serum concentrations (P < 0.05; Fig. 10D). Hence, alike in intestinal compartments, *C. jejuni* infection of hma and SAB mice was associated with differentially enhanced pro-inflammatory mediator secretion in the systemic compartment.

Bacterial translocation following *C. jejuni* infection of hma versus SAB IL-10−/− mice

We further addressed whether viable *C. jejuni* translocated from the infected intestinal tract to other tissue sites. Our cultural analyses of respective organ homogenates revealed comparable bacterial cell numbers in the MLN, liver, kidneys, lungs, and spleen taken from hma and SAB mice
IL-10−/− mice on day 6 p.i., which also held true for cardiac blood cultures (n.s.; Fig. 11A). When calculating the bacterial translocation frequencies, C. jejuni could be detected in 63.0% and 37.9% of MLN, in 18.5% and 6.9% of the livers, in 3.7% and 0% of the kidneys, in 37.0% and 13.8% of the lungs, in 7.4% and 3.4% of the spleens, and finally, in 7.4% and 0% of blood samples derived from in SAB and hma mice on day 6 p.i. (Fig. 11B). Hence, C. jejuni translocated less frequently from the infected intestines to extra-intestinal tissue sites of hma as compared to SAB IL-10−/− mice.

DISCUSSION

Results of the present study provide evidence that hma IL-10−/− mice constitute a valuable C. jejuni infection model.
to unravel the interactions between the enteropathogen, the commensal human gut microbiota and host immunity. Both, culture and molecular analyses of fecal samples immediately before and after infection confirmed an established complex human gut microbiota within the intestinal tract of the murine host. However, it became evident that the engraftment of the human gut microbiota varied considerably between individual mice. In some murine fecal samples, for instance, lactobacilli and bifidobacteria but also enterobacteria were beyond the detection limit, whereas high numbers of respective bacteria could be detected in others (Fig. 4). An earlier study revealed that following hFMT bifidobacteria, for instance, were eliminated from the intestinal tract of some hma mice which was depending on the respective human fecal donor suspension [29]. This might be explained by inter-individual differences in the intraluminal milieu favoring or counteracting successful establishment of distinct bacterial species in the intestinal tract. Differences in engraftment are without a doubt a limitation of FMT in general and also of the hma mice as infection

![Graphs showing extra-intestinal pro-inflammatory cytokine secretion following C. jejuni infection of human microbiota associated versus secondary abiotic IL-10−/− mice.](image-url)

**Fig. 9.** Extra-intestinal pro-inflammatory cytokine secretion following C. jejuni infection of human microbiota associated versus secondary abiotic IL-10−/− mice. Human microbiota associated (hma; squares) and secondary abiotic (SAB, circles) IL-10−/− mice were perorally infected with C. jejuni strain 81-176 on days 0 and 1 (grey symbols). On day 6 post-infection, (A,C) IFN-γ and (B,D) TNF-α concentrations were measured in ex vivo biopsies taken from (A,B) liver and (C,D) kidneys. Respective naive mice were used as uninfected counterparts (white symbols). The box plots indicating the 25th and 75th percentiles of the median (black bar within box), the total range, the significance levels (P values) determined by the Kruskal-Wallis test with Dunn’s post-correction, and the numbers of included mice (in parentheses) are indicated (n.s., not significant). Outliers have been identified by the Grubb’s test (α = 0.001)
model in particular. It is further a major challenge to assure persistence of the human gut microbial communities in the murine host over time especially when mice are not subjected to a human diet or reassociated with further human fecal samples over time. Nevertheless, others and we have shown to date that hma mice can be used for dissecting pathogen-host interactions [15, 21, 30–37].

Following oral challenge, *C. jejuni* could stably establish within the intestinal tract of *IL-10−/−* mice with relatively high numbers indicating that the human gut microbiota does not cause colonization resistance against the pathogen. Whereas the *C. jejuni* loads were only slightly lower in the distal intestines of hma versus SAB mice (i.e., approximately one order of magnitude), the differences in pathogen densities became more overt in proximal parts of the gastrointestinal tract given two and five orders of magnitude lower *C. jejuni* numbers in the small intestines and the stomach of the former versus the latter, respectively (Fig. 3). Even though not addressed in this study, it is highly likely that the abundance of distinct human gut bacterial members might

Fig. 10. Systemic pro-inflammatory mediator secretion following *C. jejuni* infection of human microbiota associated versus secondary abiotic *IL-10−/−* mice. Human microbiota associated (hma; squares) and secondary abiotic (SAB, circles) *IL-10−/−* mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1 (grey symbols). On day 6 post-infection, (A) IFN-γ, (B) TNF-α, (C) IL-6, and (D) MCP-1 concentrations were measured in serum samples. Respective naive mice were used as uninfected counterparts (white symbols). The box plots indicating the 25th and 75th percentiles of the median (black bar within box), the total range, the significance levels (*P* values) determined by the Kruskal-Wallis test with Dunn’s post-correction, and the numbers of included mice (in parentheses) are indicated (n.s., not significant). Outliers have been identified by the Grubb’s test (*α* = 0.001)
have had direct implications for their competition with *C. jejuni* for limited ecological niches alongside the gastrointestinal tract referred to as competitive exclusion [38] and/or provided a luminal milieu within the intestines of individual mice towards rather hostile conditions countering successful enteropathogenic growth. In addition, differences in gut microbial colonization might result in a different repertoire of secreted antimicrobial peptides such as bacteriocins, for instance, which could in turn, exert anti-*C. jejuni* directed effects [39].

Similar to SAB counterparts, also hma IL-10−/− mice developed overt clinical signs of acute enterocolitis such as bloody diarrhea and wasting symptoms within 6 days following *C. jejuni* infection. As early as 24 h after the latest of two consecutive oral challenges, however, hma mice displayed less severe clinical signs when compared to SAB animals, and the better overall clinical outcome in the former versus the latter became most evident at the end of the observation period (Fig. 5). It is noteworthy that at day 6 p.i., hma mice exhibited a broad range of clinical signs of pathogen-induced disease, varying from very mild to severe symptoms of campylobacteriosis, which is also observed in infected humans [7, 8]. On one hand side, the high standard deviation of clinical scores were rather unexpected given relatively comparable prerequisites and conditions in infected murine individuals since inbred, age- and sex-matched litter mate mice were included into the study. However, as already stated above, inter-individual differences in human

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**Fig. 11.** Bacterial translocation following *C. jejuni* infection of human microbiota associated versus secondary abiotic IL-10−/− mice. Human microbiota associated (hma; squares) and secondary abiotic (SAB, circles) IL-10−/− mice were perorally infected with *C. jejuni* strain 81-176 on days 0 and 1. (A) The pathogen loads were assessed in distinct compartments (as indicated; MLN, mesenteric lymph nodes) on day 6 post-infection by culture and expressed as colony-forming units per gram (CFU/g). Medians (black bar) and numbers of *C. jejuni* culture-positive mice out of the total number of analyzed animals (in parentheses) are indicated. (B) The bacterial translocation rates (bars; in %) to respective compartments (as indicated) are expressed as percentage of the numbers of *C. jejuni* culture-positive samples out of the total number of analyzed animals (in parentheses).
gut bacterial engraftment in the murine gastrointestinal tract could be a plausible explanation for this observation. Of note, in particular potentially probiotic bacterial members of the commensal gut microbiota such as lactobacilli and bifidobacteria could not be detected in some mice, which also held true for enterobacteria (Fig. 4). Furthermore, enterobacterial species such as commensal Escherichia coli have been shown to facilitate C. jejuni colonization given that conditions associated with increased enterobacterial loads and additionally, feeding of live E. coli to conventionally colonized wildtype mice could overcome murine colonization resistance against C. jejuni [14, 40]. Furthermore, enterobacterial overgrowth of the inflamed intestinal lumen was shown to be accompanied with a loss of commensal species diversity and decreased numbers of lactobacilli, bifidobacteria and clostridia [22, 27, 41, 42] that contribute to intestinal homeostasis due to production of short chain fatty acids, for instance [36, 43–45]. One needs to take into consideration, however, that the rather subtle differences in colonic C. jejuni loads in the context of relatively high median enteropathogenic numbers of between 10^8 and 10^9 CFU/g do not sufficiently explain the wide range in clinical signs. Furthermore, at the end of the observation period distinct macroscopic and microscopic inflammatory sequelae of infection were comparable in hma and SAB mice as indicated by similarly pronounced colonic shrinkage as well as histopathological and apoptotic cell responses in the colon on day 6 p.i., respectively (Fig. 6). These results were further supported by similarly enhanced innate and adaptive immune cell responses upon C. jejuni infection of mice with and without a human gut microbiota (Fig. 7). Overall, hma and SAB mice displayed enhanced pro-inflammatory mediator secretion upon C. jejuni infection not only in the intestinal tract (Fig. 8), but also in extra-intestinal organs (Fig. 9) and strikingly, even systemically (Fig. 10). With respect to individual pro-inflammatory mediators, however, secretion was differentially increased at defined tissue sites of mice in the presence or absence of a human gut microbiota. We further tested for translocation of viable C. jejuni from the infected intestines to organs beyond the gastrointestinal tract and found less frequent enteropathogenic translocation events to extra-intestinal including systemic organs in hma when compared to SAB IL-10−/− mice (Fig. 11). It is tempting to speculate that the intestinal epithelial barrier was less distinctly compromised in the former versus the latter at day 6 p.i. which needs to be further addressed by functional tests such as transepithelial resistance measurements in future studies.

CONCLUSION

The here applied hma IL-10−/− mouse model provides a valuable tool to dissect the complex interplay between C. jejuni, the human commensal gut microbiota and immunity of a vertebrate host.

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 (IP7/01K1725D) 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: NWS: Performed experiments, analyzed data, co-wrote the paper.
MSF: Performed experiments.
LQL: Performed experiments.
RB: Performed experiments.
KD: Performed experiments.
SM: Performed experiments, analyzed data, edited the paper.
SB: Provided advice in experimental design, critically discussed results, edited the paper.
MMH: Designed and performed experiments, analyzed data, wrote the paper.

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

ACKNOWLEDGMENTS

We thank Alexandra Bittroff-Leben, Ines Puschendorf, Ulrike Fiebig, 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, respectively.

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