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THE ROLE OF IL-23, IL-22, AND IL-18 IN *CAMPYLOBACTER JEJUNI* INFECTION OF CONVENTIONAL INFANT MICE

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We have recently shown that, within 1 week following peroral *Campylobacter jejuni* infection, conventional infant mice develop self-limiting enteritis. We here investigated the role of IL-23, IL-22, and IL-18 during *C. jejuni* strain 81-176 infection of infant mice. The pathogen efficiently colonized the intestines of IL-18$^{-/-}$ mice only, but did not translocate to extra-intestinal compartments. At day 13 postinfection (p.i.), IL-22$^{-/-}$ mice displayed lower colonic epithelial apoptotic cell numbers as compared to wildtype mice, whereas, conversely, colonic proliferating cells increased in infected IL-22$^{-/-}$ and IL-18$^{-/-}$ mice. At day 6 p.i., increases in neutrophils, T and B lymphocytes were less pronounced in gene-deficient mice, whereas regulatory T cell numbers were lower in IL-23p19$^{-/-}$ and IL-22$^{-/-}$ as compared to wildtype mice, which was accompanied by increased colonic IL-10 levels in the latter. Until then, colonic pro-inflammatory cytokines including TNF, IFN-γ, IL-6, and MCP-1 increased in IL-23p19$^{-/-}$ mice, whereas IL-18$^{-/-}$ mice exhibited decreased cytokine levels and lower colonic numbers of T and B cell as well as of neutrophils, macrophages, and monocytes as compared to wildtype controls. In conclusion, IL-23, IL-22, and IL-18 are differentially involved in mediating *C. jejuni*-induced immunopathology of conventional infant mice.

Keywords: *Campylobacter jejuni, in vivo* infection model, conventional infant mice, IL-23/IL-22/IL-18 axis, Th17 cytokines, pro-inflammatory immune responses, translocation, colonization resistance, intestinal microbiota, apoptosis

Introduction

Human gastroenteritis cases caused by the zoonotic gram-negative bacteria *Campylobacter jejuni* are emerging worldwide [1, 2]. As part of the commensal gut microbiota in a plethora of wild and domestic animal species, transmission to humans occurs from livestock animals via consumption of contaminated meat products or water for instance [3, 4]. Infected patients present with symptoms of considerable variability ranging from mild, non-inflammatory, watery diarrhea to severe, inflammatory, bloody diarrhea associated with abdominal pain that might last for a few weeks, but mostly resolve spontaneously. In rare cases, however, infected patients develop post-infectious sequelae including reactive arthritis and peripheral neuropathies such as Guillain–Barré and Miller–Fisher syndromes later on [5, 6]. Histological changes such as apoptosis, crypt abscesses, ulcerations, and pronounced influx of pro-inflammatory immune cell populations including lymphocytes and neutrophils into the intestinal mucosa and lamina propria can be observed in intestinal tissues derived from infected patients [7, 8]. Despite the global importance of human *C. jejuni* infection, our understanding of the molecular mechanisms underlying campylobacteriosis is limited due to the scarcity of appropriate *in vivo* models. Whereas newborn piglets, weanling ferrets, chicken, gnotobiotic canine pups, and primates have been more or less successfully used for studying *C. jejuni*–host interactions [6], our group has recently shown that, upon peroral *C. jejuni* infection immediately after weaning, 3-week-old conventional infant mice develop acute enteritis within 1 week that resolves thereafter [9–11]. *C. jejuni*-induced immune responses were characterized by increased colonic abundances of effector cells and innate as well as adaptive immune cell subsets that were accompanied by increased colonic secretion of pro-inflammatory

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mediators including TNF, IFN-γ, IL-6, MCP-1, and nitric oxide [9–13]. Interestingly, as compared to adult mice, infant mice harbored higher intestinal loads of commensal enterobacteria such as *Escherichia coli* in their intestines facilitating *C. jejuni* colonization [9–11]. Overall, the infant mouse model displayed key features of human campylobacteriosis and can be regarded as well suitable in order to investigate *Campylobacter*–host interactions in more detail [6, 14]. Very recently, we were able to show that IL-23p19, IL-22, and IL-18 were upregulated in the large intestines of not only *C. jejuni*-infected conventional infant [13] but also of gnotobiotic (i.e., secondary abiotic) mice generated by broad-spectrum antibiotic treatment [15]. Moreover, IL-22 and IL-17 were upregulated in *C. jejuni*-infected IL-10-deficient mice [16]. IL-22 is a cytokine of the IL-10 family and well known not only for its antimicrobial and tissue-protective but also pro-inflammatory properties [17, 18]. Particularly in the intestinal tract, IL-22 exerts its dichotomous actions in a tissue-dependent fashion. Whereas in the large intestinal tract, IL-22 has been shown to act as an anti-inflammatory mediator [18], we have recently shown its pro-inflammatory properties within the small intestines. In acute *Toxoplasma gondii*-induced ileitis, IL-23p19-dependent IL-22 induction resulted in small intestinal necrosis [19–21]. In addition, IL-22 induced the expression of IL-18 mRNA in intestinal epithelial cells following *T. gondii* infection, whereas, conversely, IL-18 amplified IL-22 production from innate lymphoid cells (ILCs) and T helper (Th) -1 cell mediated intestinal inflammation [21].

In the present study, we aimed to shed further light onto the impact of cytokines belonging to the IL-23/IL-22/IL-18 axis during *C. jejuni* infection. To address this, we infected 3-week-old conventional infant IL-23p19$^{-/-}$, IL-22$^{-/-}$, IL-18$^{-/-}$, and corresponding wildtype (WT) mice perorally with *C. jejuni* strain 81-176 immediately after weaning and investigated 1) the gastrointestinal colonization and translocation properties of *C. jejuni* as well as of commensal *E. coli* facilitating pathogenic infection, 2) the clinical outcome of infection, 3) the histopathological changes in the colon including apoptosis, 4) the abundances of distinct immune cell populations in the colonic mucosa and lamina propria, and, furthermore, 5) the large intestinal expression of pro- and anti-inflammatory cytokines.

Methods

Mice and C. jejuni *infection*

Female IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ mice (all in C57BL/6j background) as well as age- and sex-matched C57BL/6j wildtype (WT) control mice were bred and maintained within the same specific pathogen-free (SPF) unit in the Forschungseinrichtungen für Experimentelle Medizin (FEM, Charité – University Medicine Berlin). In order to confirm absence of IL-23p19, IL-22, or IL-18 gene expression, genomic DNA was isolated and disruption of either gene was confirmed by polymerase chain reaction (PCR) [19]. Immediately after weaning, 3-week-old conventional infant mice were perorally infected with 10^9 colony forming units (CFU) of viable *C. jejuni* strain 81-176 in a volume of 0.3 ml phosphate buffered saline (PBS) on two consecutive days (day 0 and day 1) by gavage as described earlier [22].

Clinical score

To assess clinical signs of *C. jejuni*-induced infection on a daily basis, a standardized cumulative clinical score (maximum 12 points), addressing the occurrence of blood in feces (0: no blood; 2: microscopic detection of blood by the Guaiac method using Haemoccult, Beckman Coulter/PCD, Krefeld, Germany; 4: macroscopic blood visible), diarrhea (0: formed feces; 2: pasty feces; 4: liquid feces), and the clinical aspect (0: normal; 2: ruffled fur, less locomotion; 4: isolation, severely compromised locomotion, prefinal aspect) was used [23, 24].

Sampling procedures

Mice were sacrificed at day 6 or day 13 p.i. by isoflurane treatment (Abbott, Greifswald, Germany). Cardiac blood and tissue samples from the gastrointestinal tract (i.e., stomach, duodenum, terminal ileum, and colon), mesenteric lymphnodes (MLN), spleen, liver, and kidney were asserved under sterile conditions. Colonic *ex vivo* biopsies were collected in parallel for immunohistochemical, microbiological, and immunological analyses. Immunohistopathological changes were assessed in colonic samples that were immediately fixed in 5% formalin and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E) or respective antibodies for *in situ* immunohistochemistry as described earlier [13, 25].

Histopathological grading of large intestinal lesions

Histopathological changes were quantitatively assessed in H&E-stained large intestinal paraffin sections, applying a histopathological scoring system by two independent double-blinded investigators as described previously [26]. In brief:

Colonic histopathology (max. 4 points; according to ref. [27]): 0: no inflammation; 1: single isolated cell infiltrates within the mucosa; no epithelial hyperplasia; 2: mild scattered to diffuse cell infiltrates within the mucosa and submucosa; mild epithelial hyperplasia; starting loss of goblet cells; 3: cell infiltrates within mucosa, submucosa, and sometimes transmural; epithelial hyperplasia; loss of goblet cells; 4: cell infiltrates within mucosa, submucosa, and transmural; severe inflammation; loss of

goblet cells, loss of crypts; ulcerations; severe epithelial hyperplasia.

Immunohistochemistry

In situ immunohistochemical analysis of colonic paraffin sections was performed as described previously [26]. Primary antibodies against cleaved caspase-3 (Asp175, Cell Signaling, Beverly, MA, USA, 1:200), Ki67 (TEC3, Dako, Denmark, 1:100), myeloperoxidase (MPO-7, no. A0398, Dako, 1:500), F4/80 (no. 14-4801, clone BM8, eBioscience, San Diego, CA, USA, 1:50), CD3 (no. N1580, Dako, 1:10), FOXP3 (FJK-16s, eBioscience, 1:100), and B220 (eBioscience, 1:200) were used. For each animal, the average number of positively stained cells within at least six high power fields (HPF, 0.287 mm^2, 400× magnification) were determined microscopically by a double-blinded investigator.

Quantitative analysis of bacterial colonization and translocation

Viable *C. jejuni* were detected in feces over time p.i. or at time of necropsy (day 6 or day 13 p.i.) in luminal samples taken from stomach, duodenum, terminal ileum, and colon, by culture of serial dilutions in PBS on Karmali- and Columbia-Agar supplemented with 5% sheep blood (Oxoid) for 2 days at 37 °C under microaerobic conditions using CampyGen gas packs (Oxoid). To quantify bacterial translocation, *ex vivo* biopsies derived from MLN, spleen, liver, and kidney were homogenized in 1 ml sterile PBS, whereas cardiac blood (≈100 µL) was directly streaked onto Karmali-Agar and Columbia-Agar supplemented with 5% sheep blood and cultivated accordingly. Numbers of viable *E. coli* were quantitatively assessed by culture as described earlier [28]. The respective weights of fecal or tissue samples were determined by the difference of the sample weights before and after asservation. The detection limit of viable *C. jejuni* by direct plating was 100 CFU per gram of sample.

Cytokine detection in supernatants of colonic ex vivo *biopsies*

Colonic *ex vivo* biopsies were cut longitudinally and washed in PBS. Strips of approximately 1 cm^2 intestinal tissue were placed in 24-well flat-bottom culture plates (Nunc, Wiesbaden, Germany) containing 500 µL serum-free RPMI 1640 medium (Gibco, Life Technologies, Paisley, UK) supplemented with penicillin (100 U/ml) and streptomycin (100 µg/ml; PAA Laboratories). After 18 h at 37 °C, culture supernatants or serum samples were tested for TNF, IFN-γ, IL-6, MCP-1, and IL-10 by the Mouse Inflammation Cytometric Bead Assay (CBA; BD Biosciences) on a BD FACSCanto II flow cytometer (BD Biosciences).

Statistical analysis

Medians and levels of significance were determined using Mann–Whitney *U* test (GraphPad Prism v6.05, La Jolla, CA, USA) as indicated. Two-sided probability *(p)* values of <0.05 were considered significant.

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, registration number G0135/10). Animal welfare was monitored twice daily by assessment of clinical conditions.

Results

Gastrointestinal colonization and translocation of C. jejuni *in infant mice lacking IL-23p19, IL-22, or IL-18 upon peroral infection*

In the present study, we aimed to dissect the impact of the cytokines IL-23p19, IL-22, and IL-18 in murine campylobacteriosis and applied the infant mouse model of *C. jejuni* infection. Immediately after weaning, 3-week-old IL-23p19$^{-/-}$, IL-22$^{-/-}$, IL-18$^{-/-}$, and corresponding WT mice were perorally infected with 10^9 CFU *C. jejuni* strain 81-176 on two consecutive days (namely, day 0 and day 1) by gavage. We then performed a kinetic survey of colonization densities in individual fecal samples. At day 2 p.i. (i.e., as early as 24 h after the latest infection), 72.7%, 81.0%, 78.9%, and 78.3% of infected infant WT, IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ mice, respectively, harboured *C. jejuni* with a median load of approximately 10^4 CFU per gram fecal samples, whereas mice of the former three genotypes successively expelled the pathogen from their intestinal tract *(Fig 1)*. In fact, fecal *C. jejuni* could be isolated in two thirds of infected IL-18$^{-/-}$ mice, but only in 18.2%, 23.8%, and 27.8% of WT, IL-23p19$^{-/-}$, and IL-22$^{-/-}$ mice, respectively, at day 6 p.i. *(Fig 1)*. Interestingly, median pathogenic loads in fecal samples taken from IL-18$^{-/-}$ mice were even one order of magnitude higher at day 6 as compared to days 2 or 3 p.i. ($p < 0.05$; *Fig 1D*). Mice were then sacrificed at two different time points, namely, day 6 and day 13 p.i., and the *C. jejuni* infection efficiencies were determined alongside the gastrointestinal tract. At day 6 p.i., IL-18$^{-/-}$ mice exhibited higher pathogenic loads in their stomach ($p < 0.05$; *Fig 2A*) and colonic lumen ($p < 0.05$−0.001; *Fig 2D*) as compared to mice of the remaining genotypes. Also the small intestinal tract of IL-18$^{-/-}$ mice could be colonized by *C. jejuni,* with higher loads in the duodenum as compared to IL-23p19$^{-/-}$ and IL-22$^{-/-}$ mice ($p < 0.01$; *Fig 2A*) and higher ileal pathogenic numbers versus WT and IL-22$^{-/-}$ animals ($p < 0.05$;

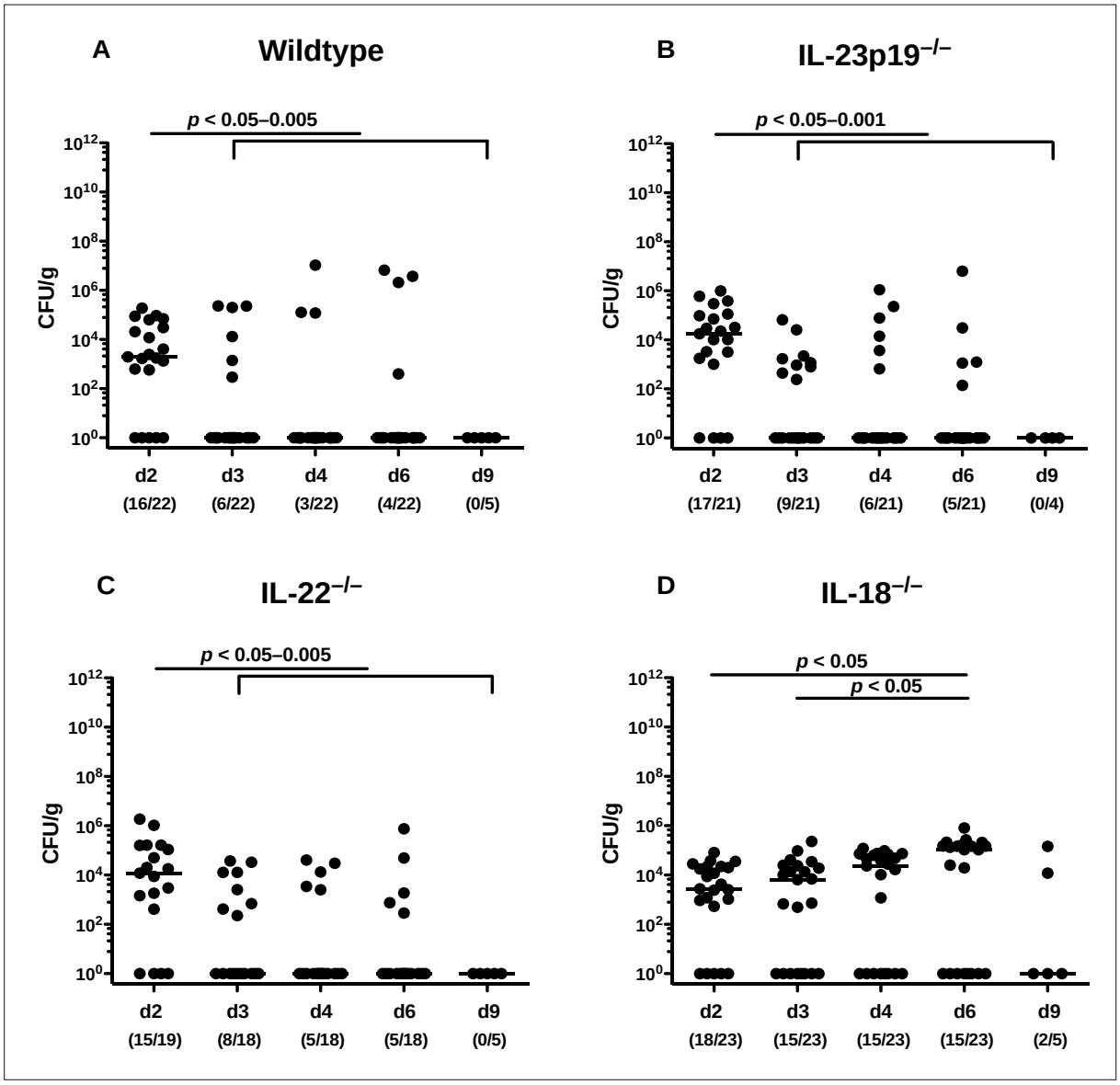

Fig. 1. Kinetic survey of intestinal *C. jejuni* strain 81-176 colonization in perorally infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old A) wildtype, B) IL-23p19$^{-/-}$, C) IL-22$^{-/-}$, and D) IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Pathogenic loads were determined in fecal samples (CFU, colony forming units per gram) at distinct time points (d, day) postinfection as indicated by culture. Medians and numbers of mice harbouring *C. jejuni* strain 81-186 out of the total number of analyzed animals are given in parentheses. Data were pooled from four independent experiments

Fig 2C). Until day 13 p.i., however, conventional infant mice had completely expelled the pathogen from their gastrointestinal tract, except for single IL-18$^{-/-}$ animals *(Fig 2)*. Hence, the gastrointestinal tract of infant IL-18$^{-/-}$ contrary to WT, IL-23p19$^{-/-}$, and IL-22$^{-/-}$ mice could be efficiently colonized by *C. jejuni* following peroral infection. We further investigated whether *C. jejuni* was able to translocate from the intestinal to extra-intestinal tissue sites. Whereas viable *C. jejuni* could be isolated from MLN in single cases only (i.e., 5.9% of WT mice at day 6 p.i. and 20% of IL-18$^{-/-}$ mice at day 13 p.i.; *Fig S1*), homogenates of spleen, liver, and kidney as well as blood samples were all *C. jejuni* negative as determined by culture *(Fig S2)*.

Commensal gastrointestinal E. coli *loads and bacterial translocation in infant mice lacking IL-23p19, IL-22, or IL-18 upon peroral* C. jejuni *infection*

We next addressed the question whether observed differences in pathogenic colonization efficiencies might be due to different gastrointestinal loads of commensal *E. coli* known to facilitate murine *C. jejuni* infection [9]. Surprisingly, before infection, naive IL-22$^{-/-}$, but not IL-18$^{-/-}$, mice exhibited the highest fecal *E. coli* densities with median loads of more than 10^9 CFU per g feces ($p < 0.01$−0.001 vs. remaining groups of mice; *Fig 3*). Furthermore, naive IL-23p19$^{-/-}$, but not IL-18$^{-/-}$, infant mice exhibited

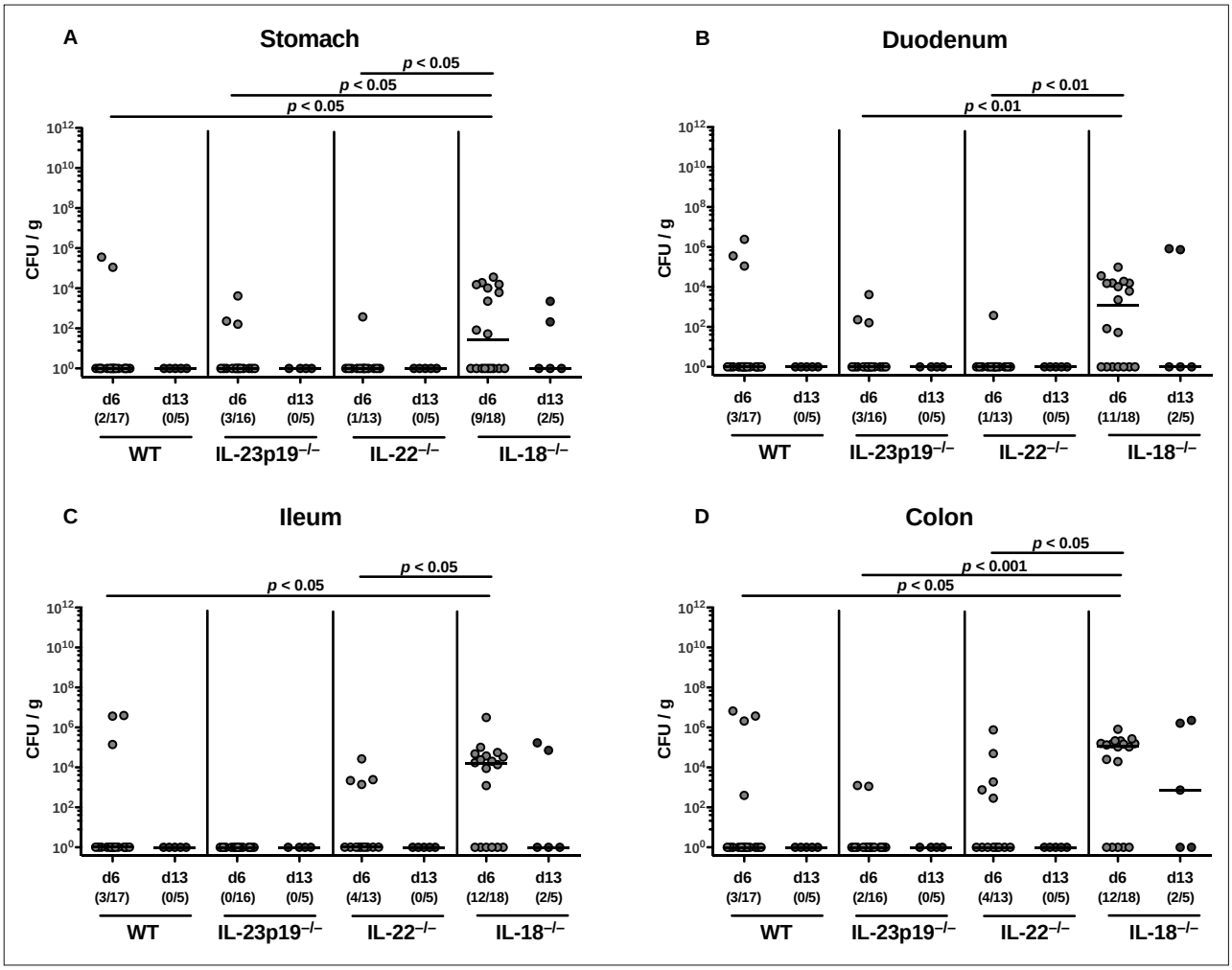

Fig. 2. Gastrointestinal *C. jejuni* loads in perorally infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Pathogenic loads (CFU, colony forming units per gram) were determined in luminal samples taken from the A) stomach, B) duodenum, C) terminal ileum, and D) colon at day (d) 6 (grey circles) or 13 (black circles) postinfection as indicated by culture. Numbers of mice harbouring *C. jejuni* strain 81-176 out of the total number of analyzed animals are given in parentheses. Medians (black bars) and level of significance (*p* value) determined by Mann–Whitney *U* test are indicated. Data were pooled from three independent experiments

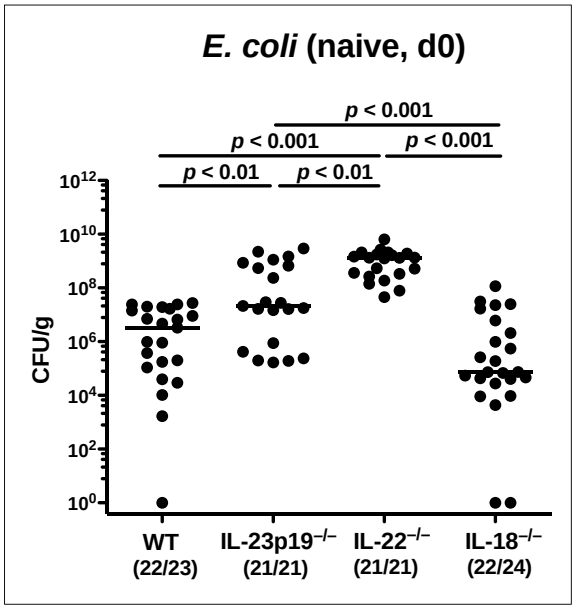

Fig. 3. Fecal commensal *E. coli* loads in uninfected (naive) infant mice lacking IL-23p19, IL-22, or IL-18. Commensal *E. coli* loads (CFU, colony forming units per gram) were quantitated in fecal samples derived from 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice immediately after weaning and before infection (d0). Numbers of mice harbouring *E. coli* out of the total number of analyzed animals are given in parentheses. Medians (black bars) and level of significance (*p* value) determined by Mann–Whitney *U* test are indicated. Data were pooled from four independent experiments

higher fecal *E. coli* numbers as compared to WT controls ($p < 0.01$; *Fig 3*). We further assessed *E. coli* colonization properties in the gastrointestinal tract at days of necropsy. At days 6 and 13 p.i., IL-22$^{-/-}$ mice exhibited higher *E. coli* loads in the duodenum, ileum, and colon ($p < 0.001$ and $p < 0.01$, respectively), but not stomach as compared to respective WT mice *(Fig 4)*. In IL-23p19$^{-/-}$ mice, *E. coli* numbers were higher in the duodenum and colon at day 6 p.i. ($p < 0.001$; *Fig 4B* and *D*) and at either time point in the ileum as compared to WT controls ($p < 0.05$−0.01; *Fig 4C*). In MLN, *E. coli* could be isolated in 12.5% and 25.0% of IL-23p19$^{-/-}$ and 61.5% and 20.0% of IL-22$^{-/-}$ mice at day 6 and day 13 p.i., respectively *(Fig S1B)*. At day 6 p.i., *E. coli* loads in MLN derived from IL-22$^{-/-}$ mice were higher as compared to the remaining genotypes of mice ($p < 0.05$−0.001; *Fig S1B*). Hence, IL-23p19$^{-/-}$ and IL-22$^{-/-}$, but not IL-18$^{-/-}$, infant mice exerted the highest small and large intestinal *E. coli* loads, even though the latter, but not the former two, were stably infected by *C. jejuni*. As for *C. jejuni*, no viable commensal *E. coli* could be isolated from extra-intestinal tissue sites such as spleen, liver, kidney, and cardiac blood *(Fig S3)*.

Macroscopic and microscopic aspects of campylobacteriosis in C. jejuni*-infected infant mice lacking IL-23p19, IL-22, or IL-18*

During the course of *C. jejuni* infection, we surveyed clinical conditions of mice, applying a standardized scoring system. At days 6 and 13 p.i., mice of either genotype (except for IL-22$^{-/-}$ mice during late course of infection) displayed higher clinical scores as compared to day 0 (i.e., immediately before infection) *(Fig 5A),* but were suffering from only minor *C. jejuni*-induced clinical sequelae as ruffling fur and/or microscopic detection of occult blood in fecal samples. Infant IL-23p19$^{-/-}$ mice, however, displayed slightly higher clinical scores as compared to WT and IL-18$^{-/-}$ mice at day 6 p.i. ($p < 0.05$ and $p < 0.01$, respectively; *Fig 5A*). In addition to macroscopic aspects of

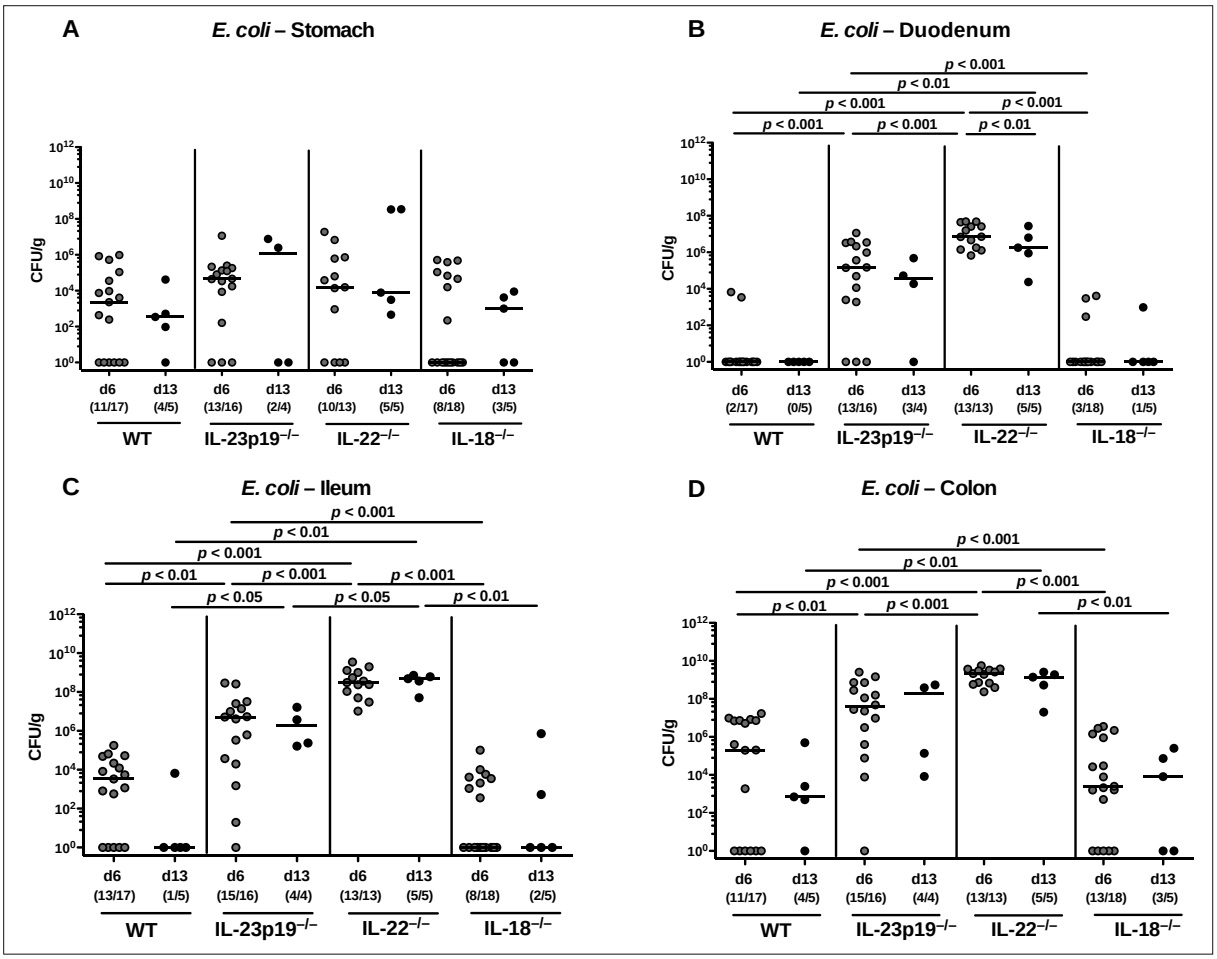

Fig. 4. Gastrointestinal commensal *E. coli* loads in perorally infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Commensal *E. coli* loads (CFU, colony forming units per gram) were determined in luminal samples taken from the A) stomach, B) duodenum, C) terminal ileum, and D) colon at day (d) 6 (grey circles) or 13 (black circles) postinfection as indicated by culture. Numbers of mice harbouring *E. coli* out of the total number of analyzed animals are given in parentheses. Medians (black bars) and level of significance (*p* value) determined by Mann–Whitney *U* test are indicated. Data were pooled from three independent experiments

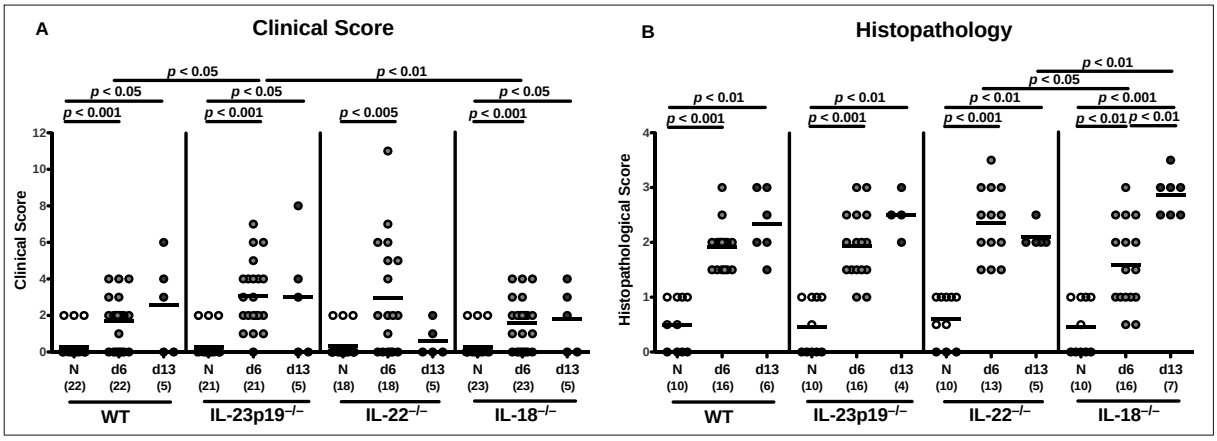

Fig. 5. Clinical and histopathological colonic changes in *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. At day (d) 6 (grey circles) and day 13 (black circles) postinfection, A) clinical conditions and B) histopathological changes in H&E-stained colonic paraffin sections were assessed, applying a standardized clinical and histopathological scoring system, respectively. Naive (N) mice served as uninfected controls (white circles). Medians (black bars), level of significance (p value) determined by Mann–Whitney U test, and numbers of analyzed animals (in parentheses) are indicated. Data were pooled from at least three independent experiments

infected mice, we assessed *C. jejuni*-induced microscopic (i.e., histopathological) changes of H&E-stained colonic paraffin section, applying an established histopathological scoring system. Irrespective of the genotype, histopathological scores were higher in infected as compared to naive mice at either time point and indicative of comparable moderate colonic inflammation. In IL-18$^{-/-}$ mice, however, histopathological sequelae were even more severe at day 13 as compared to day 6 p.i. ($p < 0.01$; *Fig 5B*).

Since apoptosis is a commonly used diagnostic marker for histopathological grading of intestinal inflammation [22], we further stained colonic paraffin sections against caspase-3. Six days following *C. jejuni* infection, WT, IL-23p19$^{-/-}$, and IL-18$^{-/-}$ mice displayed higher apoptotic cell numbers in the colonic epithelial layer as compared to naive controls ($p < 0.05$−0.001; *Fig 6A*). In IL-22$^{-/-}$ mice, however, apoptotic cell numbers tended to increase from day 0 until day 6 p.i. (n.s.), but were lower at days 13 p.i. than 7 days before ($p < 0.05$; *Fig 6A*). At the later time point of necropsy, colonic apoptotic cell numbers were lower in IL-22$^{-/-}$ as compared to WT and IL-23p19$^{-/-}$ mice ($p < 0.05$; *Fig 6A*). In IL-18$^{-/-}$ mice, a trend towards lower apoptotic cell numbers in the colonic epithelium could be observed at both days 6 and 13 p.i. as compared to respective WT mice, but did not reach statistical significance due to high standard deviations within the respective groups (n.s.; *Fig 6A*). Given that Ki67 is a well-known nuclear protein necessary for cellular proliferation [29], we stained

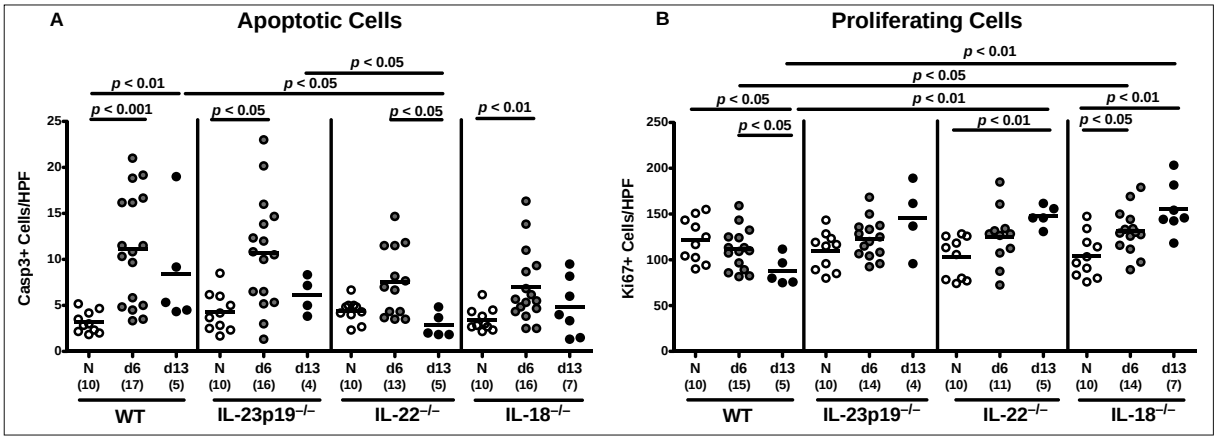

Fig. 6. Apoptotic and proliferating cells in the colonic epithelium of *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. The average number of colonic A) apoptotic cells (positive for caspase-3, Casp3) and B) proliferating cells (positive for Ki67) from at least six high power fields (HPF, 400× magnification) per animal was determined microscopically in immunohistochemically stained colonic paraffin sections at day (d) 6 (grey circles) and day 13 (black circles) postinfection. Naive (N) mice served as uninfected controls (white circles). Medians (black bars), level of significance (p value) determined by Mann–Whitney U test, and numbers of analyzed animals (in parentheses) are indicated. Data were pooled from three independent experiments

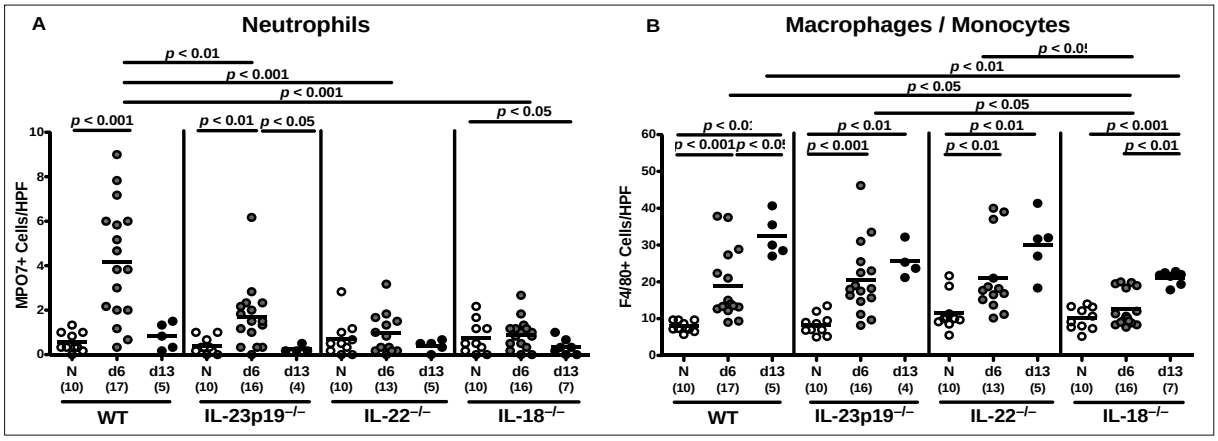

Fig. 7. Colonic innate immune cell responses in *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. The average number of colonic cells positive for A) MPO7 (neutrophils) and B) F4/80 (macrophages and monocytes) from at least six high power fields (HPF, 400× magnification) per animal was determined microscopically in immunohistochemically stained colonic paraffin sections at day (d) 6 (grey circles) and day 13 (black circles) postinfection. Naive (N) mice served as uninfected controls (white circles). Medians (black bars), level of significance (p value) determined by Mann–Whitney U test, and numbers of analyzed animals (in parentheses) are indicated. Data were pooled from three independent experiments

colonic paraffin sections against Ki67 to assess proliferative measures of the colonic epithelium counteracting apoptosis following *C. jejuni* infection. Upon C. *jejuni* infection and conversely to apoptotic cell numbers, Ki67-positive cell numbers increased in the colonic epithelial layer of infant IL-18$^{-/-}$ mice ($p < 0.05$−0.01, *Fig 6B*), and were higher than in WT controls at days 6 and 13 p.i. ($p < 0.05$ and $p < 0.01$, respectively; *Fig 6B*). An increase of colonic proliferating cells was also determined in infant IL-22$^{-/-}$ mice at day 13 p.i. versus WT animals ($p < 0.01$; *Fig 5B*). Hence, despite stable and highest *C. jejuni* colonization densities in IL-18$^{-/-}$ mice, overall, rather subtle macroscopic and moderate microscopic *C. jejuni*-induced sequelae could be observed which did not significantly differ between genotypes of infected mice.

Immune cell responses in C. jejuni-*infected infant mice lacking IL-23p19, IL-22, or IL-18*

Recruitment of pro-inflammatory immune cells to sites of inflammation is a key event in intestinal pathogenic infection including campylobacteriosis [22]. We therefore quantitatively assessed effector cell as well as innate and adaptive immune cell numbers within the large intestinal mucosa and lamina propria of infected mice by *in situ* immunohistochemical staining of colonic paraffin sections. Six days following *C. jejuni* infection, colonic MPO7-positive neutrophilic granulocyte numbers increased in WT and IL-23p19$^{-/-}$ ($p < 0.001$ and $p < 0.01$, respectively), but neither in IL-22$^{-/-}$ nor IL-18$^{-/-}$ mice, and were significantly lower in respective gene-deficient animals as compared to WT controls ($p < 0.01$−0.001; *Fig 7A*). Until day 13 p.i., neutrophil numbers decreased back to naive levels in IL-23p19$^{-/-}$ mice *(Fig 7A)*.

We next stained another subset of effector cells, namely, F4/80-positive macrophages and monocytes. Colonic F4/80-positive cells increased 6 days upon *C. jejuni* infection in WT, IL-23p19$^{-/-}$, and IL-22$^{-/-}$ ($p < 0.01$−0.001), but not IL-18$^{-/-}$, mice *(Fig 7B)*. Seven days later, however, F4/80-positive cell numbers were higher in colons of infant mice, irrespective of their genotype, as compared to respective naive controls ($p < 0.01$−0.001; *Fig 7B*). Moreover, IL-18$^{-/-}$ mice exhibited lower numbers of colonic macrophages and monocytes at both day 6 and day 13 p.i. as compared to WT mice ($p < 0.05$ and 0.01, respectively; *Fig 7B*).

We next investigated *C. jejuni*-induced changes in intestinal adaptive immune cell numbers, namely, T and B lymphocytes as well as Tregs, by staining colonic *ex vivo* biopsies with antibodies directed against CD3, B220, and FOXP3, respectively. Whereas colonic T lymphocytes increased in WT, IL-23p19$^{-/-}$, and IL-18$^{-/-}$ until day 6 p.i., elevated CD3+ cell counts could be observed in IL-22$^{-/-}$ mice later on at day 13 p.i. ($p < 0.001$; *Fig 8A*). Interestingly, already in the naive state, IL-22$^{-/-}$ infant mice displayed higher T cell numbers in their large intestines than WT controls ($p < 0.05$; *Fig 8A*). At day 6 p.i., however, mice of either genotype exhibited lower colonic T as well as B lymphocyte numbers as compared to WT animals ($p < 0.01$−0.001; *Fig 8A* and *C*), whereas, at day 13 p.i., B cells were lower in the colonic mucosa and lamina propria of IL-18$^{-/-}$ than WT controls ($p < 0.05$; *Fig 8C*). Interestingly, FOXP3+ Treg numbers increased successively in the course of *C. jejuni* infection in WT mice ($p < 0.05$ vs. naive mice; *Fig 8B*), whereas this increase was rather delayed in IL-22$^{-/-}$ and IL-18$^{-/-}$ mice as indicated by higher colonic FOXP3+ cell numbers at day 13, but not day 6 p.i. ($p < 0.05$ and $p < 0.01$, respectively vs. naive controls; *Fig 8B*). Furthermore, at day 6 p.i., Treg counts were lower

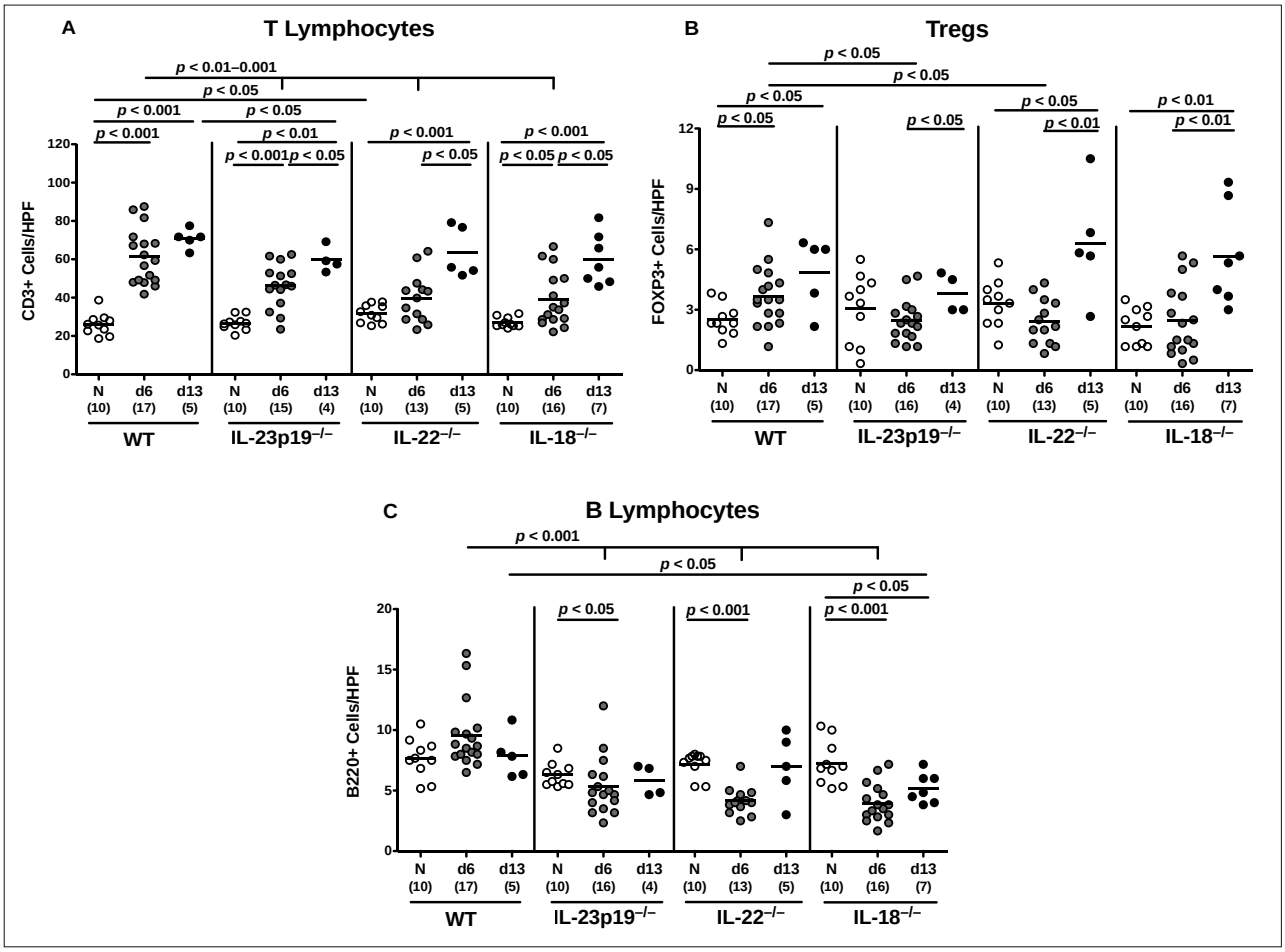

Fig. 8. Colonic adaptive immune cell responses in *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. The average number of colonic cells positive for A) CD3 (T lymphocytes), B) FOXP3 (regulatory T cells, Tregs), and C) B220 (B lymphocytes) from at least six high power fields (HPF, 400× magnification) per animal was determined microscopically in immunohistochemically stained colonic paraffin sections at day (d) 6 (grey circles) and day 13 (black circles) postinfection. Naive (N) mice served as uninfected controls (white circles). Medians (black bars), level of significance (*p* value) determined by Mann–Whitney *U* test, and numbers of analyzed animals (in parentheses) are indicated. Data were pooled from three independent experiments

in the large intestines of IL-23p19$^{-/-}$ and IL-22$^{-/-}$ as compared to WT mice ($p < 0.05$; *Fig 8B*), whereas, at the same time point, colonic B lymphocytes were lower in IL-18$^{-/-}$ versus WT mice ($p < 0.05$; *Fig 8C*).

In the following, we measured pro- and anti-inflammatory cytokine secretion in colonic *ex vivo* biopsies taken from *C. jejuni*-infected infant mice. Until day 6 p.i., TNF, IFN-γ, IL-6, and MCP-1 concentrations increased in large intestines of IL-23p19$^{-/-}$ mice only ($p < 0.05$−0.01; *Fig 9*), whereas respective pro-inflammatory cytokines were significantly lower in colons of IL-18$^{-/-}$ as compared to WT mice at day 6 p.i. ($p < 0.05$−0.01; *Fig 9*). Notably, IL-23p19$^{-/-}$ and IL-22$^{-/-}$ mice exhibited even higher colonic IFN-γ concentrations as compared to respective WT controls at both day 6 and day 13 p.i. ($p < 0.05$−0.01; *Fig 9B*). Furthermore, only in IL-22$^{-/-}$ mice, colonic secretion of the anti-inflammatory cytokine IL-10 was elevated at either time point following *C. jejuni* infection and higher at day 6 p.i. when compared to WT mice, whereas basal IL-10 expression, however, was lower in naive IL-22$^{-/-}$ vs. WT animals (all $p < 0.05$; *Fig 10*). Taken together, during the early stage of *C. jejuni* infection of infant mice, pro-inflammatory cytokines such as TNF, IFN-γ, IL-6, and MCP-1 were increased in large intestines of IL-23p19$^{-/-}$ mice only, whereas IL-18$^{-/-}$ mice, however, exhibited lower colonic pro-inflammatory cytokine levels at day 6 p.i., which is well in line with observed less distinct increases in colonic macrophages and monocytes.

Discussion

In the present study, we investigated the role of mediators belonging to the IL-23/IL-22/IL-18 axis during *C. jejuni* strain 81-176 infection of conventional infant mice that were gene-deficient for the respective regulatory and inflammatory cytokines. Infant mice displayed rather subtle clinical sequelae of infection, whereas moderate histopath-

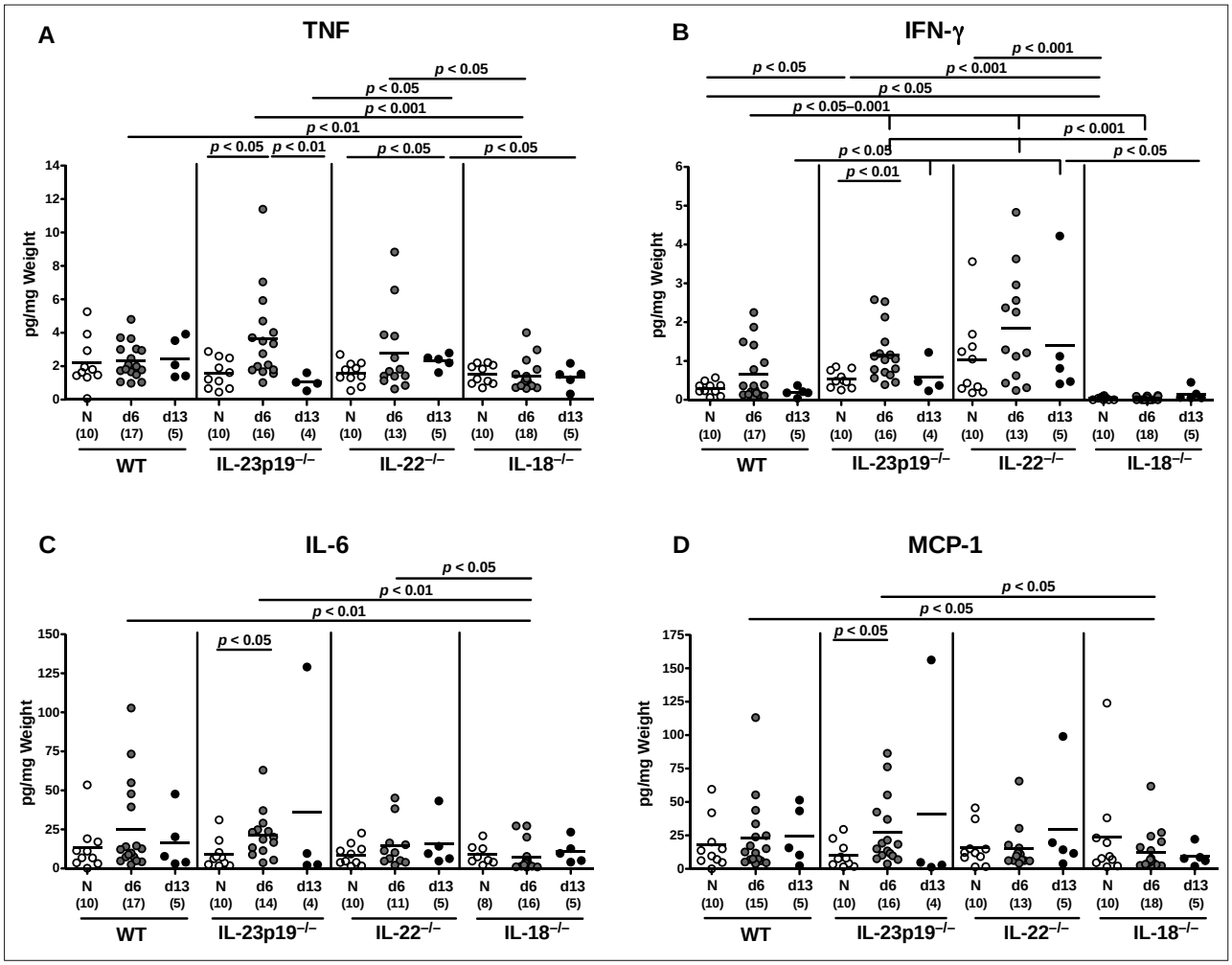

Fig. 9. Pro-inflammatory cytokine secretion in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. A) TNF, B) IFN-γ, C) IL-6, and D) MCP-1 concentrations were determined in supernatants of colonic *ex vivo* biopsies derived at day (d) 6 (grey circles) and day 13 (black circles) postinfection. Naive (N) mice served as uninfected controls (white circles). Medians (black bars), level of significance (*p* value) determined by Mann–Whitney *U* test, and numbers of analyzed animals (in parentheses) are indicated. Data were pooled from three independent experiments

ological changes of the colonic mucosa and lamina propria could be observed, but irrespective of the murine genotype. Overall, differences in distinct *C. jejuni*-induced pro-inflammatory immune responses in infant mice were inconsistent between genotypes. For instance, in IL-22$^{-/-}$ mice, lower numbers of colonic epithelial apoptotic cells, but increased levels of the anti-inflammatory cytokine IL-10, could be observed upon *C. jejuni* infection as compared

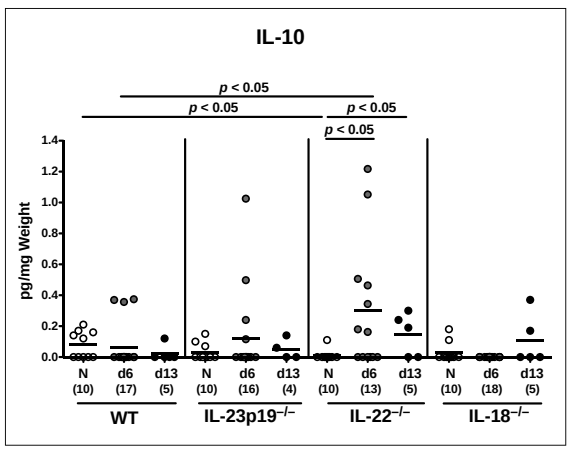

Fig. 10. Anti-inflammatory cytokine secretion in colonic *ex vivo* biopsies derived from *C. jejuni* strain 81-176 infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. IL-10 concentrations were determined in supernatants of colonic *ex vivo* biopsies derived at day (d) 6 (grey circles) and day 13 (black circles) postinfection. Naive (N) mice served as uninfected controls (white circles). Medians (black bars), level of significance (*p* value) determined by Mann–Whitney *U* test, and numbers of analyzed animals (in parentheses) are indicated. Data were pooled from three independent experiments

to WT controls. Infected IL-18$^{-/-}$ mice exhibited a trend towards less distinct large intestinal apoptosis, but significantly lower colonic numbers of innate immune cells such as macrophages and monocytes that were accompanied by lower colonic pro-inflammatory cytokines including TNF, IFN-γ, IL-6, and MCP-1 as compared to WT controls. Furthermore, higher numbers of regenerating/proliferating cells within the colonic epithelium counteracting potential *C. jejuni*-induced epithelial damage could be observed in IL-18$^{-/-}$ than in WT mice. Immune cell populations such as T and B cells, and also neutrophilic granulocytes, were less abundant in the colonic mucosa and lamina propria of IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ as compared to WT mice, whereas regulatory T cells were decreased in IL-23p19$^{-/-}$ and IL-22$^{-/-}$ mice.

Recently, IL-23 was highlighted as a master regulator of mucosal immune responses upon intestinal infection and inflammation [30]. Furthermore, IL-22 was shown to exert effective antimicrobial defence mechanisms against *C. jejuni* including enhanced β-defensin production [31] and was upregulated following *C. jejuni* infection of human intestinal *ex vivo* biopsies [32]. These *ex vivo* results were supported by increased IL-22 concentrations in large intestines and MLNs following *C. jejuni* infection of IL-10$^{-/-}$ mice [16]. To date, however, further data regarding the role of IL-18 in *C. jejuni*–host interaction are lacking. *C. jejuni* infection of three different cell lines (derived from premalignant Barrett's esophagus) resulted in upregulated IL-18 gene expression [33]. Moreover, transcriptomic and proteomic analyses revealed that genes encoding IL-23 and IL-18, but not IL-22, were regulated in differentiated THP-1 macrophages following infection with an adherent and invasive strain of *Campylobacter concisus* [34]. Our data are further supported by results from our previous infection studies in gnotobiotic IL-10$^{-/-}$ mice with a different gram-negative bacterial species, namely, *Arcobacter butzleri,* sharing taxonomic relationship with *C. jejuni*. Results revealed that, in the colon, IL-18 was upregulated upon *A. butzleri* infection during both the early and late phase of infection, whereas colonic IL-22 mRNA increased until day 6 p.i. [35].

In the present study, *C. jejuni* strain 81-176 was able to readily colonize the intestines of infant IL-18$^{-/-}$ mice only, whereas the pathogen was virtually expelled from the intestinal tract of IL-23p19$^{-/-}$, IL-22$^{-/-}$, and WT mice within the first 4 days postinfection. In a previous infection study where we had infected infant mice with a different pathogenic strain, namely, *C. jejuni* strain B2, the pathogen was also cleared rather early in the course of infection [13]. Nevertheless, infected mice exerted infection-induced phenotypes that were depending on the respective genotype of infant mice. The pathogenic colonization kinetics observed in IL-23p19$^{-/-}$, IL-22$^{-/-}$, and WT mice here were virtually comparable. Hence, genotype-dependent differences in immune responses cannot be attributed to differences in intestinal *C. jejuni* densities. It is rather the initial hit of infection that tips the balance towards immunopathological responses [13]. *C. jejuni* colonization is facilitated under conditions of elevated intestinal enterobacterial (i.e., *E. coli*) loads as shown not only in conventional infant mice [10, 11], but also in conventional mice fed viable *E. coli* or a Western diet [9, 36], in mice with a human microbiota [23], and in conventional mice suffering from *T. gondii*-induced acute ileitis [9, 37] or from chronic IL-10$^{-/-}$ colitis [38]. Unexpectedly, not infant IL-18$^{-/-}$, but IL-22$^{-/-}$ mice exhibited the highest gastrointestinal *E. coli* loads. This is well in line with a previous study showing that IL-22$^{-/-}$ mice harboured an altered colonic microbiota towards a higher abundance of the phylum Proteobacteria such as commensal gram-negative bacterial species including *E. coli* [39]. The altered micobiota composition that predisposed IL-22$^{-/-}$ mice to enhanced colitis susceptibility was attributed to the lacking regulatory properties of IL-22 including expression of antimicrobial peptides that are key components for epithelial barrier maintenance [39]. We are finally unable to explain why particularly infant IL-18$^{-/-}$, but not mice of the remaining genotypes, were readily colonized by *C. jejuni* strain 81-176 in our present study. It is, however, highly likely that, so far, unidentified host-related factors might predispose infant IL-18$^{-/-}$ for *C. jejuni* infection.

In summary, our study indicates that cytokines belonging to the IL-23/IL-22/IL-18 axis are differentially involved in mediating and orchestrating pro- and anti-inflammatory immune responses in the large intestinal tract of *C. jejuni*-infected infant mice. We conclude that the regulatory pathways of IL-23, IL-22, and IL-18 following *C. jejuni* infection need to be further unravelled in future studies in order to improve our understanding of the distinct molecular mechanisms underlying campylobacteriosis.

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Competing interests

The authors declare that no competing interests exist.

References

1. Young KT, Davis LM, Dirita VJ: *Campylobacter jejuni:* molecular biology and pathogenesis. Nat Rev Microbiol 5(9), 665–679 (2007)
2. Dasti JI, Tareen AM, Lugert R, Zautner AE, Gross U: *Campylobacter jejuni:* a brief overview on pathogenicity-associated factors and disease-mediating mechanisms. Int J Med Microbiol: IJMM 300(4), 205–211 (2010)
3. Lane JA, Mehra RK, Carrington SD, Hickey RM: The food glycome: a source of protection against pathogen colonization in the gastrointestinal tract. Int J Food Microbiol 142(1–2), 1–13 (2010)
4. Guerry P, Szymanski CM: *Campylobacter* sugars sticking out. Trends Microbiol 16(9), 428–435 (2008)
5. Wakerley BR, Uncini A, Yuki N, Group GBSC, Group GBSC: Guillain–Barre and Miller Fisher syndromes–new diagnostic classification. Nat Rev Neuro 10(9), 537–544 (2014)
6. Masanta WO, Heimesaat MM, Bereswill S, Tareen AM, Lugert R, Gross U, Zautner AE: Modification of intestinal microbiota and its consequences for innate immune response in the pathogenesis of campylobacteriosis. Clin Dev Immunol 2013, 526860 (2013)
7. van Spreeuwel JP, Duursma GC, Meijer CJ, Bax R, Rosekrans PC, Lindeman J: *Campylobacter* colitis: histological immunohistochemical and ultrastructural findings. Gut 26(9), 945–951 (1985)
8. Walker RI, Caldwell MB, Lee EC, Guerry P, Trust TJ, Ruiz-Palacios GM: Pathophysiology of *Campylobacter* enteritis. Microbiol Rev 50(1), 81–94 (1986)
9. Haag LM, Fischer A, Otto B, Plickert R, Kuhl AA, Gobel UB, Bereswill S, Heimesaat MM: Intestinal microbiota shifts towards elevated commensal *Escherichia coli* loads abrogate colonization resistance against *Campylobacter jejuni* in mice. PloS One 7(5), e35988 (2012)
10. Haag LM, Fischer A, Otto B, Grundmann U, Kuhl AA, Gobel UB, Bereswill S, Heimesaat MM: *Campylobacter jejuni* infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses. Eur J Microbiol Immunol (Bp) 2(1), 2–11 (2012)
11. Heimesaat MM, Haag LM, Fischer A, Otto B, Kuhl AA, Gobel UB, Bereswill S: Survey of extra-intestinal immune responses in asymptomatic long-term *Campylobacter jejuni*-infected mice. Eur J Microbiol Immunol (Bp) 3(3), 174–182 (2013)
12. Heimesaat MM, Fischer A, Alutis M, Grundmann U, Boehm M, Tegtmeyer N, Gobel UB, Kuhl AA, Bereswill S, Backert S: The impact of serine protease HtrA in apoptosis, intestinal immune responses and extra-intestinal histopathology during *Campylobacter jejuni* infection of infant mice. Gut Pathog 6, 16 (2014)
13. Alutis ME, Grundmann U, Hagen U, Fischer A, Kuhl AA, Gobel UB, Bereswill S, Heimesaat MM: Matrix metalloproteinase-2 mediates intestinal immunopathogenesis in *Campylobacter jejuni*-infected infant mice. Eur J Microbiol Immunol (Bp) 5(3), 188–198 (2015)
14. Heimesaat MM, Bereswill S: Murine infection models for the investigation of *Campylobacter jejuni*–host interactions and pathogenicity. Berl Muench Tierarztl Wochenschr 128(3–4), 98–103 (2015)
15. Alutis ME, Grundmann U, Fischer A, Hagen U, Kuhl AA, Gobel UB, Bereswill S, Heimesaat MM: The role of gelatinases in *Campylobacter jejuni* infection of gnotobiotic mice. Eur J Microbiol Immunol (Bp) 5(4), 256–267 (2015)
16. Malik A, Sharma D, St Charles J, Dybas LA, Mansfield LS: Contrasting immune responses mediate *Campylobacter jejuni*-induced colitis and autoimmunity. Mucosal Immunol 7(4), 802–817 (2014)
17. Ouyang WJ, Rutz S, Crellin NK, Valdez PA, Hymowitz SG: Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 29, 71–109 (2011)
18. Eidenschenk C, Rutz S, Liesenfeld O, Ouyang W: Role of IL-22 in microbial host defense. Curr Top Microbiol Immunol 380, 213–236 (2014)
19. Munoz M, Heimesaat MM, Danker K, Struck D, Lohmann U, Plickert R, Bereswill S, Fischer A, Dunay IR, Wolk K, Loddenkemper C, Krell HW, Libert C, Lund LR, Frey O, Holscher C, Iwakura Y, Ghilardi N, Ouyang W, Kamradt T, Sabat R, Liesenfeld O: Interleukin (IL)-23 mediates *Toxoplasma gondii*-induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17. J Exp Med 206(13), 3047–3059 (2009)
20. Munoz M, Liesenfeld O, Heimesaat MM: Immunology of *Toxoplasma gondii*. Immunol Rev 240(1), 269–285 (2011)
21. Munoz M, Eidenschenk C, Ota N, Wong K, Lohmann U, Kuhl AA, Wang X, Manzanillo P, Li Y, Rutz S, Zheng Y, Diehl L, Kayagaki N, van Lookeren-Campagne M, Liesenfeld O, Heimesaat M, Ouyang W: Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. Immunity 42(2), 321–331 (2015)
22. Bereswill S, Fischer A, Plickert R, Haag LM, Otto B, Kuhl AA, Dasti JI, Zautner AE, Munoz M, Loddenkemper C, Gross U, Gobel UB, Heimesaat MM: Novel murine infection models provide deep insights into the "menage a trois" of *Campylobacter jejuni*, microbiota and host innate immunity. PloS One 6(6), e20953 (2011)
23. Haag LM, Fischer A, Otto B, Plickert R, Kuhl AA, Gobel UB, Bereswill S, Heimesaat MM: *Campylobacter jejuni* induces acute enterocolitis in gnotobiotic IL-10$^{-/-}$ mice via toll-like-receptor-2 and -4 signaling. PloS One 7(7), e40761 (2012)
24. Alutis ME, Grundmann U, Fischer A, Kuhl AA, Bereswill S, Heimesaat MM: Selective gelatinase inhibition reduces apoptosis and pro-inflammatory immune cell responses in *Campylobacter jejuni*-infected gnotobiotic IL-10 deficient mice. Eur J Microbiol Immunol (Bp) 4(4), 213–222 (2014)
25. Heimesaat MM, Lugert R, Fischer A, Alutis M, Kuhl AA, Zautner AE, Tareen AM, Gobel UB, Bereswill S: Impact of *Campylobacter jejuni* cj0268c knockout mutation on intestinal colonization, translocation, and induction of immunopathology in gnotobiotic IL-10 deficient mice. PloS One 9(2), e90148 (2014)
26. Heimesaat MM, Alutis M, Grundmann U, Fischer A, Tegtmeyer N, Bohm M, Kuhl AA, Gobel UB, Backert S, Bereswill S: 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 4, 77 (2014)
27. Paclik D, Berndt U, Guzy C, Dankof A, Danese S, Holzloehner P, Rosewicz S, Wiedenmann B, Wittig BM, Dignass AU, Sturm A: Galectin-2 induces apoptosis of lamina propria T lymphocytes and ameliorates acute and chronic

experimental colitis in mice. J Mol Med 86(12), 1395–1406 (2008)
28. Heimesaat MM, Bereswill S, Fischer A, Fuchs D, Struck D, Niebergall J, Jahn HK, Dunay IR, Moter A, Gescher DM, Schumann RR, Gobel UB, Liesenfeld O: Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with *Toxoplasma gondii*. J Immunol 177(12), 8785–8795 (2006)
29. Scholzen T, Gerdes J: The Ki-67 protein: from the known and the unknown. J Cell Physiol 182(3), 311–322 (2000)
30. Buonocore S, Ahern PP, Uhlig HH, Ivanov, II, Littman DR, Maloy KJ, Powrie F: Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. Nature 464(7293), 1371–1375 (2010)
31. Zilbauer M, Dorrell N, Boughan PK, Harris A, Wren BW, Klein NJ, Bajaj-Elliott M: Intestinal innate immunity to *Campylobacter jejuni* results in induction of bactericidal human beta-defensins 2 and 3. Infect Immun 73(11), 7281–7289 (2005)
32. Edwards LA, Nistala K, Mills DC, Stephenson HN, Zilbauer M, Wren BW, Dorrell N, Lindley KJ, Wedderburn LR, Bajaj-Elliott M: Delineation of the innate and adaptive T-cell immune outcome in the human host in response to *Campylobacter jejuni* infection. PloS One 5(11), e15398 (2010)
33. Mozaffari Namin B, Soltan Dallal MM, Ebrahimi Daryani N: The Effect of *Campylobacter concisus* on expression of IL-18, TNF-alpha and p53 in Barrett's cell lines. Jundishapur J Microbiol 8(12), e26393 (2015)
34. Kaakoush NO, Deshpande NP, Man SM, Burgos-Portugal JA, Khattak FA, Raftery MJ, Wilkins MR, Mitchell HM: Transcriptomic and proteomic analyses reveal key innate immune signatures in the host response to the gastrointestinal pathogen *Campylobacter concisus*. Infect Immun 83(2), 832–845 (2015)
35. Heimesaat MM, Alter T, Bereswill S, Gölz G: Intestinal expression of genes encoding inflammatory mediators and gelatinases during *Arcobacter butzleri* infection of gnotobiotic IL-10 deficient mice. Eur J Microbiol Immunol (Bp) 6(1), 56–66 (2016)
36. Bereswill S, Plickert R, Fischer A, Kuhl AA, Loddenkemper C, Batra A, Siegmund B, Gobel UB, Heimesaat MM: What you eat is what you get: Novel *Campylobacter* models in the quadrangle relationship between nutrition, obesity, microbiota and susceptibility to infection. Eur J Microbiol Immunol (Bp) 1(3), 237–248 (2011)
37. Heimesaat MM, Plickert R, Fischer A, Gobel UB, Bereswill S: Can microbiota transplantation abrogate murine colonization resistance against *Campylobacter jejuni*? Eur J Microbiol Immunol (Bp) 3(1), 36–43 (2013)
38. Otto B, Haag LM, Fischer A, Plickert R, Kuhl AA, Gobel UB, Heimesaat MM, Bereswill S: *Campylobacter jejuni* induces extra-intestinal immune responses via Toll-like-receptor-4 signaling in conventional IL-10 deficient mice with chronic colitis. Eur J Microbiol Immunol (Bp) 2(3), 210–219 (2012)
39. Zenewicz LA, Yin X, Wang G, Elinav E, Hao L, Zhao L, Flavell RA: IL-22 deficiency alters colonic microbiota to be transmissible and colitogenic. J Immunol 190(10), 5306–5312 (2013)

Supplemental Material

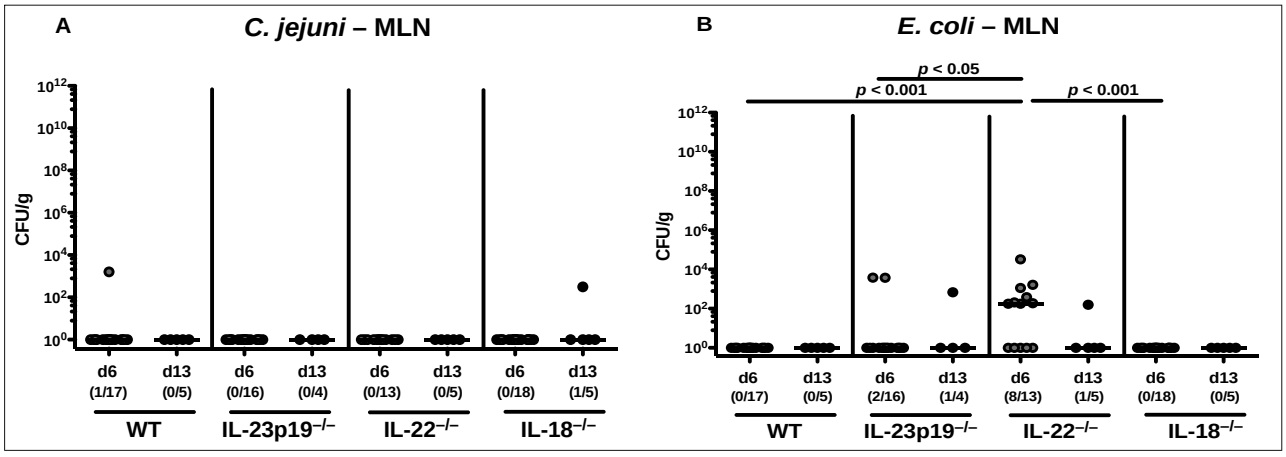

Fig. S1. *C. jejuni* strain 81-176 and commensal *E. coli* translocation to mesenteric lymphnodes in infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. A) *C. jejuni* strain 81-176 and B) commensal *E. coli* loads (CFU, colony forming units per gram) were determined in mesenteric lymphnodes (MLN) at day (d) 6 (grey circles) or 13 (black circles) postinfection as indicated by culture. Numbers of mice harbouring the respective bacteria out of the total number of analyzed animals are given in parentheses. Medians (black bars) and level of significance (p value) determined by Mann–Whitney U test are indicated. Data were pooled from three independent experiments

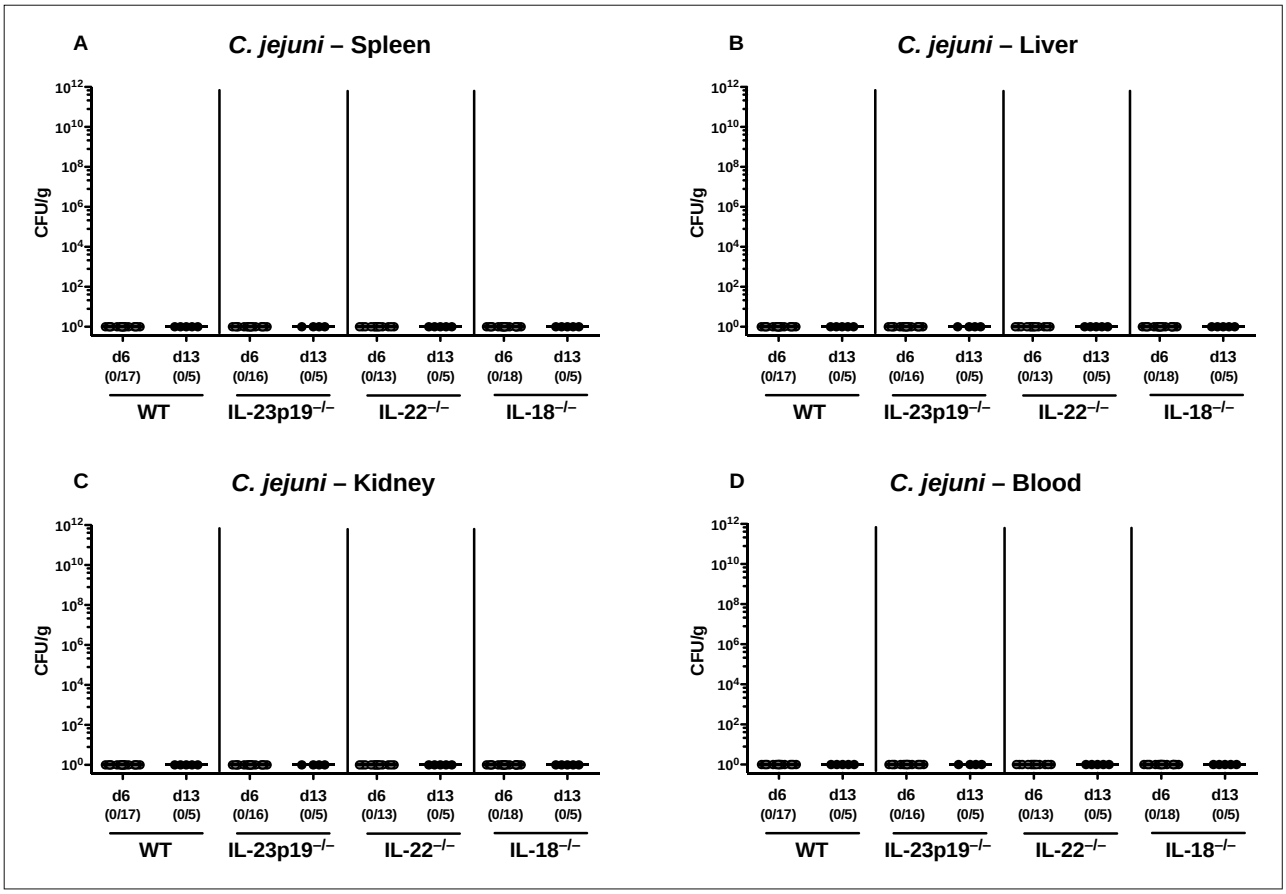

Fig. S2. Extraintestinal translocation of viable intestinal *C. jejuni* strain 81-176 in perorally infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Pathogenic translocation to extraintestinal compartments was assessed by determining *C. jejuni* strain 81-176 loads (CFU, colony forming units per gram) in A) spleen, B) liver, C) kidney, and D) cardiac blood at day (d) 6 (grey circles) or 13 (black circles) postinfection as indicated by culture. Numbers of mice harbouring the pathogen out of the total number of analyzed animals are given in parentheses and medians (black bars) are indicated. Data were pooled from three independent experiments

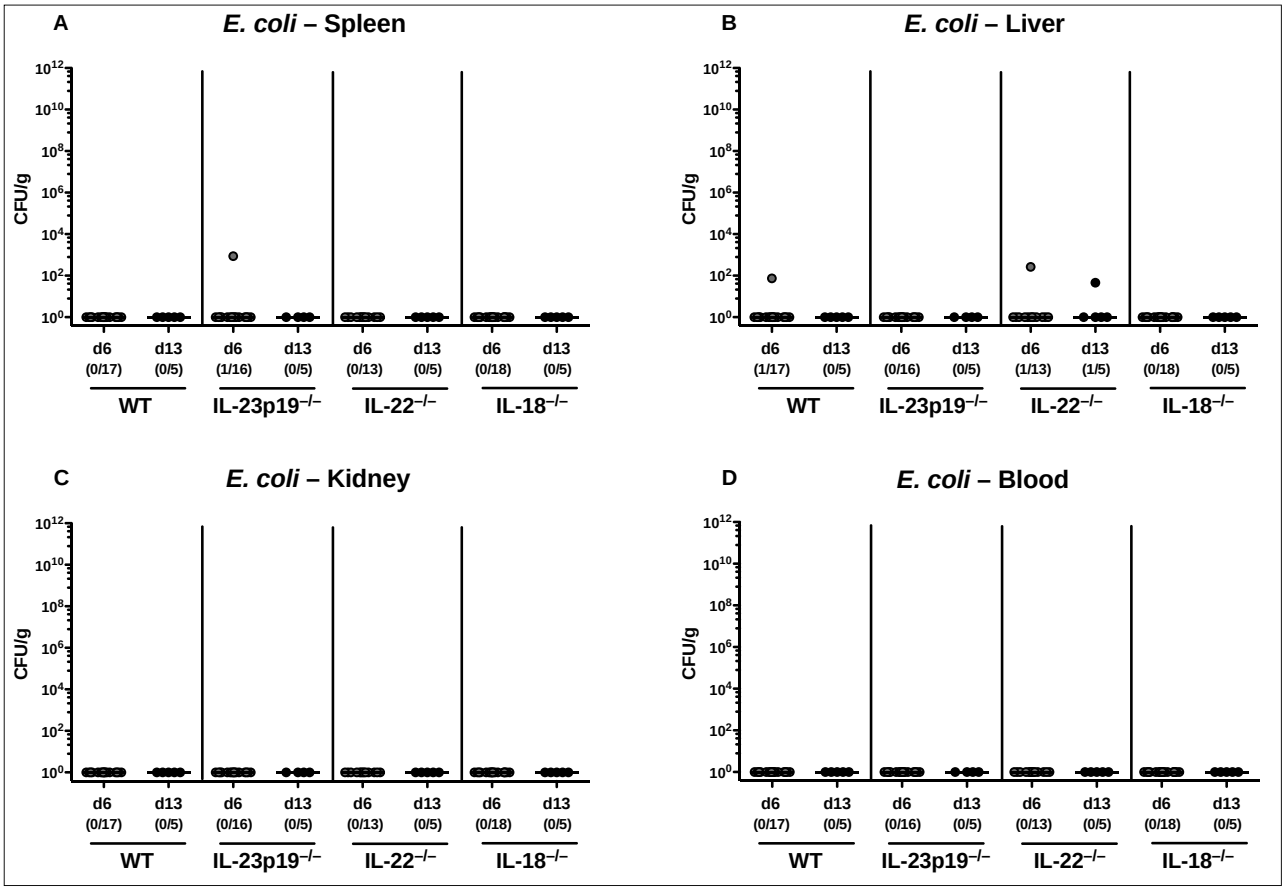

Fig. S3. Extraintestinal translocation of viable commensal intestinal *E. coli* in perorally infected infant mice lacking IL-23p19, IL-22, or IL-18. Immediately after weaning, 3-week-old wildtype (WT), IL-23p19$^{-/-}$, IL-22$^{-/-}$, and IL-18$^{-/-}$ infant mice were perorally infected with *C. jejuni* strain 81-176 by gavage at day 0 and day 1. Translocation of commensal intestinal *E. coli* was assessed by determining bacterial loads (CFU, colony forming units per gram) in A) spleen, B) liver, C) kidney, and D) cardiac blood at day (d) 6 (grey circles) or 13 (black circles) postinfection as indicated by culture. Numbers of mice harbouring *E. coli* out of the total number of analyzed animals are given in parentheses and medians (black bars) are indicated. Data were pooled from three independent experiments